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CONTENTS

Angiotensinogen M235T and angiotensin-converting enzyme I/D gene polymorphism and their association with type 2 diabetes in Egypt

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Abstract: Objectives: Type2 diabetes (T2DM) is the most common subtype of diabetes mellitus. A number of studies have examined the role of genetic polymorphisms on the risk of T2DM, and several variants have been identified as potential susceptibility genes, of those angiotensin-converting enzyme (ACE I/D) and angiotensinogen (AGT M235T). The aim of the current study was to investigate the association between genetic polymorphisms of ACE I/D and AGT M235T genes and T2DM in Egyptian population. Design and methods: A case control study was performed on 138 Egyptian subjects, 58 T2DM patients without hypertension with mean disease duration 7.8 ± 2.1 years and 80 age, and sex matching unrelated healthy controls. Genotyping of the candidate genes were performed by polymerase chain reaction technique (PCR) for ACE I/D gene and by PCR followed by restriction fragment length polymorphism (RFLEP) for AGT M235T gene and the presence or absence of the genotypes was analyzed by gel electrophoresis .We examined the association between each polymorphism and the risk of T2DM by odds ratio (OR) with 95% confidence intervals (95% CI). Results; Individuals who carried the two risk genotypes ACE (DD)/AGT (TT) had 14.5 times (95%CI 1.783-118.083, p=0.012) higher risk of developing T2DM than those who carry one risk genotype. ACE (DD) genotype OR=2.444, 95% CI 1.140-5.240, p=0.022 and AGT (TT) genotype OR=3.9, 95% CI 1.773-8.597, p<0.001. Conclusion: These data indicate an evident association between genetic polymorphisms of ACE I/D and AGT M235T genes and T2DM in Egyptian population.
[Soheir Badr, Naglaa Raafat Abd Raboh and Samia Ali. **Angiotensinogen M235T and angiotensin-converting enzyme I/D gene polymorphism and their association with type 2 diabetes in Egypt**. *Life Sci J* 2012;9(2):1226-1233]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 183

Key words: Type2 diabetes, genetic polymorphisms, angiotensin-converting enzyme and angiotensinogen (AGT M235T).

1. Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent, chronic condition associated with extensive morbidity, decreased quality of life, and increased utilization of health services. T2DM is a worldwide epidemic with a prevalence that is expected to double by the year 2025, affecting over 5% of the adult population [1]. The polygenic nature of T2DM has been a major challenge to identifying genes involved in the pathogenesis of this disease, knowledge that could give rise to new treatments modalities [2,3]. Screening of candidate genes for nucleotide variants that are associated with T2DM is a core component of much diabetes genetics research. Several studies have been analyzed to select the candidate genes because of known or presumed biological or physiological functions. The choice of candidates is inevitably limited by incomplete understanding of the regulation of the processes and the pathophysiology of T2DM [4].

The angiotensinogen (AGT) gene encodes the precursor of the vasoactive hormone angiotensin II, which is the effector peptide of the renin-angiotensin system. The protein encoded by this gene, pre-angiotensinogen or angiotensinogen precursor, is expressed in the liver and is cleaved by the enzyme renin in response to lowered blood pressure. The resulting product, angiotensin I, is then cleaved by angiotensin converting enzyme (ACE) primarily within the kidney to generate the physiologically active enzyme angiotensin II. ACE found in other tissues of the body has no physiological role (ACE has a high density in the lung, but activation here promotes no vasoconstriction, angiotensin II is below physiological levels of action). Angiotensin II acts as an endocrine, autocrine/paracrine, and intracrine hormone.

A polymorphism in exon 2 of the AGT gene a threonine to methionine substitution at position 235 (M235T), has been associated with essential hypertension [5], diabetic nephropathy [6] and coronary heart disease [7]. The ACE polymorphism identified in 1990 by Rigat and co-workers [8] is one of the best researched polymorphisms. This polymorphism of the ACE gene is based on the presence (insertion) or absence (deletion) of a 287-bp Alu1 element inside intron 16 of the ACE gene on chromosome 17 producing three genotypes (DD, II homotypes and ID heterotype) [9].

The highest serum ACE activity was seen in the DD genotype while and the lowest was seen in the II genotype. Since then it has been speculated that these differences in plasma ACE activity associated with the ACE genotype might affect the therapeutic response of ACE inhibitors explaining interindividual variability in cardiovascular or renal response to equivalent doses of ACE inhibitors. Several studies have investigated extent of this effect modification on response to ACE inhibitors for different indications such as hypertension [10], diabetic nephropathy [11] and coronary artery disease [12].

The aim of this study was to investigate the association between genetic polymorphisms of ACE I/D and AGT M235T genes and T2DM in Egyptian population.

2. Subjects and Methods:

Subjects

A total of 58 adult Egyptian T2DM patients (mean age 46.53±6.53 years) were recruited from the outpatient diabetes clinic of Ain Shams University Hospital in addition to 80 age, sex and BMI matched healthy unrelated Egyptian adult subjects (mean age 44.76 ± 7.21 years). Informed consent was obtained from all subjects with approval granted by Ain shams Research and Ethics Committee "Ain-shams Faculty of Medicine federal number IRB00006444, prior to sample collection. Inclusion criteria: fasting plasma glucose≥ 126 mg/dl, Haemoglobin A1c ≥6.5% and/or treatment for diabetes included diet and/or oral antidiabetic drugs to achieve glycemic control. Duration of the disease on diabetic patients was 7.8 ±2.1 years. Recruitment of patients was restricted by the following criteria: the presence of hypertension/or taking antihypertensive drugs, diabetic nephropathy defined by persistent microalbuminuria (Albumin: Creatinine Ratio in spot urine sample: 2.5–25 mg/mmol in males, 3.5–35 mg/mmol in females) checked at least on two consecutive occasions over the previous six months. Renal or liver failure, retinopathy diagnosed by funduscopy examination, cardiovascular disease and intake of hormonal replacement therapy. Taking hypoglycemic drugs and lipid lowering drugs were not considered as exclusion criteria.

Detailed medical history for each group was obtained. Weight and height were measured to calculate the body mass index (BMI), systolic BP (SBP) and diastolic BP (DBP) was measured in a sitting position using mercury column sphygmomanometer after at least five minutes of rest; two readings of SBP and DBP were taken. Using NICE hypertension guideline 2011, standards for hypertension was systolic blood pressure (SBP) ≥140 mmHg and diastolic blood pressure (DBP) ≥90mmHg [13]. Clinical and biochemical measurements of patients with type 2 diabetes were performed after eight hours of fasting. Diagnosis of diabetes was according to the criteria of American diabetes association (Fasting plasma glucose; FPG ≥126 mg/dl. Fasting is defined as no caloric intake for at least 8 hours) [14]. Control subjects did not have any abnormalities regarding their physical examination, blood pressure, family history, urinalysis, and routine laboratory blood tests; none of them were receiving any medications at the time of participation.

Method:

Venous blood samples were collected from each subject in two separate test tubes: one was use for biochemical analysis. The other was collected on EDTA for HBA1c and DNA extraction.

2.1-Biochemical measurements: Serum urea, creatinine, total Cholesterol, high density lipoprotein, low density lipoprotein, triglyceride and fasting blood sugar were done using C111 analyser Commercial kits (Roch-Diagnostics, Swizerland).

2.2-DNA Extraction: Genomic DNA was extracted from white blood cell pellets by salting out extraction method [15] using wizard genomic DNA extraction kit from Promega. Red blood cell lysis was done by using red cell lysis buffer (20 mM tris-HCL pH 7.6) followed by centrifugation. Nuclei lysis was carried by cell lysis buffer (10 mM tris-HCL pH 8.0, 1 mM EDTA pH 8.0, 0.1% (w/v) SDS) and proteinase K (20 mg/ mL) followed by centrifugation. Protein was precipitated by protein precipitation solution (60 mL of 5 M potassium acetate, 11.5 mL of glacial acetic acid, 28.5 mL of water) followed by centrifugation. Finally DNA was precipitated by isopropanol and then ethanol 70% and rehydrated in Tris EDTA buffer (10mM tris, 1mM EDTA pH 8.0) and stored at -20°C. DNA purity and concentration were determined by spectrophotometer measurement of absorbance at 260 and 280 nm.

2.3-Genotyping:

2.3.1- ACE I/D polymorphism by PCR:

100 ng of DNA was amplified using Gene Amp PCR system 9700 from applied biosystem. The primers used were Sense 5'CTGGAGACCA CTCCCATCCT TTCT3' and antisense 5'GATGTGGCCATCACATT CGTCAGAT3'. PCR conditions were optimized for initial heating for 3 minutes at 94°C followed by 30 cycles of denaturation for 30 seconds at 94°C, annealing at 58°C for 45 seconds, extension at 68 °C for 2 minutes and final extension was done at 68°C for 7 minutes. Reaction mixtures consisted of 1.25 units of thermostable Taq polymerase, 0.2 mM of each dNTP, 1.5 mM mgCl , 2 mM dimethylsulphoxide (DMSO) and 0.5 µmol of each primer, made up to a final volume of 50 µL The product was separated on 2% agarose gel and visualized by ethidium bromide staining.

Subjects were classified according to the presence or absence of a 287 base pairs insertion at intron 16 of ACE gene as II, ID, DD. Preferential amplification of the smaller 190 base pairs deletion allele (D) in ID heterozygote has led to their mistyping as DD homozygote so they were retyped using an insertion (I) specific sense primer 5'-TGGGACCACAGCGC CCGCCACTAC-3'; antisense: 5'GCCAGCCCTCC CATGC CCATAA-3' (primers were supplied by Alpha DNA, 4401 Notre-Dame St.w) and were then subjected to denaturation at 94°C for 3 minutes followed by 32 cycles of 94°C for 30 seconds, 67°C for 30 seconds and 72°C for 30 seconds, followed by 67°C for 30 seconds and 72°C for 10 minutes [16]. The products were separated on separated on 2% agarose gel and visualized by ethidium bromide staining. (Figures 1,2).

3.3.2- AGT M235T polymorphism by RT-PCR followed by RFLP:

Amplification was done using the following primer sequences: sense- 5'CCGTTTGTGCAGGGCC TGGCTCTCT3' and antisense: 5'CAGGGTGCTGT CCACACTGGACCCC3'. Reactions were carried out in 25 µL volumes under standard conditions (1.5 mmol MgCl2, 50 µmol for each dNTP, 10 mmol tris/HCl, 50 mmol KCl, 1 µmol primers, 1 U Taq DNA polymerase per sample. PCR conditions were optimized for initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 1 minutes denaturation at 94°C, 1 minutes annealing at 68°C and 1 minutes extension at 72°C, then final extension at 72 for 10 minutes was done. The product of PCR was subjected to digestion by restriction enzyme Tth 111I (New England Bio Labs, Missisauga, ON, Canada) for 3 hours at 65°C. The M to T point mutation creates a detection site at position 235 [17] and the digested fragments were separated on 2% agarose gel and visualized by ethidium bromide staining (Figure3).

4-Statistical analysis:

Data was analyzed using SPSS win statistical package version 15. Numerical data were expressed as mean, standard deviation and range.

Qualitative data were expressed as frequency and percentage. Results were considered statistically significant at $p<0.05$. Continuous variables are presented as mean ± standard deviation and an ANOVA was performed. AGT M235T and ACE I/D genotype frequencies were estimated by gene counting, and the differences between the studied groups were evaluated by Pearson's $\chi 2$ test and Anova test. Odds ratios (OR) and 95% confidence interval (CI) were calculated in according to Woolf's method. The distribution of alleles and genotypes between patients and controls were determined using the (goodness-of-fit) test, and statistical significance was determined at $p<0.05$. Chi-square test was used in order to test whether the frequency distribution of genotypes in T2DM or control group was in Hardy-Weinberg equilibrium (HWE).

3. Results:

Demographic data of studied groups are shown in table (1). The frequencies of the polymorphic variants of ACE I/D and AGT M235T genes were significantly different between T2DM patients and controls ($p<0.001$). Significant differences were found in DD genotype (OR=2.444, 95% CI 1.140-5.240, $p=0.022$) and TT genotype (TT: OR=3.9, 95% CI 1.773-8.597, $p<0.001$). ACE- DD/AGT-TT combined genotype was significantly higher in T2DM patients than control with marked increase in risk to disease occurrence (OR =14.510 p=0.012). Moreover, the frequencies D allele of ACE gene was not associated with increased risk of T2DM facing a high significant association with T allel of AGT gene (D: OR=1.599, 95% CI 0.986-2.59, p =0.057 and T: OR=2.104, 95% CI 1.291-3.428, p =0.003) (Table 2). Applying HWE at the ACE I/D locus and AGT M/T locus revealed that: Regarding ACE I/D locus, no deviation from Hardy-Weinberg equilibrium (HWE) was detected in both T2DM ($p^2 = 0.158$; $2pq = 0.397$; $q^2 = 0.360$) or control group ($p^2 = 0.260$; $2pq = 0.489$; $q^2 = 0.230$). Also, in AGT M/T locus no deviation from HWE was detected in both T2DM ($p^2 = 0.149$; $2pq = 0.471$; $q^2 = 0.384$) and control group ($p^2 = 0.313$; $2pq = 0.492$; $q^2 = 0.193$).

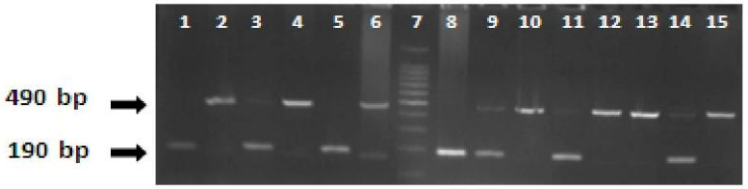

Figure 1: Gel electrophoresis of ACE I/D : lanes1,3,5,8,11 are DD homozygote with single band at 190, lanes 2,4,10,12,13 are II homozygote with one band 490, Lanes 6,9,14 are ID heterozygotes with two bands, and lane 7 is 100 bp marker.

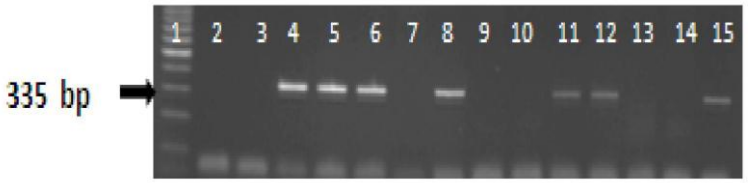

Figure 2: Gel electrophoresis using insert specific primers: lanes 4,5,6,8,11,12,15 show single bands of the insertion fragment of 335 bp so considered ID heterozygote, while lanes 2,3,7,9,10, 13, 14 no bands indicating DD homozygote, lane 1 shows 100 bp marker.

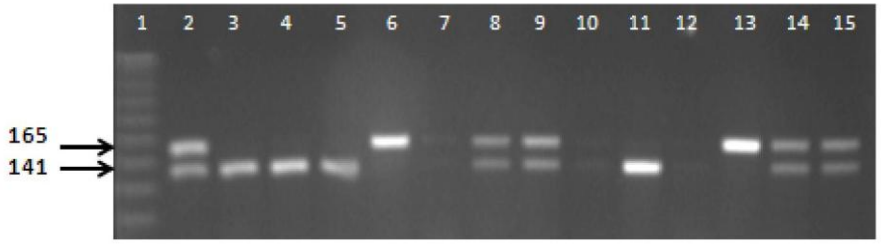

Figure 3: Gel electrophoresis of AGT T235M: samples after digestion with Tth 111 I, lanes 3,4,5,11 homozygote TT with one band 141 bp, lanes 6,13 are homozygote MM with one band 165 bp, lanes 2, 8,9,14,15 heterozygote MT with two bands at 141, and 165, lane 1 is 50 bp marker.

Table 1: demographic data of study participants—T2DM patients versus Controls

Characteristics	T2DM	Controls	p-value
Gender Male (%) Females (%)	 35 (60.34%) 23 (39.66%)	 56(70%) 24 (30%))	 0.085
Age (year)	46.53 ± 6.53	44.76 ± 7.21	0.141
Duration of diabetes	7.8 ± 2.1 years	-	-
Systolic blood pressure	136.90±18.02	132.57±13.52	0.191
Diastolic blood pressure	82.54±6.59	82.37±7.40	0.913
BMI(Kg/m^2)	28.83±3.38	27.27±3.54	0.246
Biochemical measurements:			
Fasting blood sugar (mg/dl)	163.62 ± 61.45	98.57 ± 10.19	<0.001*
HB A1C(%)	6.89±0.89	4.88±0.65	<0.001*
Cholesterol (mg/dl)	197.26 ± 41.18	187.98 ± 49.02	0.242
Triglycerides (mg/dl)	167.53 ± 91.48	128.76 ± 68.3	0.005*
HDL cholesterol (mg/dl)	38.40 ± 8.78	41.79 ± 15.06	0.299
LDL cholesterol (mg/dl)	128.85 ± 40.09	123.14 ± 43.67	0.103
Urea (mg/dl)	21.85 ± 9.08	22.22 ± 8.75	0.835
Creatinine (mg/dl)	0.91 ± 0.14	0.92 ± 0.16	0.502

***Significant p-value <0.05**

Table 2: The frequency of ACE and AGT genotypes in T2DM patients and controls.

Genotypes	T2DM No(%)	Controls No(%)	OR	95%CI	p-value
ACE I/D					
ACE -I/I	10(17.3%)	18(22.5%)	0.717	0.303-1.696	0.449
ACE -I/D	26(44.8%)	46(57.5%)	0.600	0.303-1.187	0.142
ACE -D/D	22(37.9%)	16(20%)	2.444	1.140-5.240	0.022*
D allel	0.603	0.497	1.599	0.986-2.596	0.057
AGT M235T					
AGT -M/M	11(19%)	23(28.8%)	0.580	0.257-1.311	0.191
AGT -M/T	22(37.9%)	44(55%)	0.500	0.251-0.996	0.048*
AGT -T/T	25(43.1%)	13(16.2%)	3.904	1.773-8.597	0.0007*
T allel	0.621	0.437	2.104	1.291-3.428	0.003*
Combined genotypes					
ACE -II/ AGT -MM	0(0%)	4(5%)	0.145	0.008-2.753	0.199
ACE -II/ AGT -MT	6(3%)	10(12.5%)	0.808	0.276-2.364	0.697
ACE -II/ AGT –TT	4(6.9%)	4(5%)	1.407	0.337-5.876	0.639
ACE -ID/ AGT -MM	6(10.3%)	17(21.3%)	0.428	0.157-1.163	0.096
ACE -ID/ AGT -MT	8(13.9%)	21(26.3%)	0.449	0.183-1.103	0.081
ACE -ID/ AGT –TT	12(20.6%)	8(10%)	2.348	0.892-6.182	0.084
ACE -DD/ AGT -MM	5(8.5%)	2(2.5%)	3.679	0.689-19.673	0.128
ACE -DD/ AGT -MT	8(13.8%)	13(16.3%)	0.824	0.318-2.141	0.692
ACE -DD/ AGT -TT	9(15.5%)	1(6.3%)	14.510	1.783-118.083	0.012*

*Significant p-value <0.05

4. Discussion:

Type 2 diabetes is a complex disorder accounting for about 90–95% of all diabetes syndromes. Despite numerous reports suggesting a substantial genetic contribution to the susceptibility of type 2 diabetes, no major susceptibility genes have been identified so far [18]. The aim of the current study was to investigate the association between genetic polymorphisms of ACE I/D and AGT M235T genes and T2DM in Egyptian population. To achieve this goal, ACE I/D and AGT M235T genotyping was performed by PCR and PCR-RFLP technique respectively.

In the present study, ACE I/D genotyping in T2DM revealed that 37.9% of patients had ACE DD homotype and 44.8% had ACE ID heterotypes. ACE-II genotype was higher in controls (22.5%) than T2DM patients (17.3%) suggesting a protective role for the ACE gene with decreasing incidence for T2DM. This result goes in accordance with the result of Yan Feng [19] on Chinese population with a frequency of 39.8% in diabetic patients and 44.8% in controls.

ACE-ID heterotype and D allel were detected in (44.8% and 60.3%) of T2DM compared to (57.5% and 49.7%) of controls with no statistically significant difference between the two groups p=0.142 and 0.057 respectively in agreement with the results of Yan Feng [19] and Einas et al. [20] who reported that the effect of the ID genotype did not reach a statistically significant level suggesting a recessive effect of the D allele and considering the high frequency of D allele in diabetic group an independent risk factor. Other population studies reported association of ACE I/D polymorphism with T2DM, thereby demonstrating geographical and racial/ethnic variations of ACE I/D polymorphism with T2DM [21–26].

ACE-DD homotype was higher in T2DM patients (37.9%) compared to controls (20%) with 2.4 fold risk for T2DM (OR=2.444, 95% CI 1.140-5.240, p =0.022). Similar results was obtained in previous study on Egyptian population which revealed higher frequency of DD genotype in diabetic patients (68 %) compared to controls (33.3 %) p =0.01 [27]. The positive association of ACE-DD genotype with T2DM was demonstrated in patients with diverse ethnic backgrounds such as Malaysian [28], British Caucasian [21], Arab Tunisians [29], Taiwanese [25] and a marginally significant association in a recent meta-analysis of a total of 41

studies (4708 cases and 5368 controls) in a Chinese population [30]. In contrast, negative association was found in Turkish [22] and Thailand [26]. The DD genotype is associated with twice the normal level of serum ACE activity [29]. Renin–angiotensin system (RAS) plays a central role in the regulation of sodium metabolism, vascular tone, blood pressure, renal hemodynamics, and vascular modeling. In diabetes mellitus, activation of the RAS by hyperglycemia may be the key mechanism and effects seem to be amplified with adverse consequences such as atherosclerosis and occlusive microangiopath. Suggestive evidence for this notion is the impressive beneficial effect of pharmacological interference with the RAS in large vessel disease as well as in renal and retinal microangiopathy [31].

In the current study regarding AGT/M235T gene polymorphism, AGT/MM homotype was significantly higher in control (28.7%) than in T2DM patients (19%) raising a protective role against occurrence of diabetes.

Our results have shown an association between AGT M235T polymorphism and T2DM. Then, the risk of developing diabetes seems to be higher for TT genotype and T allele compared to MM genotype and M allele. AGT-TT homotype was significantly higher in T2DM patients (43.1%) compared to controls (16.2%) p =0.0007 with 4 folds increased risk for T2DM OR=3.9, 95% CI 1.773-8.597 confirming a previous study on a Chinese population [32]. However, some studies failed to find this association [33,34]. Moreover, Chang et al. [35] reported an association between the AGT allele and diabetic nephropathy in Taiwan. But some researchers reported no obvious association between a specific AGT genotype and diabetic nephropathy [32,36]. The AGT T allele has been functionally related to increased AGT plasma levels [37]. Plasma AGT level is rather stable in one individual, but is under the long-term control of several hormones, such as glucocorticoids, estrogens, thyroid hormones, and Angiotensin II, which are known to induce AGT expression [38]

Some DNA polymorphisms may affect gene expression at both translational and transcriptional levels. However, the effect of a single polymorphism may be masked by interaction with environmental and genetic factors. AGT and ACE two important genes of RAS belonging to the same metabolic pathway may interact with each other. AGT and ACE polymorphisms affect the amount of their final products and, because of their different frequencies in various populations, may jointly contribute to a higher risk of T2DM and other diseases that is not seen with either polymorphism alone[29]. In the present study, ACE-DD/AGT-TT combined genotype was significantly higher in T2DM patients than control with marked increase in risk to disease occurrence (OR =14.510 p =0.012). This was in accordance with the results of Mehri et al.[29] and Young et al. [32] who reported a significant interaction between the AGT and ACE genes in T2DM patients. Also, previous studies revealed significant combinational effect of ACE ID and AGT M235T polymorphisms on conferring susceptibility to diabetic nephropathy in Japanese [39] and Asian Indian populations [40].

ACE-II/AGT-MM combined genotype was not present in T2DM patients and found in 22.2% of controls with decreased risk to diabetes. Thus it may have a protective role against susceptibility to T2DM. No statistically significant difference could be encountered between the patients and the controls regarding the frequency of the combined genotypes; ACE-DD/AGT-MM, ACE-DD/AGT-MT, ACE-ID/AGT-TT, ACE-ID/AGT-MM, ACE-ID/AGT-MT, ACE-II/AGT-MT and ACE-II/AGT-TT. These data strengthen the hypothesis that genotypic combinations are more important than single gene polymorphism alone. It is likely that there is an important interaction among these two RAS gene polymorphisms and T2DM since they are both part of the same metabolic pathway. However, because of their independent segregation in different populations, their contributions to the physiological and pathological processes in diabetes are likely to vary, depending on the genotypic combinations formed.

The limitation of our study was the relatively small sample size. The present study could have yielded more consistent results if included more patients and if treatment results were followed in relation to the genotyping. In conclusion, our study provides further evidence of a role for genetic variation in the ACE I/D and AGT M253T polymorphism with risk of T2DM in Egyptian population. ACE I/D and AGT M253T polymorphic markers may raise the hope to individualize ACE inhibitor therapy in order to optimize its effectiveness and to reduce adverse effects for genetically different subgroups. Diabetes remains an important focus of investigation.

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Study of the Thermal Behavior of the Complexation between Norfloxacin Drug with ZrO^{2+} and UO_2^{2+} Ions: TGA and DTG Investigations

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Abstract: The interactions of ZrO^{2+} and UO_2^{2+} ions with the second-generation fluoroquinolone, norfloxacin (NFX) were studied. The obtained complexes were characterized by elemental analysis, electrical conductivity and infrared spectra. The IR spectra of the isolated solid complexes indicated that NFX act as deprotonated bidentate ligand bound to the metal through one of the oxygen atom of the carboxylic group and the ring carbonyl oxygen atom, forming five and six atoms ring with ZrO^{2+} and UO_2^{2+} ions, respectively. The thermal behavior of these two complexes were investigated on the basis of thermogravimetric (TGA) and differential thermogravimetric (DTG) analyses, as well as kinetic thermodynamic parameters estimated from Coats-Redfern and Horowitz-Metzger integral methods. Infrared and thermogravimetric studies reveal the presence of coordinated water molecules. Based on the reported data, the proposed structure of the obtained complexes are $[ZrO(NFX)_2]\cdot 4H_2O$ and $[UO_2(NFX)_2]\cdot 9H_2O$.

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Key words: Norfloxacin, Zr(IV), U(VI), Thermal analysis, Kinetic parameters.

1. Introduction

Norfloxacin (NFX) is a second-generation fluoroquinolone, chemically named as 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline-3-carboxylic acid (Formula I) **[1].** In recent years, NFX has been ranked as the second most prescribed fluoroquinolone antibacterial just next to levofloxacin in China. It is the first fluoroquinolone, which was synthesized by converting nalidixic acid into a quinolone structure, adding a fluorine atom and piperazine ring **[2].** NFX is the first choice drug for the treatment of diseases caused by *Campylobacter*, *E. coli*, *Salmonella*, *Shigella*, and *V. colera*. It is used for the therapy of gonorrhea as well as eye and urinary tract infections **[3-5].** NFX has a broad spectrum with antibacterial activity against both Gram-positive and Gram-negative through inhabitation of DNA gyrase **[6,7],** thus it extensively used in both human and veterinary medicine. NFX is active *in vitro* against many Gram-positive and Gram-negative bacteria because it urine concentration is 100-300 times higher than that in serum which largely exceeds its minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). It was discovered that the fluorine atom at the 6th position provides increased potency against Gram-negative organisms and the piperazine moiety at the 7th position is responsible for the antipseudomonal activity of NFX **[8].**

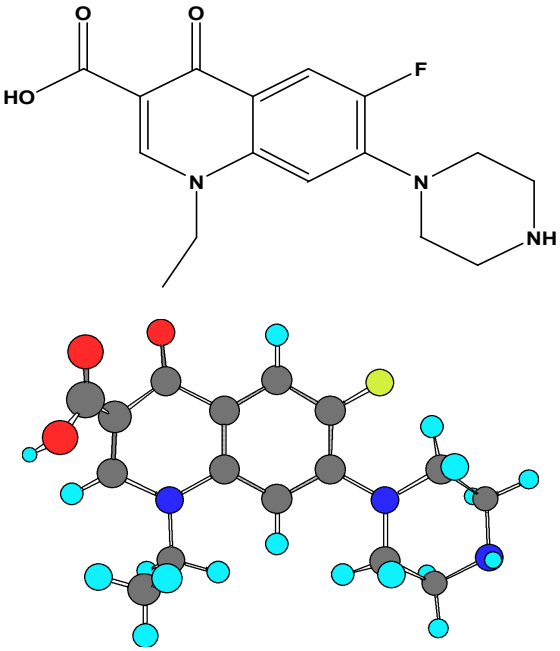
Recently, many studies and research were conducted on NFX drug such as, determination of NFX in biological samples and medicine **[9-12],** synthesis and structure of NFX metal complexes **[13-20],** the antimicrobial evaluation of NFX and its derivatives **[21-24],** sorption behavior **[25]**, and photodegradation of NFX **[26].** The aim of this work is to synthesize and investigate systematically the thermal behavior of norfloxacin complexes with ZrO^{2+} and UO_2^{2+} ions. The interpretation of the thermo-kinetic behavior of these complexes were carried out through the mathematical analysis and evaluation of kinetic parameters of thermogravimetric (TGA) and its differential (DTG), such as entropy of activation, pre-exponential factors, activation energy evaluated by using Coats-Redfern and Horowitz-Metzger equations. The elemental analysis and infrared spectra were used to elucidate the mode of coordination properties of NFX with these two ions.

2. Materials and Methods

2.1 Materials

All chemicals used were of analytical reagent grade, commercially available, and were used without further purification. Norfloxacin used in this work was supplied from Egyptian International Pharmaceutical Industrial Company

(EIPICO). $ZrOCl_2 \cdot 6H_2O$ and $UO_2(NO_3)_2 \cdot 2H_2O$ were purchased from Aldrich Chemical Company.



Formula I. Structure of Norfloxacin (NFX) drug.

2.2 Synthesis of NFX metal complexes

Norfloxacin (2 mmol) in mixed solvent (50/50 %) of methanol/acetone was stirred at room temperature for 20 min. A solution of 1.0 mmol of $ZrOCl_2 \cdot 6H_2O$ and $UO_2(NO_3)_2 \cdot 2H_2O$ in 5 ml of methanol was added to the NFX solution with constant stirring. The pH was adjustment at 7~8, then the resulting mixture was heated ~50 °C under reflux on a water bath for about 12 h and then cooled. The resulted solid complexes were separated from the reaction mixture by filtration, washed with methanol and dried under vacuum to a constant weight. The yields were found around 75% based on the metals salts. The compounds resulted have low solubility in water and in common organic solvents. The obtained two complexes were characterized by their elemental analysis, electrical conductivity, infrared spectra, as well as thermal analysis.

2.3 Instrumentation

The elemental analyses of carbon, hydrogen and nitrogen contents were performed by the micro analytical unit at Cairo University, Egypt, using a Perkin-Elmer CHN 2400 (USA), and the metal contents were found gravimetrically by ignition-weighted samples in atmospheric air to constant weight and definite structure. Decomposition of complexes was performed in concentrated HNO_3. After decomposition, the sample was diluted with water to 100 ml, and qualitative presence of Cl^- ions (in ZrO^{2+} complex) was determined by means of $AgNO_3$. Molar conductivities of freshly prepared 10^{-3} mol dm^{-3} DMSO solutions were measured on a Jenway 4010 conductivity meter. Infrared (IR) spectra (KBr discs) within the range of 4000-400 cm^{-1} for the resulted CT-complexes were carried out on a Genesis II FT-IR spectrophotometer with 30 scans and 2 cm^{-1} resolution. Thermogravimetric measurements (TG and DTG) were carried out in dynamic nitrogen atmosphere (30 ml/min.) between room temperature and 900 °C with a heating rate of 10 °C/min. using a Shimadzu TGA –50H thermal analyzers.

3. Results and discussion

3.1 Elemental analysis

The results of the elemental analysis and some physical characteristics of NFX ligand and the obtained complexes are given in Table 1. The complexes were synthesized using 1:2 (metal: ligand) mole ratio of all reactants. The elemental analysis of the complexes indicates a 1:2 metal to ligand stoichiometry, too. The analytical data indicate that the two prepared complexes contain water molecules, and the number of bound water molecules in these compounds begin different. The IR spectroscopic and thermogravimetric data also confirm water in the composition of the complexes. All of the resulted complexes are stable in air, hygroscopic, with high melting points. Investigation of the solubility of these complexes showed that they were insoluble in water, methanol, ethanol, diethylether, benzene, chloroform and carbon tetrachloride, but soluble in dimethylformamide (DMF) and dimethylsulfoxide (DMSO) with gently heating. All complexes have been prepared at high yield (76-77%), and melting points of the complexes (>300 °C) are higher than that of the ligand revealing that the complexes are much more stable than ligand. The molar conductance values of the complexes in DMF solvent lay in the range of 12-15 Ω^{-1} cm^2 mol^{-1} (at 25 °C), which indicates that the complexes are non-electrolytes, which agreed with the elemental analysis data and the absence of nitrate and chloride ions. The low conductivity values are in agreement with the low solubility of NXF complexes in water, ethanol, chloroform, acetone and most organic solvents. On the other hand, they are soluble in DMSO and DMF and concentrated acids. The proposed structures of the obtained complexes are shown in Formula II. These structures were confirmed by its analytical data.

3.2 IR spectral analyses

The full tentative assignments of infrared bands of NFX and the obtained complexes are presented in Table 2 and Fig. 1, and have been

compared with those of the free ligand NFX, in order to determine the site of coordination that may be involved in chelation. Table 3 gives the characteristic IR peaks of NFX and its complexes. The infrared spectrum of free NFX exhibits two characteristic bands at 1727 and 1716 cm^{-1} which are assigned to the stretching vibration of the carboxylic group, $\nu(C=O)_{carb}$ **[27,28].** These bands are shifts or disappears in the complexes which indicative of the involvement of the carboxyl group in the interaction with metal ion. The spectral region between 1630 and 1350 cm^{-1} is very congested which complicate the assignment of the stretching modes of ligated carboxylato group. The band around 1630 cm^{-1} in the spectra of the two complexes can be assigned to the asymmetric stretching vibration ($\nu C=O_{asym.}$) of the ligated carboxylato group. The two complexes also show another strong or medium strong intensity bands at 1390 and 1430 cm^{-1}, these bands are absent or weak in its intensity in the spectrum of NFX and may be assigned to the symmetric vibration of the ligated COO^- group. The $\nu(C=O)$ in the spectrum of NFX appears at 1630 cm^{-1} but in the spectra of the complexes, the $\nu(C=O)$ is effected by the interaction with the metal ion. **Deacon and Phillips 1980 [29],** investigate the asymmetric and symmetric stretching vibration of large number of carboxylato complexes with known crystal structure, and they have studied the criteria that can be used to distinguish between the three binding states of the carboxylate complexes. These criteria are:

(a) Uindentate complexes; $\Delta\nu>200$ cm^{-1} (where $\Delta\nu$ is the frequency separation between the asymmetric and symmetric stretching of carboxylato group, $[\nu_{asym.}(COO^-)-\nu_{sym.}(COO^-)]$). This relation was found in case of monodentate carboxylato complexes.

(b) Bidentate or chelating carboxylato complexes; exhibit $\Delta\nu$ significantly smaller than ionic values ($\Delta\nu<100$ cm^{-1}).

(c) Bridging complexes; show $\Delta\nu$ comparable to ionic values ($\Delta\nu\sim150$ cm^{-1}).

Therefore, the difference value $\Delta\nu$ is a useful characteristic for determining the coordination mode of the carboxylate group of the ligands. The observed $\Delta\nu$ for ZrO^{2+} and UO_2^{2+} complexes is around 200 cm^{-1} (Table 3) which suggest a monodentate coordination mode of the carboxylato group. The most important changes during the transformation of NFX into metal complexes take place within the range 650-400 cm^{-1}. The spectra of the synthesized complexes display a group of bands with different intensity which characteristics for $\nu(M-O)$ stretching vibrations of coordinated carboxylato oxygen atom and carbonyl oxygen atom. The $\nu(Zr-O)$ and $\nu(U-O)$ bands observed at 537 and 536 cm^{-1}, respectively. The spectra of the two complexes show broad water bands around 3500 cm^{-1} confirm the presence of water molecules. The $\nu_{as}(Zr=O)$ and $\nu_{as}(U=O)$ absorption band occurs as a medium singlet band at 930 cm^{-1}, where the $\nu_s(Zr=O)$ and $\nu_s(U=O)$ absorption bands occur as a medium-weak singlet band at 830 cm^{-1}. These assignments for the stretching vibrations of the zirconyl (ZrO) and uranyl group (UO_2) agree quite well with those known for many oxo-zirconium and dioxo-uranium complexes.

According to the IR spectral data, the NFX is coordinated to the metal ions as a bidentate ligand via one-carboxylato oxygen atoms and the oxygen atom of the pyridine carbonyl group **[30].** The most probably structures of the obtained complexes are shown in Formula II. In the $[UO_2(NFX)_2]\cdot9H_2O$ complex, the four oxygen atoms of the two NFX ligands occupy equatorial positions around the central metal atom U(VI), forming a plane containing the four-member ring and the two oxygen atoms of the uranyl group occupy axial positions.

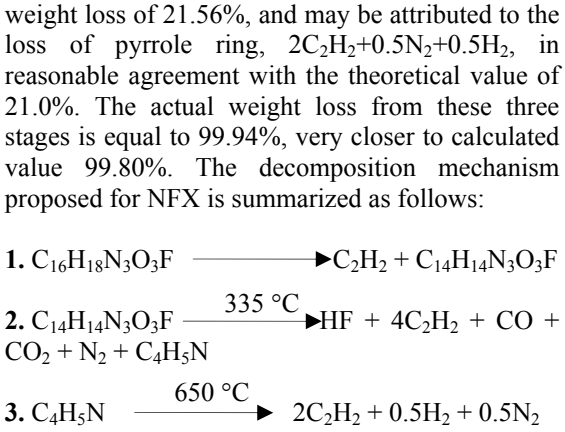
3.3 Thermal analysis

The NFX complexes with ZrO^{2+} and UO_2^{2+} are stable at room temperature and can be stored for several months without any changes. The obtained complexes were studied by thermogravimetric (TG) and differential thermogravimetric (DTG) analysis form ambient temperature to 900 °C N_2 atmospheres. The TG curves were redrawn as mg mass loss versus temperature (TG) curves and as the rate of loss of mass versus temperature (DTG) curves. Typical TG and DTG curves are presented in Fig. 2, and the thermoanalytical results are summarized in Table 4. The overall loss of mass from TG curves is 99.94% for NFX, 70.38% for $[ZrO(NFX)_2].4H_2O$, 75.54% for $[UO_2(NFX)_2].9H_2O$. All the complexes show two or three stages of mass loss in their TG/DTG curves, and all mass loss in these stages is due to the decomposition of counter ions and NFX molecules. The found weight loss associated with each step of decomposition for each complex agrees well with the calculated weight loss. The results of TG-DTA agreed with that of elemental analysis. The final products of the complexes obtained at 900°C were confirmed with infrared spectra.

3.3.1 NFX ligand

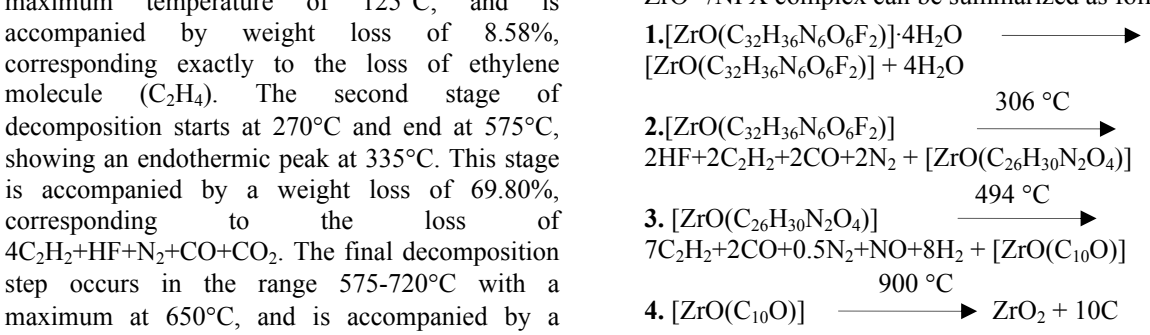
The data obtained indicate that the NFX is thermally stable in the temperature range 25-50°C. Decomposition of NFX start at 50°C and finished at

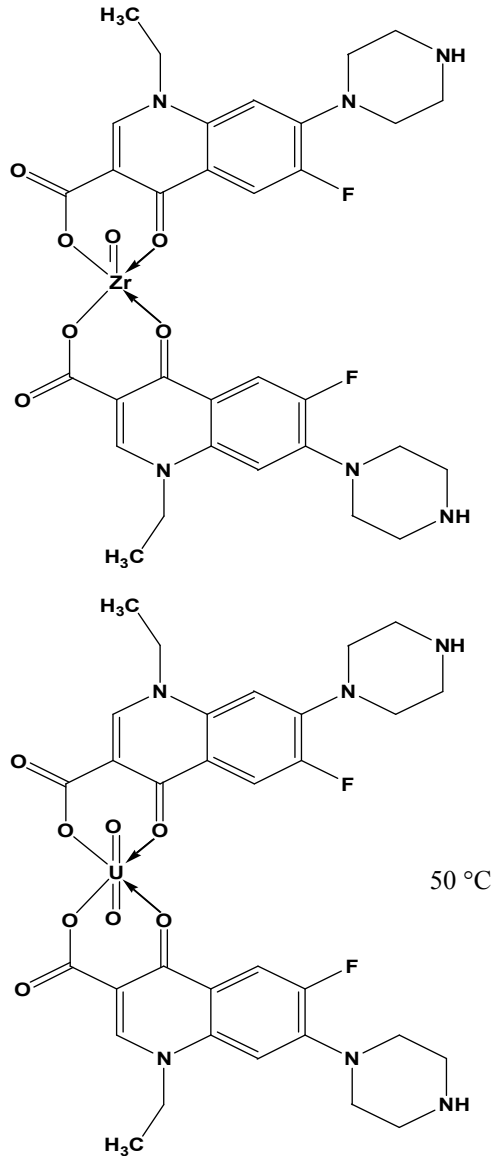
726°C with three stages. The first stage of decomposition occurs between 25 and 270°C, at maximum temperature of 125°C, and is accompanied by weight loss of 8.58%, corresponding exactly to the loss of ethylene molecule (C_2H_4). The second stage of decomposition starts at 270°C and end at 575°C, showing an endothermic peak at 335°C. This stage is accompanied by a weight loss of 69.80%, corresponding to the loss of $4C_2H_2+HF+N_2+CO+CO_2$. The final decomposition step occurs in the range 575-720°C with a maximum at 650°C, and is accompanied by a weight loss of 21.56%, and may be attributed to the loss of pyrrole ring, $2C_2H_2+0.5N_2+0.5H_2$, in reasonable agreement with the theoretical value of 21.0%. The actual weight loss from these three stages is equal to 99.94%, very closer to calculated value 99.80%. The decomposition mechanism proposed for NFX is summarized as follows:



3.3.2 $[ZrO(NFX)_2]\cdot 4H_2O$

The thermal decomposition of $[ZrO(NFX)_2]\cdot 4H_2O$ complex proceeds approximately in three main degradation steps in 25-900°C temperature range, one is dehydration process and the two others is decomposition process. The first dehydration stage of degradation is accompanied by endothermic peak occurs at maximum temperature of 50°C (DTG), in the temperature range 25-180°C. The mass loss in this stage is found to be 9.53%, corresponding to the loss of four water molecules. This is followed by another mass loss (25.21%) in the temperature range 180-400°C, by giving an endothermic effect (DTG_{max}, 306°C), corresponding to the loss of $2HF+2C_2H_2+2CO+2N_2$. The third decomposition step occurs at the maximum temperature of 494°C and is accompanied by a weight loss of 35.64%, corresponding to the loss of $7C_2H_2+2CO+0.5N_2+NO+8H_2$, then the final thermal product obtained at 900°C is ZrO_2 with some carbon atoms. Reported data on thermal analysis studies in the nitrogen atmosphere indicate that ZrO^{2+}/NFX complex decompose to give metal oxide as final product, with few carbon atoms indicates that no sufficiently of oxygen atoms helps to evolved carbon as carbon monoxide or dioxide. Accordingly, the proposed mechanism for the decomposition of ZrO^{2+}/NFX complex can be summarized as follows:





Formula II. Suggested structure of Zr(IV) and U(VI)-NFX complexes.

3.3.3 $[UO_2(NFX)_2]\cdot 9H_2O$

The thermal analysis curves of $[UO_2(NFX)_2]\cdot 9H_2O$ complex show that decomposition takes places in two stages. The first stage is exothermic stage occurs at maximum temperature 45 °C. The weight loss in this stage is 15.49% in the 25-195 °C range corresponding to the loss of $9H_2O$. The following stage occurs at maximum temperature of 380 °C in the temperature range 195-900°C corresponding to the decomposition of two molecules of NFX. The final thermal product obtained at 900 °C is UO_2. Accordingly, the proposed mechanism for the decomposition of UO_2^{2+}/NFX complex can be summarized as follows:

1. $[UO_2(C_{32}H_{36}N_6O_6F_2)]\cdot 9H_2O \xrightarrow{45\ °C} [UO_2(C_{32}H_{36}N_6O_6F_2)] + 9H_2O$

2. $[UO_2(C_{32}H_{36}N_6O_6F_2)] \xrightarrow{380\ °C} 2HF+14C_2H_2+4CO+2N_2+2NO+3H_2+UO_2$

3.4 Kinetic data

In recent years, there has been increasing interest in determining the rate-dependent parameters of solid-state non-isothermal decomposition reactions by analysis TG curves. Several equations **[31-38]** have been proposed as means of analyzing a TG curve and obtaining values for kinetic parameters. The most commonly used methods for this purpose are the differential method of **Freeman and Carroll 1958 [31]**, integral method of **Coats and Redfern 1964 [33],** and the approximation method of **Horowitz and Metzger 1963[36].** In the present study the general thermal behaviors of the NFX ligand and the two complexes in terms of stability ranges, peak temperatures and values of kinetic parameters, are shown in Fig. 3, and Table 5. The kinetic parameters have been evaluated using the following methods and the results obtained by these methods are compared with one another. The following two methods are discussed in brief.

3.4.1 Coats-Redfern (CR) method

The Coats-Redfern equation (1), which is atypical integral method, can be represented as:

$$\int_0^{\alpha} d\alpha/(1-\alpha)^n = (A/\varphi)\int_{T1}^{T2} e^{-E^*/RT}\,dT \qquad (1)$$

For convenience of integration, the lower limit T_1 is usually taken as zero. This equation on integration gives:

$$Ln[-\ln(1-\alpha)/T^2] = -E^*/RT + \ln[AR/\varphi E] \qquad (2)$$

where α is the fraction of the sample decomposed at time T, T is the derivative peak temperature, A is frequency factor, R is the gas constant, E^* is the activation energy and φ is the linear heating rate. A plot of left-hand side (LHS) against $1/T$ was drawn. E^* is the energy of activation in KJ mol^{-1} and calculated from the slop and A in (s^{-1}) from the intercept. The entropy of activation ΔS^* in (JK^{-1} mol^{-1}) was calculated by using the following equation:

$$\Delta S^* = R\ln(Ah/kT_s) \qquad (3)$$

where k is the Boltzmann constant, h is the Plank's constant and T_s is the DTG peak temperature.

3.4.2 Horowitz-Metzger (HM) approximation method

The Horowitz-Metzger equation (4) was written in the form as follows:

$$Log[\log(w_\alpha/w_\gamma)] = E^*\theta/2.303RT_s^2 - \log 2.303 \qquad (4)$$

where $\theta = T - T_s$, $w_\gamma = w_\alpha - w$, w_α = mass loss at the completion of the reaction; w = mass loss up to time t. The plot of Log[log (w_α/w_γ)] versus θ was drawn and found to be linear from the slope E^* was calculated. The pre-exponential factor, A, was calculated from the Eg. (5):

$$E^*\theta/RT_s^2 = A/[\varphi\exp(-E^*/RT_s)] \qquad (5)$$

From the TG curves, the activation energy, E^*, entropy of activations, ΔS^*, enthalpy activations, ΔH^*, and Gibbs free energy, ΔG^*, were calculate from;

$\Delta H^* = E^* - RT$

$\Delta G^* = \Delta H^* - T\Delta S^*$

From Table 5, the following outcomes are draw:

1. The results show that the kinetic data obtained by the two methods are comparable and in harmony with each other.
2. Higher value of activation energy suggests the higher thermal stability. The ZrO^{2+}/NFX show higher value of activation energy than UO_2^{2+}/NFX, indicating higher thermal stability of this complex.
3. The activation energy of ZrO^{2+} and UO_2^{2+} complexes is expected increase with decreasing metal ion radium **[39-42].** The calculated ΔE^* values using Coats-Redfern method for the first decomposition stage of the complexes are found to be E^* Zr(IV)= 1.57×10^4 $kJmol^{-1}$ > E^* U(VI)= 1.36×10^4 $kJmol^{-1}$, which is in accordance with r U(VI)= 156 pm < r Zr(IV)= 160 pm,
4. The ΔS^* values of the main stage for the two complexes were found to be negative which indicates that the reaction rates are slower than normal **[43]**. Furthermore, these data indicate that the activated complexes have more ordered structure than the reactants.
5. The satisfactory values of correlation coefficients of the Arrhenius plots (r) of the thermal decomposition steps were found to be (r ~ 0.9) in all cases indicate good fit with linear function and reasonable agreement between

experimental data and the values of kinetic parameters.

6 It is clear that the thermal decomposition process of the two complexes is non-spontaneous, i.e., the complexes are thermally stable.

Table 1. Elemental analysis and physical properties of the NFX and its complexes.

Compound	Molecular weight	Yield (%)	Color	mp (°C)	Analysis (%) found (calculated)				Λ ($\Omega^{-1}cm^2mol^{-1}$)
					C	H	N	M	
NFX	319	-	Yellow	232	59.45 (60.18)	5.54 (5.68)	12.89 (13.16)	-	10.16
$[ZrO(NFX)_2]\cdot 4H_2O$	817	76	White	> 300	47.23 (47.00)	5.28 (5.39)	10.50 (10.28)	11.46 (11.14)	12.5
$[UO_2(NFX)_2]\cdot 9H_2O$	1070	77	Yellow	> 300	35.34(35.89)	5.21(5.05)	8.03 (7.85)	29.87 (30.65)	15.0

Table 2. Infrared frequencies[a] (cm^{-1}) and tentative assignments[b] for NFX and its complexes.

NFX ligand	ZrO^{2+} complex	UO_2^{2+} complex	Assignments
3399 ms	3747 ms, 3712 vw, 3674 m, 3649 m 3613 vw, 3588 vw, 3502 br, ms	3712 w, 3674 w, 3650 w, 3503 br	ν(N-H) + ν_{as}(O-H); H_2O
3267 vw, 3228 vw, 3189 vw, 3130 vw 3021 w, 2927 m, 2823 w, 2796 w 2764 w, 2723 m, 2696 vw, 2654 w 2617 w, 2511 w, 2468 m	3040 br, ms, 2850 mw, 2736 mw 2516 mw, 2364 ms, 2338 sh, ms	3023 br, 3023 br, 2925 w, 2850 w 2486 w, 2366 m, 2340 sh, m	ν(C-H), ν(N-H), ν(-$\overset{+}{N}H_2$)
1727 sh, 1716 ms	1794 vw, 1719 vs	1720 vs	ν(C=O): (OCO^-)
1630 vs, 1620 sh	1629 vs	1630 vs	ν(C=O) + $\delta_b(H_2O)$
1595 w, 1552 w	1541 w	1541 m	Phenyl breathing modes
1482 vs, 1454 m	1497 vw	1495 vs,	CH; deformation of -CH_2-
1396 s	1460 vs , 1393 m	1459 vs , 1390 m, 1340 m	$\nu_s(COO^-)$ + ν(N-O): NO_3^-
1307 vw	1339 m, 1303 mw	1304 mw	$\delta_b(CH_2)$
1277 vw, 1263 s	1271 s	1271 s	ν(C-C)
1248 vw, 1201 m	1215 mw	1213 w	ν(C-O)
1192 m	1178 mw	1178 m	ν(C-N)
1153 vw, 1142 w 1132 w, 1115 w, 1095 m, 1076 m 1051 vw, 1036 ms, 1024 w, 1005 m 982 m	1144 mw, 1105 mw, 1077 vw, 1029 m	1143 m, 1104 w, 1076 w, 1030 ms	$\delta_r(CH_2)$
972 w, 935 ms, 916 m, 899 m 887 m, 858 w, 823 ms, 804 ms	939 m, 892 ms, 831 mw, 805 mw	939 ms, 896 s, 832 mw, 805 m	CH- bend; phenyl
750 s, 706 m	775 mw, 746 m, 705 w	776 mw, 747 m, 706 w	$\delta_b(COO^-)$
667 w, 631 w, br 569 ms, 524 w, 499 m, 474 m 453 vw, 430 ms	668 w, 621 w, 537 mw 470 mw, 417 mw	667 w, 621 w, 536 m 409 w, 415 w	ν(M-O) ring; δ_r, rocking deformation

[a] s, strong; w, weak; m, medium; sh, shoulder; v, very; br, broad.
[b] ν, stretching; δ_b, bending; δ_r, rocking.

Table 3. Characteristic IR peaks (cm^{-1}) of NFX and its complexes

Compound	ν(N-H)	$\nu(C=O)_p$	$\nu(COO^-)$ (asym.)	$\nu(COO^-)$ (sym.)	Δν (asym.-sym.)	(M-O)
NFX	3399	1727	1620	-	-	-
NFX/ZrO^{2+}	3502	1719	1629	1430	199	537
NFX/UO_2^{2+}	3503	1720	1630	1429	201	536

Table 4. Thermo analytical data for NFX and its complexes

Compound	Decomposition	TG results temp. range (°C)	T_{max} (°C)	Weight loss (%)		Lost species
				Found	Calculated	
NFX $C_{16}H_{18}N_3O_3F$	First step	25-270	125	8.58	8.78	C_2H_4
	Second step	270-575	335	69.80	70.22	$HF+4C_2H_2+CO+CO_2+N_2$
	Third step	575-726	650	21.56	20.80	$2C_2H_2+0.5H_2+0.5\ N_2$
	Total loss, Residue	-	-	99.94, 0.0	99.80, 0.0	-
$[ZrO(NFX)_2]\cdot 4H_2O$	First step	25-180	50	9.53	8.81	$4H_2O$
	Second step	180-400	306	25.21	24.97	$2HF+2C_2H_2+2CO+2N_2$
	Third step	400-900	494	35.64	36.47	$7C_2H_2+2CO+0.5N_2+NO+8H_2$
	Total loss, Residue	-	-	70.38, 29.62	70.25, 29.74	ZrO_2, 10C
$[UO_2(NFX)_2]\cdot 9H_2O$	First step	25-195	45	15.49	15.14	$9H_2O$
	Second step	195-900	380	60.05	59.63	$2HF+14C_2H_2+4CO+2N_2+2NO+\ 3H_2$
	Total loss, Residue	-	-	75.54, 24.46	74.77, 25.23	UO_2

Table 5. Thermo analytical data for NFX and its complexes determined using Coats-Redfern (CR) and Horowitz-Metzger (HM).

Compound	Stage	Method	Parameters [a]					*r*
			E^*	A	ΔS^*	ΔH^*	ΔG^*	
NFX	1st	CR	9.36×10^4	2.00×10^1	-5.01×10^1	9.03×10^4	1.10×10^5	0.98530
		HM	9.66×10^4	8.68×10^1	-3.79×10^1	9.33×10^4	1.08×10^5	0.98170
ZrO^{2+}/NFX	1st	CR	1.57×10^4	0.678	-2.15×10^2	1.25×10^4	1.08×10^5	0.89296
		HM	2.23×10^4	5.380	-2.33×10^2	1.91×10^4	1.07×10^5	0.90901
UO_2^{2+}/NFX	1st	CR	1.36×10^4	0.296	-2.57×10^2	1.06×10^4	1.02×10^5	0.88324
		HM	1.72×10^4	1.340	-2.44×10^2	1.42×10^4	1.01×10^5	0.90922

[a] Units of parameters: E in kJ mol^{-1}, A in s^{-1}, ΔS in J mol^{-1}K^{-1}, ΔH and ΔG in kJ mol^{-1}.

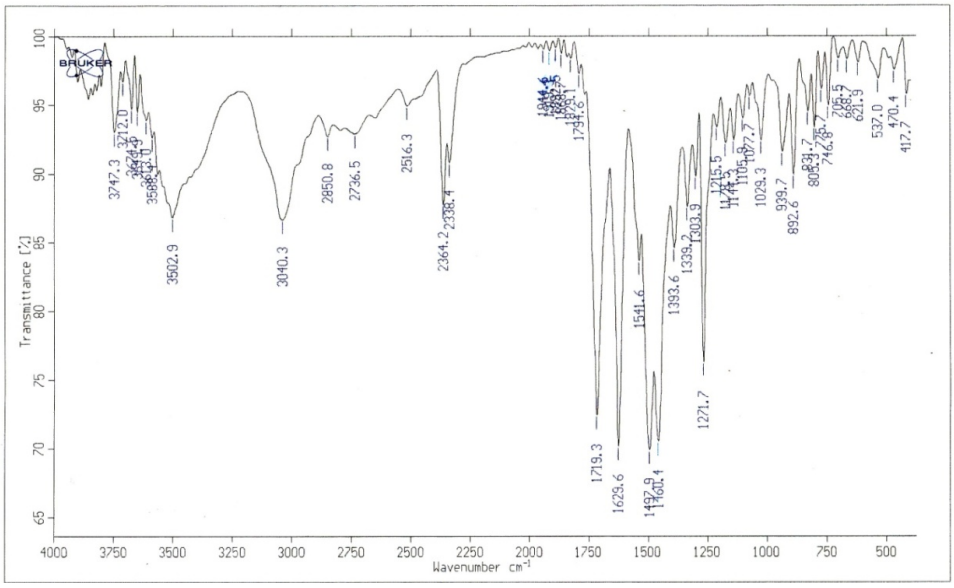


Fig. 1 (A) Infrared spectra of $[ZrO(NFX)_2]\cdot4H_2O$ complex.

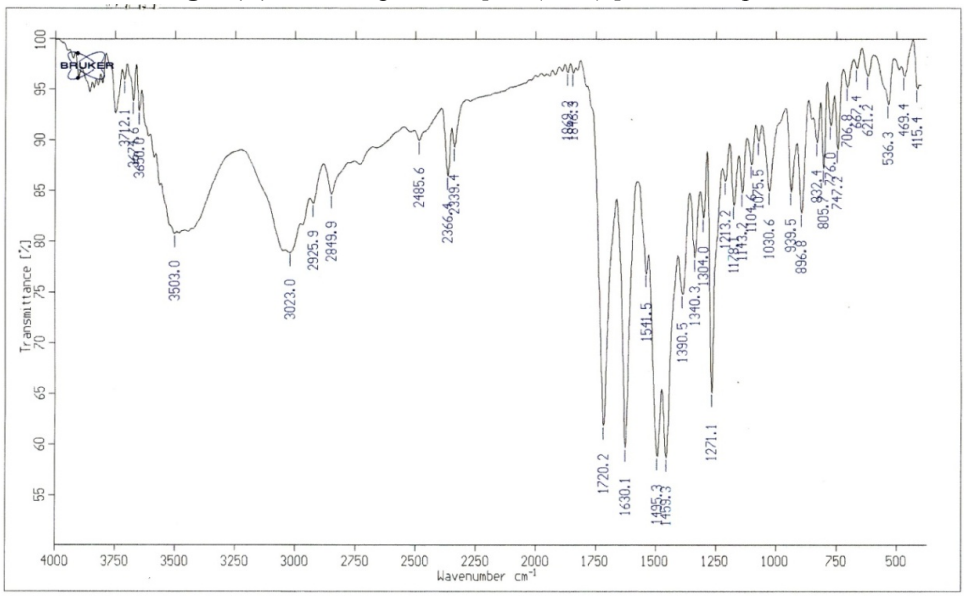


Fig. 1 (B) Infrared spectra of $[UO_2(NFX)_2]\cdot9H_2O$ complex.

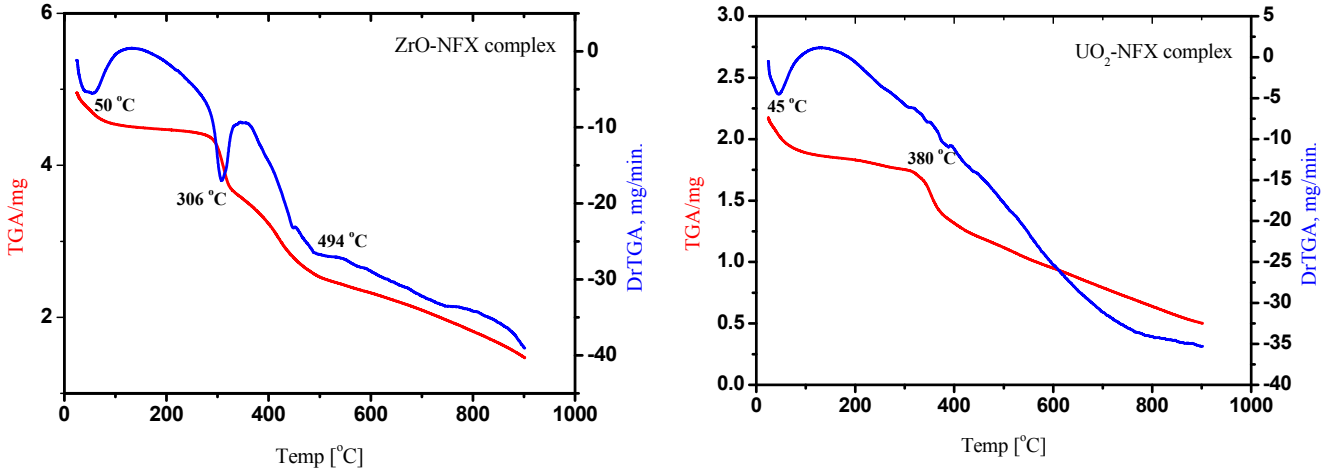


Fig. 2 TG and DTG curves of NFX complexes.

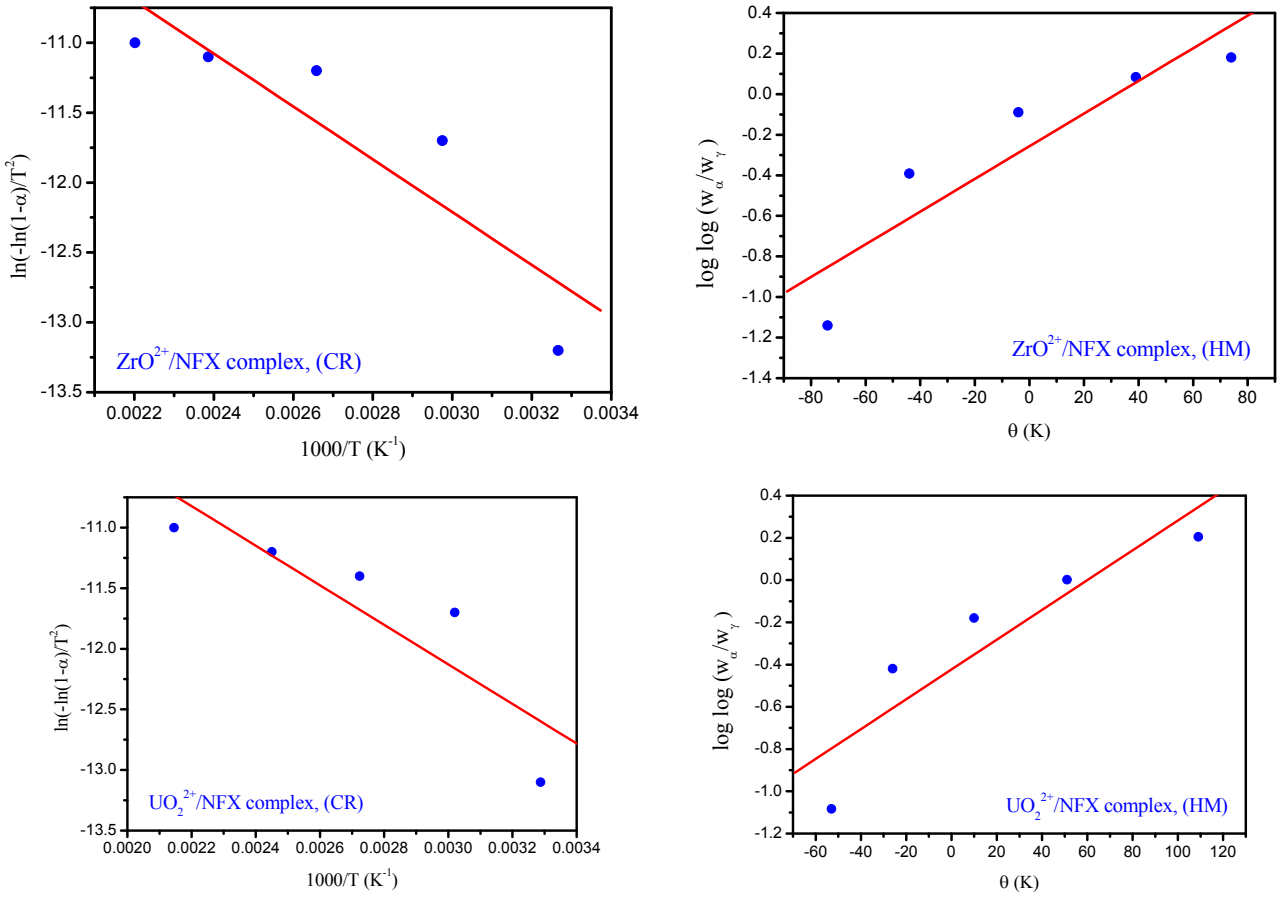


Fig. 3 The diagrams of kinetic parameters of NFX complexes using Coats-Redfern (CR) and Horowitz-Metzger (HM) equations.

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Synthesis a New Series of Methenamine Complexes with Some Different Metal Ions: Spectral, Thermal and Biological Investigations

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Abstract: In this paper methenamine (HMTA) ligand acts as monodentate with each central atom in all isolated complexes although the ligand molecule possesses four potential donor atoms through three fused rings in the chair configuration with four bridges – head nitrogen atoms. The nitrogen atoms of this donor may permeate the coordination towards multinucleous of metal atoms. This behavior may be due to the shape of the ligand distribute the donor atoms by the shape leads to this coordination. Ag(I), Cd(II), Cu(II), Hg(II), Sn(II), Sn(IV), Sb(III), Bi(III), Ce(III), Sm(III) and La(III) are the metal ions concerned this study. In the infrared spectra the shift in υ(C-N) bands proposed the type of coordination mode. The ^{1}HNMR spectra of Sn(II) and Bi(III) complexes are further supported for the proposed chelation. The XRD study for some selective complexes reflects their amorphous nature. The thermal behavior of these chelates shows that the hydrated complexes losses water molecules of hydration and coordination at relatively higher temperature. This is due to the presence of hydrated molecules introduced by H – bonding inside the coordination sphere with the active ligand centers followed immediately by decomposition of anions and ligand molecules in the subsequent steps. The activation thermodynamic parameters as; E*, ΔH*, ΔS* and ΔG* are calculated from the TG curves using Coats – Redfern method for suitable TG curves in between the all, which display peaks in between sharpness and broadness. The ligand in comparison to some of complexes was screened for their antibacterial and antifungal activities. The application was concerned in this study with focusing on some special complexes chosen referring to their history of their distinguish activity. The activity data show the investigated metal complexes to be more potent than the parent organic against most species.
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Key words: Methenamine ligand, Lanthanides, Heavy metals, thermal, spectral, biological study

1. Introduction

Crystal engineering and the design of solid-state architectures of coordination polymer are very attractive fields in literatures , due to their potential applications in catalysis, separations and optoelectronics [1-6]. Assembly of such extended supramolecular architectures by selecting the coordination geometry of metal ions and the chemical structure property of organic ligands can give rise to new and varied topological types [7–24]. Methenamine (HMTA), a potential tetradentate ligand, has been used to assemble new supramolecular architectures with metal ions via various possible coordination modes, namely, involving one to four N atoms of HMTA in coordination [13–34]. However, previous studies were mainly limited in using low coordination metal ion Ag(I) as spacers to connect HMAT [13-24], or using high coordination metal ions, such as Cd(II), Mn(II), as spacers to connect HMTA [25–31]. HMTA-based coordination polymers containing complex multidentate organic anions and high coordination metal ions remain rather rare [32-34]. Here we focus on some metal ions full a shortage in previous studies. In between these ions which are characterized by their ability to form highly coordinated building. A deliberate investigation of new complexes prepared is the first aim in this study. Some special complexes were chosen for biological investigation. In which the complexes of methenamine are widely distributed in different application fields in betweens the biological activity.

2. Experimental

All chemicals used were analytical grade and were used without further purification.

2.1. The synthesis of some HMTA complexes

Ag(I), Cd(II), Hg(II), Cu(II), Ce(III), Sm(III) and La(III) complexes are prepared by adding a saturated aqueous solution of the respective metal salt to an ethanolic solution of HMTA by a molar ratio of 1:4, respectively. The product obtained were filtered, washed with ethanol and dried under vacuum at room temperature.

2.1.1. Sn (IV) – HMTA complex:

9 mmol (1.260 g) methenamine in methylenechloride (50 ml), was added drop wise with constant stirring to a freshly prepared solution of 3 mmol (0.7818 g) tin (IV) tetrachloride by a molar ratio 3 : 1, respectively. The resulting clear solution was left for 12 hrs. at room temperature with continuous stirring; the white precipitated was then filtered out, and washed several times and dried under vacuum over P_2O_5.

2.1.2. Sn (II) – HMTA complex:

The Sn(II) – HMTA complex was prepared by the addition of 0.896 g (4 mmol) of $SnCl_2.2H_2O$ to 1.12 g (8 mmol) of HMTA, both of them were dissolved in 50 ml ethanol. The reaction mixture was left for 8 hrs. with continuous stirring at room temperature. The white cream precipitated complex was then filtered out, washed several times with minimum ethylalchol and then dried under vacuum over P_2O_5.

2.1.3. Sb (III) and BiO (I) HMTA complexes:

Similar procedures were operated to prepare the two complexes. The antimony (III) trichloride and bismuth oxynitrate are reacted with HMTA in methanol as a solvent with a metal : ligand ratio by 1 : 3 at room temperature for about 8 – 10 hrs.

2.2. The Biological studies

The antimicrobial activity was applied by diffusion disc method. A filter paper sterilized disc saturated with measured quantity of the sample is placed on plate containing solid bacterial medium (nutrient agar broth) or fungal medium (Dox s medium) which has been heavily seeded with the spore suspension of the tested organism according to the method of Barry [35]. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism. The antibacterial activities of some investigated compounds were tested against Escherichia Coli and Staphylococcus Aureus as well as some kinds of fungi; Aspergillus Flavus and Candida Albicans. The complexes, the free HMTA and the pure solvent (DMSO) were tested with the antibacterial and antifungal investigation at the same time.

2.3. Physical and Instrumentation

Carbon, hydrogen and nitrogen contents were determined using a Perkin-Elmer CHN 2400 in the Micro-analytical Unit at the Faculty of Science, Cairo University, Egypt. The percentage of tin cations was determined using atomic absorption method. An atomic absorption spectrometer PYE – UNICAM SP 1900 fitted with a tin lamp was used for this purpose. The percentage of tin(IV) and tin (II) were also determined gravimetrically for conformation by transforming the product into the corresponding metal for tin (IV) and metal oxide for tin (II), while the antimony and bismuth contents are also determined as metals. The lanthanides were determined by complexometric titration using EDTA by xylenol orange [36]. The obtained analytical data are summarized in Table 1. FT IR spectra were investigated in the range 4000 – 400 cm^{-1} using a Gensis II FT IR spectrophotometer and samples were prepared as KBr discs. ^{1}HNMR spectra were recorded on a Varian Gemini 200 MHZ, at room temperature using dimethylsulphoxide as a solvent. Thermal studies of the prepared complexes were measured using a Shimadzu TGA -50H. The samples were heated in platinum crucible in a statistic nitrogen atmosphere to 600 ^oC at a heating rate 10 ^oC min^{-1}. The biological studies were carried out in Micro-Analytical Center, Faculty of Science, Cairo University, Egypt.

3. Results and Discussion

Methenamine reacts with, Ag(I), Cd(II), Hg(II), Cu(II), Sn(II), Sn(IV), Sb(III), Bi(III), Ce(III), Sm(III) and La(III) ions in non aqueous medium to form the obtained HMTA complexes. The physical and analytical data of the complexes are summarized in Table 1. The complexes are thermal stable in air and soluble in common organic solvents like DMSO. All the prepared complexes are non colored (white / white cream color) except for copper(II) complex. The colorless appearance observed for the isolated complexes devoted us to excluding the UV/ Vis study due to there is no significant bands characterized the complex geometry. So, the geometry proposed here referring to the most stable one for each metal ion, as well as the excepted attachment with the corresponding ligand and their conjugated anions (Fig. 1). In order to verify the presence of chloride is coordinated or ionic forms, the complex solution was tested with an aqueous $AgNO_3$ solution.

3.1. Spectroscopic Studies

3.1.1. IR Spectral Analysis

The characteristic spectral bands and their assignments for the isolated complexes in comparing with their relative ligand are presented in Table 2. The compounds are scanned over the range 4000 – 400 cm^{-1} which offer information regarding the coordination mode in the complexes. HMTA is a potentially tetradentate ligand. It may display different mode as a mono -, bi- , tri – or tetradentate. Framework molecular models show that it is more likely to act as monodentate ligand. This is through only one nitrogen atom from the four towards mononucleosis central atom or multidentate bridging ligand towards multi central atoms [37]. The fundamental bands in the ligand spectrum at 1250 and 1000 cm^{-1} are assigned to υC-N vibrations and split into doublets in all the complexes. This splitting elucidates the participation of some donor atoms in between the four for the same complex nucleolus. The IR spectra of

$[Cu_3(NO_3)_6(HMTA).3(H_2O)]$, $[Ag(NO_3)(HMTA).2(H_2O)]$, $[CdCl_2(HMTA).(H_2O)]$ and $[Hg(NO_3)_2(HMTA).H_2O]H_2O$ complexes (Fig. 2, some examples) display a splitting in υC-N bands at ≈ 1250 cm^{-1} and ≈ 980 cm^{-1}. This is due to the differentiate behavior of the donor atoms and the non coordination of one or more in betweens. The minor splitting formed closely spaced doublets or triplets. The IR spectra associated with coordinated HMTA in the complexes reported here are almost super imposable on the IR spectrum of the uncoordinated ligand. This may be attributed to the fact that the chair configuration of the uncoordinated HMTA is retained in all these complexes [38]. The coordinated nitrate groups are terminally monodentate bonded towards Ag(I) and Hg(II) complexes and bidentated in multinuclear Cu(II) complex. This is through the appearance of new bands for υ_{as} and υ_s NO_3 by150 cm^{-1} difference, which may assigned for monodentate and 50 cm^{-1} difference for bidentate nature. The supplementary coordinating ligand (H_2O) was proposed based on the presence of new bands at lower frequency region (600 - 800 cm^{-1}) for δ_r and δ_w H_2O and over 3400 cm^{-1} for υOH, as well as the other supporting bands for υM-N and υM-OH_2. The IR spectra of $[SnCl_2(HMTA)_2]2H_2O$, $[SnCl_4(HMTA)_2]2H_2O$, $[SbCl_3(HMTA)_2]3H_2O$ and $[BiO(NO_3)(HMTA)_2.H_2O]$ $3H_2O$ as heavy metal complexes. These complexes were investigated concerning the same bands (υC-N). A band appeared at ≈ 1250 cm^{-1} and ≈ 1012 cm^{-1}, concerning 1012 cm^{-1} band displays a lower shift than the ligand. The lower appearance supports the participation of nitrogen atom in coordination with the central atoms or with the hydrated water molecules through H- Bonding. The latter proposal is strongly supported through the relative thermal stability of these complexes during the TG investigation. The broad band in the range around 3500 cm^{-1} as well as new bands at the region ≈ 690 – 820 cm^{-1} for υOH, δr and δw, respectively. The rocking vibration associated to the CH_2 of HMTA is observed at 840 and 808 cm^{-1}, while the corresponding vibration of its complexes is observed as a strong absorption and found as group of bands lying in the 986 – 798 cm^{-1} region. The presence of these collected bands in the complexes under study, suggests that HMTA coordinates through its some donor atoms [39-42]. Finally other new bands appeared in all spectra at 499, 499, 510 and 515 cm^{-1} for Sn(II), Sn(IV), Sb(III) and BiO(I) complexes attributed to υM-N vibration. The IR spectra of $[CeCl_3(HMTA)_3]3H_2O$, $[SmCl_3(HMTA)_3]$ and $[La(NO_3)_3(HMTA)_3]3H_2O$ as inner transition metal complexes are investigated in comparing with the ligand spectrum. The significant υC-N bands appeared at ≈ 1250 and 980 cm^{-1} also reflects the presence of different behavior concerning the donor atoms. One or more in between donors may be coordinated towards the central metal ions the others may be non coordination or H – Bonded with hydrated water molecules. This proposal was confirmed through thermal analysis. The rocking vibration bands of CH_2 are also suffered shift (≈ 560 - 790 cm^{-1}) due to the coordination of its neighboring atoms. Also, the appearance of new bands at ≈ 510 cm^{-1} in the complexes spectra are expected for υM-N band.

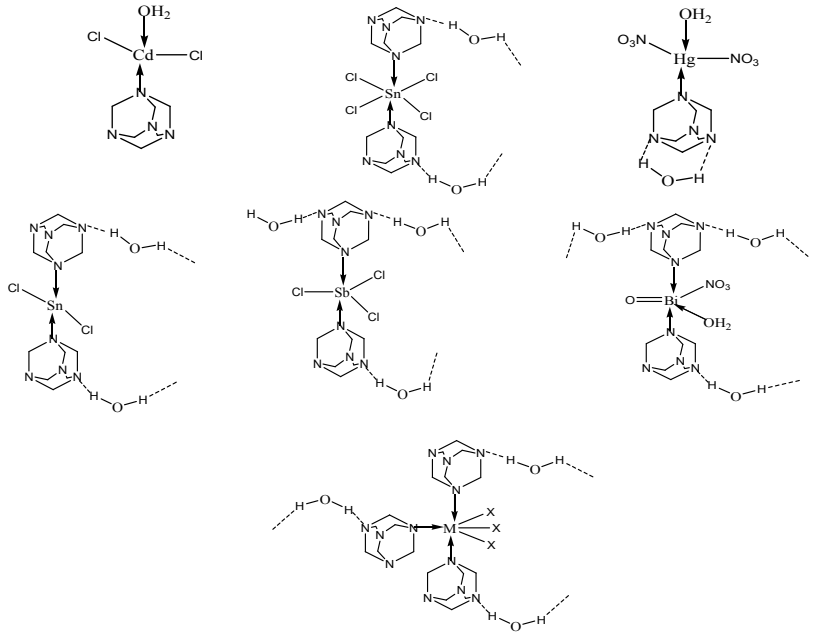

Fig. 1: The proposed structural formulae for most isolated complexes where as: M = Ce (X = Cl) or La (X = NO_3)

3.1.2. ^{1}HNMR Spectra

The ^{1}HNMR spectra of $[SnCl_2(HMTA)_2]2H_2O$ and $[BiO(NO_3)(HMTA)_2.H_2O]3H_2O$ complexes in DMSO $-d_6$ were carried out. Their spectra display whatever difference in comparing with their free ligand spectrum (standard spectrum), a shifted in $-CH_2-$ groups peaks to downfield (range from δ 4.41 to δ 5.11ppm for Sn (II) complex and from δ 4.25 to δ 5.08 ppm for Bi (III) complex) in comparing with the free HMTA ligand. Also, according to the ^{1}HNMR spectral data for $[SnCl_2(HMTA)_2]2H_2O$; δ = 3.45 [H , H_2O] and for $[BiO(NO_3)(HMTA)_2.H_2O]$ $3H_2O$ complex ; δ = 3.38 ,3.47 [H , H_2O] which not found in the spectrum of free HMTA compound , indicate the presence of water molecules in the two complexes. However, the presence of two peaks for H_2O in the Bi (III) complex may indicate that the water molecules found as coordinated lattice. The little difference proposed the presence of lattice molecules inside the coordination sphere through H – Bonding as previously proposed since these data are in agreement with the infrared and thermal analysis.

3.1.3. X – Ray diffraction study

X – Ray powder diffraction of the ligand and some of its complexes were done. The XRD of free ligand reflects its relative amorphous nature (Fig. 3) but the other investigated complexes (Hg(II), Ag(I), Sn(II) and BiO(I)) reveal a highly amorphous nature for the isolated solid complexes. This devoted us to exclude the idea of trying to isolate a single crystal from such complexes. This is may be due to the complexes are heavily hydrated through a strong physical bond with coordinated ligand which may deform crystalline structure in all isolated complexes.

3.2. Thermal analysis aspects

The correlations between the different decomposition steps of the complexes with the corresponding weight losses are reported in Table 3. All the new complexes prepared are thermally investigated. The TG and DTG curves of some complexes are shown in Figure 4. The thermogravimetric analysis is an essential for supporting the presence of solvent molecules coordinated with central atoms or in crystal lattice. The TG curves of multi nuclear $[Cu_3(NO_3)_6(HMTA).3H_2O]$ complex was degraded firstly at a high temperature (midpoint 234.2˚C) by a mass loss 33.83 (Calcd. 33.85 %) for the decomposition of $3H_2O + HMTA + NO_2 + 0.5O_2$. The final residue by 66.17 (Calcd. 66.15%) as a major part of a complex $[2Cu(NO_3)_2 + CuO + NO_2]$. The TG of $[Ag(NO_3)(HMTA).\ 2H_2O]$ complex displays two degradation stages ended at ≈ 550 ˚C. The first stage mid point at 226.25˚C may be for a decomposition of $2H_2O + NO_2 + CO + N_2 + 6H_2$ by loss 50.7 (Calcd. 51.48%) weights. The second stage mid point at 458.89˚C may be corresponding to the removal of 3C atoms oxidized to their oxides by 10.51 (Calcd. 10.41%) weight losses. The final residue is AgC + C by weight percentage of 38.78(Calcd. 38.11%). The TG of $[CdCl_2(HMTA).H_2O]$ complex displays four degradation stages ended at ≈ 600 ˚C. The first stage mid point at 107.05˚C by 5.07 (Calcd. 5.27%) weight losses for the decomposition of H_2O coordinated with central atom. The following steps conclude the gradual decomposition of the coordinated ligand. The final residue at 600˚C by 10.02 (Calcd.10.55%) weights may be for the removal of 3C atoms. The TG curve of $[Hg(NO_3)_2(HMTA)(H_2O)]H_2O$ starts degradation at temperature mid point 88.75˚C may be assigned to the removal of $2(H_2O) + L + 2NO_2 + O_2$ by 60.09 (Calcd. 59.94%) weight losses. The residue recorded at 246.6˚C assigned for Hg by 39.89 (Calcd. 40.05%) weights. The TG curve of $[SnCl_4(HMTA)_2]2H_2O$ complex starting the decomposition at first stage mid point at 140˚C corresponding to the removal of hydrated molecules bonded through H – Bonding inside the coordination sphere by 6.2 (Calcd. 6.24%) weight percentage. The final residue assigned to Sn atom by 20.19(Calcd. 20.58%). The TG curve of $[SnCl_2(HMTA)_2]2H_2O$ complex is starting the decomposition at the mid point temperature 240˚C may be corresponding to the removal of $2H_2O + Cl_2 +$ 2HMTA by 76.32(Calcd. 76.54%) weight losses and the residual part assigned for Sn by 23.68(Calcd. 23.36%). The TG curve of $[SbCl_3(HMTA)_2]3H_2O$ complex displays a decomposition step by a major fragments weights by 78.81(Calcd. 78.89%) and the residual part includes the Sb only by 21.19(Calcd. 21.103%). The TG curve of $[BiO(NO_3)(HMTA)_2.H_2O]$ $3H_2O$ complex displays two degradation stages starting firstly at temperature mid point 150˚C which corresponding to the removal of hydrated water molecules by 8.51 (Calcd. 8.45%) weight loss. The second decomposition stage at mid point temperature 305˚C is corresponding to the removal of coordinated water molecule beside the ligands completely by 46.6 (Calcd. 46.67%) weight losses. The final residue may be corresponding to $BiO(NO_3)$. The TG curve of $[CeCl_3(HMTA)_3.3H_2O]$ complex displays two degradation stages starting at ≈ 300˚C (mid point) may be corresponding to the removal of $1.5\ Cl_2 + 3H_2O$ by 22.00 (Calcd. 22.24%). The second stage at 513˚C may be corresponding to the removal of 2HMTA + $C_6H_{12}N$ by 51.18(Calcd. 52.49%). The final residue may be for CeN_3 by 26.8(Calcd. 25.26%). The TG curve of $[SmCl_3(HMTA)_3]$ complex displays two degradation stages starting at 270˚C (mid point) may be corresponding to the removal of $1.5Cl_2$ +HMTA by 36.1 (Calcd. 36.4%). The second stage mid point at ≈ 430˚C by 40.0(Calcd. 41.39%) may be corresponding to removal of 2HMTA molecules. The residual part is

representing to Sm by 23.89(Calcd. 22.21%). The TG curve of $[La(NO_3)_3(HMTA)_3]3H_2O$ complex displays three degradation stages. The first stage mid point at 95˚C by 2.19 (Calcd. 2.25%) weight losses assigned for the removal of hydrated H_2O molecule. The second stage at 135˚C is corresponding to the removal of $2H_2O$ by 4.59(Calcd. 4.50%). The third stage at 321˚C by 75.29(Calcd. 75.8%) may be corresponding to the removal of 3HMTA + $3NO_2$ + $1.5O_2$. The residual part represents La by 17.93(Calcd. 17.37%). All the data abstracted from the thermal study supporting the proposal of presence of hydrated and coordinated water molecules. Also, the trend of decomposition in relatively higher temperature supports the interaction of solvent molecules inside the coordination sphere through the H- Bonding with the active donor atoms in the ligand as the same as coordinated molecules. Such by the relative thermal stability recorded for most complexes. Most complexes display the same trend in degradation by showing 1 to 2 degradation steps and corresponding to expel of all surrounds of central metal atoms in most investigated complexes except some of them which containing other coherent with the central atoms. This may be due to the recording of residual at relatively lower temperature as well as the strength of bonds coherently attached with metal atoms. This H-Bonding stabilizing the complexes towards the thermal decomposition at relatively higher temperature.

3.3. Thermal decomposition kinetics

Recently, there has been increasing interest in determining the rate-dependent parameters of solid-state non-isothermal decomposition reactions by analysis of TG curves [43,44]. The kinetic analysis parameters such as activation energy (E*), enthalpy of activation (ΔH*), entropy of activation (ΔS*), free energy change of decomposition (ΔG*) were evaluated graphically by employing the Coats–Redfern relation [45] for Cu(II), Ag(I), Cd(II) and Hg(II) complexes (Table 4).

$$\ln\left[\frac{1-(1-\alpha)^{1-n}}{(1-n)T^2}\right] = \frac{M}{T} + B \qquad \text{for n} \neq 1 \quad (1)$$

$$\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right] = \frac{M}{T} + B \qquad \text{for n} = 1 \quad (2)$$

Where $M=-E/R$ and $B = \ln AR/\Phi E$; E, R, A, and Φ are the heat of activation, the universal gas constant, pre-exponential factor and heating rate, respectively. The correlation coefficient, r, was computed using the least square method for different values of n, by plotting the left-hand side of Eqs. (1) or (2) versus $1000/T$ (Fig. 5). A plot of left hand side of Eq. (1) against 1/T gives a slope from which E* was calculated and A (Arrhenius constant) was determined from the intercept. From relevant data, linearization plots confirm first order kinetics. It has been found that E* values for complexes > ligand. The entropy of activation (ΔS*) and the free energy change of activation (ΔG*) were calculated using Eqs. (3) and (4):

$$\Delta S^* \ (JK^{-1}\ mol^{-1}) = R\ [\ln(Ah/kT)-1] \quad (3)$$

$$\Delta G^* (J\ mol^{-1}) = \Delta H^* - T\Delta S^* \quad (4)$$

Where; k and h are the Boltzman and Plank constants, respectively. The calculated values of ΔE*, A, ΔS* and ΔG* for the decomposition steps of the complexes are given in Table 4. According to the kinetic data obtained from the TG curves, all the complexes have negative entropy, which indicates that the complexes are formed spontaneously. The negative entropy also indicates a more ordered.

3.4. Biological Studies

From the antimicrobial profile (Table 5), Hg-HMTA displays a significant inhibitory effect on the growth of tested fungal and bacterial species followed by Cd-HMTA and Ag- HMTA, regarding to the standard antibacterial and antifungal agents. A relative inhibitory effect on the growth of tested bacterial and fungal isolates was observed by Ag-HMTA. Unlike, the slightly antibacterial activity of Cu has relatively no antifungal activity. The plausible antifungal and antibacterial activities by Hg-HMTA and Cd – HMTA complexes may be attributed to the toxicity of Hg and Cd ions on plasma membrane functions, by interference with the ATP binding domains or amino acid transporter channels, causing a negative effect on some metabolic pathways, especially metalloproteinase dependent. According to Overtone's concept [46] of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to the liposolubility is an important factor that controls antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a great extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. The reduction in positive charge of the central metal ion is greater for oxidation states I and II than the oxidation state III, so here we focus on some special complexes which may serve based on the previous concepts. The increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking the metal binding sites on the enzymes of the microorganism. Also, however the metal salts alone may exhibit a higher activity than the complexes but cannot be used as antibacterial agents because of their toxicity and the probability of binding to the free ligands presented in the biological systems such as the nitrogen bases of nucleic acid and proteins.

Table 1: Analytical and Physical data for HMTA metal complexes

Compound Empirical formula (M. Wt.)	Color (geometry)	Elemental analysis (%) Calcd. (Found)				
		C	H	N	M	Cl
1) $[Cu_3 (NO_3)_6 (C_6H_{12}N_4) 3H_2O]$ (756.89)	Blue (distorted octahedral)	9.52(9.60)	2.39(2.35)	-----	25.2(25.1)	------
2) $[Ag (NO_3) (C_6H_{12}N_4). 2H_2O]$ (346.09)	Buff (tetrahedral)	20.82(20.80)	4.66(4.60)	20.24(20.27)	31.17(31.14)	------
3) $[Cd Cl_2 (C_6H_{12}N_4) H_2O]$ (341.52)	White (Tetrahedral)	21.1(21.20)	4.13(4.14)	16.40(16.50)	32.91(32.87)	20.76(20.65)
4) $[Hg (NO_3)_2(C_6H_{12}N_4) (H_2O)] (H_2O)$(500.82)	White (Tetrahedral)	14.39(14.40)	3.22(3.30)	16.78(16.60)	40.05(40.15)	----
5) $[Sn Cl_4 (C_6H_{12}N_4)_2]2H_2O$ (576.93)	White (Octahedral)	24.98(24.96)	4.89(4.87)	19.42(19.40)	20.58(20.57)	24.58(24.57)
6) $[SnCl_2(C_6H_{12}N_4)_2]2H_2O$ (506.02)	White (Tetrahedral)	28.48(28.45)	5.58(5.57)	22.14(22.19)	23.46(23.44)	14.01(14.00)
7) $[Sb Cl_3(C_6H_{12}N_4)_2]3H_2O$ (562.53)	White (Trigonal bipyramidal)	25.62(25.60)	5.37(5.36)	19.92(19.87)	21.64(21.64)	18.91(18.90)
8) $[BiO(NO_3)(C_6H_{12}N_4)_2(H_2O)]$ $3H_2O$(639.42)	Whit (Trigonal bipyramidal)	22.54(22.53)	5.04(5.10)	19.71(19.65)	32.68(32.66)	-----
9) $[Ce Cl_3(C_6H_{12}N_4)_3]3H_2O$ (721.09)	White (Octahedral)	29.98(30.10)	5.87(5.88)	23.31(23.32)	19.43(19.42)	14.75(14.76)
10) $[SmCl_3(C_6H_{12}N_4)_3]$ (677.29)	White (Octahedral)	31.92(31.88)	5.36(5.34)	24.82(24.80)	22.20(22.20)	15.70(15.60)
11) $[La(NO_3)_3(C_6H_{12}N_4)_3]3H_2O$ (799.53)	White (Octahedral)	27.04(27.03)	5.29(5.28)	----	17.37(17.38)	-----

Table 2: Assignments of the IR Spectral bands (cm^{-1}) of HMTA and its metal complexes

Compound	υ (C-N)		$\upsilon(H_2O)$	$\delta_r(H_2O)$	$\delta_w(H_2O)$	$\upsilon(NO_3)$	ν_{M-N}	ν_{M-O}
1) $[C_6H_{12}N_4]$	1250	1000	---	---	---	----	----	---
2) $[Cu_3 (NO_3)_6 (C_6H_{12}N_4) 3H_2O]$	1100	900	3550	800	700	1400 1350	470	550
3) $[Ag (NO_3) (C_6H_{12}N_4). 2H_2O]$	1000	980	3500	800	650	1450 1300	500	520
4) $[Cd Cl_2 (C_6H_{12}N_4) H_2O]$	1250	1000 980	3500	800	650	----	500	520
5) $[Hg (NO_3)_2(C_6H_{12}N_4) (H_2O)] (H_2O)$	1050	1000	3500	800	700	1450 1350	500	550
6) $[Sn Cl_4 (C_6H_{12}N_4)_2]2H_2O$	1250	1018	3480	818	798	---	499	---
7) $[SnCl_2(C_6H_{12}N_4)_2]2H_2O$	1253	1012	3475	818	693	---	499	----
8) $[Sb Cl_3(C_6H_{12}N_4)_2]3H_2O$	1269	1018	3433	800	690	---	510	----
9) $[BiO(NO_3)(C_6H_{12}N_4)_2(H_2O)]3H_2O$	1264	1018	3422	818	745	1468 1295	515	-----
10) $[Ce Cl_3(C_6H_{12}N_4)_3]3H_2O$	1259	1012	3564	818	792	----	520	----
11) $[SmCl_3(C_6H_{12}N_4)_3]$	1250	1012	3427	800	712	----	525	----
12) $[La(NO_3)_3(C_6H_{12}N_4)_3]3H_2O$	1243	1012	3532	818	750	1468 1290	509	----

Table 3: Thermogravimetric data of the investigated complexes

Complex	Steps	DTG peak (°C)	Decomposed assignments	Weight loss Found (Calcd. %)
(2)	1st residue	234.22	$-NO_3 + L + 3H_2O$ $-2[Cu(NO_3)_2] + Cu + NO_2 + 0.5O_2$	33.83 (33.85) 66.17 (66.15)
(3)	1st 2nd residue	226.25 458.89	$-2H_2O + NO_2 + 0.5O_2 + 2N_2 + 6H_2 + C$ $- 3C$ $Ag + 2C$	50.70 (51.48) 10.51 (15.41) 38.78 (38.11)
(4)	1st 2nd 3rd 4th residue	107.05 208.93 241.4 329.13	$-H_2O$ $- Cl_2 + N_2$ $-C + 3H_2$ $-Cd + 2C + 3H_2 + N_2$ $3C$	5.07 (5.27) 28.29 (28.96) 5.56 (5.28) 51.06 (49.92) 10.02 (10.55)
(5)	1st residue	88.75 246.6	$- 2H_2O + L + 2NO_2 + O_2$ Hg	60.09 (59.94) 39.89 (40.05)
(6)	1st 2nd residue	140 240	$- 2H_2O$ $- 2L + 2Cl_2$ Sn	6.20 (6.24) 73.61 (73.18) 20.19 (20.58)
(7)	1st residue	240	$- 2H_2O + 2L + 2Cl_2$ Sn	76.32(76.54) 23.68(23.36)
(8)	1st residue	270	$- 3H_2O + 2L + 1.5Cl_2$ Sb	78.81(78.89) 21.19(21.103)
(9)	1st 2nd residue	150 295	$- 3H_2O$ $- H_2O + 2L$ $BiO(NO_3)$	8.51(8.45) 46.60(46.67) 44.89(44.88)
(10)	1st 2nd residue	307 513	$- 1.5Cl_2 + 3H_2O$ $- 3C_6H_{12}N + 2L +$ CeN_3	22.00(22.24) 51.18(52.49) 16.81(25.26)
(11)	1st 2nd residue	272 433	$- 1.5Cl_2$ $2L$ Sm	36.10(36.40) 40.00(41.39) 23.89(22.21)

Table 4: Kinetic parameters using the Coats – Red fern (CR)operated for the HMTA complexes

Complex	Step	Method	Kinetic Parameters					
			E ($Jmol^{-1}$)	A (S^{-1})	ΔS ($Jmol^{-1}K^{-1}$)	Δ H ($Jmol^{-1}$)	Δ G ($Jmol^{-1}$)	r
(2)	1st	CR	1.02×10^5	1.5×10^2	-2.08×10^2	9.76×10^4	2.03×10^5	0.9746
(3)	1st	CR	4.81×10^5	7.11×10^2	-1.9×10^2	4.77×10^5	5.74×10^5	0.9859
	2nd	CR	2.85×10^5	4.22×10^2	-2.02×10^2	2.79×10^5	4.27×10^5	0.9402
(4)	1st	CR	1.22×10^5	1.80×10^2	-2.04×10^2	1.19×10^5	1.96×10^5	0.9177
	2nd	CR	1.43×10^5	2.11×10^2	-2.05×10^2	1.39×10^5	2.44×10^5	0.9594
	3rd	CR	1.84×10^5	2.72×10^2	-2.07×10^2	1.77×10^5	3.53×10^5	0.9935
(5)	1st	CR	1.17×10^5	1.73×10^2	-2.06×10^2	1.13×10^5	2.11×10^5	0.9941
	2nd	CR	1.16×10^5	1.71×10^2	-2.07×10^2	1.12×10^5	2.20×10^5	0.9750

Table 5 :The inhibition zone values of bacteria and fungi for the ligand and its metal complexes

Compound	Escherichia coli (G^-)	Staphylococcus Aureus (G^+)	Aspergillus flavus (Fungus)	Candida albican (Fungus)
DMSO	0.0	0.0	0.0	0.0
Tetracycline	32	26	--	--
Amphotricin B	--	--	17	20
$[Cu_3(NO_3)_6(C_6H_{12}N_4)3H_2O]$	13	14	0.0	13
$[Ag(NO_3)(C_6H_{12}N_4).2H_2O]$	25	24	13	17
$[CdCl_2(C_6H_{12}N_4)H_2O]$	31	35	18	24
$[Hg(NO_3)_2(C_6H_{12}N_4)(H_2O)](H_2O)$	35	36	23	29

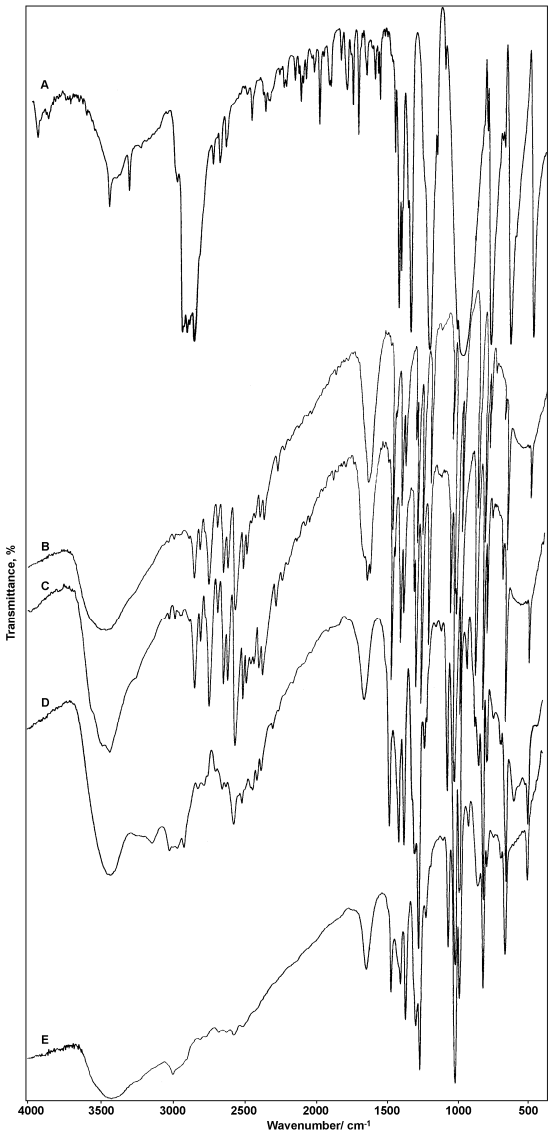

Fig. 2: Infrared spectra of (A) Free HMTA, (B) Sn(IV)-HMTA, (C) Sn(II)-HMTA, (D) Sb(III)-HMTA and (E) Bi(III)-HMTA complexes.

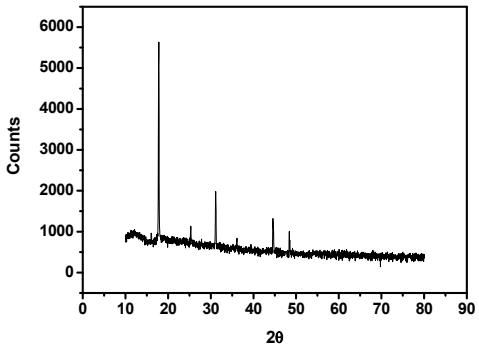

Fig. 3: XRD of HMTA in free State

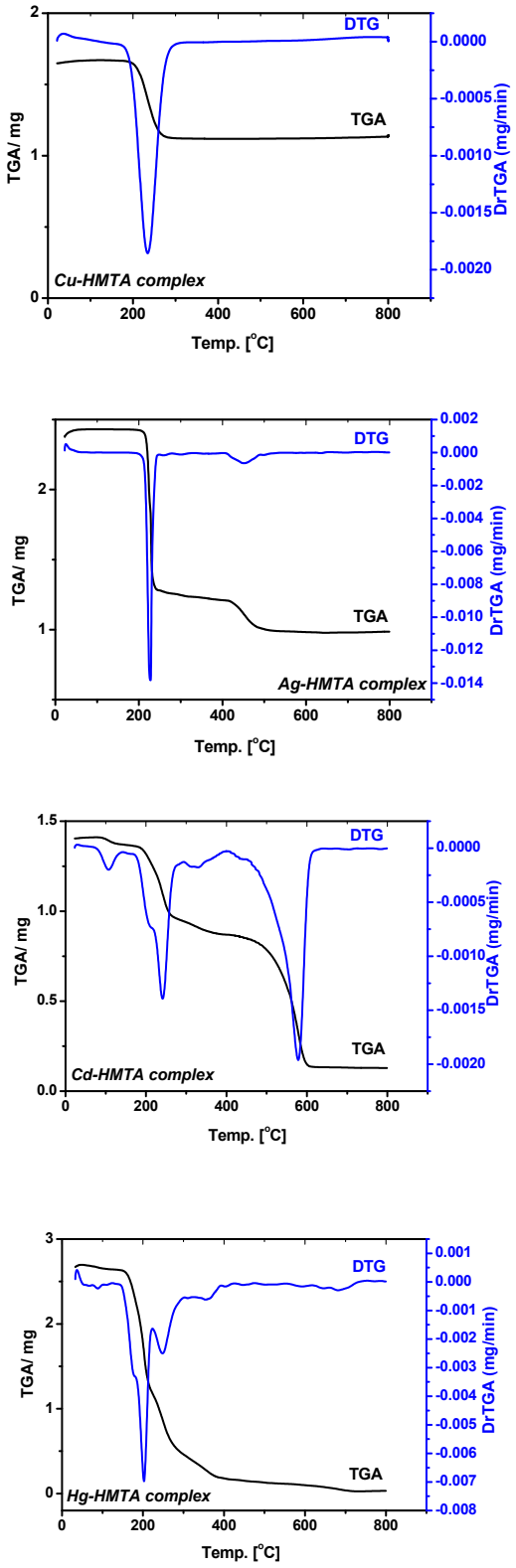

Fig. 4: Thermogravimetric analysis TG and DTG of HMTA complexes.

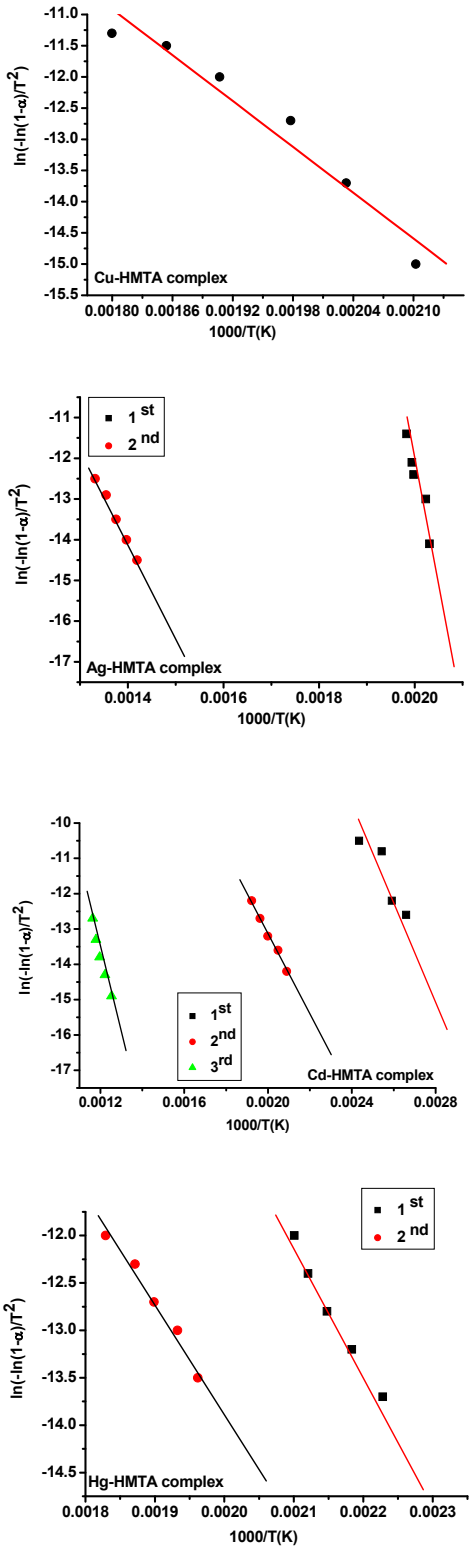

Fig. 5: Coats–Redfern curves of Cu(II), Ag(I), Cd(II) and Hg(II) HMTA complexes.

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Synthesis and thermal studies of Mn^{II}, Cr^{III} and Fe^{III} methionine complexes

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Abstract: The resulted complexes produced between Mn^{II}, Cr^{III} and Fe^{III} ions and biological molecules like amino acids play an important role in human life. Mn^{II}, Cr^{III} and Fe^{III} complexes are synthesized with methionine (MIE). These complexes were characterized by elemental analysis, molar conductance, infrared and UV-Vis spectra as well as thermogravimetric analysis (TGA/DTG). The elemental analysis introduce that the chelation ratio between metal ions and free methionine moiety behaves as bidentate ligand forming chelates with 1:2 (metal: ligand) stoichiometry for Mn^{II} ion and 1:3 for Cr^{III} and Fe^{III} ions. The molar conductance measurements of the products in DMSO indicate that the complexes are non-electrolyte nature. The activation energies and other kinetic parameters were calculated from the Coats-Redfern and Horowitz-Metzger equations.
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Keywords: Methionine; Infrared spectra; Thermal analyses; transition metals.

1. Introduction

Amino acids are the principal building blocks of proteins and enzymes. They exist naturally in a zwitterionic state where the carboxylic acid moiety is ionized and the basic amino group is protonated [1]. Methionine with molecular formula, $C_5H_{11}NO_2S$, (Fig. 1) is one of the two sulfur containing amino acid, cysteine being the other [2]. Methionine helps to initiate translation of messenger RNA by being the first amino acid incorporated into the N-terminal position of all proteins. It is considered as an essential amino acid for normal Metabolism, growth and maintenance of body tissue. It is used as nutritional supplement and act as antioxidant in biological system [3-5]. Some metal complexes of DL–methionine were prepared in aqueous medium and characterized by different physico-chemical methods [6]. Methionine forms 1:2 complexes with metal, M(II). The general empirical formula of the complexes is proposed as $[(C_5H_{10}NO_2S)_2M^{II}]$; where M^{II} = Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II). All the complexes are extremely stable in light and air and optically inactive. These transition metals are essential trace elements and used as nutritional supplement. They act as cofactors in various enzyme systems i.e. as metalloenzymes or as enzymatic activators [7-10]. Cd(II) and Hg(II) are toxic elements, that methionine is a biological chelating agent may lower the degree of toxicity for the formation of chelate with toxic metals [11]. The infrared and laser Raman spectra of L-methionine, L-methioninium perchlorate monohydrate were recorded at room temperature and the vibrational assignments of the observed wave numbers were made [12, 13]. A new palladium(II) complex with methionine was synthesized and characterized by a set of chemical and spectroscopic techniques [13] which indicate coordination of the amino acid to Pd(II) through the carboxylate and amino groups in a square planar geometry [14, 15]. In view of literature, the coordination chemistry of amino acids with different metal ions is a very interesting subject, so, in this paper, we report the formation of three methionine complexes in alcoholic solution. The aim of this study is to investigate the coordination behavior of methionine with Mn(II), Cr(III) and Fe(III) salts. Their synthesis, isolation and speculation by different spectroscopic studies are demonstrated.

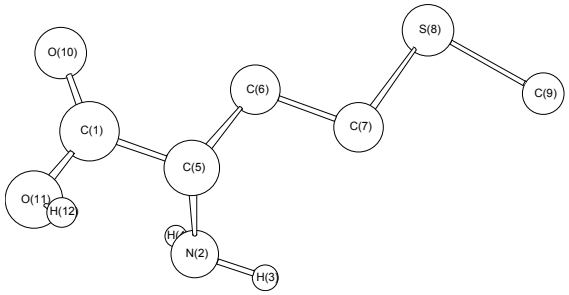


Fig. 1: Structure of methionine

2- Experimental

2-1- Material and instrumentation

All chemicals used were of the purest laboratory grade (Merck). Carbon and hydrogen percentages were determined using a Perkin–Elmer CHN 2400. Infrared spectra were recorded on Bruker FT-IR Spectrophotometer (4000–400 cm^{-1}) in KBr pellets. The UV–vis, spectra were studied in dimethylsulfoxide (DMSO) solvent with concentration (1.0×10^{-3} M) for the methionine and their complexes by help of Jenway 6405 Spectrophotometer with 1.0 cm quartz cell within the range of 800–200 nm. Thermogravimetric analysis (TGA and DTG) were carried out in dynamic nitrogen atmosphere (30 ml/min) with a heating rate of 10 °C/min using a Schimadzu TGA– 50 H thermal analyzer. Molar conductance measurements of the methionine free ligand and their complexes with 1.0×10^{-3} mol/l in DMSO were carried out using Jenway 4010 conductivity meter.

2-2- Preparation of the complexes

The Mn(II) complex was synthesized by adding 2 mmol of $MnCl_2.H_2O$ in alcoholic solution to an 4 mmol alcoholic solution of methionine with molar ratio metal: ligand equal 1:2, at room temperature under stirring. The Mn(II) complex was precipitated as a brown powder, after constant stirring, at pH 8. The compound was filtered, washed with methanol and dried at 80 °C in an oven for about 2 hrs and the product was kept in a desiccate. The Cr(III) complex was synthesized by adding 2 mmol of $CrCl_3.6H_2O$ in alcoholic solution to an alcoholic solution of methionine containing 6 mmol of the ligand (molar ratio metal: ligand equal to (1:3), at room temperature and under stirring. The Cr(III) complex was precipitated as a green powder, after constant stirring, at pH 8-9. The compound was filtered, washed with methanol and dried at 80°C in an oven for about 2 hrs and the product was kept in a desiccate. The Fe(III) brown powder complex was synthesized by the same procedure of Cr(III) complex.

3- Results and Discussion

The complexes were characterized by different physicochemical techniques and some properties were investigated. The methionine Mn^{II}, Cr^{III} and Fe^{III} complexes were investigated in this study, are very stable at room temperature in the solid state. These complexes are insoluble in common organic solvents in cold or hot conditions except for DMSO solvent. No suitable crystals of the complexes were obtained in order to perform an X-ray structure determination. The elemental analyses and molar conductance of the free ligand and its complexes are given in Table 1. The analytical data are in a good agreement with the proposed stoichiometry of the complexes. The metal-to-ligand ratio in Mn^{II}, Cr^{III} and Fe^{III} complexes were different molar ratio, that found to be 1:2 or 1:3 (metal: ligand) in the coordination behaviors. All Mn^{II}, Cr^{III} and Fe^{III} complexes melted with decomposition far from the melting point of the free ligand (281 °C) owing to the formation of new compound with fitted with metal ions.

Conductivity measurements in non-aqueous solutions have frequently been used in structural studies of metal chelates within the limits of their solubility. They provide a method of testing the degree of ionization of the complexes, the molar ions that a complex liberates in solution, the higher will be its molar conductivity and vice versa. The non-ionized complexes have negligible value of molar conductance. The molar conductivities of the solid chelates are measured for 1.0×10^{-3} mol solution of 1:2 and 1:3 complexes in DMSO. The conductivity data reported for these complexes are given in Table 1. It is clear from the conductivity data that the complexes present behave as non-electrolytes [16] behavior. The molar conductivity values for all the complexes in organic solvent (DMSO) with 10^{-3} mol were in rang of (15-24) Ω^{-1} cm^{-1} mol^{-1} (Table 1).

Table 1: Elemental analysis and conductivity data for methionine complexes

Complexes	Mwt.	Λm ($\Omega^{-1}cm^{-1}$ mol^{-1})	Content (found) calculated		
			%C	%H	%N
$[Mn(C_5H_{10}No_2S)_2]$	351	15	34.18 (34.6)	5.74 (5.70)	7.97 (8.10)
$[Cr(C_5H_{10}No_2S)_3]$	496	25	36.28 (36.40)	6.09 (6.11)	8.46 (8.53)
$[Fe(C_5H_{10}No_2S)_3]$	500	22	36.00 (35.94)	6.04 (6.13)	8.40 (8.53)

The IR spectra in the (4000–400 cm^{-1}) region have provided information regarding the coordination mode in the methionine complexes (Fig. 2) and were analyzed by comparison with data for the free methionine ligand. The most relevant bands and proposed assignments for all the complexes are mentioned in Table 2. In the FT-IR spectra, extensive coupling occurs for several vibrations, making qualitative deductions about the environment around metal ions difficult. However, the IR spectral data (Table 2) shown changes in the position and profiles of some bands, as compared to those of free methionine, suggesting participation of the groups that produce these bounds in the coordination with Mn^{II}, Cr^{III} and Fe^{III} ions. Major changes are related to the carboxylate and amine bands. ligand methionine; amino acid physical properties indicate a "salt-like" behavior. In solution, the amino acid molecule appears to have a change which changes with pH. As intermolecular neutralization reaction leads to a salt-like ion called a Zwitterion. The amino group can lose a hydrogen ion to become negative charged, and also can accept a hydrogen ion to become positive charged. Assignment of observed frequencies; In all spectra, the characteristic band of NH_2 group vibration appears at 3300–3400 cm^{-1} corresponding to $\nu_s(NH_2)$ and $\nu_{as}(NH_2)$. The band due to the NH_3^+ group $\nu(NH_3^+)$ at 3100, 2950 and 2500 cm^{-1} [17], which are very intense in the free ligand, appears as a weak shoulder or disappear in the spectra of the complex compounds. The tentative assignments have been done on the basis of standard references and some published papers [17, 18]. The characteristic bands of the complexes are listed in Table 2. The pattern of the IR spectra of other complexes is almost similar with each other with some exceptions. Most of the important bands in all the complexes were shifted significant compare to that of the free ligand which indicates the formation of new compound. The intensity of bands in the complexes has been reduced in most of the cases comparing the intensity of bands found in the free ligand. The reduction of absorption frequencies may be due to the formation of coordination bond through oxygen

atom of –COO group with metal ion. Since there is no significant absorption band at about 3450 cm^{-1} for ν(O–H) absorption in any of the complexes, we can conclude that there is no water molecule in the complexes as coordinated water or as water of crystallization. In Cr(III) and Fe(III) complexes a weak significant absorption band is observed at about 3422, 3419 cm^{-1} which may be due to the O–H stretching vibration for the presence of moisture in the sample .

Table 2: The most significant FT-IR bands (cm^{-1}) of the methionine complexes

Complexes	ν(N–H)(str.)	ν(COO) (asym)	ν(COO) (sym)	$\Delta(\upsilon_{as}-\upsilon_{s})$
$[Mn(MIE)_2]$	2917	1584	1408	176
$[Cr(MIE)_3]$	2947	1580	1413	167
$[Fe(MIE)_3]$	2947	1580	1413	167

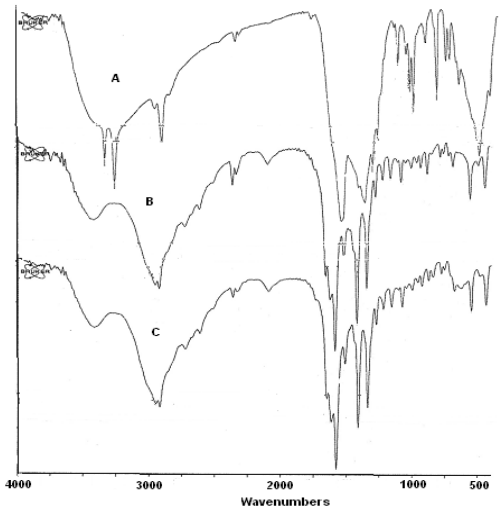
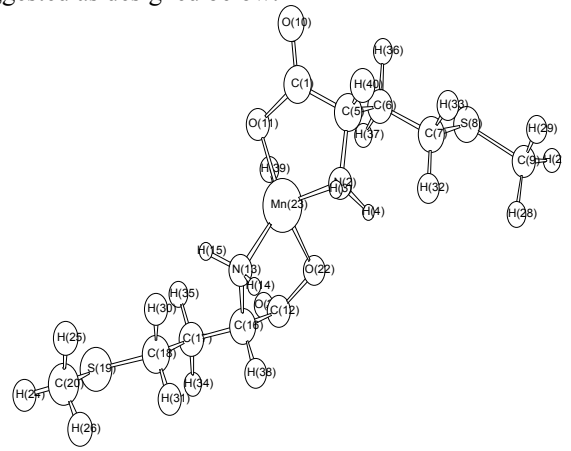


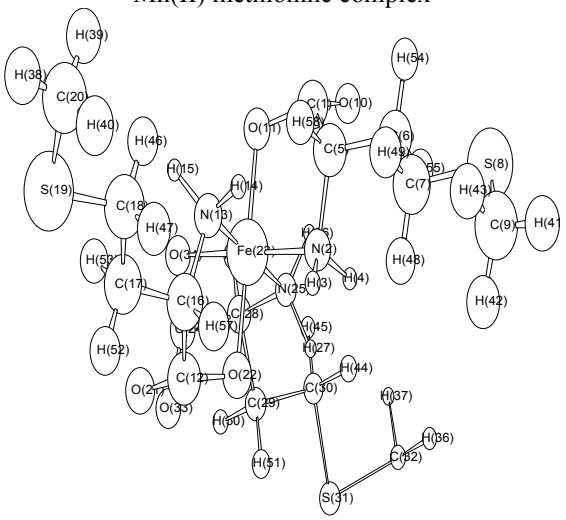
Fig. 2: IR spectra of (A): Mn(II), (B): Cr(III) and (C): Fe(III) methionine complexes

The characteristics absorption bands with tentative assignments of electronic absorption spectra of methionine complexes were demonstrated. The assignments have been done on the basis of some standard references [19-21]. The absorption bands located between 200 and 400 nm were assigned to the organic moiety and peaks above 400 nm was due to chelation. The bands due to π→π* transition in all metal complexes at 236–257 nm have broadening behavior, whereas in methionine it was found at about 236 nm. The presence of absorption band within 295–299 nm in methionine complexes was due to n→σ* transitions that was observed at 297 nm in ligand. The n→π* transition bands were observed at 318–365 nm (338 and 350 nm in free ligand) in almost all the metal complexes. The presence of π→π*, n→π* and n→σ* bands in all methionine complexes indicate the presence of the functional groups of the parent ligands (e.g. C=O, $–NH_2$ and C–S). A large shifting of the absorption bands in the complexes and appearing of a new band for d–d transitions also indicate the probability of forming M→L coordination bonds in the complexes. Transition metal complexes are generally colored and this color arises due to the absorption of light in visible region. Therefore, the band's centering around 413 nm in Mn(II), Cr(III) and Fe(III) complexes are clearly due to the d–d electronic transitions, which causes color of the complexes.

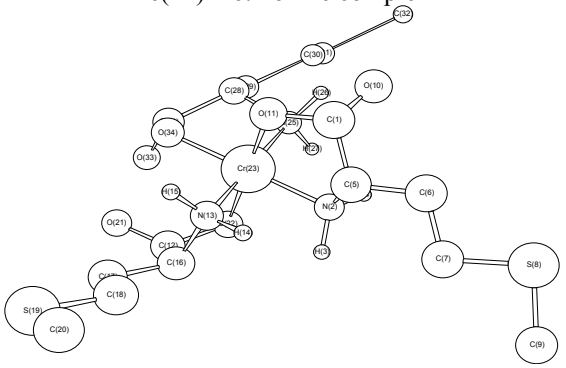
The structures of the methionine complexes (Fig. 3) accordingly the above interpretation using elemental analysis, molar conductance, (infrared and electronic) spectra as well as thermogravimetric analysis can be suggested as designed below.



Mn(II) methionine complex



Fe(III) methionine complex



Cr(III) methionine complex

Fig. 3: Suggested structures of the methionine complexes

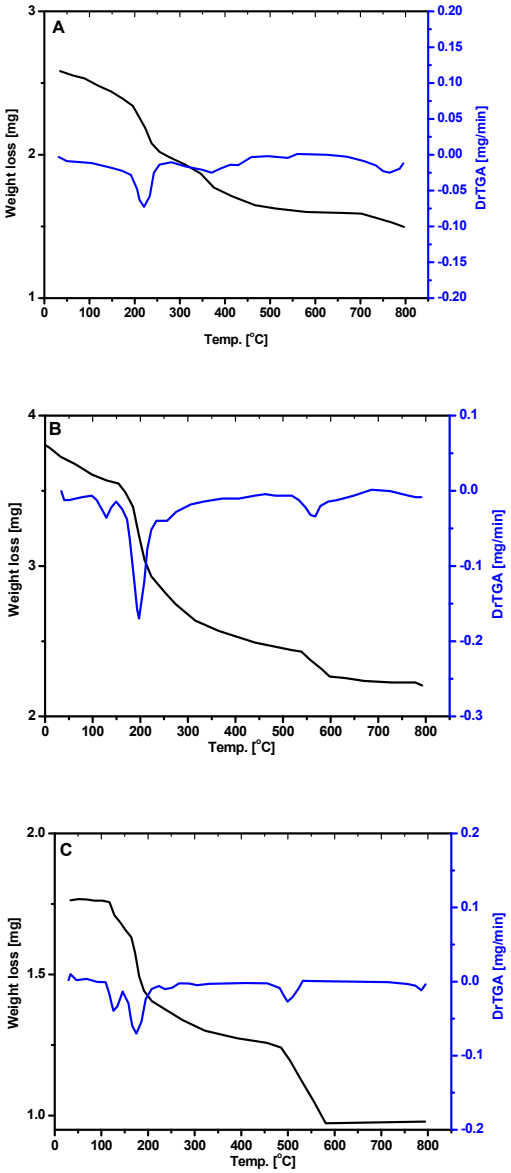


Fig. 4: TG/DTG curves of A- Mn(II)/methionine, B- Cr(III)/methionine and Fe(III)/methionine complexes.

The complexation between metal ions like (Mn(II), Cr(III) and Fe(III)) with methionine produced 1:2 and 1:3 molar ratio (metal: methionine) as a bidentate through its nitrogen of amino group and oxygen of carboxylate group which give general formula: $[M(MIE)_x]$, where MIE = methionine anion; x= 2 or 3. The TGA curves (Fig. 4) for methionine Mn(II), Cr(III), and Fe(III) complexes were carried out within a temperature ranged from room temperature until 800 ^oC. The calculated mass losses were estimated based on the TG data and agree quite well with the molecular formula of the suggested complexes. The decomposition stages, temperature ranges, maximum decomposition peaks DTG_{max}, percentage in mass loss, and the assignments of decomposition moieties were discussed and assigned. The Mn(II) methionine complex gives four medium-to-weak stages of decomposition pattern. The temperature ranges within 50–230, 230–380, 380-440 and 750-800 °C with T_{max}= 230, 378, 436 and 770 °C, respectively. The total mass loss is about 40.75% due to loss of most organic moiety of methionine ligand, then leaving mixtures of manganese oxide (MnO_2) and few carbon atoms as a final residual. The thermal decomposition of chromium(III) complex is thermally decomposed in a successive four decomposition steps with definite maximum temperature at 125, 195, 560, and 780 ^oC. The found mass loss are 6.23%, 17.50%, 12.40%, and 6.90% at temperature range 50–125, 125–200, 200–560, and 560–800 ^oC, respectively. The final residual is become chromium(III) oxide polluted with unburned carbon atoms. Finally, the thermal decomposition of Fe(III) complex is thermally decomposed in a successive five decomposition steps. The total weight loss is to be 46.24% within the temperature range 50–800^oC attributed to the loss most terminal groups of three molecules of chelating methionine. The final decomposition stage was leaving metal oxide contaminated with few carbon atoms which have a lack in oxygen atoms.

In recent years there has been increasing interest in determining the rate-dependent parameters of solid-state non-isothermal decomposition reactions by analysis of TG curves. Several equations [22-29] have been proposed as means of analyzing a TG curve and obtaining values for kinetic parameters. Many authors [22-26] have discussed the advantages of this method over the conventional isothermal method. The rate of a decomposition process can be described as the product of two separate functions of temperature and conversion [23], using

$$d\alpha/dt = k(T)f(\alpha) \quad (1)$$

Where α is the fraction decomposed at time t, k(T) is the temperature dependent function and f(α) is the conversion function dependent on the mechanism of decomposition. It has been established that the temperature dependent function k(T) is of the Arrhenius type and can be considered as the rate constant k.

$$k = A\, e^{-E^*/RT} \quad (2)$$

Where, R is the gas constant in ($Jmol^{-1}K^{-1}$). Substituting equation (2) into equation (1), we get,

$$d\alpha/dT = (A/\varphi\, e^{-E^*/RT})f(\alpha)$$

Where, φ is the linear heating rate dT/dt. On integration and approximation, this equation can be obtained in the following form

$$\ln g(\alpha) = -E^*/RT + \ln[AR/\varphi E^*]$$

Where, g(α) is a function of α dependent on the mechanism of the reaction. The integral on the right hand side is known as temperature integral and has no closed for solution. So, several techniques have been used for the evaluation of temperature integral. Most commonly used methods for this purpose are the differential method of Freeman and Carroll [22] integral method of Coats-

Redfern [24], the approximation method of Horowitz-Metzger [27].

In the present investigation, the general thermal behaviors of the methionine complexes in terms of stability ranges, peak temperatures and values of kinetic parameters are tabulated Table 3. The kinetic parameters have been evaluated using the following methods and the results obtained by these methods are compared with one another. The following two methods are discussed in brief.

i- Coats- Redfern equation

The Coats-Redfern equation, which is a typical integral method, can be represented as:

$$\int_0^{\alpha} \frac{d\alpha}{(1-\alpha)^n} = \frac{A}{\varphi}\int_{T_1}^{T_2} \exp(-\frac{E^*}{RT})dt$$

For convenience of integration the lower limit T_1 is usually taken as zero. This equation on integration gives;

$$\ln[-\frac{\ln(1-\alpha)}{T^2}] = -\frac{E^*}{RT} + \ln[\frac{AR}{\varphi E^*}]$$

A plot of left-hand side (LHS) against 1/T was drawn. E^* is the energy of activation in kJ mol^{-1} and calculated from the slop and A in (s^{-1}) from the intercept value. The entropy of activation ΔS^* in ($JK^{-1}mol^{-1}$) was calculated by using the equation:

$$\Delta S^* = R \ln(Ah/k_B T_s) \quad (3)$$

Where, k_B is the Boltzmann constant, h is the Plank's constant and T_s is the DTG peak temperature [29].

ii- Horowitz-Metzger equation

The Horowitz-Metzger equation is an illustrative of the approximation methods. These authors derived the relation:

$$\log[\{1-(1-\alpha)^{1-n}\}/(1-n)] = E^*\theta/2.303RT_s^2 \quad \text{for } n\neq 1 \quad (4)$$

When n = 1, the LHS of equation 4 would be log[-log (1-α)]. For a first-order kinetic process the Horowitz-Metzger equation may be written in the form:

$$\log[\log(w_\alpha / w_\gamma)] = E^*\theta/2.303RT_s^2 - \log 2.303$$

Where, $\theta = T - T_s$, $w_\gamma = w_\alpha - w$, w_α = mass loss at the completion of the reaction; w = mass loss up to time t. The plot of $\log[\log(w_\alpha / w_\gamma)]$ vs θ was drawn and found to be linear from the slope of which E^* was calculated. The pre-exponential factor, A, was calculated from the equation:

$$E^*/RT_s^2 = A/[\varphi \exp(-E^*/RT_s)]$$

The entropy of activation, ΔS^*, was calculated from equation 3. The enthalpy activation, ΔH^*, and Gibbs free energy, ΔG^*, were calculated from; $\Delta H^* = E^* - RT$ and $\Delta G^* = \Delta H^* - T\Delta S^*$, respectively.

Table 3: Kinetic parameters determined using the Coats–Redfern (CR) and Horowitz–Metzger (HM) of the methionine complexes.

Complex	Stage	Method	Parameter					r
			E (kJ mol^{-1})	A (s^{-1})	ΔS (J mol^{-1} K^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ $mol^{-1)}$	
Mn(II)	1st	CR	1.01E+5	3.98E+8	-8.45E+1	9.71E+4	1.39E+5	0.9919
		HM	1.09E+5	4.45E+9	-6.44E+1	10.5E+4	1.37E+5	0.9888
Cr(III)	1st	CR	1.03E+5	3.21E+9	-6.67E+1	4.42E+4	1.31E+5	0.9980
		HM	1.13E+5	6.00E+10	-4.23E+1	10.9E+4	1.29E+5	0.9985
Fe(III)	2nd	CR	1.10E+5	5.41E+10	-4.28E+1	1.07E+5	1.26E+5	0.9764
		HM	1.15E+5	5.10E+11	-2.41E+1	1.11E+5	1.22E+5	0.9742

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Role of Vitamin E in Combination with Methionine and L- carnosine Against Sodium Fluoride-Induced Hematological, Biochemical, DNA Damage, Histological and Immunohistochemical Changes in Pancreas of Albino Rats.

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Abstract
Excessive fluoride ingestion has been identified as a risk factor for fluorosis and oxidative stress. The present study was aimed to evaluate vitamin E in combination with methionine and L- carnosine as a potential natural antioxidant to mitigate the effects of sodium fluoride on hematological indices, DNA damage, pancreatic digestive enzyme activities and histological structure of pancreas through light, electron microscopic and immunohistochemical studies. Thirty-six of adult male albino rats were divided into six groups (6 rats in each group). Oral administration of sodium fluoride caused a statistical significant decrease in RBC, HCT, MCV, RDW, MCH, MCHC and PLT and increase in WBC, lymphocytes and granulocytes The levels of the these parameters were significantly reversed in the groups pretreated with vitamin E in combination with methionine and L- carnosine prior to NaF. Animals treated with NaF showed significant decrease in pancreatic digestive enzyme activities and protein levels as compared to the control group, while significant increase in animals treated with vitamin E in combination with methionine and L- carnosine prior to NaF. Also, NaF resulted in a significant decrease in serum total protein, albumin and blood glucose levels, while pretreated with vitamin E in combination with methionine and L- carnosine prior to NaF resulted in a significant increase in these parameters. Plasma malondialdehyde levels were significantly increased and the activities of erythrocyte superoxide dismutase were significantly decreased in the NaF treated group. However, vitamin E in combination with methionine and L- carnosine prior to NaF reduced the process of lipid peroxidation and increased the activity of SOD. NaF reduced DNA, RNA contents of the liver and significant increase DNA damage in liver and the frequencies of micro nucleated polychromatic erythrocytes (MN-PCE) in bone marrow. But, concurrent administration of NaF and vit. E in combination with methionine and L- carnosine for 35 days caused significant amelioration in all parameters was studied. Histologically, multiple vacuoles of variable size were observed in the cytoplasm of pancreatic acinar cells together with inflammatory cells infiltration in the stroma of pancreas of Na F treated group. Pancreas of animals treated with vit. E in combination with methionine and L- carnosine prior to NaF displayed amelioration in toxic effects of NaF. Intensive positive immunoreactivity for caspase- 3 was observed in the cytoplasm of most pancreatic acinar cells of NaF treated group which was of significant value. On the other hand the cytoplasmic acinar cells of vit. E in combination with methionine prior to NaF treated group and L-carnosine prior to NaF treated groups showed apparent reduction of caspase-3 immunoreactivity which were also of significant values. Dilatation and globular- shaped rER, intra-cisternal granules, few or even absence of zymogen granules and irregular shaped, pyknotic and heterochromatic nuclei were observed ultrastructurally in the cytoplasm of pancreatic acinar cells of NaF treated group. Ultrathin sections of serous cells of vit. E in combination with methionine prior to NaF treated group and L-carnosine prior to NaF treated group showed preservation of acinar cytoplasmic contents. These results indicate that sodium fluoride can inhibit pancreatic digestive enzyme activities and cause histological and immunohistochemical changes, which may lead to a series of biochemical and pathological abnormalities and concurrent administration of NaF and vit.E in combination with methionine and L-carnosine for 35 days to these animals alleviated the adverse effects of fluoride. [Fatma E. Agha[1], Mohamed O. El-Badry[2], Dina A. A. Hassan[3], Amira Abd Elraouf .**Role of Vitamin E in Combination with Methionine and L- carnosine Against Sodium Fluoride-Induced Hematological, Biochemical, DNA Damage, Histological and Immunohistochemical Changes in Pancreas of Albino Rats]**. *Life sci J 2012; 9(2):1260-1275].(ISSN:1097-8135*). http://www.sciencesite.com. **187**

Key Words: Albino rat; Sodium Fluoride; Heamotological Parameters; Digestive enzymes; Pancreatic acinar cells; vitamin E; Methionine and L-carnosine .

1.Introduction

Fluoride is widely distributed in nature in many forms and its compounds are being used extensively. Fluoride in small doses has remarkable prophylactic influence by inhibiting dental caries while in higher doses it causes dental and skeletal fluorosis (Shanthakumari et al., 2004). However, detrimental effects of high-fluoride intake are also observed in soft tissues (Monsour and Kruger, 1985). Fluoride enters the body through drinking water, food, toothpaste, mouth rinses, and other dental products; drugs and fluoride dust and fumes from industries using fluoride containing salt and hydrofluoric acid (Shulman and Wells, 1997). The fluorosis of human beings is mainly caused by drinking water; burning coal and drinking tea while that of animals is mainly by drinking water and supplementing feed additives such as calcium monohydrogen phosphate containing high levels of fluoride (Liu et al., 2003). Intake of high levels of fluoride is known to cause structural cha biological activities of some nges, altered activities of enzymes, metabolic lesions in the brain and influence the metabolism of lipids (Shivarajashankara et al., 2002). Acute poisoning can terminate in death due to blocking cell metabolism since fluorides inhibit enzymatic processes, particularly metalloenzymes responsible for important vital processes (Birkner et al., 2000). Recent studies revealed that fluoride induces excessive production of oxygen free radicals, and might cause the depletion in biological activities of some antioxidant enzymes like super oxide dismutase (SOD), antioxidant enzymes like

super oxide dismutase (SOD), catalase and glutathione peroxidase (GP*X*) (Chlubek, 2003; Shanthakumari et al.,2004). Toxic effects of fluoride on various biochemical parameters are known (Singh, 1984; Chlubek, 2003). Increased free radical generation and lipid peroxidation (LPO) are proposed to mediate the toxic effects of fluoride on soft tissues (Rzeuski et al., 1998) ;Shivarajashankara et al., (2001a,b) reported increased lipid peroxidation and disturbed antioxidant defense systems in brain, erythrocytes and liver of rats exposed to fluoride.

Although fluorosis has been investigated for many years, there are relatively few studies about its effect on the digestive system such as the pancreas. Enzyme secretions of the exocrine pancreas are required for hydrolysis of nutrients present in food and feed (Rinderknecht, 1993). Studies have shown that excess fluoride can cause DNA damage, trigger apoptosis and change cell cycle (Wang et al., 2004; Ha et al., 2004). The effects of fluoride on hematological parameters have been studied well in experimental models (Khandare et al., 2000; Cetin et al., 2004; Eren et al., 2005; Karadeniz and Altintas, 2008; Kant et al., 2009). However, there are limited studies about effects of chronic fluorosis on hematological parameters in human subjects living in endemic fluorotic areas (Uslu, 1981; Choubisa, 1996).

Excessive exposure to fluorides can evoke several oxidative reactions as induction of inflammation (Stawiarska-Pieta et al., 2007; 2008 & 2009; Shashi et al., 2010; Gutowska et al., 2011), cell cycle arrest and apoptosis in different experimental system (Thrane et al., 2001). Apoptosis is a complex process that involves a variety of different signaling pathways and results in multitude of changes in the dying cells. Many of events that occur during apoptosis are mediated by a family of cysteine proteases called caspases (Kumar et al., 2004). Sequential activation of caspase 3 plays a central role in the execution-phase of apoptosis (Gu et al., 2011). The antioxidative vitamins such as A, E and C and selenium or methionine (Met) and coenzyme Q have been shown to protect the body against many diseases which characterized by disruptive activity of free radicals (Littarru and Tiano, 2007).

Among the non-enzymatic antioxidants, vitamin E is listed; its activity has been studied to a reasonable extent. The anti-oxidative ability of methionine has been recognized to a lesser degree. Methionine may play the role of endogenous scavenger of free radicals (Stadtman and Levine, 2003). Cyclic oxygenation of Met and the reduction of methionine sulfoxide (MetO) may be an important antioxidative mechanism; perhaps, it influences the enzyme activity control (Stadtman et al., 2003). It is presumed that because of those processes, the methionine residues of proteins perform the function of reproducible scavengers of reactive oxygen and nitrogen species (Levine et al., 2000; Stadtman et al., 2002).

Methionine reduces the ototoxic, hepatotoxic, and nephrotoxic activity of some drugs (Abdel-Wahhab et al., 1999; Reser et al., 1999). It also demonstrates a protective influence upon the organism in the course of exposure to sodium fluoride (Błaszczyk et al., 2009&2010). It has been found that joint administration of vitamin E and methionine to rabbits is more efficient in protecting cells against disadvantageous influence of oxidative stress than administration of vitamin E only. This may suggest that methionine takes part in regeneration of the tocopherol radical (Birkner, 2002).

Carnosine is a well known antioxidant acting as a scavenger of active oxygen radicals and peroxynitrite radical implicated in cell injury (Fontana et al., 2002). Carnosine also has SOD-like activity (Guney et al., 2006), and acts indirectly by preserving GSH which is an antioxidant itself playing a pivotal role in reducing lipid peroxides. In addition, the cytosolic buffering activity of carnosine prevents proton and lactate accumulation which is involved in the pathogenesis of oxidative tissue injury (Gariballa and Sinclair, 2000). Improved microcirculation in the injured tissue by carnosine is another factor responsible for reduced lactate accumulation (Stvolinsky and Dobrota, 2000). Recently, it was found that carnosine could also exhibit antioxidant activity by acting at the molecular level causing a dose-dependent up-regulation of hepatic catalase mRNA expression (Liu et al., 2008). The present study was undertaken to assess whether vitamin E plus methionine or L- carnosine may prevent or alleviate the effects of sodium fluoride on hematological indices, pancreatic digestive enzyme activities, DNA damage and histological structure of pancreas through histological and immuno-histochemical studies.

2.Materials and Methods

2.1.Materials:

2.1.1. Chemicals:

Sodium fluoride, methionine and L-carnosine powder (Fluka, Switzerland) were procured from Sigma Chemical (USA). All other chemicals were analytical reagent grade and chemicals required for all biochemical assays were obtained from Sigma–Aldrich Chemicals Co (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

2.1.2. Experimental animals

Thirty-six of adult male Wister albino rats weighing 120–130 g were obtained from animal house of Helwan farm, Egypt. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (24.3°C) during the experimental period. The rats were provided ad libitum with tap water and fed with standard commercial rat chow. Animal procedures were performed in-accordance with Guidelines for Ethical Conduct in the Care and Use of Animals

Experimental design

After one week of acclimation, animals were divided into six groups (6 rats in each group).

Group (1) served as control, received distilled water orally by gavages once daily for 35 days.

****Group (2)*** received vit.E (3 mg /rat/day) in combination with methionine (2 mg /rat/day) orally by gavages for 35 days (Stawiarska-Pieta et al., 2009 & 2007).

****Group (3)*** received L-carnosine in a dose of 5 mg/kg bw orally by gavages once daily for 35 days (Soliman et al., 2001).

Group (4) received NaF in a dose of 10 ml/kg bw orally by gavages once daily for 35 days **(**Blaszczyk et al., 2008).

Group (5) received vit. E (3 mg /rat/day) in combination with methionine (2 mg /rat/day) followed by NaF in a dose of 10 mg/kg bw orally by gavages once daily for 35 days.

Group (6) received L-carnosine in a dose of 5 mg/kg bw followed by NaF in a dose of 10 ml/kg bw orally by gavages once daily for 35 days.

2.2.Methods:

2.2.1. Blood collection and tissue homogenate

At the end of the treatment, blood samples were collected from the anaesthetized rats, by direct puncture of the right ventricle and from the retro-orbital vein plexus. Whole blood was used to assay hematological variables, while the serum was used to assay glucose, total protein, and albumin level. Malondialdehyde (MDA) was determined in plasma and superoxide dismutase (SOD) activities in erythrocyte. In addition, pancreas was collected for the estimation of pancreatic digestive enzyme activities and protein concentration.

2.2.2. Biochemical analysis

A-Clinical Hematological Variables

White blood cells (WBC), red blood cells (RBC), haematocrit (Hct), haemoglobin (Hb), mean cell volume (MCV), red cell distribution width (RDW), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet (PLT) were measured on a Sysmex Hematology Analyzer (model K4500).

B-Pancreatic digestive enzyme activities

The pancreas from rats was homogenized and centrifuged. The supernatant was saved for determining the activities of lipase (EC 3.1.1.3), protease, and amylase (EC 3.2.1.1). Lipase was determined at 37°C by a pH-stat titration using tributyrin as substrate according to the method of Erlanson-Albertsson et al., (1987). Protease activity was analyzed with the modified method of Lynn and Clevette-Radford, (1984) using azocasein as substrate. One lipase or protease unit is defined as the amount of enzyme that hydrolyses 1 μmol of substrate per minute. Amylase was determined by the iodometric method (Harms and Camfield, 1966). One amylase unit is the amount of enzyme that hydrolyses 10 mg of starch in 30 min.In pancreatic homogenates; protein concentration was determined using Lowry's method (Lowry et al., 1951)

C-Other serum biochemical analyses

Glucose, total protein and albumin levels were determined using Johnson & Johnson label kits and a Vitros750 model autoanalyser

D-Determination of plasma malondialdehyde (MDA) levels and erythrocyte superoxide dismutase (SOD) activities

Plasma MDA levels, and erythrocyte SOD activities were determined as described by Yoshioka et al., (1979); Sun et al., (1988) respectively.

2.2.2. Determination of nucleic acid (DNA and RNA) contents

Total DNA and RNA contents in the liver were determined according to pears, (1985).

DNA fragmentation was quantified by Di-phenyl Amine l (DPA) method according to Gibb et al., (1997).

2.2.3. Micronucleus assay

Rats were sacrificed 24 h after treatment. Rat's femora were removed through the pelvic bone. The epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with fetal calf serum (Valette et al., 2002). The cell suspension was centrifuged and a small drop of the re-suspended cell pellet was spread onto slides and air-dried. The bone marrow smears were made in five replicates and fixed in absolute methanol and stained with May-Grünwald/Giemsa (D'Souza et al., 2002). Scoring the nucleated BMCs and the percentage of micronucleated BMCs (polynucleated MN-BMCs) was determined by analyzing their number in 3000 BMCs per rat.

2.2.4.Histological,immunohistochemical,and morphometric studies:

- For ***routine histological examination***, the pancreatic specimens were fixed in 10% neutral buffered formaldehyde and processed for paraffin sections of 5μm thickness. Sections were stained with Hematoxylin and Eosin **(**Bancroft and Stevens, 1996).

- *Additional sections were prepared :*

-**For *caspase- 3 immunohistochemical staining*** for detection of apoptosis in pancreatic acinar cells**.** A standard avidin-biotin complex method with alkaline phosphatase detection was carried out. Formalin-fixed paraffin-embedded sections were dewaxed in xylene and rehydrated through graded alcohol to distilled water. The sections were subjected to antigen retrieval by boiling in a microwave for 20 min in 0.01 M sodium citrate buffer (pH 6.0). The primary antibody to caspase-3 (Transduction Laboratories, Lexington, KY) was applied at a dilution of 1:1000 and incubated overnight at 4°C. After incubation, the slides were treated with biotinylated rabbit antimouse immunoglobulin (1:600 for 30 min; Dako Ltd., Ely, UK) washed as before, and then treated with streptavidin and biotinylated alkaline phosphatase according to the manufacturer's instructions (Dako). The slides were then washed, and the signal was visualized using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. A negative control reaction with no primary antibody is always carried out alongside the reaction containing sample. The specificity of the caspase-3 antibody was confirmed by comparison with control antibodies (Ansari et al., 1993).

- For ***electron microscopic examination*** pancreatic tissue specimens were immediately fixed in 2.5 % phosphate buffered gluteraldehyde (ph 7.4) at 4ºC for 24 hours and post fixed in 1% osmium tetraoxide for one hour, then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. Semithin sections (1μm thickness) were cut, stained with toluidine blue and examined by light microscopy. Ultrathin sections (80-90nm thickness) were stained with uranyl acetate and lead citrate **(**Bozzola and Russell, 1998) and were examined and photographed with JEOL 1010 transmission electron microscope.

- For ***quantitative morphometric measurement*** the number of caspase3 positive pancreatic acinar cells were counted in five non overlapping fields of vision from each slide of all animals of each group at X 400 magnification using Leica Qwin 500 C image analyzer computer system and were expressed as cell number per μm^2.

2.2.5. Statistical analysis:

Data were computerized and expressed as mean ± standard deviation using Microsoft office excel 2007 soft ware, where the differences between the four groups were analyzed using student 's t-test . The results were considered statistically significant if $p<0.05$.

3.Results:

3.1 Biochemical Results:

There was a statistical significant decrease in RBC, HCT, MCV, RDW, MCH, MCHC and PLT and a statistical significant increase in WBC, lymphocytes and

granulocytes in sodium fluoride (NaF) treated group as a compared to control group. The levels of the above parameters were significantly reversed in the groups pretreated with vitamin E in combination with methionine and L- carnosine prior to NaF (Table1)

As shown in Table (2): Animals treated with NaF showed significant decrease ($P < 0.001$) in pancreatic digestive enzyme activities and protein levels as compared to the control group, while animals treated with vitamin E in combination with methionine and L- carnosine prior to NaF showed statistical significant increase in pancreatic digestive enzyme activities and protein levels as compared to NaF treated group. The results also indicated that, treatment with NaF resulted in a significant decrease in serum total protein, albumin and blood glucose levels as compared to the control group. On the other hand, pretreated with vitamin E in combination with methionine and L- carnosine prior to NaF resulted in a significant increase in serum total protein, albumin and blood glucose levels (Table 3). Plasma malondialdehyde levels were significantly increased and the activities of erythrocyte superoxide dismutase were significantly decreased in the NaF group compared to the control group. The administration of vitamin E in combination with methionine and L- carnosine prior to NaF reduced the process of lipid peroxidation and increased the activity of SOD (Table 4)

3.2. Determination of nucleic acid (DNA and RNA) contents:

Oral administration of sodium fluoride for 35 days resulted in a significant reduction in the DNA, RNA contents of the liver a and significant increase DNA damage in liver and the frequencies of micro nucleated polychromatic erythrocytes (MN-PCE) in bone marrow. However, concurrent administration of NaF and vitamin E in combination with methionine and L- carnosine for 35 days caused significant amelioration in all parameters was studied (Table 5).

3.3.Histological Results:-

3.3.1.Light microscopic results:

Light microscopic examination of pancreas of control group showed rounded to oval serous acini, the exocrine portion of the pancreas, and pancreatic islets of Langerhans, the endocrine portion of the pancreas, that were packed in a connective tissue stroma (fig.1A). Each acinus was lined by pyramidal cells. Each had a spherical, vesicular and basal located nucleus containing a prominent nucleolus and surrounded by a basophilic cytoplasm. However the apical part was occupied by acidophilic zymogen granules (fig.1B). The pancreatic acini of vit.E in combination with methionine and L-carnosine treated groups were more or less similar in histological structure to those of control group (figs.1C&1D). Histological alternations were observed only in pancreas of NaF treated group. These changes occurred in the form of multiple vacuoles of variable size in the cytoplasm of pancreatic acini (figs.2A&2B). The serous cells had deeply stained pyknotic and peripheral situated nuclei (figs.2B&2C). Furthermore inflammatory cells infiltrations were commonly observed in the stroma of pancreas (fig.2A) especially in area around blood vessels (fig. 2C). Pancreas of vit. E in combination of Na fluoride as evidenced histologically by complete absence of vacuoles and normal appearance of nuclei of pancreatic acinar cells and absence of inflammatory cells infilterations in the stroma of pancreas.. Thus serous cells of pancreatic acini and stroma of pancreas in the last two groups regained its normal structure to be more or less similar to those of control group (figs.2D, 2E&2F)

3.3.2-Immunohistochemical Results:

Pancreatic acinar cells of control group, vit.E in combination with methionine treated group and L-carnosine treated group revealed negative caspase- 3 immunohistochemical reactivity. However few scattered cells exhibited faint light brown granules in their cytoplasm (figs.3A, 3B&3C). Intensive positive immunoreactivity for caspase- 3 was observed in the cytoplasm of most pancreatic acinar cells of NaF treated group (fig.3D). On the other hand the cytoplasmic acinar cells of vit. E in combination with methionine prior to NaF and L-carnosine prior to NaF treated groups showed apparent reduction of caspase-3 immunoreactivity to be more or less similar to the control group (figs.3E&3F) **.**

3.3.3-Morphometric and statistical Results:

As regards the statistical study concerning caspase- 3 immunoreactivity, there was a statistically significant increase in the mean number of caspase- 3 positive pancreatic acinar cells in NaF treated group, when compared with the control group. There was also significant decrease in the mean number of caspase- 3 positive pancreatic acinar cells in vit. E in combination with methionine prior to NaFand L-carnosine prior to NaF treated groups when compared with NaF treated group. On the other hand, vit.E in combination with methionine and L-carnosine treated groups showed no significant differences in the mean number of caspase- 3 positive pancreatic acinar cells in comparison to the control group the mean number of caspase- 3 positive pancreatic acinar cells in comparison to the control group.

3.3.4-Electron microscopic Results:

In control group, electron microscopic examination of exocrine pancreatic cells revealed well developed rough endoplasmic reticulum (rER) in their basal regions and great amount of zymogen granules in the apical parts. Each cell had a large basal, spherical and euchromatic nucleus containing a prominent nucleolus. Multiple mitochondria were also obviously scattered in the pancreatic acinar cell cytoplasm (fig.4A). The cytoplasm of pancreatic acinar cells of vit.E in combination with methionine and L-carnosine treated groups showed normal ultrastructure, that were more or less similar to those of control group (figs.4B&C). In NaF treated group, few or even absence of zymogen granules were observed in the cytoplasm of pancreatic acinar cells. The rER saccules showed dilatation and exhibited a globular- shape in some parts. Intra-cisternal zymogen granules were contained in multiple globular- shaped rough endoplasmic reticulum. Most acinar cells had irregular shaped, pyknotic and heterochromatic nuclei (figs4D&E).

Ultrathin sections of serous cells of vit. E in combination with methionine prior to NaF treated group.showed preservation of acinar cytoplasmic contents to be more or less similar to those of control group. The cytoplasm of pancreatic acinar cells of vit.E in combination with methionine prior to NaF treated group regained its normal structure. Rough endoplasmic reticulum showed neither dilatation nor globular shape and

Table (1): Effect of vitamin E in combination with methionine and L- carnosine on sodium fluoride-induced changes in blood parameters.

Parameters	Control group	Vit.E + Meth. treated group	L-Carnosine treated group	NaF treated group	Vit.E and Meth. +NaF treated group	L-Carnosine + NaF treated group
WBC (10^3/ul)	7.55±0.035[a]	8.05±0.336[a]	8.0±0.353[a]	10.85±0.318**[b]	9.15±0.388*[c]	8.5±0.212**[c]
Lymph (10^3/ul)	5.36±0.091[a]	5.76±0.243[a]	6.1±0.604[a]	8.6±0.049**[b]	6.16±0.113**[c]	5.48±0.011***[c]
GRA (10^3/ul)	1.18±0.07[a]	1.28±0.195[a]	1.27±0.215[a]	2.58±0.02**[b]	1.20±0.11**[c]	1.16±0.15**[c]
Lymph%	73.6±0.282[a]	73.95±0.247[a]	74.6±0.565[a]	81.25±0.318**[b]	74.45±0.388**[c]	73.5±0.494**[c]
GRA%	17.1±0.63[a]	17.35±2.43[a]	17.25±2.72[a]	26.45±1.52*[b]	17.65±0.318*[c]	17.4±0.282*[c]
RBC (10^6/ul)	6.59±0.049[a]	6.4±0.082[a]	6.5±0.127[a]	5.23±0.049***[b]	6.49±0.12*[c]	6.47±0.102**[c]
HCT (%)	43.4±0.282[a]	42.85±0.53[a]	42.95±0.318[a]	41.05±0.03**[b]	43.0±0.353*[c]	43.7±0.353*[c]
MCV(fL)	67±0.707[a]	64.5±0.35[a]	65±0.707[a]	63.5±0.212*[b]	66.5±0.282*[c]	67.0±0.707*[c]
RDW (%)	27.95±0.459[a]	27.05±0.035[a]	26.7±0.565[a]	25.8±0.707*[b]	27.15±0.176*[c]	26.5±0.141*[c]
MCH(pg)	23.7±0.212[a]	22.5±0.848[a]	23.25±0.388[a]	20.8±0.212**[b]	23.3±0.282**[c]	22.35±0.03**[c]
MCHC (g/dl)	33.85±0.813[a]	32.55±0.247[a]	32.7±1.34[a]	30.05±0.035*[b]	32.5±0.353**[c]	32.4±0.212**[c]
HGB (g/dl)	13.65±0.106[a]	13.25±0.167[a]	13.55±0.247[a]	12.95±0.035*[b]	13.65±0.106*[c]	13.7±0.071*[c]
PLT (10^3/ul)	544±0.707[a]	539±1.41[a]	540.5±1.06[a]	537±0.707*[b]	543.5±0.212*[c]	542.5±0.494*[c]

Data are presented as mean ± SE of the six animals *P<0.05, **P<0.01and*P<0.001**
(b) Significantly different from control group. (c) Significantly different from NaF treated group.
Within each row, means superscript with the same letter are not significantly different.

Table (2): Pancreatic digestive enzyme activities (U/g tissue) and Protein levels (mg/g tissue) in control and experimental groups.

Parameters	Control group	Vit.E +Meth. treated group	L-Carnosine treated group	NaF treated group	Vit.E and Meth. + NaF treated group	L-Carnosine + NaF treated group
Pancreatic lipase (U/g tissue)	342.5 ± 2.25[a]	341.75±2.41[a]	340.25±0.65[a]	319.5±2.93***[b]	345.25±1.24***c	336.0±2.35**[c]
Pancreatic amylase (U/g tissue)	470±2.96[a]	469±3.47[a]	463.5±1.29[a]	423±3.95***[b]	489±2.03***[c]	455.5±1.6***[c]
Pancreatic Protease (U/g tissue)	50.33±1.97[a]	47.53±0.67[a]	48.35±0.78[a]	39.4±0.272**[b]	50.25±0.754***[c]	48.05±1.89**[c]
Pancreatic Protein levels (mg/g tissue)	4.683±0.242[a]	4.478±0.158[a]	4.833±0.05[a]	3.633±0.147**[b]	4.448±0.221**[c]	4.768±232**[c]

Data are presented as mean ± SE of the six animals. **P <0.01 and ***P<0.001.
(b) Significantly different from control group. (c) Significantly different from NaF treated group.
Within each row, means superscript with the same letter are not significantly different.

Table (3): Serum total protein, albumin and blood glucose levels (g/dl) in control and experimental groups

Parameters	Control group	Vit.E +Meth. treated group	L-Carnosine treated group	NaF treated group	Vit.E and Meth. + NaF treated group	L-Carnosine + NaF treated group
Total protein(g/dl)	7.32±0.08[a]	7.34±0.052[a]	7.35±0.04[a]	7.04±0.03*[b]	7.323±0.023***[c]	7. 298±0.02***[c]
Albumin(g/dl)	4.79±0.01[a]	4.81±0.16[a]	4.72±0.17[a]	3.99±0.07***[b]	4.32±0.01**[c]	4. 294±0.031**[c]
Glucose(g/dl)	123±0.35[a]	123.3±0.96[a]	124.5±1.43[a]	97±0.35***[b]	123.8±0.4***[c]	117±0.62***[c]

Data are presented as mean ± SE of the six animals. *P<0.05, **P<0.01and*P<0.001**
(b) Significantly different from control group. (c) Significantly different from NaF treated group.
Within each row, means superscript with the same letter are not significantly different.

Table (4): Plasma MDA (nmol/mL) and erythrocyte SOD (U/mL) in control and experimental groups.

Parameters	Control group	Vit.E +Meth. treated group	L -Carnosine treated group	NaF treated group	Vit.E an Meth. + NaF treated group	L-Carnosine + NaF treated group
MDA (nmol/mL)	3.443±0.154a	3.085±o.129a	3.035±0.13a	6.232±0.177***b	4.222±0.164***c	3.845±0.11***c
SOD (U/mL)	6.693±0.174a	6.958±0.093a	7.012±0.062a	3.592±0.176***b	5.9±0.149***c	6.267±0.319***c

Data are presented as mean ± SE of the six animals. ***P<0.001
(b) Significantly different from control group. (c) Significantly different from NaF treated group.
Within each row, means superscript with the same letter are not significantly different.

Table (5): Hepatic DNA, RNA contents (mg/g tissue weight), % of hepatic DNA fragmentation and frequency of micro nucleated polychromatic erythrocytes in bone marrow of control and experimental groups.

Parameters	Control group	Vit.E +Meth. treated group	L –Carnosine treated group	NaF treated group	Vit.E and Meth. + NaF treated group	L-Carnosine + NaF treated group
DNA(mg/g tissue weight)	0.499±0.012a	0.536±0.016a	0.51±0.01a	0.345±0.01***b	0.517±0.01***c	0.578±0.008***c
RNA(mg/g tissue weight)	0.281±0.007a	0.290±0.006a	0.293±0.005a	0.193±0.004***b	0.251±0.009***c	0.267±0.009***c
% of hepatic DNA fragmentation	0.779±0.013a	0.746±0.01a	0.743±0.019a	1.65±0.17***b	0.721±0.013***c	0.709±0.012***c
MN-PCE/3000 in bone marrow	19.5±0.969a	18.167±0.523a	17.833±0.658a	78.166±1.707***b	23.167±0.867***c	21.667±0.658***c

Data are presented as mean ± SE of the six animals. ***P<0.001
(b) Significantly different from control group. (c) Significantly different from NaF treated group.
Within each row, means superscript with the same letter are not significantly different.

and complete absence of intra-cisternal granules. Increases in number of zymogen granules were also observed. The nucleus regained its normal spherical and euchromatic contents with a prominent nucleolus (fig.4F).

Most acinar cells of L-carnosine prior to NaF treated group also regained their normal ultrastructre. However, few pancreatic acinar cells showed mild dilatation and globular shape of rER (fig.4G) .

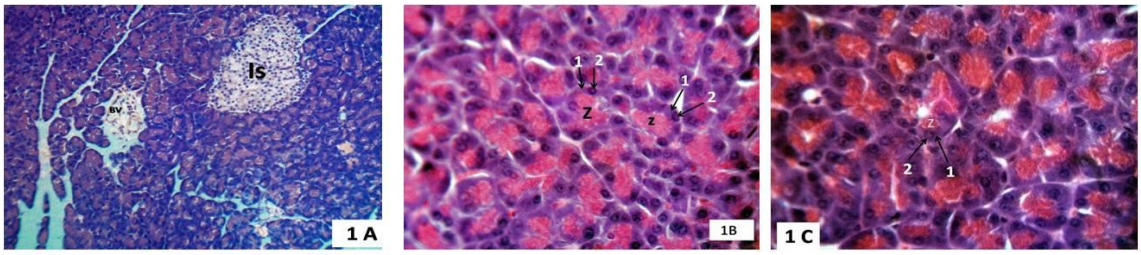


Fig. (1):- Representative photomicrographs of pancreatic sections stained by H & E. A: A section taken from a pancreas of control group shows normal pancreatic architecture, pancreatic islet (IS) and a blood vessel(BV)(X 50). B: A section taken from a pancreas of control group, C: A section taken from a pancreas of vit. E in combination with meth. treated rat and D: A section taken from a pancreas of L-carnosine treated rat shows normal structure of pancreatic acinar cells, each has basal basophilic cytoplasm (1) containing a single, spherical, vesicular and basal nucleus(2) containing a prominent nucleolus and apical acidophilic zymogen granules(Z)(X400).

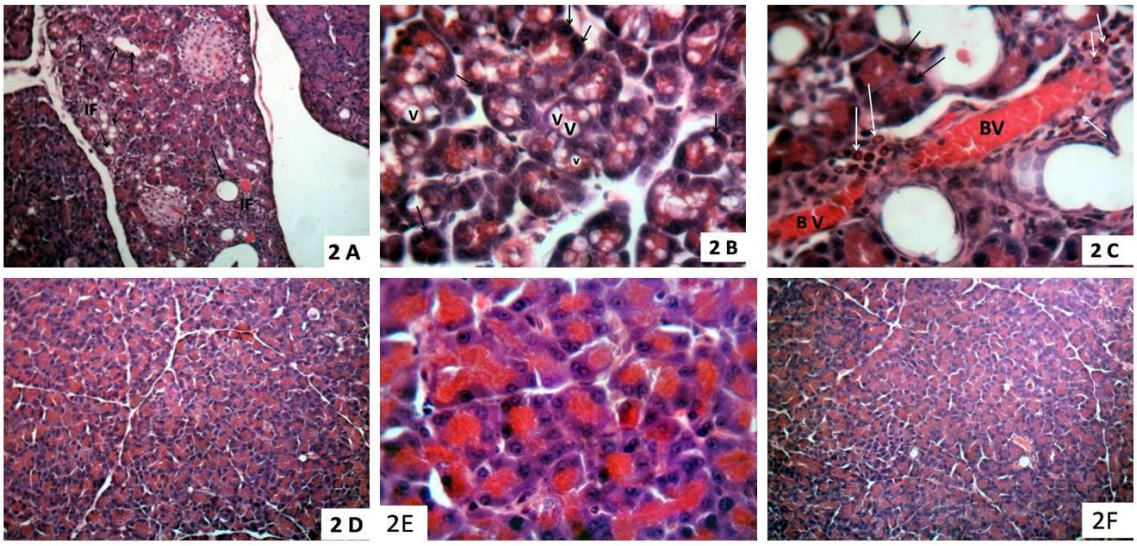


Fig.(2):- Representative photomicrographs of pancreatic sections stained by H & E : A , B and C: Sections taken from pancreas of NaF treated rats. A: Shows multiple vacuoles (arrows) of variable size in the cytoplasm of pancreatic acinar cells and inflammatory cells infiltrations (IF) in the stroma of pancreas (X 50). B: Shows multiple vacuoles (V) in the cytoplasm of pancreatic acinar cells and the peripheral pyknotic and darkly stained nuclei (arrows) of pancreatic acinar cells (X400). C: Shows inflammatory cells infiltrations (white arrows) in the stroma of pancreas around blood vessels (BV) and the peripheral, pyknotic and darkly stained nuclei (black arrows) of pancreatic acinar cells (X400). D and E: Sections taken from pancreas of vit.E in combination with meth. prior to NaF treated rats (D: X 50, E: X 400) and F: A section taken from a pancreas of L- carnosine followed by Na F treated rat show the apparent normal looking of pancreatic acini and stroma (X 400).

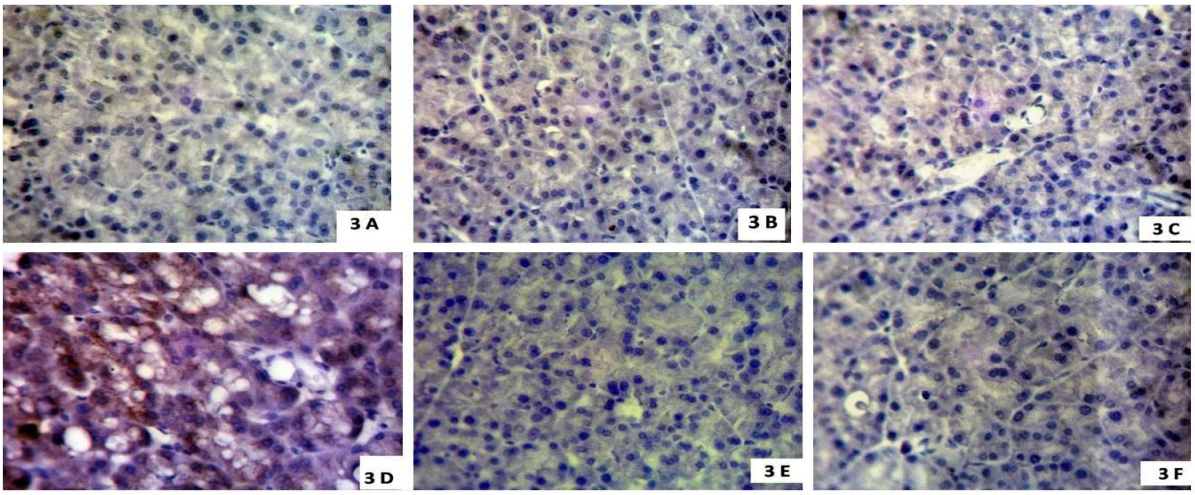
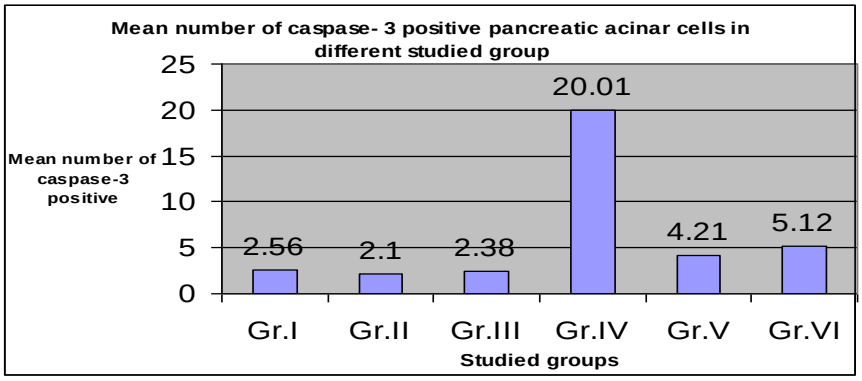


Fig. (3):- Expression of caspase- 3 immunohistochemical staining (X 400). A: A section obtained from a pancreas of control rat, B: A section obtained from a pancreas of vit. E in combination with meth. treated rat and C: A section obtained from a pancreas of L-carnosine treated rat show faint cytoplasmic reactions for caspase-3 in few pancreatic acinar cells. D: A section obtained from a pancreas of Na F treated rat shows strong cytoplasmic reactions for caspase-3 immunoreactivity in the cytoplasm of most pancreatic acinar cells. E: A section obtained from a pancreas of vit. E in combination with meth. prior to Na F treated rat and F: A section obtained from a pancreas of L-carnosine prior to Na F treated rat shows week cytoplasmic immuno- reactions for caspase-3 in pancreatic acinar cells.

Table (6): The mean number of caspase 3 positive pancreatic acinar cells in the different studied group

Groups	Control group (I)	Vit.E +meth. treated group (II)	L –Carnosine treated group (III)	NaF treated group (IV)	Vit.E and meth.+ NaF treated group (V)	L-Carnosine + NaF treated group (VI)
Mean± SD	2.56±0.32	2.10±0.42	2.38±0.66	20.01±0.5	4.21±0.53	5.12±0.14
P. value		P1>0.05 NS	P2>0.05 NS	P3 < 0.05 *	P4<0.05 *	P5<0.05 *

P1, P2, P3 = P. value of each group versus control group P4, P5= P. value in group V and VI versus group IV
NS= Non significant * = Significant



Histogram (1): The mean number of caspase 3 positive pancreatic acinar cells in the different studied groups.

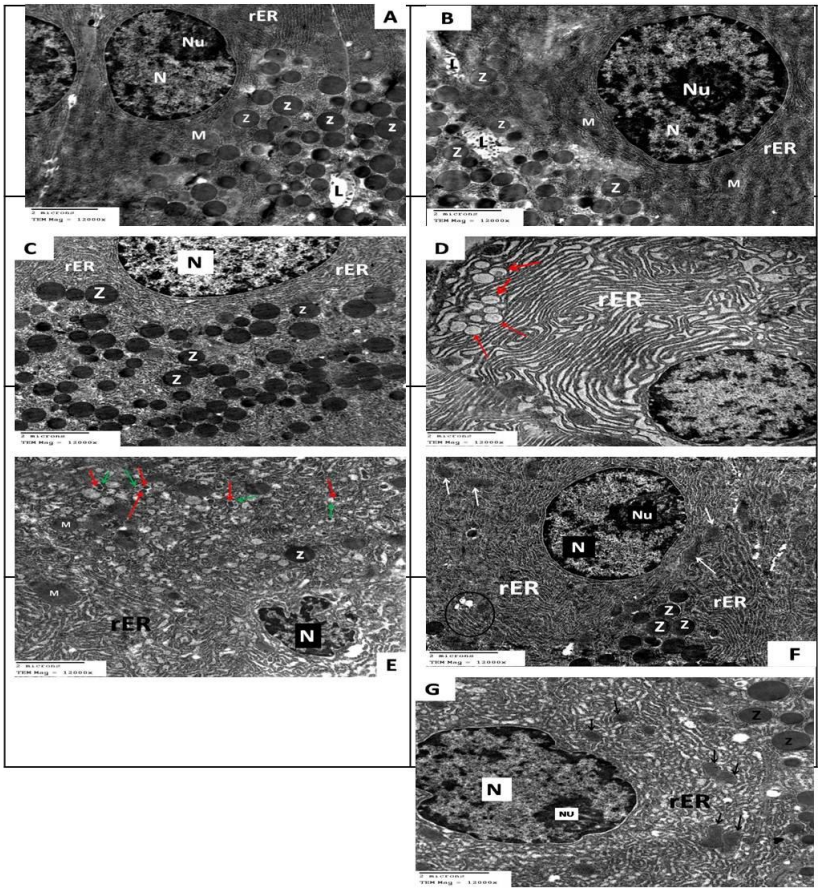


Fig. (4):- Representative electronmicrographic ultrathin sections of pancreatic acinar cells cytoplasm (O.M. X 12000). A: A section obtained from a pancreas of control rat, B: A section obtained from a pancreas of vit. E in combination with methionine treated rat and (C) A section obtained from a pancreas of L-carnosine treated rat. A and B: Show zymogen granules(Z) facing lumen(L), rough endoplasmic reticulum(rER), mitochondria(M) and an euchromatic nucleus(N) containing a prominent nucleolus(Nu) . C: Shows zymogen granules(Z), rough endoplasmic reticulum(rER) and a nucleus(N). D and E: Sections obtained from pancreas of Na F treated rats. D: Shows marked dilatation of rough endoplasmic reticulum(rER) , globular shape of rough endoplasmic reticulum(arrows) and complete absence of zymogen granules and E: shows dilatation of rough endoplasmic reticulum(rER), intra-cisternal zymogen granules (red arrows) in the globular endoplasmic reticulum (green arrows), few zymogen granules(Z), mitochondria(M) and an irregular shape, pyknotic and heterochromatic nucleus(N). F: A section obtained from a pancreas of vit. E in combination with meth. prior to fNa F treated rat and G: A section obtained from a pancreas of L-carnosine prior to Na F treated rat show preservation of cytoplasmic contents in pancreatic acinar cells to be more or less similar to the control group. F: Shows normal rough endoplasmic reticulum(rER), multiple apical zymogen granules(z), multiple mitochondria(arrows), an euchromatic nucleus(N) containing a prominent nucleolus(Nu) and Golgi apparatus(circle). G: Shows multiple zymogen granules (Z), mitochondria (arrows), an euchromatic nucleus (N) containing a prominent nucleolus(Nu), mild dilatation and globular shape in the rough endoplasmic reticulum(rER).

4.Discussion:-

In the present study**,** we evaluated the protective effects of vitamin E in combination with methionine and L- carnosine against the pancreatic toxicity induced by NaF in rats. Treatment with NaF resulted in a significant decrease in RBC, HCT, MCV, RDW, MCH, MCHC and PLT and a statistical significant increase in WBC, lymphocytes and granulocytes as compared to control group. The levels of the above parameters were significantly reversed in the groups pretreated with vitamin E in combination with methionine and L-carnosine prior to NaF. These results were in-agreement with Cetin et al., (2004); Karadeniz and Altintas, (2008); Tiwari & Pande (2009); Kant et al., (2009) who reported significant increase in WBC and decrease in RBC, packed cell volume, WBC count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration and neutrophil counts, as well as the hemoglobin and hematocrit values in mice, goats and rabbits with chronic fluorosis. Rao and Vidyunmala, (2009) observed that a significant decrease in RBC, hemoglobin, PCV, MCH relative to the respective controls and with no change in WBC and MCHC. Changes of these values in mice have been attributed to rate of bioaccumulation of fluoride in blood. Banu Priya et al., (1997) reported that fluoride intoxication depressed bone marrow activity in cattle. Machalinska et al., (2002) revealed that fluoride induced disorders in hematopoietic organs in mice and in human hematopoietic progenitor cells. Machalinski et al., (2000); Mittal and Flora, (2007) reported that sodium

fluoride at 50 mg/L, in drinking water caused significant depletion in blood δ--aminolevulinic acid dehydratase (ALAD) activity and platelet counts (PLT). Vitamin E supplementation during sodium fluoride exposure had limited beneficial effects in restoring altered biochemical variables. The fluoride-induced anemia may result from inhibition of globulin synthesis, depression of erythropoiesis, or a decrease in the level of blood folic acid (Cetin et al., 2004). Aydogan et al., (2008) suggested that L-carnosine supplemention can be used to improve the RBC quality or to protect them from oxidative damage in survival of RBC in the circulation. Caylak et al., (2008) reported that, Hb levels in lead–methionine were significantly higher than the lead group ($p<0.01$). In the present study, animals treated with NaF showed significant decrease in pancreatic digestive enzyme activities and protein levels as compared to the control group, while animals treated with in the vitamin E in combination with methionine and L- carnosine prior to NaF showed statistical significant increase in pancreatic digestive enzyme activities and protein levels as compared to NaF treated group. These results were in-accordance with Zhan et al., (2005b) who reported the activities of pancreatic lipase, protease and amylase were significantly decreased when exposed to 100, 250, and 400 mg NaF/ kg in their diets for 50 days. These effects might be an important reason for growth depression induced by fluorosis. Excessive production of free radicals induced by fluoride may damage the structures of digestive enzymes and reduce their activities (Liu et al., 2003). Hara and Yu, (1995) indicated that salivary amylase was inhibited by NaF. Also, Mulimani and Gopal, (1989) mentioned that pancreatic amylase activity has been found to be inhibited by sodium fluoride. Some enzymes, such as peptidases, alpha amylases, phosphatases, and ATPases, are activated by calcium ions and are inhibited by fluoride due to calcium binding to fluoride in the catalytic centre (Machoy, 1987).

In the present study, treatment with NaF resulted in a significant decrease in serum total protein, albumin and blood glucose levels as compared to the control group. On the other hand, pretreated with vitamin E in combination with methionine and L- carnosine prior to NaF resulted in significant increase in serum total protein, albumin and blood glucose levels. These results were in-agreement with Kanbur et al., (2009) who observed a decrease in total protein and albumin levels in the group that was administered fluoride. Stawiarska-Pieta et al ., (2009) showed that an increase in protein level in the pancreas of rats receiving antioxidants with NaF an increase was statistically significant in comparison with the controls and amounted to 92.31%. Bouaziz et al., (2006) who showed a significant decrease in serum levels of total protein and albumin and marked hypoglycemia in fluoride-treated mice. Some researcher has reported fluoride to change blood glucose (Eraslan et al., 2007). Cenesiz, et al., (2005) reported that, total protein levels were decreased ($p<0.01$ in the NaF group compared to the control group.

Verma and Guna Sherlin, (2002) indicated that NaF treatment from day 6 of gestation throughout lactation caused a significant reduction in glucose and protein content in the serum of both P- and F1-generation rats than control. Compared with the vitamin E co-treated animals, amelioration in protein concentrations was significantly higher with vitamins C and C+D+E in both P- and F1-generation rats. Zang et al., (1996) reported a significant decrease in serum proteins in individuals with poor nutrition and living in high-fluoride areas. The significant recovery on co-treatment with vitamins C, E and C+D+E could be attributed to the action of these vitamins as free radical scavengers. Wilde and Yu, (1998) opined that the toxicity of free radicals is greater if fluoride can impair the production of free radical scavengers such as ascorbic acid and glutathione and this can be prevented by the additional supplementation with vitamins C and E. The antidotal effect of vitamin E is by preventing the oxidative damage caused by fluoride, which increases peroxides and free radicals of reactive oxygen species in tissues. The protective role of free radical scavenger is by the hydrogen donor ability of tocopherol. Vitamin E channels the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH), which in turn helps compression of mono- and dehydroascorbic acid to maintain ascorbic acid levels. It also has an inhibitory effect on the conversion of free or protein-bound -SH to –SS groups, thus maintaining -SH groups (Basu and Dickerson, 1996) .

Fluoride is well known to affect protein synthesis by causing impairment in polypeptide chain initiation (Rasmussen, 1982), weak incorporation of amino acids into proteins (Helgeland, 1976), abnormal accumulation or inhibition of RNA synthesis (Holland, 1979). Decreased protein synthesis during fluorosis has been attributed to a decrease in activity of a group of enzymes catalyzing the key process of cellular metabolism (Chinoy et al., 1993). The enzymes are glutamine synthetase catalyzing certain stages of amino acid biosynthesis and methionine activating enzymes of the liver (Zahvaronkov and Strochkova, 1981).

In the present study, plasma malondialdehyde levels were significantly increased and the activities of erythrocyte superoxide dismutase were significantly decreased in the NaF group compared to the control group. The administration of vitamin E in combination with methionine and L- carnosine prior to NaF reduced the process of lipid peroxidation and increased the activity of SOD. These results were in-accordance with Błaszczyk et al., (2008) who reported NaF caused increased concentration MDA and decreased activity of ant oxidative enzyme (SOD). The administration of vitamin E increased the activity of SOD; it also reduced the process of lipid peroxidation. It has been found that combined doses of vitamin E and methionine were most effective in inhibiting lipid peroxidation process. The results confirmed the antioxidative properties of methionine. He and Chen, (2006) reported that the contents of MDA in oral mucosa and liver tissue of fluoride group were significantly higher than those of control group ($P < 0.01$). Zhan et al., (2005a) found that Serum malondialdehyde and the activities of superoxide dismutase were significantly decreased in the NaF group compared to the control group. Vani and Reddy, (2000) showed that fluoride increases free radical production and at the same time inhibits the ant oxidative enzyme

SOD which probably make the tissue more susceptible to biochemical injury. Patel and Chinoy, (1998) concluded that fluoride impaired the functioning of the SOD enzyme in ovary of mice. Sun et al., (1998) also reported a decrease in SOD activity in the liver, kidney, and heart of fluoridated mice. Abdel-Kader et al., (2011) observed that significant reduction in MDA concentrations in lead- Methionine and lead –vit E groups when compared to lead group, Błaszczyk et al., (2010) suggested that fluoride reduces the efficiency of the enzymatic antioxidative system in the liver and kidney. The slight increase of the activity of superoxide dismutase after administration of methionine may indicate its protective influence upon that enzyme. Caylak et al., (2008) reported that MDA levels in lead–methionine were significantly reduced. Methionine influences the metabolism of elements participating in the biosynthesis of superoxide dismutase isoenzymes. The reduced availability of any of them would then cause an inhibition of the synthesis and a diminished activity of SOD (Bourdon et al., 2005). Corrales et al., (2002) observed that administration of methionine should protect the liver from oxidative stress caused by fluorine. However Patra et al., (2001) found that in rats methionine administration may lead to an increased activity of SOD in the liver. Superoxide dismutase catalyses a reaction, in which the superoxide anionic radical is rendered harmless and molecular oxygen as well as hydrogen peroxide, get formed. The total activity of SOD depends upon the activity of iso-enzymes MnSOD and CuZnSOD (Oyanagui, 1984).

Sharonov et al., (1990); Hipkiss and Chana, (1998) reported the advantage of carnosine as a universal antioxidant, relating its ability to give efficient protection against oxidative stress. Also, carnosine was able to diminish the level of the primary products of lipid peroxidation previously accumulated (Dupin et al., 1988). Therefore, it appears that carnosine exerts multipotent antioxidant ability by inhibiting the injurious action of free radicals and diminishing previously accumulated toxic products. Thus carnosine could be recommended as an antioxidant, beside the classical antioxidants, such as vitamins E and C, flavonoids and carotenoids (Babizhayev and Steven, 1996). In addition, the fact that carnosine is water-soluble and located at the site of primary free-radical generation makes it even more valuable.

In the present study, oral administration of sodium fluoride for 35 days resulted in significant reduction in the DNA, RNA contents of the liver and significant increase DNA damage in liver and the frequencies of cells with MN in bone marrow. However, concurrent administration of NaF and vitamin E in combination with methionine and L- carnosine for 35 days caused significant amelioration in all parameters studied. These results were in-agreement with Trivedi et al., (2008) who observed oral administration of sodium fluoride for 30 days resulted in significant reduction in the DNA, RNA, and protein contents of the liver and kidneys. However, concurrent administration of NaF and black tea extract for 30 days caused significant amelioration in all parameters studied. Shashi, (2003) revealed significant ($P<0.001$) decline in the DNA and RNA Content and total proteins in adrenal gland of fluoridated animals of both sexes compared to the control. The significant decline of DNA and RNA in the adrenal gland of fluoridated rabbits in this investigation may be due to alterations in DNA polymerase activity and changes in enzymes involved in nucleic acid synthesis. Shashi, (1993); Shashi et al., (1994); Patel and Chinoy, (1998) reported that sodium fluoride (5 mg/kg body weight) was effective from the 45th day of treatment in causing a significant decline in DNA and RNA levels of mice ovary and uterus, indicating alterations in nucleic acid and protein metabolism in these organs.

Jacinto-Aleman et al., (2010) suggested that excessive fluoride ingestion has been identified as a risk factor for fluorosis and oxidative stress. The oxidative stress results from the loss of equilibrium between oxidative and antioxidative mechanisms that can produce DNA fragmentation, resulting in apoptosis. Individual exposure to fluoride leads to DNA damage in brain may have occurred via two mechanisms (i) indirectly, in which free radicals generated by fluoride attack hydrogen bond of DNA molecule to give various DNA adducts and (ii) directly, fluoride attacks free -NH group. Supplementation of vitamin E with fluoride protected fluoride induced oxidative stress and DNA damage in brain. These protective values of vitamin E could be attributed to its ability to efficiently scavenge lipid peroxyl radicals before they attack membrane lipids leading to the removal of cell-damaging free radicals. Clarke et al.,(2008); He and Chen, (2006) revealed that DNA damage rate in fluoride group was 50.20% in oral mucosal cells and 44.80% in hepatocytes higher than those in the control group ($P < 0.01$). Zhang et al., (2006) found that fluoride can induce DNA damage in osteoblasts. Wang et al., (2004) observed that fluoride can cause lipid peroxidation, DNA damage, and apoptosis in human embryo hepatocytes. Oxidative stress, DNA damage and modifications of membrane lipids can be induced in hepatocytes by excess fluoride (Shanthakumari et al., (2004). Ha et al., (2004**)** reported that the rate of DNA damage of group treated with sodium fluoride was significantly higher than the control grouped.

Patel et al., (2009) observed that a significant increase in mean frequency of micronuclei induction in NaF treated groups when compared to control. Li et al., (2000) found that the micronucleus rate of adults from the high-fluoride area and mice drinking high-fluoride water was higher than that of the control group ($P<0.05$). Fluoride may be mutagenic, Meng and Zhang, (1997) showed the frequencies of cells with MN in peripheral blood lymphocytes of workers exposed mainly to fluoride were statistically significantly higher than controls. Wu and Wu, (1995) stated that in persons with fluorosis as well as those considered "healthy", the MN rate was significantly higher than in a neighboring control group drinking low-fluoride water. Suzuki et al., (1991) revealed that a significant increase in micronucleated polychromatic erythrocytes was observed 24 H after intraperitoneal injection of sodium fluoride at a dose of 30 mg/kg body weight. In the in vitro micronucleus test, the frequency of micronucleated polychromatic erythrocytes was increased significantly at concentrations of 2 and 4 MM. These results indicate

that the micronucleus test may be useful in evaluating the cancer risk of sodium fluoride.

Jordao et al., (2009) demonstrated the increased DNA damage induced by acute ethanol administration and DNA protection when ethanol is administered together with methionine. Kang et al., (2002) revealed that L - Carnosine prevented both TPA and hydrogen peroxide induced DNA fragmentation Pretreatment with vitamin C (400 mg/kg/day) or vitamin E (100 mg/kg/day) for 5 days before acute ethanol administration (5 g/kg) inhibits the generation of the hydroxyl-ethyl radical by 30 and 50%, respectively, and prevents oxidative DNA damage, which is high in groups receiving no antioxidants Navasumrit et al., (2000). Bagchi et al., (1998) reported that pretreatment of animals with antioxidants such as vitamin C, vitamin E succinate and grape seed proanthocyanidin decreased O- tetradecanoylphorbol-13-acetate (TPA) induced DNA fragmentation in the liver and brain.

Histological examination of the exocrine portion of pancreas of fluoridated rats of the present study exhibited structural alternations. The most prominent alternation was the occurrence of multiple vacuoles in the cytoplasm of pancreatic acini. This finding was in consistence with the histological findings of many investigators who reported the occurrence of vacuolar degeneration in pancreas of sodium fluoride treated animals (Stawiarska-Pieta et al., 2007, 2008 &2009; Shashi et al., 2010). The same finding was attributed by Cicek et al., (2005); Shashi et al., (2010) who explained that fluoride toxicity leads to loss of selective permeability of the cell membrane, resulting in dilatation of cytoplasmic component secondary to intracellular fluid and electrolyte redistribution.

Light microscopic examination of pancreas of sodium fluoride treated group of the present work showed also inflammatory cells infiltrations in the stroma of pancreas especially around blood vessels. This coincided with the work of previuos studies, Stawiarska-Pieta et al., (2007, 2008 &2009); Shashi et al., (2010) who reported during their histological examination, that fluorosis induced inflammation in pancreas. Gutowska et al., (2011) also reported that excessive exposure to fluoride can result in inflammatory reactions involving macrophages and their differentiation. Fluoride has been considered to play an important role in pathogenesis of chronic fluoride toxicity as it increases the production of reactive oxygen species (ROS) and lipid peroxidation (Machoy, 1987; Chinoy&Patel,1998; Chlubek, 2003). Other studies reported reduction of anitioxidant defense system (enzymatic and non enzymatic) in different tissues of animals treated with fluoride (Shivarajashankara et al., 2001a; Zhan et al., 2005a).

In rats of vit. E in combination with methionine prior to NaF and L-carnosine prior to NaF treated groups, no histological changes were observed, concerning vacuolar degeneration and inflammation in the pancreas that were clearly observed in Na F treated group, during the present light microscopic study. The light microscopic results obtained in this study correlated with the previous studies concerning the influence of different antioxidants on pancreas (Stawiarska-Pieta et al., 2007, 2008, & 2009). Vitamin E prevents the organism from the activity of free oxidative radicals (Stawiarska-Pieta et al., 2009). Stawiarska-Pieta et al., (2007) during their experimental studies stated that simultaneous administration of vitamin E in animal intoxicated with fluoride increases the activity of antioxidative enzymes and added that vitamin E is usually administrated along with methionine or vitamin C. The functional role of L-carnosine is still not completely understood, however several studies indicate that the dipeptide content of L-carnosine exerts protective actions against metal- and or antibodies-mediated toxicity by acting as anti-oxidant and free-radical scavenger (Kohen et al., 1988; Preston et al., 1998*;* Horning et al., 2000; Trombley et al., 2000; Carlo et al., 2011). Littarru & Tiano, (2007) reported that antioxidants have a beneficial influence upon oxidation-reduction equilibrium. Different studies confirmed that administration of antioxidants to animals intoxicated with sodium fluoride inhibits the alterations observed in exposure to fluorides, which clearly shows their preventive action on brain (Chinoy and Patel, 2000) and testis (Krasowska et al., 2004).

The pyknotic and darkly stained nuclei that were observed during light microscopic examination, the irregular shaped and heterochromatic nuclei that were also seen during the electron microscopic study and the intensive expression of caspase- 3 that were demonstrated immunohistochemically in the cytoplasm of pancreatic acinar cells of Na F administrated group of the present work which was of a statistical significant value when compared to the control group, confirmed the induction of apoptosis by Na F administration, Gomez et al., (2001) revealed through their immunohistochemical study that inflammation of pancreas causes activation of apoptosis. Apoptosis is an active regulated cell death. The characteristics of apoptosis include; a series of biochemical and morphological changes, such as caspase family activation, nucleosomal DNA fragmentation, cell volume loss and chromatin condensation (Vaskivuo et al., 2000). Chronic fluorosis induces oxidative stress leading to generation of free radicals and alterations in antioxidants or reactive oxygen species (ROS) scavenging enzymes (Sharma and Chinoy, 1998; Shivarajashankara & Shivashankara, 2002). ROS have been implicated as potential modulators of apoptosis. It has been suggested that oxidative stress plays a role as a common mediator of apoptosis. Some studies revealed that LPO is one of the molecular mechanisms involved in chronic fluoride- induced toxicity. Fluoride ion, although a non oxidant ion, causes oxidative stress indirectly leading to increase in lipid peroxide levels. Lipid peroxidation impairs a variety of intra and extra mitochondrial membrane system that may contribute to apoptosis (Shivarajashankara et al., 2001a,b).

The present histological study revealed regaining of the normal appearance and contents of nuclei of pancreatic acinar cells in last two groups. The present immunohistochemical and statistical studies of the same groups showed also marked reduction in caspase 3 expressions in pancreatic acinar cells which indicated reduction of apoptosis. It has been reported that methionine has an important role in inhibition of apoptosis and methionine restriction induces apoptosis of prostate cancer cells (Lu et al., 2002). Vitamin E is

also the most important lipophilic antioxidant and resides mainly in the cell membrane, thus helping to maintain membrane stability (Stoyanovsky et al., 1995). As mentioned before, several studies indicate that the dipeptide content of carnosine exerts protective actions against metal- and or antibodies-mediated toxicity by acting as anti-oxidant and free-radical scavenger (Kohen et al., 1988; Preston et al., 1998*;* Horning et al., 2000; Trombley et al., 2000; Carlo et al., 2011). Consequently antioxidants could reduce cell death in different cellular structures caused by oxidants and free radicals effects of sodium fluoride , for example, blocking apoptosis induced by changes in mitochondrial membrane permeability and subsequent release of cytochrome c and caspase activation (Ramanathan et al., 2003). Reduction of apoptosis in last two groups could be also attributed by Patel & Chinoy, (1997); Chinoy & Patel, (1998); Sharma & Chinoy, (1998) who stated that antioxidant reduce LPO caused by fluoride in rat resulting in stability in mitochondrial membrane system and reduction in apoptosis.

The present electron microscopic examination of pancreatic acinar cells of fluoridated animals revealed dilatation and globular shape of rough endoplasmic reticulum. Some of these globular shape rough endoplasmic reticulum contained intra-cisternal zymogen granules. Zymogen granules were markedly decreased in number or even absent. These findings could be attributed by Quijeq et al., (2002) through their several studies which showed that fluoride inhibits protein synthesis. These findings were previously studied in sodium fluoride treated animals (Matsuo et al., 2000; Zhan et al., 2005b) and were attributed by Matsuo et al., (2000) to be due the toxic effect of fluoride that disrupts the export of zymogens from the rough endoplasmic reticulum. During the secretary process, incompletely assembled and aggregated products are selectively retained in the rough endoplasmic reticulum. Excessive protein accumulation in the rough endoplasmic reticulum results in formation of intra-cisternal granules and decreases in number of zymogen granules (Pfeffer and Rothman, 1987; Arias & Bendayan, 1993).

Stawiarska-Pieta et al., (2009) stated that application of antioxidants leads to an increase in the protein level, which may confirm our electron microscopic results in last two groups that showed apparent increase in the number of zymogen granules and no or mild dilatation in rough endoplasmic reticulum.

In conclusion, the combined administration of antioxidants showed beneficial effects upon hematological, biochemical, histological and immunohistochemical alterations which developed in pancreas following sodium fluoride intoxication. This proves that oxidation processes play a significant role in fluoride toxicity, and that the administration of antioxidants in response to an increased risk of exposure to fluoride compounds may protect the human body against their harmful effects.

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Assessing of distance learning in adult education

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Abstract: The adult education process drives towards the achievement of the capability to individually use reason on the subject matter in question. Adult education is oriented at the use, at any age, of attitudes and skills prone to clarifying any distortions in communication, favouring "why," "how," "when" and "where" as well as the "what for" in all situations. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge.

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Introduction:

Adult who is able to recognize their needs? He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult illiteracy is like a disease that infects virtually every dimension of Kentucky life. Adult illiteracy saps the energy and capability of Kentucky's people and its economy. Adult illiteracy feeds the state's unemployment, its welfare rolls, and the correctional institutions. Adult illiteracy severely hinders the life chances of young children, undermines school reform, and limits the opportunities for postsecondary education. Despite landmark reforms in public schools, too many Kentuckians continue to drop out of school, thereby perpetuating the chronic problem of adult illiteracy. Too many young Kentucky parents are unable to read and lack the basic literacy necessary to provide the necessary stimulating, supportive family environments for young children. It is known that children's literacy levels are strongly linked to the educational level of their parents and that children of parents who are unemployed and have not completed high school are five times more likely to drop out. To be successful, the Commonwealth's strategies must energize and gain the commitment of all the state's political, education, business, and civic leaders. No strategy will succeed unless it engages leaders in each community and county to identify needs and develop programs and services appropriate to the community's unique circumstances. The most serious challenge will be to motivate low-skilled, under-educated adults within the working age population to seek further education. Simply expanding the number of providers and programs will not necessarily increase demand from the populations and communities where the needs are greatest. Deepseated social, economic and cultural barriers—many dating back generations—lead people to undervalue education. In addition, in many counties it is difficult for people to see a direct relationship between better education and better-paying jobs. Either there are no jobs available or many existing employers do little to emphasize the connection between better education and the possibilities for getting a job, keeping a job, or earning a higher wage. For many, getting more education and earning a high school diploma or a college degree has little positive meaning.

Only the negative consequences are obvious: getting more education often means leaving one's family and community for jobs and opportunities for advancement somewhere else. The future of Kentucky depends on uplifting the quality of life and economy of all of Kentucky. The social and economic costs of neglect of large parts of the state will drag down the rest of the state and seriously hinder its capacity to compete in the global economy.

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular population) with a particular means (e.g., adult basic education) to achieve the goal. Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. "Literacy" refers to the knowledge, skills, and competencies of individuals. The federal Adult Education and Family Literacy Act (Title II of the Workforce Investment Act)1 defines literacy as "an individual's ability to read, write, speak in English, compute and solve problems, at levels of proficiency necessary to function on the job, in the family of the individual, and in society." Literacy is often defined in

terms of specific domains such as "basic academic skills," "workplace skills," "life skills," "parenting skills," or skills necessary to exercise one's rights and responsibilities for citizenship. Different dimensions of literacy are often categorized by terms that cluster several dimensions of literacy important for different clients. Examples include workplace literacy (combining both basic academic skills and workplace skills), and family literacy (combining basic academic skills and other skills essential for successful parenting).

2. "Education attainment" usually refers to the numbers of years of schooling completed or the level of credential (e.g., high school diploma or associate degree) an individual has obtained. Despite concerns about the meaning of credentials, there is a strong correlation between educational attainment and literacy.

3. "Literacy initiatives" often are defined in terms of the needs of a particular target group. These may be parents of young children, youth who have dropped out of high school without earning a high school diploma, welfare recipients, persons with limited English-speaking ability, incarcerated adults, or adults in the workforce.

Getting a college education can be difficult for people with inflammatory bowel disease (IBD). Frequent trips to the restroom, exhaustion, doctor visits, and medication side effects are all barriers to the traditional college experience.

What if you could get the degree without ever setting foot on a campus? You can do just that through distance or virtual learning.

Distance learning has been around for a long time (we've all seen the commercials on TV). While there is still prejudice surrounding some distance learning, it is increasingly being accepted as an alternative to traditional classroom learning. Courses can be offered via the Internet, where students are able to interact with instructors and other students without physically being in the same room.

Before considering if distance learning is a viable option for you, there are several questions you should ask yourself:

- What course of study would you pursue?
- Are you interested in pursuing a degree? Brushing up on existing skills?
- Would your course of study require some traditional classroom time (such as laboratory or field work)?
- After obtaining a degree, would you be able to obtain employment that allows for your illness (such as telecommuting or flexible hours)?

There are two types of programs offered by distance education schools: synchronous learning programs and asynchronous learning programs. With synchronous learning, distance education students must log on to the school's website at a set time. Often, they interact with their peers and professors via group chats, web seminars, video conferencing, and phone call-ins. With asynchronous learning, distance education students complete all coursework on their own time. They often learn via assignment sheets, message boards, email, pre-recorded video lectures, mp3s, and traditional mail correspondence. Many students find that distance education courses give them the freedom to complete a degree while meeting their personal and professional obligations. Motivated learners are often able to complete distance education degrees in a fraction of the time often required. Distance education courses also allow students to network with participants from all over the nation. On the downside, distance education courses do not offer the face-to-face interaction found in traditional classrooms. Some students find that they struggle to stay motivated and meet deadlines due to the independent nature of distance education courses.

What Is Adult Learning?

Adult learners have a different approach to learning. By the time you reach adulthood, you're most likely responsible for your own success and you're perfectly capable of making your own decisions once you have the information you need.

Adults learn best when learning is focused on them, not the teacher. This is called andragogy, the process of helping adults learn.

Malcolm Knowles, a pioneer in the study of adult learning, observed that adults learn best when:

1. They understand why something is important to know or do.
2. They have the freedom to learn in their own way.
3. Learning is experiential
4. The time is right for them to learn.
5. The process is positive and encouraging.

Choosing a Distance Learning Program:

Distance learning is one of the fastest-growing components of higher education. Almost 3.5 million students were enrolled in at least one distance learning course in the fall of 2006 and online enrollments are increasing every year. The convenience of taking classes at any time from any location appeals to today's adult learner, especially those who work, have families or live in rural areas.

Today a growing number of paralegal and legal secretarial programs have a distance learning component (no law schools currently grant credit for distance learning studies). However, not all distance learning programs are of equal quality. Moreover, the increasing popularity of distance learning programs have led to "diploma mills" or "accreditation mills" that offer bogus degrees and certificates. Choosing a distance learning program requires careful research and evaluation. Below are several important factors to consider in choosing a distance learning program.

1. **Accreditation.** Accreditation is a means of ensuring the quality and effectiveness of higher education institutions and programs in the United States. Eight regional accrediting agencies accredit most of the colleges and universities in the United States. A host of national and professional accrediting organizations also exist, including the Distance Education and Training Council (DETC), an organization that identifies and accredits distance learning programs. These twelve questions outlined by the Council for Higher Education Accreditation are helpful in examining a distance learning program's claims of acccreditation.

In evaluating distance learning paralegal programs, determine if the school is accredited by one of the regional accrediting bodies and by the American Bar Association (ABA). ABA-approval signifies that the school has met certain standards in terms of academics, facilities and instruction. Graduating from an ABA-approved school may give you an advantage in the legal job market.

2. **Reputation.** The reputation of the distance learning program you attend may hinder or enhance your post-graduate employment prospects. In evaluating the reputation of a distance learning program, you should not solely rely on the school's website or marketing materials. Other ways to investigate the reputation of a distance learning program include:

- Visiting the school.
- Talking to alumni (contact the career services department for alumni names and contact information).
- Researching the distance learning program's record with the Better Business Bureau.
- Talking to paralegals, attorneys and legal employers about the reputation of the school you are considering.
- Researching the school in print publications, news articles and on the Internet.

1. **Academic Offerings.** When evaluating distance learning programs, it is also important to consider the program's academic offerings. A quality distance learning program offers a comprehensive curriculum with a variety of options, electives and advanced coursework. Talk to professors or an academic dean regarding the content and delivery of courses. The American Association for Paralegal Education (AAfPE) recommends that paralegal instructional content include courses in legal research and writing, litigation, ethics, contracts, business organizations and torts. In addition, courses should develop students' critical thinking, communication, computational, computer and organizational skills, and competency to handle ethical issues, according to the AAfPE.

Legal programs should also offer an experiential learning component such as an internship, practicum, pro bono work or clinical experience. These are great resume-building opportunities and allow you to learn practical skills and gain real-world experience.

2. **Instructional Technologies.** Distance learning courses can be delivered in a variety of ways through a growing array of technological tools including audio tapes, CD or DVD ROM's, e-mail, telephone conferences and web-based delivery systems. Questions to ask include whether the program employs a mix of instructional technology? Is hands-on training and support provided? Can students preview courses online and try out the technologies before enrolling?

3. **Teaching Staff.** The faculty is the backbone of any distance learning program. Are the courses taught by professors or are the courses pre-taped correspondence instruction? If the courses are taught by instructors, what is the background and qualifications of the teaching staff? Are classes taught by paralegals, attorneys or a mix of both?

4. **Career Services.** Another important consideration in any distance learning program is the extent and quality of its career services program. Research indicates that the greater the resources offered by the career services department, the greater the program's job placement success. You might inquire as to what percentage of graduates find related employment following graduation and whether the career center offers personalized career counseling, job placement assistance, job search seminars, online job boards or resume assistance.

Conclusion:

Distance education programs are more popular than ever. College and high school students now have hundreds of legitimate distance education schools to choose from. If you're new to the idea of learning through distance education, this article will help you understand the basics.

Distance education is any type of schooling that takes place away from a physical campus. In traditional programs that the principles of psychology and curriculum planning, less attention is the form of content presentation ie codification and providing books, original format and have the dominant form, while for adult content that could have valuable experience in addition to writing, other ways also be provided Affect the selection of pictures and images related to the concepts and content produced by including them.

Learning activities such as activities outside the classroom, dialogue, role playing and ... Another type of content is presented. Duties are placed on the learner, a resource for developing knowledge, skills and insights he considered.

Curriculum content only from the training provided to learners or not, but put together their learning through activities that can inform or does, skills and attitude to

achieve. In this case, apart from learning that the assays taught learners directly to sustainable and effective learning occurs in his.

Another way of providing content that is educational activities outside the learning environment possible for learning more and better enables adult learners. For example, hits, field trip experiences for learners or transfer is provided, develop knowledge, insight and skills they will.

To ensure that science curriculum and educational aspects, according to community needs and audiences, application form is provided or not, the content selection criteria should be considered. These criteria is being include knowledge, effectiveness, flexibility, diversity, relevance and practical learning.

Some research findings that can be a learning process for the Guidelines for training operations are applied, is given below:

1- intrinsic motivation, learning a deeper and make them sustainable. When the need is met directly by the learning itself, what is learned, but is complementary learning. Creating a training activity in adult learning needs, learning ensures stable

2- Positive reinforcement (reward) learning to reinforce the negative (punishment) is more effective. Many adults because of negative experiences at the beginning of schooling, are weak and afraid. Feeling of success in adult learning for continuous learning and adult participation is essential.

3- Learning, especially regarding skills development, will be added frequently.

4 - Duties and meaningful content than meaningless subjects are learned more easily and are later forgotten. This issue, especially for older adult learners is true. Challenges of adult learning facilitators by the way that content was significantly associated with the experiences and needs of learners is.

5- Passive than active participation in learning activities, learning increases. Adult educators are allowed to participate actively in India, a stable and meaningful learning to help

The task force's policy recommendations are guided by these principles:

1. Recognize that adult illiteracy is not an isolated problem but a fundamental barrier to every major challenge facing Kentucky. Without significant improvements in adult literacy the Commonwealth will be unable to make progress on issues such as early childhood education, education reform (elementary/secondary and postsecondary), economic development, and improving the health and well-being of Kentucky's families and communities.

2. Shift from top-down implementation of a federal or state program to leading a statewide public campaign that depends fundamentally on a bottom-up commitment of communities, employers, and educational institutions. The campaign must engage all aspects of Kentucky life—all dimensions of state and local government, all education levels, the state's business and civic leaders, voluntary organizations, and all others whose work affects—or is affected by—the problem of adult illiteracy.

3. The future of Kentucky depends on narrowing the disparities among counties by improving the adult literacy of the population in all regions of the state.

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Prepration the Sensor of Imprinting Molecular Polymer Based on Polyaniline to Recognize Agricultural Toxin Chlorpyrifos and Diazinon

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Abstract: Polyaniline as an intelligent organic polymer has been noticed since many years ago. Recently there have been some efforts on its applications such as ion selective membranes, batteries, conducting dyes, chemical and gas sensors and etc. Using of conducting polymers as sensors has opened a new field in their applications and it is at the initial steps. On the other hand detection of orgnophosphorus compounds due to their wide applications in insecticides and pesticides, chemical warefare agents are great importance. Regarding this, in present research sensing behavior of molecular imprinting polymer powder base on polyaniline in compare with organophosphorus agronomy pesticides as like as a sample via measurements and FTIR conductance by using a four-point probe method. Among sensing properties studied in this work can mention to analyte concentration, temperature, polymer response time. Molecular imprinting polymer selectivity examined in compare with structure of molecules similar to analyte. In all of the cases conductivity of molecular imprinting polymer remarkable in comparing to molecular non-imprinting polymer because of analyte effect on polymer structure. Preparation process of molecular imprinting polymer and sensing properties did with this method for like compounds namely of diazinon, chlorpyrifos at the results show that molecular imprinted surface in combination with conductometric method is a useful approach for the sensing applications.

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Keywords: Sensor; Molecular imprinted polymer (MIP); Chlorpyrifos toxin; Diazinon toxin; Conductivity;

1. Introduction

About 60 years past from the emergence of chemical sensors, devices which have different names but all are recognized by general name as "sensor" have been classified at different groups including physical and chemical sensors. After 60 years, chemical sensors enjoyed large development in human life, industry and modern technology as well [1].
The sensors based on electrical conductivity are considered as one of those sensors. Lack of selectivity is among deficiencies of these sensors. This feature has limited the application of these sensors in different industries [2]. The proposed method in this research work is to increase the selectivity of sensor based on the electrical conductivity by molecular molding in order to produce Polymer. In recent years, conductive polymers carried out applications in this field, and in this case, polyanilinehas potential capabilities [3].
Those vapors and compounds which are able to oxide and reduce conjugated chains of polyanilineshould be naturally able to impact on electrical properties of polymer. Therefore, those compounds which are subject to a kind of electron deficiency and positive charge, both can impact on electrical and conductive properties of polyanilineby separating electron from full electron chains and producing positive charge centers in these chains and compounds which are able to inject electron and producing negative charge centers on these chains [4]. With a closer look at the structure of organic phosphorous compounds and nature of bonds between P=O and/or P=S, it is noticeable that phosphor's atom in this bond has little positive charge due to bond between dπ-pπ and 2p orbitals of oxygen's atom and/or sulfur and according to electronegativity of atoms participating in bond, and also little positive charge on the phosphor's atom in bond of P=O is more rather than P=S due to the higher electro negativity of oxygen's atom rather than sulfur. Consequently, it is expected that compounds with this bond, impact polyanilinethrough oxidation mechanism and/or p-doping [5]. According to little extent of positive charge of phosphorus's atom it is expected that this impact to be higher for the organic phosphorous compounds with P=O bond rather than compounds with P=S. Also it is expected that by substituting group which are able to participate at πbond, this impact would be reduced and by substituting electron-killer groups, this impact would be increased [6]. At this part of research work, changes of polymer conductivity being exposed to agriculture toxins(pesticides), (in the role of sample) would be analyzed by electrical conductivity device with four-point method and results would be reported completely at conduct curves in terms of time, temperature and density .

It is notable that all used toxins were analyzed, in lower density level than human's toxic [7].

2. Experimetals

2.1. Instrumentals: Fourier Transform Infrared spectra (FT-IR) of the samples in KBr pellets were recorded on an (Perkin-Elmer) spectrometer. Scanning electron microscopy (SEM) was performed by a (Hitachi, S-4160) at an operating voltage of 10KV. Prior to scanning, the specimens were coated with a very thin layer of gold.

2.2. Preparation of polyaniline

In order to produce polyanilinechemically, protonic acid solution is used. Therefore, solutions (300 Ml distilled aniline and Pro-sulfate Ammonium –APS- are prepared with the same molar ratios in 1mollar acid hydrochloric) in two separate containers. Then the temperature should be decreased to -5 to -10 C by putting in container which contains ice and sodium chloride, and then while monomer container is in mixing, Ammonium persulfate should be added to 1mollar acid hydrochloric. After few moments, polymerization would be started and temperature of container would be increased to some extent. Gradually, the transparent monomer solution will be darkened that would indicate the formation of poly aniline. Solution would be stirred strongly for an hour at 5 C and four hours at room temperature. Molecular mass at lower temperature would be increased and consequently, conductivity of polyanilinewould be increased. After that, contents which contain green sediment of polyanilinewould be smoothened by Buchner funnel. The sediment would be washed by distilled water to the point that it would be colorless under filter with methanol. Soluble part in methanol which contains oligomers of polyaniline would be separated from polymer due to washing with methanol. The sediment would be washed again with distilled water in order to remove the remained methanol in polymer (solution under filter is green at this level). In the next step, polymer would be dried at 50-60 C.

2.3. Supplying the molecular form of polymers

Providing the molecular form of polymers based on polyanilinesensitive to diazinon.

In order to make polyanilinesensitive to diazinon, it is necessary to do polymerization of polyanilinein the presence of diazinon toxin and/or being immersed in desired PANi Toxin. Given the fact that there is possibility of toxin degradation in acidic solution, as a result, the second method should be used.

In order to prepare molecular form of polymer which is sensitive to diazinon, at first, 0.5g PANi in alkaline solution of ammonia 25% should be stirred for 24 hours and then it will be smooth and completely dry. Afterwards, doped polymer is solved in 25 Ml N-methyl pyrolidone (NMP) and 2g diazinon would be added to it. After that, when it was stirred for 8 hours, accordingly the uniform film would be prepared gradually by gradual temperature.

That film would be dried on the desiccator under vaccum condition for 24 hours. The resulting polymer powder can be prepared as primary powder then it can be dried to be prepared for rest of the work.

2.4. Supplying the molecular form of polymer based on polyanilinesensitive to chlorpyrifos

In order to prepare polyanilinewhich is sensitive to chlorpyrifos, it is necessary to do polymerization in the presence of chlorpyrifos toxin and/or being immersed in desired PANi Toxin. Given that there is possibility of toxin degradation in acidic solution, therefore the second method should be used. Then the doped polymer should be solved in 25 Ml N-methyl pyrolidone (NMP) and 2g chlorpyrifos would be added to it. After being stirred for 8 hours , as a result the uniform film would be prepared gradually by gradual temperature. That film would be dried on the desiccators under vaccum condition for 24 hours. The resulting polymer powder can be prepared as primary powder then it can be dried to be prepared for rest of the work.

2.5. Extracting Diazinon from Molded Polymer

In order to merge sensing operation one should be able to separate sample (toxin) from the created hole in polymer. Chloroform solvent was used in this case. Thus the prepared molecular form of polymer was grinded in porcelain mortar after being completely dried. Comminuted polymers were washed with chloroform solvent for 5 minutes. Then they were smoothened and dried. When the polymer was dried, its conductivity was increased due to the exit of samples which have been absorbed.

2.6. Extracting Chlorpyrifos from Molded Polymer

In order to merge sensing operation one should be able to separate sample (toxin) from the created hole in polymer. Chloroform solvent was used in this case. Thus the prepared molecular form of polymer was grinded in porcelain mortar after being completely dried. Comminuted polymers were washed with chloroform solvent for 5 minutes. Then they were smoothened and dried. When the polymer was dried, its conductivity was increased due to the exit of samples which have been absorbed.

3. Results and Discussion

Assessing the sensing properties of molecular form of polymer based on polyanilinefor diazinon

Affective parameters on conductivity degree and sensing principles were assessed with the order of time of reaction, density, temperature, selectivity of sensor, in

order to evaluate the sensing properties of molecular form polymers and compare it with the observant polymers.
Assessing sensing properties of molecular form polymer based on polyanilinefor chlorpyrifos Affective parameters on conductivity degree and sensing principles were assessed with the order of time of reaction, density, temperature, selectivity of sensor, in order to evaluate the sensing properties of molecular form polymers and compare it with the observant polymers.

3.1. Assessing the time of reaction in sensor

One standard solution of diazinon and acetone (50 ppm) was prepared to assess the reaction time of this sensor. 0.05g polymer powder was washed with chloroform in 2.5Ml from 50ppm and the density of diazinon was estimated at different times (15, 30,45,60,75 min) and after being dried completely , its conductivity was measured by four point device.

3.2. Assessing the effect of temperature

0.05g polymer from polymeric powder was put in 2.5Ml of 50pmm standard solution for an hour with different temperatures in order to assess the effects of temperature on penetration rate of sample to holes and create doping in polymer. After being dried, the conductivity was measured.

3.3. Assessing the extent of sensor selectivity

In order to assess the extent of selectivity of this sensor, 0.05g of polymeric powder in 2.5Ml of one standard solution from the same molecule with chlorpyrifos molecule was assessed for an hour, and then the conductivity was measured after being dried.

3.4. Assessing the durability time of conduction

In order to assess the durability time of conduction, conduction of molecular form of polymer and conduction of observant polymer were measured after one week, one month and three months respectively and compared with each other.

3.5. Assessing the molecular form of polymer: Conditions of polymerization in providing the molecular form of polymer

4 hours was considered in order to integrate the process of polymerization for preparing molecular form of polymer. Since the used monomer in this work is aniline which changes into polyanilineduring the reaction, therefore the long polymerization time for synthesis is not proper. Reaction efficiency from 1 to 24 hours is the same; however, the reduced viscosity will be appeared after 4 hours. Thus, the polymerization would be completed after 4 hours and long time leads to the slow massification of poly aniline.

In assessing the role of temperature also, it has proven that polymer viscosity would be increased by decreasing the temperature to -5C.
Used starter in this work is ammonium persulfate and is in rate of mole to mole with monomer. Starters such as $(NH_4)_2S_2O_8$ and $K_2Cr_2O_7$ in aniline polymerization , would cause high polymerization efficiency , conductivity and viscosity but by using $FeCl_3$ and KIO_3 resulting polymers would have high efficiency and in contrast have very low viscosity . Polymerization in the presence of $KClO_3$ and $KBrO_3$ leads to polymers with low efficiency and viscosity.

3.6. Extracting the analytee sample from the molecular form of polymer

One of main steps in molecular form of polymers is the step of extracting sample from molded polymer, thus it is necessary to break non-covalent bonds between doping factor and polymer, and then doping factor should be removed from the current holes in polymer. Also, the used solvent in this step should not damage the form of polymer and/or cause it to be solved; therefore the chloroform solvent was proper.
Results proved that the best time for extracting the sample is 5 minutes because more time leads to damaging the whole structure of polymer.

3.7. Molecular form of polymer sensitive to diazinon based on poly aniline

The extents of conductivity of polymer before and after extracting the analytee sample are as following:
Conductivity before extraction = 0.36 mS/cm
Conductivity after extraction = 0.08 mS/cm
The conductivity before washing with chloroform is more than after washing for the reason which was described before.

3.8. Molecular form of polymer sensitive to chlorpyrifosbased on poly aniline

The extents of conductivity of polymer before and after extracting the analytee sample are as following:
Conductivity before extraction = 0.78 mS/cm
Conductivity after extraction = 0.096 mS/cm
As it is noticeable, the conductivity before washing is much more and the reason is that some chlorpyrifos molecules exited from the surface of polymer due to absorbed molecules which have been washed.

3.9. Assessing the sensing properties of diazinon molecular form of polymer

The Sensing properties of polymer would be assessed after extracting the analytee sample when it was exposed to sample again.

3.10. Reaction time of sensor

Figure 1 has reported the conductivity changes against the reaction time of sensor for the density of diazinon 50ppm in ambient temperature. It can be observed that as time increased, penetration rate of sample has been increased within the empty hollows of polymer. The most increase would reach at the time of 60 minutes. After that time, it has been decreased due to the fillin active sites of polymer. Increasing conductivity an long reaction time would prove the presence o hollowness in polymer.

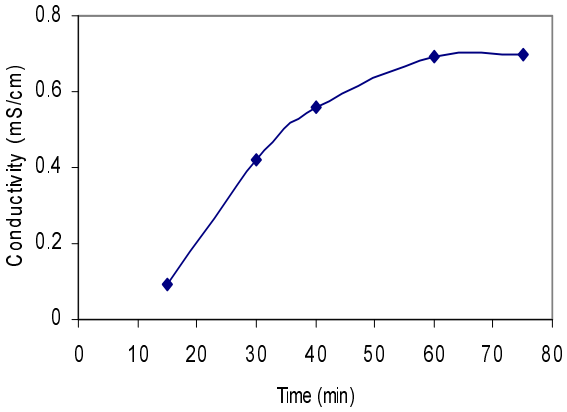

Figure 1 : Conductivity changes based on time increasing for diazinon toxin

3.11. Effect of sample density

The Relation between sample density and conductivity change in MIP of diazinon has been reported in figure 2. In this step, different densities of sample reacted with polymer for an hour at ambient temperature. It has been observed that the sample density has positive relation with conductivity which means that by increasing the density, conductivity will be increased too, where sample absorption has been stopped and polymer conductivity remains fixed due to satiating of active sites in polymer.

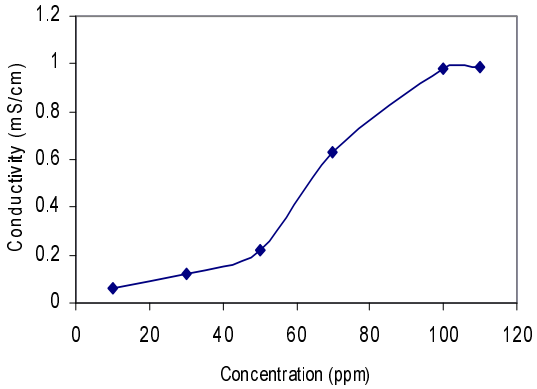

Figure 2 : Conductivity changes based on the density increase in diazinon

3.12. Effects of doping temperature

Figure 3 shows the curve of temperature changes of diazinon molecule penetration than 50ppm density for an hour against conductivity .

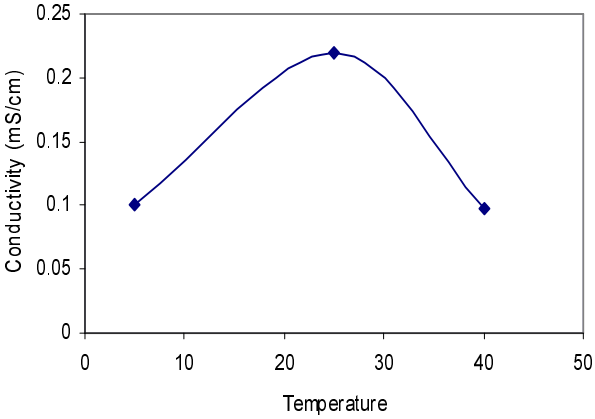

Figure 3: Conductivity changes based on time increasing for diazinon toxin

3.13. Assessing the selectivity of diazinon sensor

The ability of polymer to distinguish between sample and nuisance species which have the same structure with sample enjoys lots of importance for sensing applications. Extent of conductivity of diazinon polymer against the same molecule with diazinon equals to 0.055mS/cm. This result shows the sensitivity and selectivity of diazinon MIP .

3.14. Assessing durability time of conduction

Figure 4 shows the conductive durability of MIP after washing with chloroform and being exposed to 50 ppm diazinon density at weeks 1, 2, 3 , 4 and 5 . First, it has been observed that conductivity is fixed and then decreased finally being fixed again. This little decrease shows the destruction of those samples which have been absorbed, and fixed conductivity represents the consistency in MIP method and conductivity consistency .

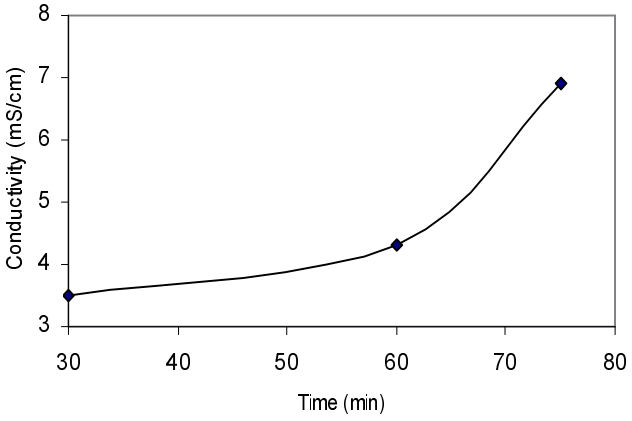

Figure 4: Conductivity changes of MIP after exposed to 50 ppm diazinon

Spectroscopy has many applications in determining the purity and also assessing the structural features and identifying quantification and qualification of phosphoric compounds.

After several years , many studies have been done on the factorial groups of phosphate esters, amidothioates phosphor, amidates phosphor, amidothioates and relative compounds , then the absorbing frequencies ranges of factorial groups such as (P=O) ،(P=S) ،(P-N-C) ،(P-X) have been represented . Starting point of these studies has been on the phosphoric toxins by IR spectroscopy in 1950's . In the following section , some of absorbing frequencies of factorial groups would be described in the phosphoric compounds.

3.15. FT-IR spectrum investigation

The band relates to stretching vibrations of P=O group is strong and appears on 1150-1400 cm^{-1} region. This band sometimes might appear as twin band which this issue is related to rotational isomers. Electronegative groups compete with oxygen atom for absorbing electron from phosphor atom and cause that absorbing frequencies to move to higher regions by structuring p^{+}-o^{-} and creating the stronger bond. Assymetric Stretching vibrations of P-O-C in aliphatic compounds cause very strong wide bands that have been appeared at 950-1070 cm^{-1} scope. P-N-C group gives one intermediate to strong band in 930-1110 cm^{-1} region that it might be due to the asymmetric stretch of P-N-C group. This group also shows one intermediate to strong band in 680-750 cm^{-1} region that is due to the symmetric stretch.

Peak of stretching vibrations of P=S bond places at 655-865 $cm^{-1\ region}$ that is intermediate peak with high intensity. With this condition, the relative peak to factorial group of P-S in places at region would be around 550-730 $cm^{-1.}$ P-CI bond , gives one intermediate to strong band at 435-610 cm^{-1} region and its location depends on the connected atoms to phosphor. Absorbing band which appeared at 1675 cm^{-1} region relates to the stretching the frequency of C=O group. It should be noted that C=O bands appear on amidate esters at 1690 cm^{-1} but in synthetic compounds , resonance between free electron pair on the nitrogen and its connected carbonyl group would cause reduction in stretching the frequency .

Figure 5 shows FT-IR spectrum of polyaniline and its significant peaks are: 3420 cm^{-1} (stretching vibration of Amine NH) , 1400 cm^{-1} (vibration of CH2 group) , 1227 cm^{-1} (stretching vibration of CN) , 3000 cm^{-1} (stretching vibration of aromatic C-H) , 1596 and 1492 cm^{-1} (aromatic ring) .

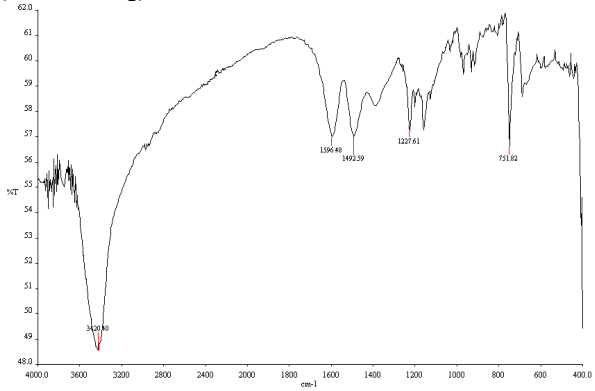

Figure 5: FT_IR spectrum related to polyaniline

Figure 6 shows FT-IR spectrum of observant polymer that its significant peaks are: 3400 cm^{-1} (stretching the vibration of amine NH and stretching vibration of OH that overlapped), 1590 cm^{-1} (scissor-like vibration of NH group), 1450 cm^{-1} (stretching vibration of C=C), 3100 cm^{-1} (stretching vibration of aromatic C-H), 2950 cm^{-1} (stretching vibration of aliphatic C-H).

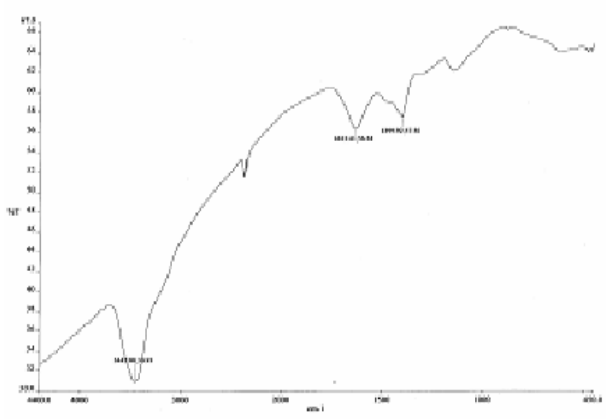

Figure 6: FT_IR spectrum related to control polymer

Infrared spectrum of molecular form of polymer (MIP) is shown before and after the exiting diazinon molecule by washing with chloroform solvent and after reconnecting (50ppm density) in Figure 7. In addition to significant groups in NIP, groups related to sample consist of: 1300

cm^{-1} (stretching vibration of P=O), 830 cm^{-1} (flexural vibration of P-O-C), 500 cm^{-1} (flexural vibration of P=S) , 2931 cm^{-1} (stretching vibration of aliphatic C-H), 3290 cm^{-1} (stretching vibration of N-H) , 1600 cm^{-1} (stretching vibration of C=O) have been observed which show the presence of sample in polymer . Now in reference to figure B, (MIP after washing) these groups have been observed milder which shows lower sample due to the surface washing. Also in Figure C, after reconnecting, the expected significant groups have been observed.

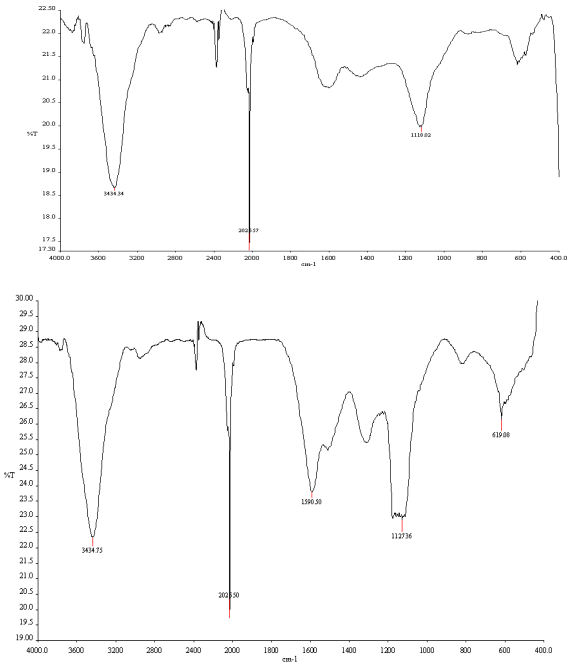

Figure 7 : FT-IR spectrums related to polymers of diazinon toxins

3.16. Studying scanning electron microscopic

Here there is scanning electron microscopic image for the observant polymer in Figure 8. The image shows the smooth surface without pores compared with MIP which is due to lack of sample in polymer.

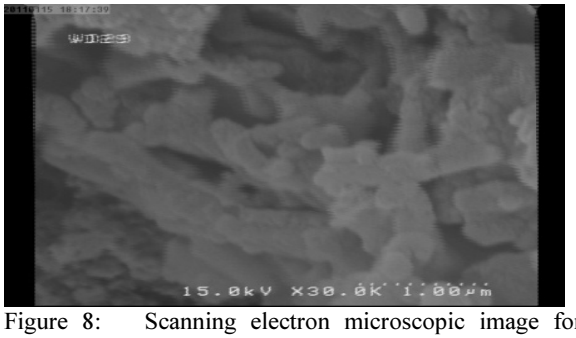

Figure 8: Scanning electron microscopic image for observant polymer

4. Conclusion

We have synthesis polyaniline as molecular imprinting polymer for sensing organophosphorus agronomy pesticides. This polymer is good candidate to sensor material for toxic agents. The sensing behavior of molecular imprinting polymer was measured for molecular imprinting polymer (MIP) and non-imprinting polymer (NIP) by FTIR and using a four-point probe method. Among sensing properties studied in this work can mention to analyte concentration, temperature, polymer response time. Sensing results for diazinon and chlorpyrifos showed that molecular imprinted surface in combination with conductometric method and IR are a useful approach for the sensing applications.

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Combined Distance-Reliability Model for Hazardous Waste Transportation and Disposal

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Abstract: A mathematical model that simultaneously locating a multiple disposal or a treatment facilities and determining a route for hazmat transportation network is presented. The objective is to minimize the distance traversed and population at risk. The route which minimizes a weighted hybrid metric path designation of accident probability and distance is significantly different from the minimum distance path. An adaption of Floyd Warshall's algorithm is used to find the hybrid path designation. An example is used to illustrate the applicability of the model.
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Keywords: Waste disposal; Location and routing; Multiobjective model.

1. Introduction

The transportation of hazardous waste from generation sites to disposal sites or treatment sites has drawn considerable public attention. Over the last few decades there has been an increasing awareness of environmental matters, both by governing bodies and by the public. This includes a realization of the importance of the sensitive disposal of waste in its various forms (nuclear, chemical, domestic, etc.), each of which poses its own peculiar problems. Such terms as 'HAZMAT' (or HAZardous MATerials), 'noxious', 'obnoxious', 'semi-obnoxious' and 'undesirable' have been associated with waste, but here we will prefer merely to speak of high-, medium- or low-level waste [3].

Hazardous materials (hazmat) comprise explosives, flammables, oxidizing substances, poisonous gases, and radioactive materials. By definition, they can be extremely harmful to environment and to human health, since exposure to their toxic ingredients may cause injury or death to plants, animals, and humans. Their negative effects are an apparently inevitable consequence of industrial processes dictated by the life style of a modern society. It follows that transporting these materials, often in populated or environmentally sensitive areas, is also inevitable. Reducing the potential negative impacts of transporting hazmat is an important task faced by communities, governments, hazmat producers and shippers. Routing hazmat wisely and designing safer networks for doing so are powerful means to achieve this end. A fundamental requirement of route design and assignment is to assess the potential risk imposed by shipments traversing each link in a network [17].

Moreover the location of an obnoxious or potentially dangerous facility usually determines either the origin or the destination of obnoxious materials shipments, and therefore interacts with the routing decisions: the facility location and transportation logistics decisions are strictly interrelated within the context of obnoxious materials management systems. The problem of simultaneously locating obnoxious facilities and routing obnoxious materials between a set of built-up areas and the facilities is addressed.

This paper presents a model that combines siting and routing for hazardous material transportation and disposal, to minimize the sum of the weighted hybrid path designation over the planning horizon, and we use an adaptation of Floyd Warshall's algorithm to find the hybrid path designation for transportation of hazardous waste.

This paper is organized as follows: we present the relevant literature in section 2. Section 3 describes the transportation network with edge attribute which is the probability of accident and Section 4 presents the accident probability-distance hybrid metric for a path. In section 5, we developed a reliable hybrid multifacility location and routing model. An adaptation of Floyd Warshall's algorithm is described in section 6. An example for locating a single disposal facility and determining the routes of transport vehicles over the highway transportation network is presented in Section 7. Section 8 gives a summery and conclusion of this article.

2. Literature Review

The hazardous waste management problem is first handled in the location literature in locating treatment or disposal facilities. The treatment facilities, such as incinerators, and the disposal facilities, such as landfills, are usually termed as 'undesirable facilities' in this literature. There is a significant amount of literature on undesirable facility location. For an extensive discussion on undesirable facility location one can refer to Erkut and Neuman [4], which is the most recent review published in this area. In the location of undesirable

facilities the aim is to minimize the nuisance and the adverse effects on the existing facilities or the population centers. Although the service cost of an undesirable facility increases when the facility is located far from the population centers, the undesirability of the facility usually seems to be more important.

Erkut et al. [6] presented an extensive study in a book chapter titled Hazardous Materials Transportation. They presented a high-level view of hazmat logistics research including a number of special issues of refereed academic journals that focus on hazmat transportation or location problems, books and book chapters, reports, web sites, and software. Also, they present different ways for risk assessment, routing and scheduling, and facility location and transportation for hazardous materials. Erkut and Verter [5] developed a review of the existing analytical approaches for strategic management of hazardous materials. ReVelle et al. [14] developed a model that locates storage facilities and selects routes for shipments of spent nuclear fuel. Zografos and Samara [18] presented a combined location and routing goal programming model for hazardous material transportation and disposal. Their model minimized travel time, transportation risk and disposal risk. List and Mirchandani [11] proposed a comprehensive model that simultaneously sited treatment facilities and made routing decisions for waste shipments. In their model, risk, cost, and equity were considered in a multiobjective framework. Stowers and Palekar [16] integrated routing decisions with the location of an undesirable facility using a single objective model, the minimized risk quantified by population exposure. This approach differed from previous work in that they considered both vertices and edges as feasible facility sites.

List et al. [12] presented a review of models for routing of obnoxious vehicles that is vehicles transporting undesirable materials. Giannikos [7] proposed a multiobjective model for locating disposal or treatment facilities and transporting hazardous waste along the links of a transportation network that minimizes the following four objectives: (1) total transportation cost and fixed cost of opening the treatment facilities; (2) total perceived risk due to the shipment of hazardous waste; (3) maximum individual risk (to force the risk equity); and (4) maximum individual disutility due to the treatment facilities.

Helander and Melachrinoudis [10] considered integrated location and routing models for minimizing the expected number of hazardous material transport accident. Two different routing policies are considered (1) most reliable route planning and (2) multiple routing with random selection. Path reliability measurements are used to derive the expected number of accidents over a given planning horizon. Also Melachrinoudis and Helander [13] presented the relisum location problem for siting a single facility on a tree in the presence of unreliable edges. Based on the objective of maximizing the expected number of reachable nodes from a service facility, they developed a number of analytical properties. They developed two polynomial algorithms for this problem a label-correcting procedure and a depth-first node traversal.

Boffey et al. [3] developed a model to locate a waste disposal site for low-level (domestic and nontoxic industrial) waste. Account is taken of nuisance caused to population along routes and of equity considerations. Not only is equity as regards effects on different population centers considered, but also between carriers of waste from different towns giving rise to the concept of routing fairness. Alumur and Kara [2] proposed a multiobjective location-routing model. The model has the objective of minimizing the total cost and the transportation risk and it includes some constraints. Sivakumar et al. [15] proposed the use of conditional risk (*i.e.,* expected consequence given the occurrence of the first accident).

3. Notation and Assumptions

It is assumed that the transportation network is described by an undirected graph $G(V,E)$, where $V=\{1,2,\dots,n\}$ is the node set and $E=\{(i,h): i,h\in V\}$ is the edge set, where edge (i,h) represents a direct travel link between nodes i and h. Associated with each edge $(i,h)\in E$ is an attribute, $0\le p(i,h)\le 1$ that denotes the probability of an accident on the edge during traversal by a hazmat transport vehicle. Throughout this paper, it is assumed that edge accident probabilities do not change over time, and that road conditions over an edge are uniform so that accident likelihood is approximately equivalent over points on the edge. In the case when this assumption is not practical, then a road segment can be represented in the transportation network $G(V,E)$ by two or more edges and connecting nodes instead of a single edge.

Hazmat transport is assumed to originate at nodes (origins) $i\in V$ and to be restricted along edges in the set E enroute to a storage facility (destination) $k\in G(V,E)$. Feasible sites for locating storage facilities are assumed to include the entire network including nodes and edges. Associated with each origin $i\in V$ is the attribute $w_i\ge 0$, denoting a weight for node i. The weights are general in the sense that they reflect frequency of transport shipments leaving the node, or the combined effect of frequency of transport shipments and virulence of the material to be transported. A number p of storage facilities are to

be located. Each origin $i \in V$ is assigned to a unique destination k that receives hazmat shipments from i throughout the planning horizon.

For each edge $(i,h) \in E$, let $A(i,h)$ denote the event that an accident occurs while traversing that edge and $A(i,h)$ denote the event that an accident does not occur on the edge. These events are related to the edge attributes introduced earlier: $Pr\{A(i,h)\} = p(i,h)$ and $Pr\{\bar{A}(i,h)\} = q(i,h)$ for all $(i,h) \in E$, where $q(i,h) = 1 - p(i,h)$

Let $\Psi_{kj} = \left\{P_{kj}^1, P_{kj}^2, P_{kj}^3, \ldots, P_{kj}^{r_{kj}}\right\}$ be the set of all r_{kj} feasible paths from $k \in G$ to node j. Let $P_{kj}^l \in \Psi_{kj}$, $l = 1, 2, \ldots, r_{kj}$ denote a subset of edges from E that represents a loopless path from node j to node k in $G(V,E)$. For cyclic networks, several distinct loopless paths may exist between j and k, each differing by at least one edge. Associated with a path P_{kj}^l is an event that an accident occurs enroute from j to k during travel along the path, as well as an event that an accident does not occur on the path. These two events are denoted by $A(P_{kj}^l)$ and $\bar{A}(P_{kj}^l)$ respectively. It assumed that accidents are serious in the sense that vehicles are not able to continue (no accident recovery). Then clearly the probability of no accident during traversal of path P_{kj}^l is

$$Pr\{\bar{A}(P_{kj}^l)\} = \prod_{(i_h, i_{h+1}) \in P_{kj}^l} q(i_h, i_{h+1})$$

and the probability of an accident during traversal of path P_{kj}^l is

$$Pr\{A(P_{kj}^l)\} = 1 - \prod_{(i_h, i_{h+1}) \in P_{kj}^l} q(i_h, i_{h+1})$$

Following Melachrinoudis and Helander [13], we model the edge operational probability as an exponential function of the physical displacement, which allows us to determine the operational probabilities of the newly created edges and the exact edge location. The underlying assumption is that failures occur completely randomly along edges, according to the Poisson process. The longer the edge length (or physical displacement from a node), the higher the probability that a failure occurs, *i.e.*, the lower the operational probability is. The exponential model also allows us to calculate the operational probabilities of the edges of the network based on their lengths and failure rates.

The term $Pr\{\bar{A}(P_{kj}^l)\}$ is referred to the path reliability [10]. The most reliable route from node j to k is the path $P_{kj}^* \in \Psi_{kj}$ such as

$$Pr\{\bar{A}(P_{kj}^*)\} = \max_{P \in \Psi_{kj}} Pr\{\bar{A}(P)\}$$

When k is on edge $e = (i,h)$, the path reliability of the most reliable route from k to node j is $Pr\{\bar{A}(P_{kj})\} = \max[Pr\{\bar{A}(P)\}: P \in \Psi_{kj}] = R_{kj}^*$. Referring to Fig. 1, it is clear that the reliability of the most reliable route from k to node j is related to the path reliabilities of the most reliable routes from nodes i and h to node $R_{jk}^* = \max\{q(k,i)R_{ij}^*, q(k,h)R_{hj}^*\}$.

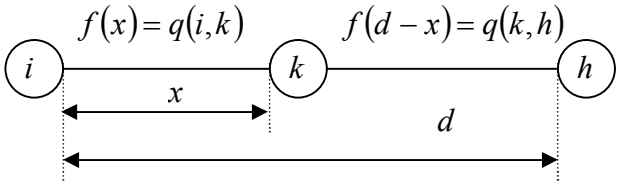

Figure 1: Facility location on an edge *(i, h)*

4 Hybrid Path Designations

ReVelle et al. [14] presented a multiobjective model in a problem dealing with storage siting and routing of spent nuclear fuel. They used two criteria: minimum transportation burden in ton-miles and minimum perceived risk as tons- past-people. Our model considers two objectives; minimizing the probability of an accident during traversal of path and minimizing the distance of that path between the origin and destination.

For each $P \in \Psi_{kj}$ define

$$\min_{P \in \Psi_{kj}} D(P) = \sum_{(i,h) \in P} d(i,h)$$

$$\max_{P \in \Psi_{kj}} R(P) = \prod_{(i,h) \in P} q(i,h)$$

where $\lambda(i,h)d(i,h) = -\ln q(i,h)$. Note that $d(i,h) \geq 0, \forall (i,h) \in E$, and $R(P) = \prod_{(i,h) \in \Psi_{kj}} q(i,h) = e^{-\sum_{(i,h) \in P} \lambda(i,h) d(i,h)}$. $D(P)$ is the sum of lengths of the edges of path P and $R(P)$ is the reliability of the path P which refers to the probability of traversal, *i.e.*, the probability that all edges along the path are operational.

We will use a constant α to relate the distance and the accident probability of any path between the origins and the destination. The accident probability-distance hybrid metric for the path $P_{kj}^l \in \Psi_{kj}$, traversed by a hazmat vehicle originating at node j with destination k, is defined as the convex combination of its accident probability and its length as follows:

$$H(\alpha, P_{kj}^l) = \alpha Pr\{A(P_{kj}^l)\} + (1 - \alpha) D(P_{kj}^l)$$

where $D(P_{kj}^l)$ is the length of path P_{kj}^l and $0 \leq \alpha \leq 1$. The parameter α reflects the trade-off between population at risk and transportation cost. Let Ψ_{kj} be the set of all admissible paths between origin i and destination k. Then under the hybrid path designation

policy, the designated path from j to k will always be $P_{kj}^* \in \Psi_{kj}$ defined by

$$H(\alpha, P_{kj}^*) = \alpha Pr\{A(P_{kj}^*)\} + (1-\alpha)D(P_{kj}^*) = \min_{P \in \Psi_{kj}} H(\alpha, P) \quad (1)$$

The location of the point k can be at any node or edge of the network. If k is located on an edge, $e = (i,h)$ then k is going to split e into two new edges: edge (k,i) with operational probability $q(k,i)$ and length x, and edge (k,h) with operational probability $q(k,h)$ and length $d-x$. For consistency we will let x be the length of the edge between the newly created node k and the vertex of the edge with the smaller index $(i.e.,i)$, as shown in Fig. 1. So, the destination node lies on edge (i,h), a distance x from node i.

The edge (i,h) is replaced with a new node labeled k and two new edges (i,k) and (k,h) connecting the endpoints of the original edge. Suppose that k is to be located x units from endpoints i where $0 \le x \le d(i,h) = d$. The probability of no accident on the new edge (i,k) is denoted by $f(x)$ and the probability of no accident on the new edge (k,h) is then $f(d-x)$. A path P_{kj}^l either includes node i or node h. Therefore the hybrid metric for path P_{kj}^l is:

$$\alpha[1 - f(x)Pr\{\bar{A}(P_{ji})\}] + (1-\alpha)[x + D(P_{ji})], \quad \text{if } P_{jk}^l = P_{ji}^l \cup (i,k) \text{ or}$$

$$\alpha[1 - f(d-x)Pr\{\bar{A}(P_{jh})\}] + (1-\alpha)[d - x + D(P_{jh})], \quad \text{if } P_{jk}^l = P_{jh}^l \cup (h,k)$$

where $D(P_{ji}^l)$ and $D(P_{jh}^l)$ are the lengths of paths P_{ji}^l and P_{jh}^l, respectively. Under the assumption that accidents are generated by Poisson Process, $f(x)$ is a convex function and the two functions above are therefore concave functions. The minimum of these functions over all $P \in \Psi_{kj}$ defined in (2) is a piecewise concave function of x. So, in case of location of a new facility on an edge (i,h) the optimal path is found by

$$H(\alpha, P_{jk}^*) = \min \begin{cases} \alpha[1 - f(x)Pr\{\bar{A}(P_{ji}^*)\}] \\ +(1-\alpha)[x + D(P_{ji}^*)] \\ \alpha[1 - f(d-x)Pr\{\bar{A}(P_{jh}^*)\}] \\ +(1-\alpha)[d - x + D(P_{jh}^*)] \end{cases} \quad (2)$$

Floyd Warshall's algorithm (see, [1]) computes shortest path distances between all pairs of network vertices. The algorithm is modified here to compute the minimum hybrid path between any two vertices in the network.

A realistic assumption regarding $q(i,h)$ is that failures that prohibit the use of the edge for traversal are generated according to a Poisson process with constant rate $\lambda(i,h), (i,h) \in E$, modeling $q(i,h)$ as an exponential function of the physical distance. Since the operational probability $q(i,h)$ is now assumed to be exponentially distributed, the random variable X is defined as the distance until the first failure occurs along an edge.

The failure rate $\lambda(i,h)$ represents the average number of failures per unit length. We represent the relationship between edge length, operational probability and failure rate, using the exponential model introduced by Melachrinoudis and Helander [13], as $q(i,h) = e^{-\lambda(i,h)d(i,h)}$, then $R(P) = e^{-\sum_{(i,h)\in P}\lambda(i,h)d(i,h)}$. We assume that the operational probabilities of the two newly created edges are also functions of the physical displacement of k from node i, as well as the original operational probability $q(i,h)$. Let us define that function as $f(x)$. The probabilities of successful traversal on the newly created edges (i,h) and (k,h) as functions of the physical displacement x, are referred to as $f(x) = q(i,k)$ and $f(d(i,h)-x) = q(k,h)$, respectively. The following four conditions were introduced by Melachrinoudis and Helander [13] with respect to a suitable function $f(x)$:

1) $f(0) = 1$
2) $f(d(i,h)) = q(i,h)$
3) $f(x)$ is monotonically decreasing in $x \in [0, d(i,h)]$ and $q(i,h) \le f(x) \le 1$ and
4) $f(x) * f(d(i,h)-x) = f(d(i,h)) = q(i,h)$

The exponential model satisfies the four conditions. Functions $f(x)$ and $f(d(i,h)-x)$ can be written as

$$f(x) = P[X > x] = e^{-\lambda_e x} \text{ and } f(d-x) = P[X > d-x] = e^{-\lambda_e(d-x)} = q(i,h)e^{\lambda_e x}$$

The Poisson Process provides a specific formula for the probability that no accident occurs while traversing x units over an edge having total length d and accident rate λ. That is, $f(x) = e^{-\lambda x}$ for $0 \le x \le d$. Similarly, the probability that an accident occurs is $1 - f(x) = 1 - e^{-\lambda x}$ for $0 \le x \le d$. Edge accident-free probabilities can be readily computed using the exponential function, $f(d) = e^{-\lambda d}$ given a road segment of length d and accident rate λ. Similarly, accident probabilities are computed as $1 - f(d) = 1 - e^{-\lambda d}$.

5 Reliable Hybrid Multifacility Locations and Routing Problem

When the storage facility or the disposal site k located on an edge $(i,j) \in E$ then k subdivides the edge into two subedges (i,k) and (k,h) where

$(i,k) \cup (k,h) = (i,h)$ and $(i,k) \cap (k,h) = k$. The safest path P_{jk}^* from node j to node $k \in (i,h)$ can be found by

$$Pr\{\bar{A}(P_{jk}^*)\} = \max[f(x)Pr\{\bar{A}(P_{ji}^*)\}, f(d - x)Pr\{\bar{A}(P_{jh}^*)\}]$$

In this expression, the two terms contained in brackets represent the probability of no accidents on path P_{ji}^* augmented by edge (i,k) and the probability of no-accident on path P_{jh}^* augmented by edge (k,h). Equivalently, the safest path P_{jk}^* can be found by

$$Pr\{\bar{A}(P_{jk}^*)\} = \max[1 - f(x)Pr\{\bar{A}(P_{ji}^*)\}, 1 - f(d-x)Pr\{\bar{A}(P_{jh}^*)\}]$$

Consider the Bernoulli random variable X_{jk}^t, whether or not there is an operational path from j to k for traversal $t = 1, 2, \ldots, w_j$ at a random instance, where $X_{jk}^t = 1$ if an accident occurs on trip t from j to k and $X_{jk}^t = 0$ otherwise. Under the most reliable route policy, the parameter associated with X_{jk}^t is $Pr\{X_{jk}^t = 1\} = Pr\{A(P_{jk}^*)\}$ and $Pr\{X_{jk}^t = 0\} = Pr\{\bar{A}(P_{jk}^*)\}$. If the node weights, w_j for all $j \in V$, reflect the frequency of hazmat shipments from j to the facility, then the random variable N_k defined by

$$N_k = \sum_{j \in V} \sum_{t=1}^{w_j} X_{jk}^t$$

reflects the total number of these trips during which an accident occurs.

The expected total number of accidents occurring over the same planning horizon

$$E[N_k] = E\left[\sum_{j \in V} \sum_{t=1}^{w_j} X_{jk}^t\right] = \sum_{j \in V} w_j Pr\{A(P_{jk}^*)\} \quad (3)$$

The problem of finding the location of the facility k on $G(V,E)$ to minimize (3) is the reliable 1-median problem which introduced by Helander and Melachrinoudis [10].

Let $S_p \subseteq G(V,E)$ be the set of p points at which storage facilities are to be located, where by a point $k \in G(V,E)$ we mean a point along any edge (i,h) of $G(V,E)$ which may or may not be a vertex of $G(V,E)$

We define the reliability $Pr\{\bar{A}(P_{jk}^*)\}, k \in S_p$ between a vertex j of $G(V,E)$ and a set S_p on $G(V,E)$ by

$$Pr\{\bar{A}(P_{jk}^*)\} = \max_{1 \le l \le p} Pr\{\bar{A}(P_{jl}^*)\}$$

$$Pr\{A(P_{jk}^*)\} = 1 - Pr\{\bar{A}(P_{jk}^*)\}$$

We are looking for the location that minimizes the sum of all weights unreachable nodes, in order to provide a maximum access to network disposal sites. The performance of the network is measured by the number of successful or unsuccessful traversals to demands originating at the nodes. The worst performance could be considered either the maximum number of successful traversals or the minimum number of unsuccessful traversals. We apply this criterion in the definition of the following version of the reliable multifacility problem.

5.1 Multifacility Problem for Hazardous Waste Disposal and Routing

Let $V_k \subseteq V$ be the set of vertices assigned to the disposal site $k \in S_p$:

$$V_k = \{j \in V : Pr\{\bar{A}(P_{jk}^*)\} = \max_{1 \le l \le p} Pr\{\bar{A}(P_{jl}^*)\}\}, \text{ for each } k \in S_p$$

Let X_{jk}^t be a Bernoulli random variable defined in the previous section. The random variable $N(S_p)$ defined by

$$N(S_p) = \sum_{k \in S_p} \sum_{j \in V_k} \sum_{t=1}^{w_j} X_{jk}^t$$

For each set S_p of p points on $G(V,E)$, we define

$$\Omega(S_p) = E[N(S_p)] = E\left[\sum_{k \in S_p} \sum_{j \in V_k} \sum_{t=1}^{w_j} X_{jk}^t\right] = \sum_{k \in S_p} \sum_{j \in V_k} w_j Pr\{A(P_{jk}^*)\}$$

If S_p^* on $G(V,E)$ such that

$$\Omega(S_p^*) = \max_{\{S_p : S_p \subset G(V,E), |S_p| = p\}} \Omega(S_p) \quad (4)$$

then S_p is called a reliable multifacility problem for hazardous materials location and routing of $G(V,E)$. The sets $V_k, 1 \le k \le p$ constitute a partition of $V, i.e., \bigcup_{1 \le k \le p} V_k = V$.

This model is simultaneously finding:

- the set S_p of p destinations $k \in G(V,E)$.
- the assignment of shipping origins to destinations, denoted by the sets V_k for each $k \in S_p$ and
- the routing from each origin j to the assigned destination k.

These routes will be shortest distance paths in case of ignoring the population impact by the hazmat accidents $i.e., \alpha = 0$, otherwise the chosen routes will compromise population impact and transportation cost.

For traditional p-median problem, Hakimi [8] has shown that there exists a set of p nodes $V_p^* \subseteq V$ that is optimal. A vertex optimality condition similar to Hakimi's [8] follows:

Lemma 1 Under the Safest Path Designation policy, there exists at least one p- node subset of V that solves (4).

5.2 Hybrid Path Designation in Case of Multifacilty Location

Then under the hybrid path designation policy, the hybrid path designation model is mathematically stated as

$$\min_{\{S_p: S_p \subseteq G(V,E), |S_p|=p\}} \sum_{k \in S_p} \sum_{j \in V_k} w_j H(\alpha, P_{jk}^*) \quad (5)$$

where $H(\alpha, P_{jk}^*)$ is defined in (1). As stated previously, weights w_j may reflect the combined effect of frequency of transport shipments and virulence of the materials being transported, in a surrogate objective function. If the disposal facility $k \in S_p$ lies on edge (i,h) a distance x from node i. The hybrid metric for optimal path P_{jk}^* is defined in (2).

For the reliable multifacility problem, there exists at least one p-node V_p^* subset of V that is optimal. A vertex optimality condition is similar to Hakimi [8].

Lemma 2 Under the hybrid path designation policy, there exists at least one p-node subset of V that solves (5).

For a fixed value of α, the method with hybrid path designation defined by (5) can be solved by methods similar to those used for the traditional p-median problem. The solution should be accompanied with appropriate sensitivity analysis on the parameter α within its range, $0 \leq \alpha \leq 1$.

6 Adaptation Floyd Warshall's Algorithm

We propose an extension of Floyd Warshall's algorithm which is a shortest path algorithm to find a minimum accident probability path. The Floyd Warshall's algorithm is used to determine the shortest path distances between every pair of nodes in a network. This is known as the all-pairs shortest path problem.

Let $A = [H(u,v)]$ be $n \times n$ weight matrix and let $P = [pred(u,v)]$ be $n \times n$ matrix, where $pred(u,v) = u$. There are n iterations during the execution of the algorithm. Iteration k begins with two $n \times n$ matrices.

In this section, we present an algorithm for finding the optimal node location on undirected network with unreliable links, *i.e.,* an optimal location is one that minimizes the objective function stated by expression (2). Our interest in a node location, as opposed to a location on an existing edge, assumes that the mathematical conditions leading to vertex optimality, presented in the last section, hold. The algorithm presented in this section is polynomial with respect to worse-case running times. The algorithm is $O(n^3)$ and is basically an adaptation of the Floyd Warshall's algorithm for finding all pairwise paths in a graph.

Algorithm

Given are: $G(V,E), d(i,j), q(i,j), (i,j) \in E, \alpha$

Begin

for all node pairs $(i,j) \in V \times V$ **do**

$Pr\{\bar{A}(i,j)\} := 0;\ D(i,j) := 0;\ H(i,j) := \infty;\ pred(i,j) := 0;$

for all $i \in V$ **do**

$Pr\{\bar{A}(i,i)\} := 1;\ D(i,i) := 0;\ H(i,i) := \infty;\ pred(i,i) := 0;$

for each edge $(i,j) \in E$ **do**

$Pr\{\bar{A}(i,j)\} := q(i,j);\ D(i,j) := d(i,j);$

$H(i,j) := \alpha[1 - q(i,j)] + (1-\alpha)d(i,j);$

$pred(i,j) := i;$

for each $k := 1\ to\ n$ **do**

for each $(i,j) \in V \times V$ **do**

$if\ H(i,j) > \alpha[1 - Pr\{\bar{A}(i,k)\}Pr\{\bar{A}(k,j)\}] + (1-\alpha)[D(i,k) + D(k,j)]$

then

begin

$Pr\{\bar{A}(i,j)\} := Pr\{\bar{A}(i,k)\}Pr\{\bar{A}(k,j)\};$

$D(i,j) := D(i,k) + D(k,j);$

$H(i,j) := \alpha[1 - Pr\{\bar{A}(i,k)\}Pr\{\bar{A}(k,j)\}] + (1-\alpha)[D(i,k) + D(k,j)];$

$pred(i,j) := pred(i,k);$

end

end

7 Computational Experiences

The following example problem is provided to demonstrate the procedure presented in the previous section. The example problem network consists of 33 nodes and 54 arcs. The nodes weights are given in Table 1. Table 2 displays the edges, edge lengths in miles, accident rates in accident per million miles and accident free probability. For accident rates, USA average rates were used from Harwood et al. [9], after adjusting them for local road

The adapted Floyd Warshall's algorithm is coded in C and implemented for different values of α. The designated routes corresponding to the solutions and the optimal locations for disposal facilities are illustrated in Figure 3. The optimal disposal facility may stay without change but the optimal spanning tree corresponding to designated routes changes.

Published accident rates can be used for various types of roadways, see for example Harwood et al. [9]. They report accident rates that are generally very small, e.g. of the order 10^{-6} accidents per mile. Rates of this order allow for linear approximation $f(d) \approx 1 - \lambda d$ instead of the exact expression $f(d) = 1 - e^{-\lambda d}$.

Table 1: Node weights of the network in Figure 2

i	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
w_i	8	0	1	1	2	0	6	1	1	1	1	3	2	1	1	0	1
i	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
w_i	1	1	3	0	1	1	2	1	6	5	0	5	6	0	1	4	

Table 2: Edges attributes of the network in Figure 2

Edge (i,h)	Distance $d(i,h)$	Accident rate $\lambda(i,h)$	Accident probability $\lambda(i,h)d(i,h) \times 10^{-6}$	Edge accident-free probability $1 - e^{-\lambda(i.h)d(i,h)} \approx 1 - \lambda(i,h)d(i,h) \times 10^{-6}$
(1,2)	26	4.0	1.04×10^{-4}	0.999896
(1,8)	22	7.0	1.54×10^{-4}	0.999846
(1,24)	21	4.0	8.40×10^{-5}	0.999916
(2,3)	17	4.0	6.80×10^{-5}	0.999932
(2,5)	18	5.0	9.00×10^{-5}	0.99991
(3,4)	41	5.0	2.05×10^{-4}	0.999795
(3,5)	17	5.0	8.50×10^{-5}	0.999915
(5,6)	30	5.0	1.50×10^{-4}	0.99985
(6,7)	11	8.0	8.80×10^{-5}	0.999912
(6,8)	13	7.5	9.75×10^{-5}	0.9999025
(7,9)	15	7.5	1.125×10^{-4}	0.9998875
(7,10)	12	7.5	9.00×10^{-5}	0.99991
(7,13)	22	8.0	1.76×10^{-4}	0.999824
(7,14)	18	8.0	1.44×10^{-4}	0.999856
(8,9)	14	7.0	9.80×10^{-5}	0.999902
(9,10)	8	7.0	5.60×10^{-5}	0.999944
(9,23)	17	5.0	8.50×10^{-5}	0.999915
(9,24)	18	3.0	5.40×10^{-5}	0.999946
(10,11)	11	7.0	7.70×10^{-5}	0.999923
(10,23)	16	4.0	6.40×10^{-5}	0.999936
(11,12)	5	4.0	2.00×10^{-5}	0.99998
(11,18)	12	7.0	8.40×10^{-5}	0.999916
(11,20)	14	6.0	8.40×10^{-5}	0.999916
(13,14)	19	4.0	7.60×10^{-5}	0.999924
(13,17)	17	5.0	8.50×10^{-5}	0.999915
(13,18)	7	7.0	4.90×10^{-5}	0.999951
(14,15)	4	7.0	2.80×10^{-5}	0.999972
(14,16)	55	9.0	4.95×10^{-4}	0.99951
(15,16)	32	7.0	2.24×10^{-4}	0.999776

(16,17)	24	4.0	9.60×10^{-5}	0.999904
(17,21)	26	4.0	1.04×10^{-4}	0.999896
(18,19)	6	4.0	2.40×10^{-5}	0.999976
(18,20)	5	5.0	2.50×10^{-5}	0.999975
(19,21)	18	4.0	7.20×10^{-5}	0.999928
(20,21)	10	6.0	6.00×10^{-5}	0.99994
(21,22)	8	4.0	3.20×10^{-5}	0.999968
(21,27)	14	6.0	8.40×10^{-5}	0.999916
(22,23)	16	4.0	6.40×10^{-5}	0.999936
(22,26)	19	5.0	9.50×10^{-5}	0.999905
(23,24)	7	4.0	2.80×10^{-5}	0.999972
(23,26)	23	5.0	1.15×10^{-4}	0.999885
(24,25)	26	3.0	7.80×10^{-5}	0.999922
(25,26)	9	2.0	1.80×10^{-5}	0.999982
(26,27)	25	3.0	7.50×10^{-5}	0.999925
(26,29)	54	5.0	2.70×10^{-4}	0.99973
(26,30)	50	3.0	1.50×10^{-4}	0.99985
(27,28)	52	6.0	3.12×10^{-4}	0.999688
(28,29)	19	2.0	3.80×10^{-5}	0.999962
(28,33)	35	6.0	2.10×10^{-4}	0.99979
(29,30)	15	3.0	4.50×10^{-5}	0.999955
(30,31)	42	2.0	8.40×10^{-5}	0.999916
(31,32)	10	6.0	6.00×10^{-5}	0.99994
(32,29)	43	5.0	2.15×10^{-4}	0.999785
(32,33)	22	6.0	1.32×10^{-4}	0.999868

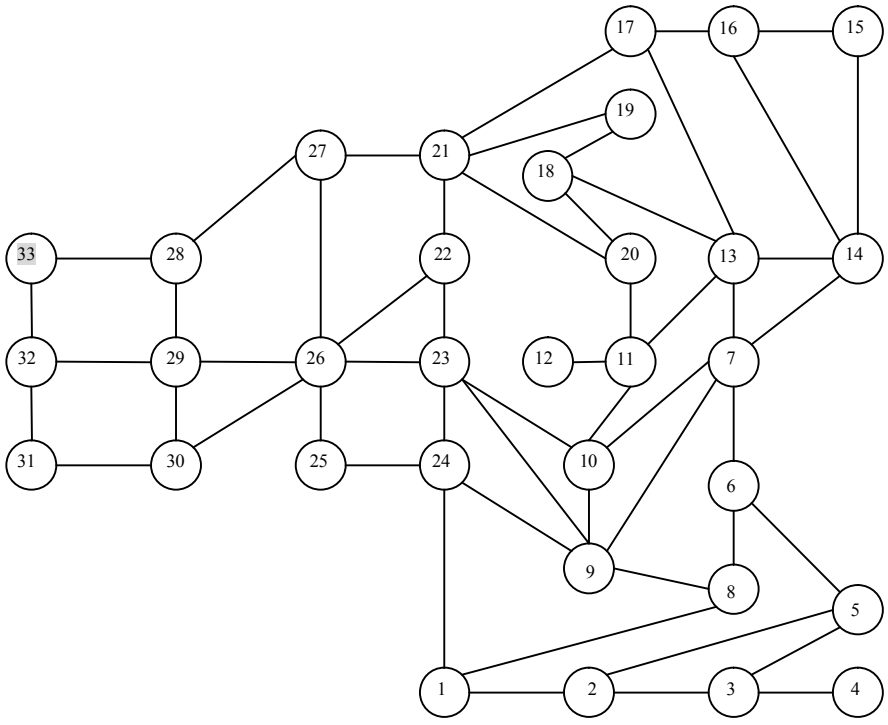

Figure 2: A hazardous wastes transportation network. Edges and nodes attributes are given in Tables 1 and 2

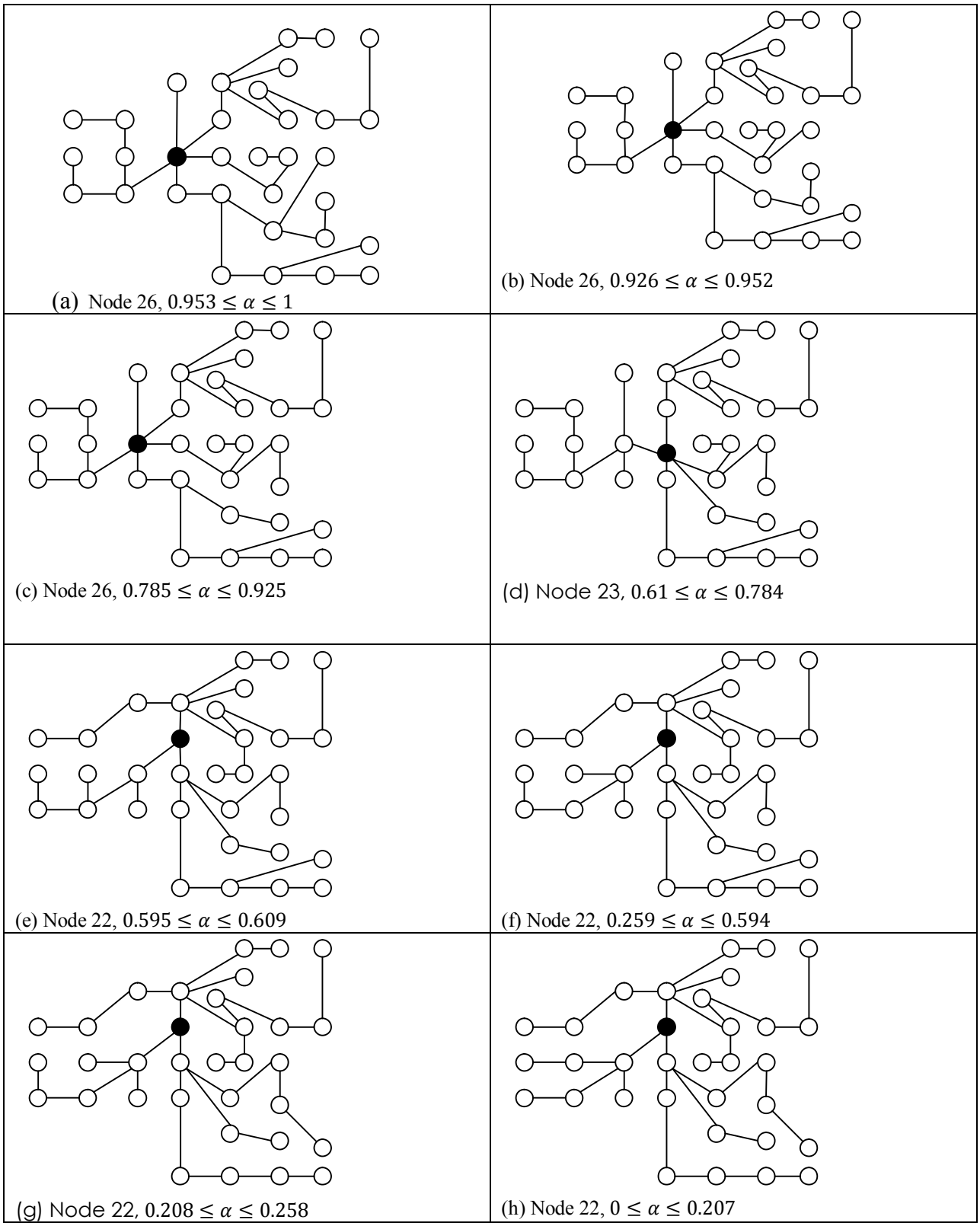

Figure 3: a, b, c, d, e, f, g, and h give the optimal facility locations and the optimal spanning trees representing routing for different values of α

8. Conclusions

In this paper a hybrid path metric designation model for locating disposal or treatment facility and routing hazardous wastes through the underlying transportation network has been presented. The model determines the location of hazardous waste facilities and the routes from given hazardous waste generation sites to the selected disposal facilities. The path reliability measures the expected number of accidents over a given planning horizon. So, reliability refers to the probability of a hazmat transport vehicle completing a journey from an origin to a destination. Two different location modeling frameworks were introduced: (1) The hybrid path designation model for locating a single facility and routing hazardous wastes to it and (2) reliable hybrid multifacility location and routing problem. A numerical example is presented to illustrate the applicability of the hybrid path designation model.

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Effect of Soft Liner Material on Retention of Complete Denture, (An *In Vitro* Study)

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Abstract: This study cover three types of materials tested on 90 patients for their effect on retention of complete denture by measuring the force of retention using force guage. Soft linings are compliant, viscoelastic materials used to form all or part of the fit surface of a denture. Their complete function is debatable, but they serve to distribute the forces of mastication.

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Keywords: material; patient; retention; force guage; viscoelastic; mastication

1. Introduction:

Resilient lining materials are used on dental prostheses to absorb some of the energy produced by masticator impact.[1] A soft liner would distribute the functional and parafunctional stresses more evenly and thus have a dampening effect due to their elastic behavior, thus acting like a "shock absorber".[2,3] Because of their ability to restore health to inflamed and distorted mucosal tissues, soft liners are used in the management of frail and chronically irritated tissues, thin and non resilient mucosal tissues, etc.[4,5] Plasticized acrylic resins, silicones, vinyl resins, polyurethane and polyphosphazines have been tried as soft liners, of which the first two were selected for this study since they have a long term successful record of clinical application. One of the most common problems encountered with the soft liners is the failure of adhesion between the liner and the denture base.[6,9] Such adhesive failure creates an environment for potential bacterial growth and accelerated breakdown of the soft liner.[8] Since failure of soft liner often results from breakdown of bond between liner and denture base, measurement of bond strength is very important.[10]

Researchers have suggested various methods to improve bond strength, e.g. mechanical roughening by sandblasting or lasers, treatment with denture base monomer, etc.[2,5,11] The effect of roughening the surface of denture base on the bond strength of soft liner is controversial, e.g. Craig *et al.*[11] advocated a roughened surface to improve the adhesive bond whereas Amin *et al.*[12] reported that roughening the acrylic resin base by sandblasting before applying a lining material had a weakening effect on the bond. Jacobsen reported that laser treatment of denture base before liner application resulted in reduction of bond strength.[5] Al-Athel *et al.* reported the effect of test methods on bond strengths of the liners. Tensile bond strength (TBS) of sandblasted surface specimens decreased when compared with the smooth surface while the shear bond strength increased, implying that the bond strength will depend on the test method used. In case of testing shear strength; the frictional force was increased when the surface was roughened, since more force was required to push the elevation of one surface through the other as compared to smooth surface.[7] When the soft liner was applied to the untreated surface, the friction between the two surfaces was reduced, and therefore, the failure was seen at a lower load. Effect of liner thickness on tensile strength was studied by Al-Athel *et al.*[7] A significant difference was noted in the TBS of specimens with 4.5 mm and 3 mm liner thickness. A positive correlation was reported between the tensile strength values and rate of deformation of specimens.[7] The tensile strength of lining material increased significantly with increasing rate of deformation to a limit of 40 mm/minute, beyond which the strength of the bond decreased. While information is available about mechanical surface roughening of denture base, there is paucity of information about the chemical treatment, particularly with denture base monomer. This study was undertaken to evaluate the effect of different three soft liner materials (visco- gel Dentsupply, Germany), (Silicone soft reline material, Pentusil Bosworth company, EU), and (Molloplast-B) on the force of retention of the complete denture.

2. Material and Methods:

Three resilient liners, visco- gel, Silicone soft reline material, and Molloplast-B Super-Soft were selected. Sample of ninety patients were used and divided into three equal groups, each of thirty patients. Each group was treated by complete denture with the different soft liner materials respectively.

Force guage was used to record the retention of the all denture bases. The device first adjusted and the unit of measure was selected to be grams. The desired adapter tension hook was attached to sensing head but

it was first painted by pressure indicating paste for every measurement. The sending end with the adapter were placed in line with the denture that being measured. Rotation of the testing head was avoided.the patient was sitting in an upright position with the occlusal plane parallel to the floor and the denture base was inserted and allowed to remain for setting time of 4 minuits for the hook of the denture base was engaged.

3. Results:

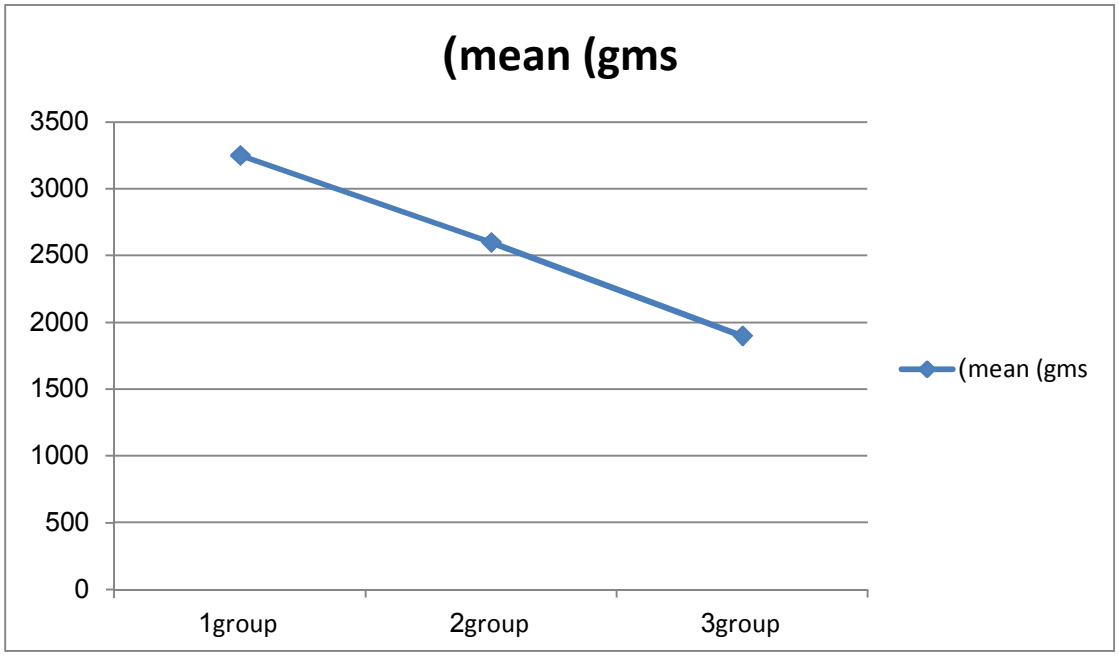

Fig (1): Displays means and standard deviations for the three soft liner materials

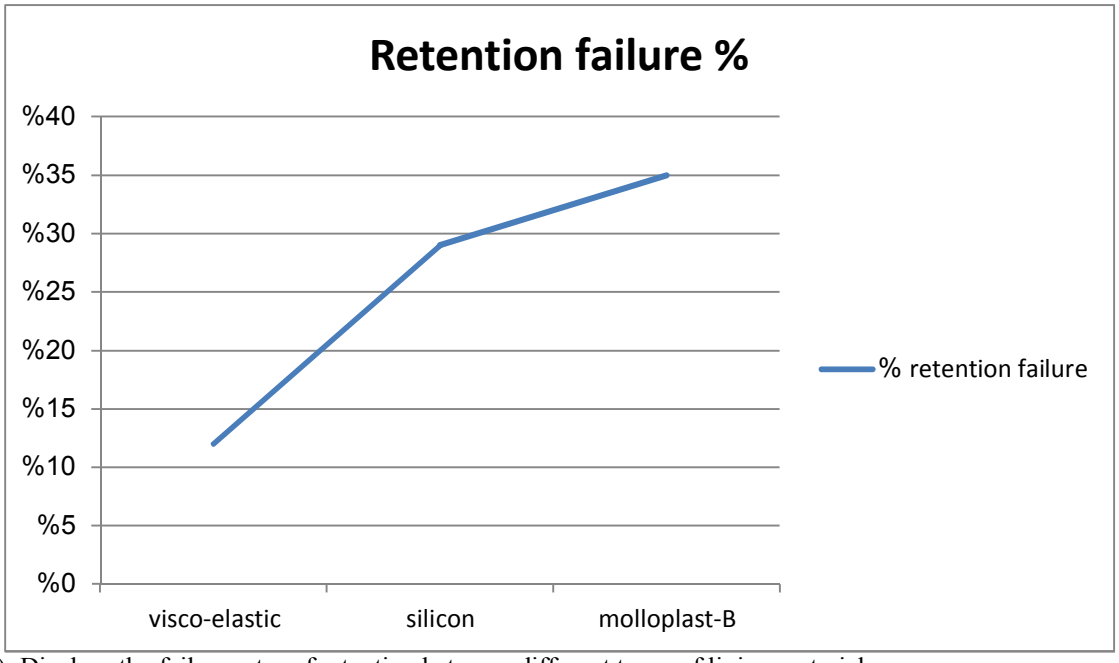

Fig (2): Displays the failure rates of retention between different types of lining materials.

Table 1: Means and standard deviations for the three soft liner materials.

Groups	Material	Mean(gm)	±SD	*P** value
Group 1	Visco- gel	3250	±1300	<0.001*
Group 2	Silicon	2600	±1120	
Group 3	Molloplast-B	1900	±1090	

*$p \leq 0.05$ significant

Table (2): Result of one-way ANOVA for tensile strength between various soft lining materials

Lining material	Source of variation	Sum of squares	df	mean	F	*P* value
Visco-gel	Between groups	6500	2	3250	9200	<0.001
Silicon		5200	2	2600	8100	
Molloplast-B		3800	2	1900	4700	

4. Discussion:

The failure of adhesion between a silicone based resilient liner and an acrylic denture base material is a significant clinical problem. Adhesive failure between the liner and the denture base resin creates a potential interface for microleakage leading to an environment for potential bacterial growth and accelerated breakdown of soft liner resulting in deteriorating prosthesis [13, 14].

To achieve better bonding between denture lining materials and denture base resin, several experimental procedures have been conducted such as mechanical surface preparation i.e., roughening of denture base resin, effect of polymerization stage at which resilient liner is packed against the acrylic resin and chemical surface treatment of denture base resin [13, 15]. In the present study, the retention strength values of the dentures according to the different lining materials.

The surface treatment of denture base by monomer enhanced bond strength of both the liners. Super-Soft forms a strong bond with acrylic resin, even without a bonding agent as both have a similar composition.[16] Molloplast-B, being a silicone based liner, requires an adhesive MMA, a solvent that dissolves the PMMA surface, and the bond strength of silicone liners will depend on tensile strength of the materials and the adhesive used.[17] Therefore, using monomer and adhesive together prior to the resilient liner application may effectively increase the dissolution of the PMMA surface. It enables added fluid to penetrate between polymer chains and become entangled when the added monomer or solvent is evaporated.[18] Sandblasting resulted in reduction of bond strength of both materials. Theoretically, sandblasting increases surface area and provides mechanical locks at bond site and should result in stronger bonds. According to Amin *et al.,*[19] lower bond strengths were due to stresses that occurred at the interface of the PMMA and soft liner.

Conclusions:

Within the limitations of this in-vitro study, the following conclusions were drawn:

1. Mean bond strength of the visco-elastic material was significantly ($P < .05$) higher than silicon liner and Molloplast, for clinical application.
2. Mixed type of failures were more predominant in the third group of Molloplast-B material, while was less in the second group using silicon liner material, and the least was in the last group using visco-elastic lining material.

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Hope and Religious Beliefs in Iranian Cancer Patients

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Abstract: Despite considerable advances in medical science, cancer remains to be as one of the most important diseases. Psychological problems of cancer patients affect on the quality of life, suicide rate, long confinement and even their life lengths. As well as the goal of this study was assess the relation between hope and religious beliefs in the cancer patients who refer to chemotherapy center. This study is a descriptive and analytic study in which 220 cancer patients who referred to the chemotherapy and radiotherapy center, through accessible random sampling method were tested. For gathering the data we use demographic particulars questionnaire, Allport religious beliefs questionnaire and the Hope Herth questionnaire. Findings showed that, 78 patients (35.5%) of the total 220 studied patients were in an age group of 51-60 and 14 (6.4%) were 41-50. As for the goal of the research, i.e., determining the relation of religious beliefs and patients' hope, the results of Man-Whitney U test indicated a significant relation, $p<0.002$. Considering the results of this study as well as other conducted studies, it seems that addressing the effective factors can result in improvement of the relevant nursing cares and will allow the families and nurses to concentrate on important and significant aspects such as religious beliefs.
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Key words: Hope, Religious Beliefs, Cancer, Patient, Iran

1. Introduction

Despite considerable advances in medical science, cancer remains to be as one of the most important diseases of the present century and the second cause of death after cardiac and vascular diseases. This disease is distinguished by abnormal deformation of cells and loss of cellular distinction. At the present time, more than 7 million people in the world lose their lives as the result of cancer. New cases of cancer are anticipated to increase from 10 million to 15 million by 2020. Cancer has different changes, pressures and effects on the patient's life as well as on his/her family's. A good reaction to cancer depends on the patient and his/her psychological structure, family and social environment, inabilities and developed deformities and may also affect all the patient's activities. The crises arising from cancer lead to imbalance and inconsistency of the mind, body and soul (Hasanpoor Dehkordi and Azari, 2006). In this regard, psychological problems of cancer patients affect on the quality of their lives, suicide rate, long confinement and even their life lengths (Palmen and Fish, 2005).

Furthermore, reactions such as denial, anger and sense of sin and guilt are observed in these patients. A group of Italian researchers studied cancer patients within an age range of 18-65 and found that anxiety was the most important mental health-related factor that affected the quality of their lives. They also found that in case of having more than 50 years of age, low level of education and unemployment, life quality of the patients will be dissatisfactory (Distefano et al., 2008; Masoudi et al., 2012).

On the other hand, if the cancer patients' anger is not expressed correctly, it is likely to become internal and to lead to depression, disappointment and suicide. Most cancer patients suffer from depression and have low concentration and attention. In other words, prevention from emotional disorders including depression in cancer patients is necessary since depression will result in loss of their life quality. Therefore, appropriate interferences to

reduce depression can improve their life quality (Juver and Verçosa, 2008).

The Behavioral Research Center of the American Cancer Society studied 739 people of the family members of cancer patients and found that their high levels of mental stress are related to physical, mental and social disorders (Spillers et al., 2008). But disappointment is the most dominant state for the patient. Depression may occur immediately after the disease or sometime later. It is quite harmful for the patients since it leads to their surrender against the disease. They will no longer endeavor to live and they will lose their opportunities to have a better life in the remaining time of their lives. Hope has a negative correlation with the individual's mental pathological indices in general and with the existing depression specifically. Hope anticipates physical and mental health in such a way as they are specified by different indices including self-reporting health, positive answer to medical interventions, mental health, positive temperament, effective adaptability and health improving behavior (Bijari et al., 2008).

Today, there is a high interest in the importance of the role of religion and spirituality in health, disease and caring methods (Yidrim et al., 2009; Shokati et al., 2012). Generally, several studies have shown a high relation between religious and mental health such as reduced depression, increased self-confidence, more support and less alcohol consumption (Koenig et al., 1992). In a study made by Konig, McKalf et al. on 850 studies conducted regarding the relation between religious beliefs and actions and mental health and social performances, they reported that religion influences on mental health by enhancing the ability to cope with stress, to provide a social support space, to provide hope and optimism in order to cause positive excitements such as a better way of living, etc (Koenig et al., 1992). Furthermore, religious tendencies and the behaviors proportional to it will reduce depression, a better feeling of health and reduced fatality (Steffen and Masters, 2005). For this reason, considering few studies made in this regard, the researchers decided to conduct a study aiming at determining the relation between hope and religious beliefs in the cancer patients.

Special objectives:

1. Determining the demographic particulars of those cancer patients who referre to chemotherapy center of Rasht city;
2. Determining the relation of hope and religious beliefs in those cancer patients who refer to chemotherapy center of Rasht city.

2. Material and Methods

This study is a descriptive and analytic study in which 220 cancer patients who referred to the chemotherapy and radiotherapy center of Razi Hospital of Rasht city through accessible random sampling method were tested. The used tool included a questionnaire which included three parts. The first part included demographic particulars questionnaire, the second part included Allport religious beliefs questionnaire and the third part was Hope Herth questionnaire. Hope Herth questionnaire consisted of 12 questions and was pointed from 1 to 3 based on the three-point scale, point 1 = I disagree, point 2 = I am not sure and point 3 = I agree. Negative materials were pointed inversely. Total points included 12 to 36 points. The higher the point is, the higher will be the level of hope. The scientific validity of the used tool was examined and confirmed by using content reliability. The validity of Hope Herth index was examined in 2000 by using a retest in the cancer patients conducted by Ghaznayn and Ghaffari and was confirmed by a Peterson correlation coefficient of 0.84 (Porghaznein and Ghafari, 2004). SPSS software, version 15 and X^2 and Man-Whitney test were used to analyze the data.

3. Results

The findings of this study are presented in 5 diagrams and 1 table as follows:

Table 1.The relation of religious beliefs and hope in cancer patients

Man Whitney Test	Total	Hope			Religious Beliefs
		High	Medium	Low	
0/002 <p	74	35	37	2	Low
	146	37	106	3	Medium
	220	72	143	5	Total

Based on the findings of this study, 78 patients (35.5%) of the total 220 studied patients were in an age group of 51-60 and 14 (6.4%) were 41-50. 103 patients (46.8%) were men and 117 patients (53.2%) were women. In view of education, 47.3% were illiterate and a minimum number of them (4.5%) had academic educations. Most of them (86.8%) were married and also most of them (62.7%) were city dwellers. Maximum suffering period of the patients who participated in the study was less than one year and only two of them had a suffering period more than 2 years. Furthermore, the results showed that in relation to religious beliefs, maximum number of the studied patients had average religious beliefs (146 patients) and none of them had high religious beliefs. In relation to hope, maximum number (72

patients) of the total 220 patients had a high hope and only 5 patients had low hope.

4. Discussions

During treatment stages, hopeful patients have a higher resistance in tolerating long and painful treatments as well as the effects of chemotherapy by radiotherapy and they more likely to follow up treatment. These patients cope better with the symptoms of treatment such as loss of hairs, weight gaining, tiredness, and nausea. They are also more likely to follow additional necessary treatments in case of recurrence of breast cancer. Also during improvement stage, more hopeful individuals show more positive thoughts about their lives. Even though the cancer is an advanced one and there is no hope for any treatment, more hopeful patients can arrange other goals for themselves such as spending more time with family and enjoying from the remaining time.

Schneider and Lopez (2002) refer to some researches including Karver researchers which showed that optimist women accepted cancer diagnosis better than the pessimist women and that their refusal from treatment was less than the latter.

In the research conducted by Mohammadi Shahbolaghi entitled "A Survey on the Relation of Hope and Efficient Campaign of Cancer Patients in the Tehran Association of Cancer" which was of descriptive correlation, the results showed that 1.62% of the patients enjoyed a high level of hope, 9.50% of them had a medium efficiency in coping with cancer and 9.25% of them enjoyed a high efficiency in coping with cancer. There was no significant relation between hope and demographic variables. Spearman correlation coefficient showed that there is a positive correlation of 0.68 between hope and efficient coping which is statistically significant as well (Shahbolaghi and Abaszade, 1998).

As for the second goal of the research, i.e., determining the relation of religious beliefs and patients' hope, the results of Man-Whitney U test indicated a significant relation, $p<0.002$.

The results of Kenneth et al (2003) showed that negative attitudes towards living related to lack of religious beliefs leads to the Increased risk of mental illness, In general, religious men and women become less anxious and anxious disorders (Kenneth et al., 2003).

In addition, a study by Tracy et al (2007) with title a religious and spiritual support for patients with advanced cancer and their association with treatment and quality of life showed that the spiritual needs support of patients and improve the quality of life is associated with clinically important outcomes, moreover there are significant relation between the religious beliefs and tendency to invasive treatment for life longevity (Tracy et al., 2007).

As well as about the relationship between religious belief and hope, the results of McClain et al (2003) showed that spiritual wellbeing has a strong effect on the end of life hopelessness in cancer patients (McClain et al., 2003., Sheikholeslami et al., 2012).

Considering the results of this study as well as other conducted studies, it seems that addressing the effective factors can result in improvement of the relevant nursing cares and will allow the families and nurses to concentrate on important and significant aspects such as religious beliefs.

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Surveying and Comparing the effect of Two Training Methods: Drug Addiction Prevention (Peer education with Teachers) on the Level of Knowledge and Attitudes on the High School Students of ShahreKord, Iran

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Abstract: Considering the increasing growth of addiction in the youth as well as the importance of training to avoid that, the effect of two training methods (by the teacher and peer education) for preventing from the addiction of high school boy students of Shahr-e-Kord were studied in this research. In this study 450 boy students in third grade at high school were included in the study from Shahr-e-Kord High Schools on a random and stratified basis. 225 of the total 450 studied students were trained by the teacher and 225 students were trained by the group of the same age. 20 days after completion of training, a posttest was made by using the same questionnaire. The collected data was analyzed by spss 11.5 and by using descriptive and analytical statistics. The studied students included 450 students with an average age of 16.78±0.7. Average pretest and posttest scores in both interventional groups were significantly different (p=/0001). Average change of pretest and posttest scores in the training group by the students of the same age (10/.7±3.6) was higher than the average change of scores before and after the training by the teacher (8.8±3.4). Bon Froni test showed these differences to be statistically significant (p=0.0001). Considering the findings of this study, training on how to avoid addiction by those of the same age will be more efficient in increasing the knowledge and views of the students.

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Keywords: Prevention, Addiction, Peer education, Knowledge and Attitudes, Peer education

1. Introduction

Drug abuse is one of the problems of human society which is increasing day by day. Few countries may be found to have remained immune from its recurrence and risks (Manshaei, 2009). Alcohol consumption and drug abuse are one of the most important problems of the modern countries. Each year, millions of dollars are spent in different countries for campaigning against the drug or for treating and caring the addicts. (Rostami et al., 2007). The experiences of successful countries in campaigning against the drugs show that such successes require scientific typological understanding of the addicts and its use for initial and secondary preventions (Heidari Pahlavan et al., 2002).

Social psychologists and researchers have introduced several reasons for drug abuse. Some believe that making effort to be accepted by the society and trying to show oneself as a mature and adult person are some of the reasons to tend in drug abuse. Some others believe that admission in social groups and receiving the suggestion from the friends to consume drugs are considered as the important factors for increased possibility of drug consumption. Moreover, incapability of the individual to control his/her impulses as well as excitement and difference seeking are also considered as the effective psychological factors for drug consumption (Mirahmadi et al., 2009).

Based on the report of WHO (1998), 70% of the fatalities occur consequent to the behaviors that had occurred during puberty which could be adjusted. The global extension of consumption of drug, alcohol, cigarettes and psychotropic drugs among the teens (Corbet, 2001) and the increasing epidemiology of smoking especially among the teen age girls warn us. Those teens that smoke and drink are very likely to begin consuming drugs (Hyde et al., 2001). Those teens who begin to smoke earlier will likely continue to smoke for several years. They are also exposed to much more risks due to the long period of their smoking. Therefore, increase of smoking age from 12

to 14, decrease of the number of those teens who drink alcohol, and decrease of smoking by the teens are declared (Parvizi et al., 2004), to be among the goals of WHO by 2010 (WHO, 1998).

People's intention for drug abuse, at least in the first times of consumption, is influenced by their view to drug consumption. Therefore, adjustment of tendency especially among the teens is one of the recognized strategies to prevent from addiction (Rafie et al., 2006). Since a high percentage of teens attend the schools (e.g., about 18 million students study throughout (Iran Statistics Center, 2001), training to prevent addiction in the schools is especially important. Training is the basis for different types of learning and its main goal is to change the behavior and comprehensive performance. Training methods are one of the important and efficient factors in the quality of training. Therefore, considering different training methods, we decided to assess two training methods in connection to addiction among the high school students. Therefore, the present study was designed and implemented aiming at examining and comparing the effects of two training methods for prevention from drug addiction by the group of the same age and teachers on the awareness levels and views and of boy high school students in Shahr-e-Kord, Iran.

2. Material and Methods

Examination method in this research includes a semi-experimental study in which after selecting two groups for study, each of them is exposed to an independent variable, in the manner that the first and second groups are trained by the group of the same age and teachers, respectively. The experimental plan used in this study is of pre and post type. In this method, first the information of students' knowledge and view in both groups is gathered simultaneously by the questionnaire before executing the training program and. Then, one of the groups is trained by the group of the same age and the second group is trained by the students. After separation of a suitable period, i.e., three weeks after execution of training program, the information of students' knowledge and view in both groups is collected by the questionnaire once again. After extracting the data and analyzing the obtained information, the students' knowledge and view in both groups concerning addiction is examined before and after executing the training program and the effects of each of the two training methods are determined and compared.

Information collection tool in this research was questionnaire which was used in performing the initial and secondary tests. This questionnaire has 36 questions and is prepared in three main parts. In the first part which contains 10 questions, the specifications of studied units (demographic information) are questioned. The second part of the questionnaire contains 12 questions designed to examine the knowledge level of studied units about addiction. The third part of the questionnaire includes 14 questions designed to examine the viewpoint of studied units on addiction.

Sample size was calculate and estimated at 222 people for each group by considering the amounts of p0=0.4, p1=0.7, à=0.05, and ß=0.15 in the sample size formula of interventional studies. Therefore, 450 boy students at third grade of high school were included in the study from Shahr-e-Kord high schools on a random and stratified basis. Training method was specified in each group using simple random method. After the declaration of the ethics in research committee of the university to the effect that there was no impediment and after issuance of a permit by the education department and coordinating with the principals of the selected schools, the pretest was held for the students by using a questionnaire containing 36 questions on the students' knowledge and view about addiction. The reliability of the questionnaire was determined by using content and formal validity method. Validity coefficient of the questionnaire on 20 students was obtained 92%. The trainers who were of the same age (3 people at each school and totally 15 people) were elected by other students. They were then trained in a one-day workshop by a physician specialized in addiction and the ways to prevent that. Before the commencement of training, the capability of trainees (students) to transfer the subjects to other students through lecturing and questioning and answering was ensured. 225 students of the total 450 students under study were trained by teachers and 225 students were trained by the group of students of their same age. 20 days after completion of training, a posttest was made by using the same questionnaire. The collected data was analyzed by spss 11.5 and by using descriptive and analytic statistics.

3. Results

The studied students included 450 students with an average age of 16.78±0.7. The education level of 74.2% of the students' mothers was lower than high school diploma and the education level of 18.1% was high school diploma and higher. 93.9% of studied students' mothers were housewives and 4.5% were employees. 225 students were trained by the teachers and the remaining 225 students were trained by other students of their same ages. K-2 test was statistically insignificant to determine the relation between the training method and mothers' educations, mothers' jobs, fathers' educations and fathers' jobs. 36.6% of the students had observed drugs and 4.3% had used

them. Based on the findings of this study, only 6.9% of the students were highly informed about the harmful effects of the drugs. It should be noted that 11 students did not give complete answers to the questions concerning their awareness. In examining the views of the studied units about the drugs, 28.1% believed that the effects of drugs were less than the effects of psychotropic pills and 52% believed that those who use drugs smoked as well. 48.7% believed that one of the family members of those who consumed drugs were addicted. 37.7% believed that drug consumers were likely to suffer from HIV and Hepatitis B. For the relation between knowledge level of students and drug abuse, the findings showed that 30.4% of the total students who smoked and consumed drug had low knowledge, 52.2% had a medium knowledge and 6.4% had a high knowledge ($p=0.002$). This finding shows the knowledge level in the students and especially in the second group. 36.6% of the students studied in this research were familiar with drugs and the average point of knowledge was 8.11 ± 3.69 vs. 3.6 ± 3.93 for those individuals who were not familiar with drugs. The average scores of pretest and posttest in both intervened groups were significantly different. In other words, both training methods were statistically significant in enhancing the levels of knowledge, view and posttest points as compared to the pretest points. The difference of average pre-training and post-training points were higher in the students who had been trained by the group of their same age as compared to the students who had trained by the teachers.

4. Discussions

It is understood from the findings of this study that the knowledge level of the students about the drug effects is quite low in the manner that only 6.9% of them had a high knowledge. These figures indicate that unfortunately one of the most susceptible classes of the society, i.e., students has a low knowledge. In 2007, Moasheri et al, made a study entitled "Knowledge Level and Attitude of the Students of Birjand Universities on the Consumption of Psychotropic Pills" and showed that low information and knowledge is an important factor in the tendency of susceptible people of the society to psychotropic pills (Moasheri et al., 2007). The results of this study indicate the significant increase of knowledge level and view from pretest to posttest in both intervention groups (teacher and the group of the same age). In other words, both interventional training methods had a positive effect in improving the level of knowledge and view of the students on the lethal effects of drugs and the ways to prevent that. The training to prevent from drug abuse especially through peers is economically considerable. The cost-saving ratio of these programs is 26 to 1 (Miler et al., 2007), They not only reduce the consumption demand, but also reduce the relevant damages (Spiser and Miler., 2005). In a study conducted by Mirahmadizadeh et al (2009) entitled "The Effect of Training for Prevention from Addiction on the Knowledge and Atittude on Soldiers in Military Places", they showed that the trainings fro prevention from addiction in the military environments not only increases the knowledge and improves view, but also it will lead to the improvement of individuals' performance (Mirahmadizadeh et al., 2009). Valente et al (2007) stated that use of peers in training programs was one of the efficient and successful methods in training for prevention from drug abuse in educational environments such as schools and workplaces like military environments (Velente et al., 2007). In the study made by Abbaspour et al, (2007) in Kerman, training by peers (peer education) to prevent from HIV transmission was introduced to be more efficient than training made by the personnel of health centers (Abbaspour et al., 2007). Furthermore, Jodati's study in Tabriz showed that training by peers was efficient in the knowledge and attitude to HIV (Jodati et al., 2007). In a study conducted by Azizi et al., (2008) entitled "Comparing the Effect of Training on HIV Infection by Peers, Physician and Pamphlet Distribution on the Knowledge of Girl Students", they showed that training to prevent from HIV by the physicians was more efficient in increasing the knowledge of students as compared to pamphlet and peers. Azizi's study is not consistent with this study as well as with Abbaspour et al. (2007) in which the physician's credit is stated to be an authentic information source (Azizi et al., 2008). The threats of addiction are not known to people and high tendency of people to addiction shows the necessity to make the society aware to prevent from addiction (Azami et al., 2004; Shokati A, 2012; Msoudi et al., 2012; Sheikholeslami et al., 2012). Considering the results of this study, training to prevent from addiction by peers is effective in increasing the knowledge and Attitude of the students though training by teachers will increase their knowledge and Attitude as well.

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Study of thermal properties of improved adhesives for medical applications

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Abstract: Adhesives are of important materials in medicine applications. These materials are used in many applications such as disposal medical devices, structural bonds, bone cement, prostheses, etc.. In order to use these materials in medicine applications, the thermal properties of these materials should be evaluated. We measured the thermal properties of the adhesive which has been modified with Hycar rubber. It is found that increasing in Hycar leads to decrease in thermal properties of adhesive. This means that we can not increase the Hycar content beyond the critical value. Because this might result in diminishing adhesive efficiency especially in human bodies where the operation temperature reaches 37 centigrade degrees.
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Keywords: Medical Adhesive, Thermal properties, Hycar

1. Introduction

Adhesives are of the most important materials for industries. Medical adhesives are often made of bio-consistent polymeric materials. The hardened, finished polymers are almost non-toxic. These materials are used in many medical applications such as disposal medical devices, structural bonds, bone cement, prostheses, etc.. In figure some medical applications of the cut-cure adhesives e.g. stopping bleeding, surgical operations are demonstrated. Among materials that are used in the manufacture of medical devices, there is a growing interest in polymers. Each medical adhesive has its own benefits and limitations and must be carefully matched with the appropriate application. The primary types of adhesives used in medical device applications includes Acrylics, Epoxies and Styrene polymers (Atefi *et al.* 2012a; 2012b; Davoodi *et al.* 2012). Challenges for medical devices that require skin contact can often be reduced to a wrestling match between adhesion and irritation.

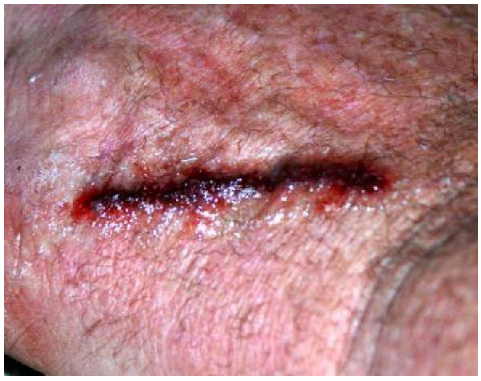
An adhesive or glue is a material, usually in a liquid or semi-liquid state, that adheres or bonds items together. Adhesives come from either natural or synthetic sources. The types of materials that can be bonded are vast but they are especially useful for bonding thin materials. Adhesives cure (harden) by either evaporating a solvent or by chemical reactions that occur between two or more constituents (www.Wikipedia.com). In this paper we investigate an adhesive that cure with chemical reactions. This adhesive hardens by mixing two or more components which chemically react. This reaction causes polymers to cross-link into acrylics, urethanes, and araldites (www.Wikipedia.com; www.adhesives.org). several commercial combinations of multi - component adhesives in use in industry. In order to use these materials in medical applications, the thermal properties of these materials should be evaluated (www.adhesives.org). Numbers of factors determining the durability of structural adhesive joints have been identified and be grouped in three categories: environment, materials and stresses by da Silva *et al.* (2004) Custódio *et al.* (2009). Ratna *et al.* (2000) modified the toughened adhesive with acrylate based liquid rubber has been investigated. Other researchers used Hycar modifier for modify the properties of adhesives (Takemura *et al.* 1985). Ratna *et al.* (2001) used an acrylate-based modifier as impact modifier for adhesives. We are going to investigate Araldite-Aradur adhesive in this paper. Improving the adhesion has been preformed using Hycar modifier. This is mixed with Araldite before mixing with Aradure. We focus on thermal behavior of the adhesion using DSC. Because thermal stability is very important for High-temperature applications of an adhesive material.

In this work improving the characteristics of adhesive used in medical external applications has been discussed. The glass transition temperature of the formulations is measured in this study. Afterwards the obtained result is reported in the following.

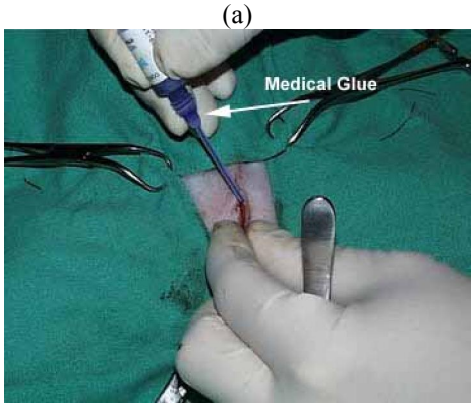
2. Experimental method

2.1 Araldite, Aradure and Hycar

Two materials have been used: one is Araldite-Aradure adhesive and the other is Hycar additive. Adhesive was made of two compounds: Araldite resin and the Aradure curing agent. These materials have been obtained from Shimi-Mobtaker-Peivand (Islamic Republic of Iran). The adhesive is mixed in the ratio 65 part of Araldite with 35 part of Aradure. The Hycar used is obtained from the similar source for modifying the adhesive material. This is a liquid state material that is added commonly to the adhesive for improving the adhesion properties.



(a)



(b)

Figure 1. Use of adhesives in medical applications in (a) stopping bleeding and (b) surgical operation

2.2 Making samples

First 0 to 15 percent of Hycar was appropriately weighted and six samples are made (We present formulations that are used in Table 1). Then these amounts are added and mixed with Araldite. Mixing is done to obtain uniform composition. Afterwards, appropriate amount of Aradure has been added to the mixture and we mixed it again for several minutes. Then mixture was cast into an appropriate small can. Afterwards, this is heated one hour at eighty centigrade degree. Specimens were cut out from the prepared by cutter.

2.3 Thermal property measurement

In this work the glass transition temperature is applied as an indicative for measuring the thermal strength of modified adhesives. A differential scanning calorimetry (DSC) has been used for measuring the glass transition temperature of the adhesives. The apparatus used in this study, was based on a Setaram DSC machine.

First, appropriate samples with weight of nearly 8 mg have been weighted. Then these samples have been appropriately pressed in some thin aluminum cans. Then these canned samples are heated in an appropriate heating cycle from the ambient temperature till the 130 centigrade degree. Finally, these are cooled back to room temperature (cooling cycle). The glass transition temperature (Tg) of the samples were measured automatically by the aparatus.

3. Results and discussion

It should be noted that the thermal strength of a modified adhesive is a crucial important factor for most applications. This factor is very crucial for engineering applications in factories such as aerospace, electrical, machinery etc.. This is especially very important for automotive industries and fluid conducting industries because in these applications there are some high temperature materials that might influence the performance of the applied adhesive.

It is well-stablished by previous researchers that using Hycar liquid modifier can improve the adhesion properties of Araldite-Aradure adhesive as well as other important mechanical characteristics of these glues (Takemura *et al.* 1985). However, it should be noted that using Hycar materials can effectively reduce the thermal stability and strength of a modified Araldite-Aradure adhesive.

Table 1. Samples

Sample / Material	Sample 0	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Araldite	65	65	65	65	65	65
Aradure	35	35	35	35	35	35
Hycar	0	3	6	9	12	15

In Figure 2, the glass transition temperature (Tg) of six Araldite-Aradure adhesive samples are presented for comparison. As seen from this figure, the glass transition temperature of a modified Araldite-Aradure adhesive decreases as the amount of Hycar increases. This is very important issue since this means that a user should not increase the Hycor content very much. Because this increased amount of Hycar might effectively diminish the thermal resistance and efficiency of Araldite-Aradure adhesive at high temperature.

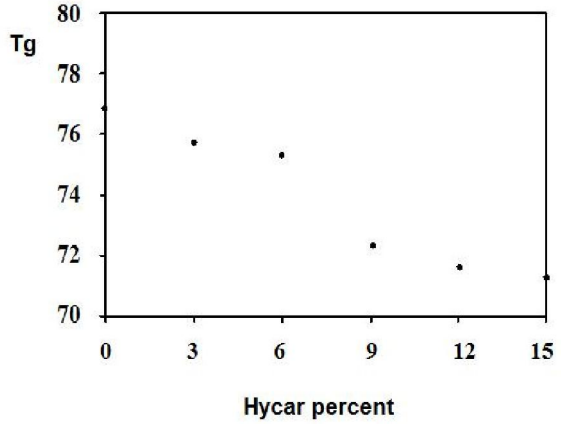


Figure 2. Tg of adhesives

4. Conclusions

Adhesives are of important materials in medicine applications such as disposal medical devices, structural bonds, bone cement, prostheses, etc. In this work we measured the thermal properties of the adhesive which has been modified with Hycar rubber. It is found that increasing in Hycar leads to decrease in thermal properties of adhesive. This means that we can not increase the Hycor content beyond the critical value. Because this might result in diminishing the efficiency of the adhesive for high-temperature application. This might causes problems when the adhesive are used in the human body temperature (37 centigrade degree).

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5/2/2012

Simulating The Cooling Of Medical Ct-Scanners: Part 2: Results

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Abstract: CT-scanning is used as an non-invasive detecting method in medical applications. The previous paper on this topic is dedicated to express the numerical method applied for simulating cooling system of CT-scanner system used in medical applications. In Part I of this paper we study the convection/diffusion mechanism of heat transfer during passing a cold fluid from the top of a hot plate of CT-scanner. This paper is concerned with the analysis of the numerical results achieved from the developing code. For verification, the approach to determining an adequately fine mesh is to perform exploratory simulations for different mesh sizes. Results obtained from this simulation describe the convective and diffusive rate of heat transfer for such CT-scanner cooling systems
[Hafshejani M K, Darasaraei A, Dadjoo F, Alimoradi F, Falavand A, Arad A. **Simulating the Cooling of Medical CT-Scanners: Part 2: Results.** *Life Sci J* 2012;9(2):1311-1315] (ISSN:1097-8135). http://www.lifesciencesite.com. 195

Keywords: CT-scanner, CFD simulation, Heat transfer

1. Introduction

In recent decades computed tomography (CT) scanners are used in medical applications and detecting disease (S. Hassan and G. Hassan, 2011). These devices can create 2-D images from patient body structures which help doctors to detect cancers, arteries etc.. Figure 1(a-c) shows the CT imaging system, the gantry and a typical produced image. As seen in figure 1(a), computed tomography imaging system primarily includes the gantry and patient table. The gantry (figure 1(b)) is a moveable frame that contains the imaging system and bears high substantial amount of heat. It is well established that the x-ray tube of CT-scanner generates large amount of heat which may causes to failing the system if no appropriate cooling has been conducted. The cooling system must transfer high heat levels generated during the high speed rotation of the anode and the bombardment of electrons upon the anode surface (Reddinger, 1997; Ahmadi and Marghmaleki, 2011). The x-ray tubes heat capacity is expressed in heat units. Modern CT scanners bear a heat capacity of 3.5-5 million heat units (Reddinger, 1997). Many CT x-ray tubes utilize a combination of oil and air cooling systems to eliminate heat and maintain continuous operational capabilities (Reddinger, 1997).

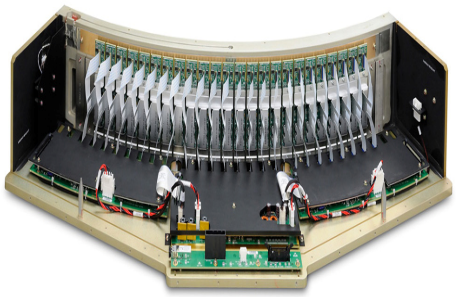
Originally, CFD was born out of the need to use in medical applications to analyze airplane components (www.ehow.com; Biao *et al.* 2012). This occurred during the 1930s, but CFD as a branch of science did not begin to take off until the 1960s due to the improvement of computing power. CFD is now studied at many major universities and is applied to many fields of science (www.ehow.com). In this work CFD is used for simulating the heat transfer in CT gantry surface via fluidic cooling system. Determining the temperature distribution in the CT gantry model is the main aspect of any heat analysis process. Technically, heat transfer analysis is important and uses numerical calculations of heat transfer coefficients for determining boundary conditions of substances (Kovtanyuk *et al.*, 2012; Wu *et al.*, 2011). Irrespective of the complexity of the problem, CFD heat transfer analysis allows engineers to calculate fluid flow properties and the effects of temperature sensitive material, non-linearity, and fluid-surface contact condition on the mechanical component. All CFD analysis and simulations are separated into three distinct parts: pre-processing, processing, and post-processing (Norton *et al.*, 2017) .

In the pre-processing phase, a problem is defined. This problem is the "what" of what is to be studied. Once the problem is defined, conditions must be defined for the problem. These are known as initial conditions. Then, boundary conditions must be defined to act as boundaries for the problem. In this phase, it is also decided how this problem will be studied, such as what method of discretization, what numerical methods to use, and what programming language to use.

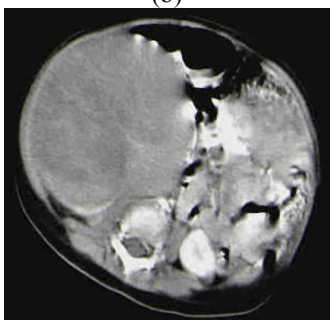
In the processing phase, the computer code used to solve the problem at hand has been written and is compiled. This phase is mainly user-free because the computer is performing hundreds and thousands of

calculations in order to simulate the problem at every step in time. The end result of this phase is a large collection of data.

(a)



(b)



(c)

Figure 1. (a) the CT-scanner (b) CT gantry anode and (c) a typical CT image.

In the post-processing phase, many thousands of calculations have been performed and data relevant to the study has been produced. This data is then filtered and converted into meaningful data.

The previous paper of this work is mostly with the per-processing stage of the performed CFD work for analyzing CT scanner cooling system. In this paper, a comprehensive analysis of the results as the stage of "pots-processing" in our CFD work is presented and discussed for the CT-scanner cooling system model.

2. Boundary conditions

There are several boundary conditions such as the inflow velocity profile, constant temperature, constant heat flux and symmetry conditions. The figure below shows the boundary conditions we considered in this problem. The boundary conditions applied for this problem is shown schematically in Figure 2. The values of L, W, T up, T left, T∞, h can be considered as input data.

3. Results

Available results can very well demonstrate dynamic variation of temperature profile with time. As seen, at the beginning, the temperature contours show the heat transfer diffusion through the bulk of the fluid. However, after passing a certain time, the impact of convective heat transfer becomes significant. The following figures (figure 3-8) presents the temperature contours through the bulk of the fluid. It should be noted that the mesh size has been reduced so that no change in temperature on each grade is demonstrated.

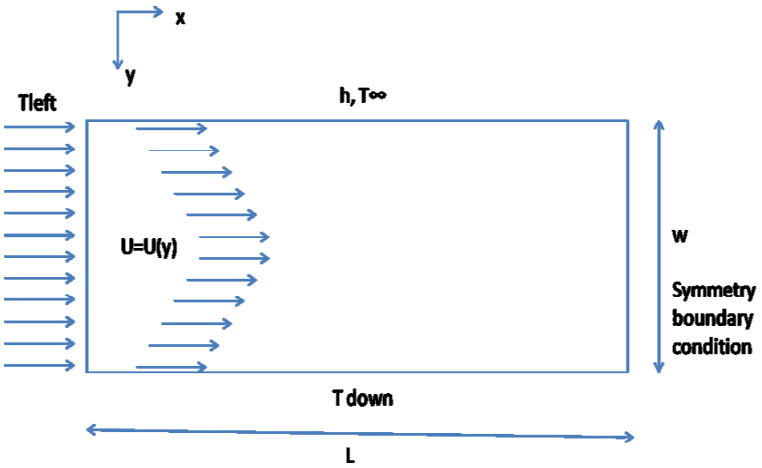


Figure 2. Boundary conditions

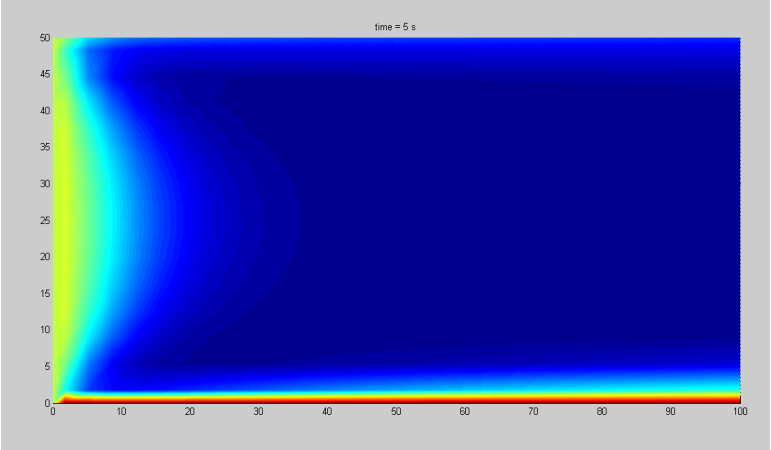


Figure 3. Temperature contour at the time 5s after the beginning of the flow

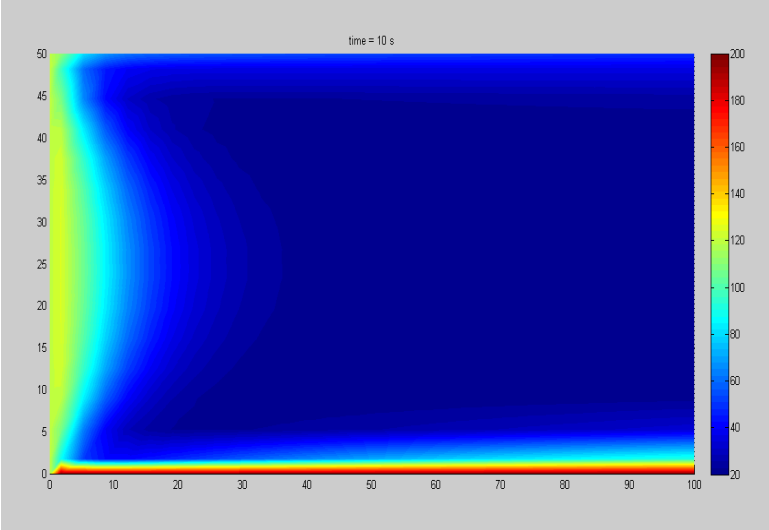


Figure 4. Temperature contour at the time 10s after the beginning of the flow

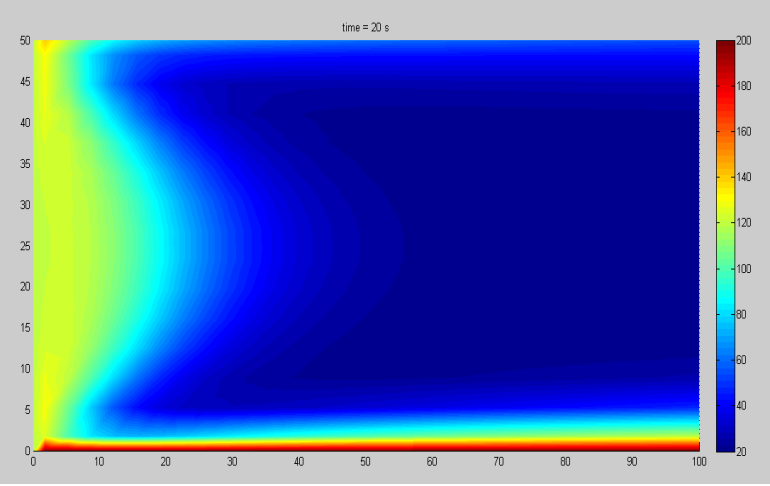


Figure 5. Temperature contour at the time 20s after the beginning of the flow

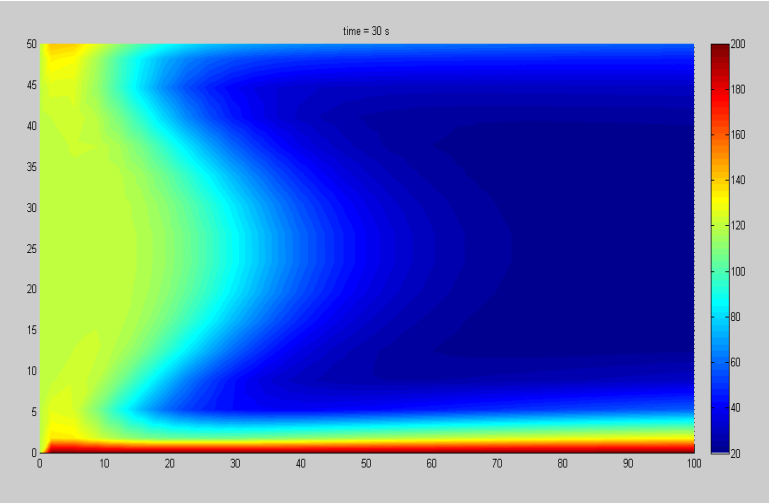


Figure 6. Temperature contour at the time 30s after the beginning of the flow

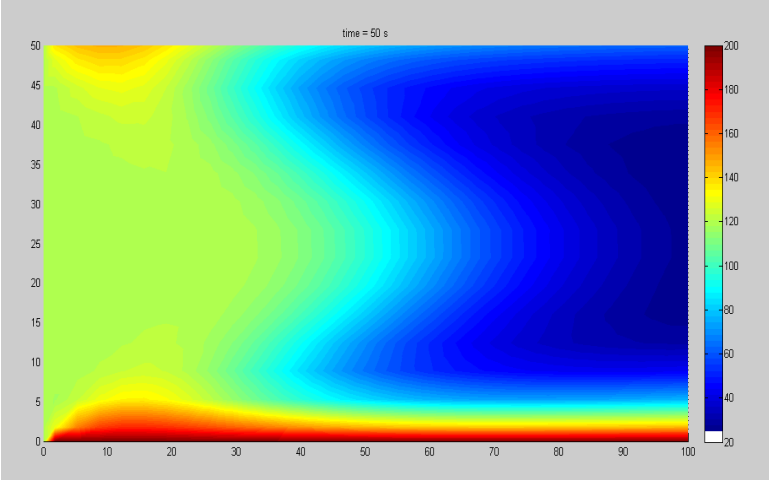


Figure 7. Temperature contour at the time 50s after the beginning of the flow

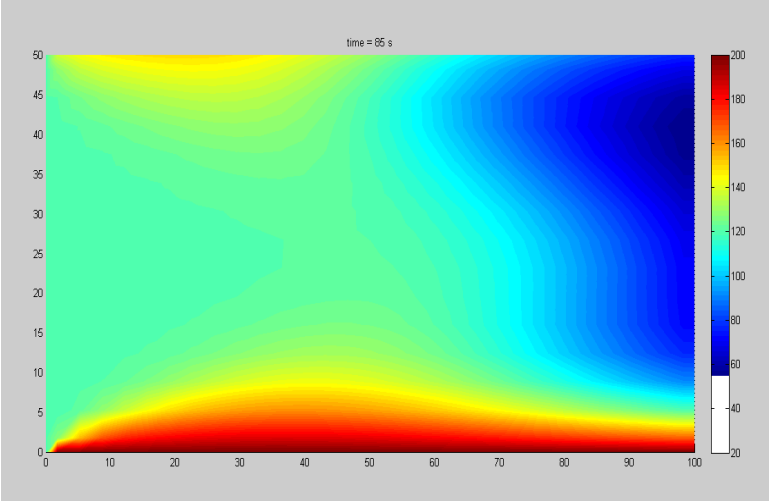


Figure 8. Temperature contour at the time 85s after the beginning of the flow

4. Conclusion

CT-scanners are used as an powerful detecting radiological devices in medical applications. The Part I of this paper we express the numerical method applied for simulating cooling system of CT-scanner system used in medical applications. This work is mostly dedicated to the study of heat transfer mechanism through the bulk of a fluid, passing above a hot plat of a CT-scanner gantry. The work is performed as a verification step in the development of our CFD code, performed based on the Finite Volume Method, to handle heat transfer problems in fluids. The obtained temperature contours very well describe the mechanism of heat transfer across the bulk of the fluid, affected by the hydrodynamic characteristics of the problem. Results show the variation of parameters during the cooling of CT-scanner.

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Health Needs Management among Patients Undergoing Day Case Cataract Surgery: A Proposed Protocol

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Abstract: Cataract is considered as a significant global health problem and represents the most important cause of visual impairment world-wide. Extraction of the cataract which accounts for a significant proportion of the surgical workload among most ophthalmologists, improving quality of the vision. **Aim:** This study aims to propose a protocol for health needs management among patients undergoing day case cataract surgery, through identifying the health needs and assessing the care given on day of the surgery. **Methods:** A descriptive explorative design was utilized to conduct this study that was carried out in the Ophthalmic Outpatient Clinic and surgical waiting room of the ophthalmic unit, at Ain Shams University Hospital and Benha University Hospital**. Sample:** A purposive sample composed of 160 patients undergoing cataract surgery, adults and old age, from both genders and were recruited from the above mentioned settings. **Tools:** 1) Patients' interviewing questionnaire, to determine patients' needs regarding day case cataract surgery (pre / post tests), 2) An observation checklist**,** to assess care given for the studied patients on the surgical day and 3) Hamilton's Anxiety Rating Scale**,** to determine patients' levels of anxiety (pre / post tests). **Results:** There are statistically significant differences between patients' health needs pre / post surgery, added to more than half of the studied patients had severe anxiety pre – surgery, compared to post – surgery. Moreover, mean percent of unsatisfactory care was higher on day of the surgery. **Conclusion:** The current study concluded that, there were more significant health needs among the studied patients pre - surgery, added to the positive effect of the surgery on the reduction of these needs and relieving patients' anxiety post – surgery. Furthermore, unsatisfactory patients' care had higher mean compared to satisfactory care on day of the surgery. **Recommendations:** Based on findings of the present study, it can be recommended that, the proposed protocol of patients health needs management that's evidence – based should be implemented and evaluated in relation to the incidence of cataract surgery complications.

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Key words: Patients' health needs management, day case cataract surgery.

1. Introduction:

Cataract is a clouding or opacity of the lens that leads to gradual painless blurring of vision and eventual loss of sight which is one of the most profound and dreaded of physical disabilities. Opacities of the lens are the leading cause of self-declared vision impairment. Mature cataract is a developed cataract that separates easily from the lens capsule. It is one of the few normal physiological changes in the aging process, therefore its incidence increase with age and occur on both eyes. Developmental cataracts are always congenital and may be hereditary. Acquired cataract may be associated with ocular disease, trauma, systemic disease or aging**.** (**Hegazy, 2004**, **Lansingh & Carter, 2009 and Agarwal & Kumar, 2011**).

Today, cataract is a greater problem, the significance of which is better understood. In the United States, more than 2.5 million people have Cataract Surgery each year and age related cataract is responsible for 48% of the world blindness, which represents about 18 million people. In one study, it was found that 30% of people with 65 years of age and over have visually impairing cataract in one or both eyes. A further 10% of them had previous cataract surgery in one or both eyes. Moreover, the prevalence of visually impairing cataract rose steadily with the age: 16% (65 - 69 yrs.), 24% (70 - 74 yrs.), 42% (75 - 79 yrs.), 59% (80 - 84 yrs.), and 71% in people of 85 years or more. Women had a higher prevalence of cataract **(Hickman *et al.,* 2010 and Sach *et al.,* 2010**). In Egypt, the prevalence of low vision for all ages is 47.9% of the population aged 65yrs. Major causes in Egypt for blindness are cataract (54.8%), corneal opacity other than trachoma (18.8%), refractive error (7%), glaucoma (4.6 %), others (7.2%) (**Hegazy *et al.,* 2008 and Mohamed *et al.,* 2011).**

Day case surgery offers a safe and cost effective alternative to inpatient care, provides patients with familiar environment on their own home, reduces the waiting list time, enhances visual rehabilitation, favoring for the doctor and the patient alike and reducing nurses to patient contact time which create

new approaches to the nursing care. Various studies found that, the patients have been very happy with the day-care surgery and reporting that 87% of them are relieved to return to their workplaces earlier and there is a strong element of "let's get it over with fast" and return home. It is imperative that the patients are deemed suitable, as the day case and the criteria of the suitability be integral to the assessment procedure **(Jakobsson *et al.,* 2009 and Lundstrom *et al.,* 2011).**

Cataract surgery has become one of the most technically advanced area of modern medicine, its types include: Intra capsular Cataract Extraction (ICCE), in which the entire lens capsule was removed with the cryoextractor and now it reserved for cases of phacoanaphylaxis and sublaxation of the lens. Extra capsular Cataract Extraction (ECCE), in which the lens capsule is incised and the lens cortex and nucleus are removed leaving the posterior capsule and lens zonule in place. Small Incision Cataract Surgery (SICS), which results in a good visual outcome. Lastly Phacoemulsification (PHACO), is a process of the lens nucleus fragment ion with ultrasonic vibrations and aspirating the lens material through a double - lumen, irrigation-aspiration system that requires only a small incision. A cataract surgery requires much less time and therefore, can be done comfortably even on a medium risk patient. In a similar manner, the basic nursing requirements to be followed are also the criteria for selection on a day care mode **(Black *et al.,* 2009 and Werner, 2010).**

In cataract extraction surgery, the eye lens was removed and replaced with an artificial lens called intraocular lens (IOL), which is a clear plastic lens that becomes a permanent part of the eye, it cannot be felt. The incision in the eye will take 4 weeks to completely heal, during that time the vision will gradually improve. It can be performed either on a day-case or in-patient basis, either under local, "twilight anesthesia" or general anesthesia, which is short- acting with rapid recovery (**Sparrow, 2007 and Timby & Smith, 2008)**. Adverse events of cataract surgery occur in about 3% of the patients. Early complications include: Corneal edema raised intraocular pressure, corneal abrasion, wound leak, suture complications, iris prolapse, incarcerated vitreous, severe anterior uveitis and displacement of the intraocular lens. Later complications include: Cystoids' macular edema, endophthalmitis, retinal detachment, posterior capsule opacification and unsatisfactory refractive error (**Gogate *et al.,* 2007 and Karaj *et al.,* 2010)**.

Moreover, almost any patients may have cataract surgery on this basis; they attend for an early postoperative examination on the following day or within a few days depending on the advice of the consultant. Later follow-up examination is usually at one to three weeks. For the majority of patients the procedure is virtually painless, with a recovery period of just a few days. The vision provided by a lens implant (an intraocular lens - IOL) is as clear as that provided by the natural lens of the eye (**Kirkwood *et al.,* 2006 and Campbell *et al.,* 2009**). Many other remedies for cataracts have been attempted, included medications, eye drops, vitamins, changes in diet and resting the eyes. Unfortunately, none of these has proven successful in dissolving or clearing cataracts. Fortunately, there have been tremendous advances in cataract surgery in the past several years. The chances for recovering good vision after surgery are now excellent **(Keenan *et al.,* 2007 and Potter & Perry, 2011).**

Significance of the study:

Today, day surgery includes concepts of care other than immediate discharge of patient after initial recovery from the anesthesia. It is becoming increasingly common throughout the world. A combination of new developments in surgical technique and technology, changes in hospital resources allocations and patient demands for quicker, added to more effective treatments have placed day surgery at the forefront of modern patient management. Moreover, Patients want treatment that is safe, efficient and effective and which provides the least possible disruption to their lives, so day surgery gives these patients -focused care. Patients' needs assessment has a positive effect by improving staff nurses' perception toward the care, helping to collect subjective data, building a trusting relationships with patient and coordinate the work with other health team members (***Belal et al., 2004, Hegazy, 2004 and* Mohamed *et al.,* 2011*)***.**

Aim of the study:

This study aims to propose a protocol for health needs management among patients undergoing day case cataract surgery**.** This aim was achieved through the following:

- Identifying needs of the studied patients pre / post surgery.
- Assessing care given for the studied patients on the day of cataract surgery.

Research questions:

- What are the health needs among patients undergoing day case cataract surgery**?**
- What are **the cares** given for patients **undergoing** day case cataract surgery**?**

2. Subjects and Methods:

Operational definitions:

Undergoing: means pre / post surgery and follow – up period.

Patients' needs: means physical, psychological, social, spiritual and educational needs.

Day case surgery: means performance of a surgical procedure that occurs without overnight admission of the patient prior to or following the intervention.

Protocol: Is a set of "RULES" and "REGULATIONS" for sending and receiving Information, by using the standard protocols.

Research design:

A descriptive exploratory design was utilized to conduct this study.

Setting:

The study was carried out in the Ophthalmologic Outpatients Clinics and Surgical waiting room of the Ophthalmologic Unit at Ain Shams University Hospital and Benha University Hospital.

Subjects:

A purposive sample was composed of 160 cataract patients, adults and old age, from both genders. They were recruited from the above mentioned settings as follows:

- Patients were taken from Ain Shams University Hospital (n = 90).
- Patients were taken from Benha University Hospital (n = 70).

Inclusion criteria:

- Patients having cataract surgery for the first time.
- Conscious patients with no speech or listening disorders.
- Patients are willing to participate in the study
- Patients are willing to take local anesthesia
- Criteria for local anesthesia_include (Patients should be able to lie flat or still and without dementia).
- Patients without ocular co – morbid conditions

Tools of data collection:

1- Patients' interviewing questionnaire, that was designed by the researchers after reviewing the related literature and consulting the experts to determine patients' health needs regarding day case cataract surgery. It was written in simple Arabic language and divided into the following parts:

- Characteristics of the study subjects namely, age, gender, marital status, income, educational level and smoking.
- Patients' medical records to identify past, present medical and surgical history, diagnosis, investigations and treatment.
- Patients' needs assessment sheet **(pre/post tests),** it composed of the following items:

Physical needs including:

Physical preparation, control of infection, relieving pain, using correct position, safe environment to prevent injury, activities limitation, assistance with physical activities, good sanitation, follow food regimen, avoid non permissible activities, perform relaxation techniques and follow prescribed medications.

Social needs including:

Positive relation with health care team, support from others, assistance with traveling / transporting, recreational activities and social relations.

Psychological needs including:

Feeling of safety and security, answers for patients' questions, awareness with the changes after the surgery, anxiety reduction and decreasing worry about health status.

Spiritual needs including:

Sense of inner peace, felling of hope fullness, praying position and enough spiritual activities and positive vision for the future.

Educational needs including:

knowledge about the surgery, anesthesia, medications, time of removing the dressing and postoperative changes, simple anatomy and physiology of the eye, causes and clinical manifestations of cataract, complications of the surgery, discharge instructions and reporting unusual signs and symptoms.

Answers of the studied Patients' regarding the presence of their needs (scored as two marks) or absence (scored as one mark), were categorized into either yes or no. The total items of patients needs = 40 item, whereas absence of the needs were considered from (1– 40) and presence of the needs from (41 - 80).

II- An observation checklist, adapted from Timby & Smith (2008), Campbell et al., (2009) and Hickman et al. (2010). It was developed and filled by the researchers to assess care given for the studied patients on day of the surgery as follows:

- **Morning of the surgery**: Medications, physical preparation, investigations.
- **Immediately post surgery:** Vital signs measurement, put patients on correct position**,** analgesics administration**,** and psychological support**.**
- **Discharge period:** Instruct patients about: activities of daily living (ADL), correct position, exercises, diet, medications, follow – up.

A correct practice was scored as (1) while the incorrect (Zero). Total score was categorized into either unsatisfactory (less than 70%) or satisfactory (70% or more).

III- Hamilton Anxiety Rating Scale: It was developed by Hamilton (1959**)** and modified by

the researchers. This scale formed of thirteen variables: anxious mood, tension, insomnia,, cognitive changes, depression, somatic(sensory), cardiovascular, respiration, gastrointestinal, Genitourinary, autonomic symptoms, somatic (muscular) and the behavior at the interview. Responses were from (0-3) scores and the total score ranged from 0-39 according to patients' responses, the following classifications were adapted: no anxiety (zero), mild anxiety (0 - less than 23), moderate anxiety (23 - less than 29) and severe anxiety (29 - 39).

Testing reliability of the scale items using alpha cronbach test = 0.92.

Validity and reliability:

Content validity was ascertained by a group of experts from Ophthalmic Surgery, Medical–Surgical Nursing and Community Health Nursing. Their opinions were elicited regarding to the tools format layout, consistency and scoring system. Contents of the tools were tested regarding to the knowledge accuracy, relevance and competence. In addition, content validity was done also for the proposed protocol to test its consistency, accuracy, applicability, relevance and feasibility.

Testing reliability of patients needs items was done using alpha cronbach test: Physical needs = 0.94, social needs = 0.84, psychological needs = 0.93, spiritual needs = 0.81 and educational needs = 0.91.

Testing reliability of the observation checklist items was done using alpha cronbach test = 0.82.

Ethical considerations:

In the planning stage approval was obtained from the directors of the above mentioned settings. All patients were informed about the study and their rights according to medical research ethics that they were free to decide whether or not they would participate in the study. Then a written informed consent was obtained from each patient who agreed to participate in the study.

Pilot study:

A pilot trial was carried out on 10% of the total study sample to test the clarity and practicability of the tools, in addition to subjects and settings. Pilot subjects were later included in the study as there were no radical modifications in the study tools.

Procedures:

- Sampling was started and completed within 3 months.
- Purpose of the study was explained to the patients who agreed to participate in the study prior to data collection.
- The researchers started to collect the data from the studied patients as follows:
  - Before the surgical technique, on the same day of diagnosis when the patients came to the out patients clinics using (needs assessment sheet and anxiety scale)
  - On day of the surgery using an observation check list to assess patients care(morning of the surgery, immediate post operatively and at discharge period).
  - On the follow - up visits within two weeks when the patients came to the out patients clinics using (needs assessment sheet and anxiety scale)
- Filling in the tools was done by the researchers according to the patients' understanding and health condition.
- The data were collected by the researchers 3 days/ week at the morning and afternoon shifts of the surgical time.

- All cataract patients were assessed individually using the previously mentioned study tools according to their physical and mental readiness.
- The proposed protocol was designed based on analysis of the actual patients' needs assessment by using the pre constructed tools.
- Content of the proposed protocol was consistent with the related literatures (national and international).
- The proposed protocol covering the following items: patients' assessment and care pre- surgery, morning care of the surgery, immediately care pre/post surgery, discharge instructions, home care and follow – up schedule.
- Testing validity of the proposed tools using face and content validity.

3. Results:

Table (1): Presents characteristics of the studied sample, this table clarified that, mean age of patients included in the study was (43.5 ± 11.6), more than half of them (59.4 %) were male and married (51.3%). As regards income, 60.0% had not enough income. In relation to education, more than one third of them (37.5%) had high level of education. Moreover, more than two fifths of them (45.0 %) were smoker.

Table (2): Shows physical needs among the studied patients pre/post cataract surgery. Results revealed a statistically insignificant difference between patients physical needs before and after the surgery, (t= 1.25, $p > 0.05$), whereas mean number of patients 'needs post the surgery was slightly higher than pre the surgery (124.8 ± 17.9 &120.8 ± 18.9 respectively).

Table (3): Shows social needs among the studied patients pre/post cataract surgery. Results revealed a statistical significant difference between

patients social needs before and after the surgery, (t= 5.1, *P*<0.05), whereas mean number of patients social needs pre the surgery was higher than post the surgery **(**115.0 ± 30.5 & 93.0 ± 45.0, respectively**).**

Table (4): Presents psychological needs among the studied patients pre/post cataract surgery. Results revealed a statistical significant difference between patients psychological needs before and after the surgery, (t= 5.6, *P*<0.05), whereas mean number of patients 'needs pre the surgery was higher than post the surgery **(**125.7 ± 9.7 & 111.7 ± 30.0 respectively**).**

Table (5): Reveals spiritual needs among the studied patients pre/post cataract surgery. Results revealed a statistical significant difference between patients spiritual needs pre and post the surgery, (t= 11.8, *P*<0.05), whereas mean number of patients 'needs pre surgery was higher than post the surgery **(**105.0 ±14.5 & 89.0 ± 8.5, respectively**).**

Table (6): Clarifies educational needs among the studied patients pre/post cataract surgery. Results revealed a statistical significant difference between patient needs pre/post surgery, (t= 19.3, *P*<0.05), whereas mean number of patients 'needs pre the surgery was higher than post the surgery **(**120.3 ± 25.2 & 64.4 ± 27.1 respectively).

Table (7): Shows care given among the studied patients on the day of cataract surgery. Results revealed a statistical significant difference between satisfactory and unsatisfactory patient care (morning of the surgery, immediate post operative period, discharge period), with t value (12.3, *P*<0.05), whereas mean number of unsatisfactory patients 'care was higher than satisfactory **(**36.0 ± 12.7 & 20.0 ± 2.8, respectively).

Figure (1): Presents patients' needs pre–surgery. Finding observed that educational needs represent the highest needs 78.1 %, followed by psychological 76.6 %, physical 72.4% then later social and spiritual (70.1 % & 68.9 % respectively).

Figure (2): Shows patients' needs post–surgery. Finding indicated that physical needs represent the highest needs 71.1 %, followed by psychological 61.9 %, social 56.1 % then later spiritual and educational (53.3 % & 41.2 % respectively).

Figure (3): Shows distribution of the studied patients according to their level of anxiety pre / post cataract surgery. Results indicated that more than two fifths of the patients (55.0%) had severe anxiety pre surgery, compared to post surgery (10.0 %). Moreover, nearly two thirds of them had mild anxiety post surgery, compared to pre surgery (10.0 %). In addition, more than one third of them (35.0%) had moderate anxiety pre - surgery.

Table (1): Characteristics of the studied patients (n = 160)

Items	No	%
Age / years ($\bar{X}$ ± SD)	43.5 ± 11.6	
Gender		
Male	95	59.4
Female	65	40.6
Marital status		
Married	82	51.3
Unmarried	78	48.7
Level of education		
High	60	37.5
Moderate	48	30.0
Low	52	32.5
Income		
Enough	64	40.0
Not enough	96	60.0
Smoking		
Present	72	45.0
Absent	88	55.0

*High education: University - Moderate education: Secondary school and technical institutions - Low education: Illiterate, read and write, primary

Table (2): Presentation of physical needs among the studied patients pre/post cataract surgery

Items	Pre - Surgery		Post - Surgery	
	No	%	No	%
Physical preparation -	147	91.9	-	-
Control of infection-	134	83.7	139	86.9
Relieving pain -	-	-	160	100.0
Using correct position -	131	81.9	139	86.9
- Safe environment to Prevent injury	122	76.2	120	75.0
Activities limitation -	118	73.7	113	70.6
Assistance with physical activities -	131	81.9	128	80.0
Good sanitation -	115	71.9	110	68.7
- Follow food regimen	108	67.5	104	65.0
-Avoid non permissible activities	72	45.0	123	76.9
- Perform relaxation techniques	81	51.0	104	65.0
Follow prescribed medications	115	71.9	126	78.7
$\overline{X}$ ± SD	120.8 ± 18.9		124.0 ± 17.9	
% of mean	75.5%		77.5%	
T - value	1.25, $p > 0.05$			

Table (3): Presentation of Social needs among the studied patients pre/post cataract surgery

Items	Pre - Surgery		Post - Surgery	
	No	%	No	%
Positive relation with health care team-	126	78.7	64	40.0
Support from others-	112	70.0	70	43.7
Assistance with traveling -	142	88.7	144	90.0
Assistance with transporting -	131	81.9	139	86.9
Recreational activities-	64	40.0	48	30.0
Social relations-	98	61.2	74	46.2
X`± SD	115.0 ± 30.5		93.0 ± 45.0	
% of mean	71.9 %		58.1%	
T - value	5.1, $P<0.05$			

Table (4): Presentation of Psychological needs among the studied patients pre/post cataract surgery

Items	Pre - Surgery		Post - Surgery	
	No	%	No	%
Feeling of safety and security -	122	76.2	110	68.7
Answer patients' queries -	128	80.0	136	85.0
- Awareness with surgical Changes	115	71.9	131	81.9
Anxiety reduction-	138	86.2	70	43.7
-Decrease worry about health status	110	68.7	48	30.0
X`± SD	125.7 ± 9.7		111.7 ± 30.0	
% of mean	78.6%		69.8 %	
T - value	5.6, $P<0.05$			

Table (5): Presentation of Spiritual needs among the studied patients pre/post cataract surgery

Items	Pre - Surgery		Post - Surgery	
	No	%	No	%
-Enough spiritual activities	91	56.9	81	50.6
-Feeling of hope fullness	104	65.0	88	55.0
-Positive vision toward future	120	75.0	98	61.2
-Sense of inner peace	126	78.7	74	46.2
X`± SD	105.0 ±14.5		89.0 ±8.5	
% of mean	65.6 %		55.6 %	
T - value	11.8, P<0.05			

Table (6): Presentation of educational needs among the studied patients pre/post cataract surgery

Items	Pre - Surgery		Post - Surgery	
	No	%	No	%
Information about cataract extraction	104	65.0	48	30.0
Signs and symptoms of cataract	67	41.9	43	26.9
Complications of cataract surgery	120	75.0	91	56.9
Investigations.	88	55.0	-	-
Discharge instructions about:				
Unusual signs and symptoms	152	95.0	72	45.0
Food regimen	115	71.9	101	63.1
Sleep position	125	78.1	54	33.7
sexual activity	123	77.0	85	53.1
Medications and eye care	147	91.9	32	20.0
Eye drop/ointment instillation	125	78.1	54	33.7
Eye dressing / irrigation	152	95.0	27	16.9
Permissible activities	126	78.7	102	63.7
Follow – up visits	122	76.2	56	35.0
X`± SD	120.3 ± 25.2		64.4 ± 27.1	
% of mean	75.2 %		40. 2%	
T - value	19.3, P<0.05			

Table 7: Presentation of the care given among the studied patients on the day of cataract surgery (n = 160)

Day of the surgery	Satisfactory Patients (n= 55)		Unsatisfactory Patients (n = 105)	
	No	%	No	%
Morning care	22	40.0	45	42.9
Immediate post operative care	18	32.7	27	25.7
Discharge instructions	15	27.3	33	31.4
X`± SD	20.0 ± 2.8		36.0 ± 12.7	
% of mean	12.5 %		22.5 %	
T-value	P<0.05 12.3,			

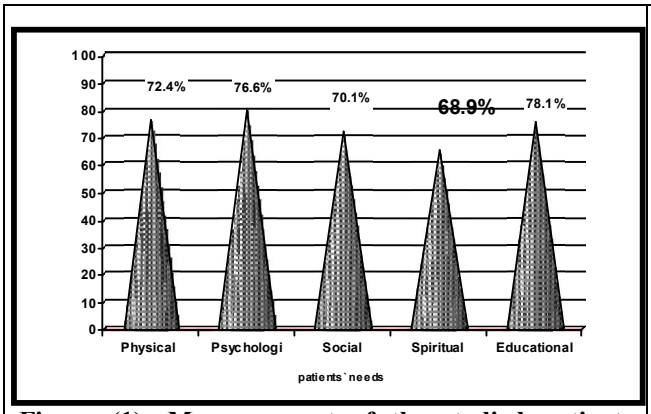

Figure (1): Mean percent of the studied patients needs pre – surgery

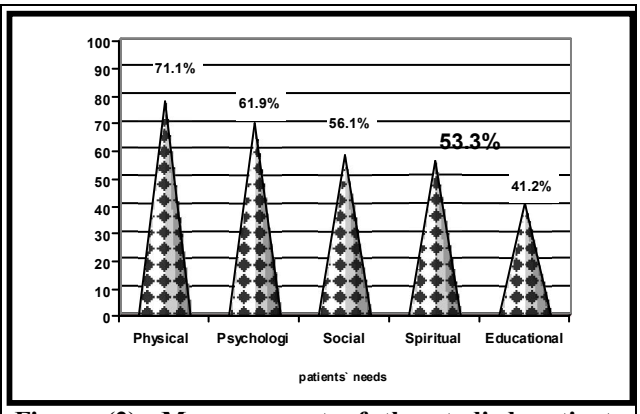

Figure (2): Mean percent of the studied patients needs post – surgery

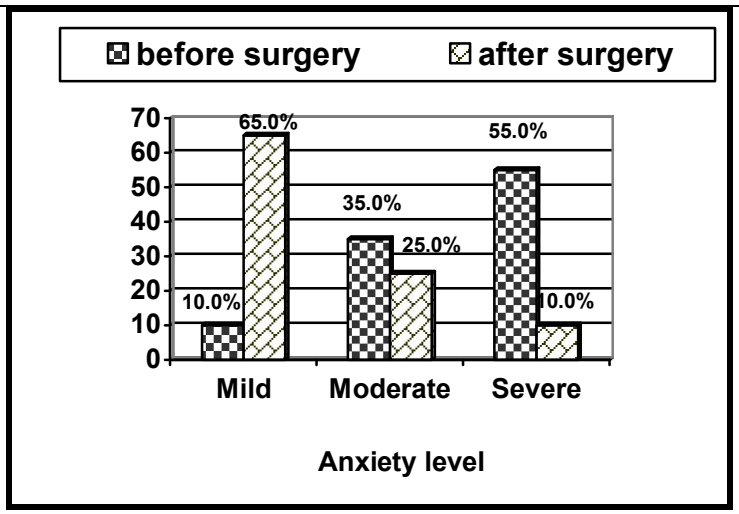

Figure (3): Percentage distribution of the studied patients according to their level of anxiety pre / post cataract surgery.

4. Discussion:

Day-care surgery has many advantages to the patient as well as to the doctor;: Patients receive treatment that is suited to their needs and which allows them to recover in their own home, cancellation of surgery due to emergency pressures in a dedicated day surgery unit is unlikely, the risk of hospital acquired infection is reduced, clinicians can provide high quality care for appropriate patients and release inpatient beds for major cases, added to reduce waiting lists (Kirkwood *et al.,* 2006 and Hegazy *et al.,* 2008). The current study aimed to propose a protocol for health needs management among patients undergoing day case cataract surgery. In the present study, exclusion criteria of patients in day care surgery involved medical history of ocular co - morbid conditions and eye infection. *Lundstrom et al. (2011)* mentioned that, to determine suitability of patients for day surgery, a process of patients' selection must take place to avoid occurrence of complications. In cataract surgery, 95% of patients experience improved vision if no other eye problem present. Effective communication is imperative in all episodes of patients' care, because patients with impaired hearing or inadequate cerebration may not clearly understand instructions relating to the operation or what to do when discharged to go home. In a similar line, patients with a stroke may have communication and comprehension difficulties so it is important to ensure that relevant information is understood and supported by hard copy and written notes (Jakobsson *et al.,* 2009).

Concerning physical needs, it is clear that insignificant difference was found between pre / post surgery. The previous findings were supported by *Keenan et al. (2007)* who stressed on the importance of physical preparation for patients with cataract surgery. He also add that day case surgery provide a major effective form of care with a high quality of services. From physical needs also food regimen instructions, *Black et al. (2009)* emphasized that, adequate diet and good nutrition are important to the conservation of sight. In relation to activities of daily living as a physical need in this study, *Gogate et al. (2007) and Campbell et al. (2009)* recommended that, the nurse must assess patients' ability to perform activities of daily living to determine level of independence in self-care and health education, added to majority of patients were in needs for helping in activities of daily living. Furthermore, medications and eye care considered as a physical need, Marsden *(2004)* claimed that, medications and eye care instructions are very important to decrease post-operative complications.

As well as, any preoperative assessment must assess the patients' ability to maintain their own safe environment. One needs to assess any problems, linked to respiratory, diabetes, heart or kidney and to evaluate in advance, drugs to be utilized. It is imperative that a pre-operative assessment include the final plan of the standby anesthetist, which must be mapped out in advance on a sheet which accompanies the patients to the theatre. Outpatient surgery is done with a high turnover and the operating room is not the place for evaluating the patients. *(Sparrow, 2007 and Karaj et al., 2010).*

In the same line, care should be taken to provide adequate information to the patients, and analytical assessment of the physical condition prior to the procedure, added to day-care staff who look after the patients following the surgery. Adequate preparation of the patients for the surgery, will

contribute to a safe surgical journey. Pre-assessment and provision of day-care services are the innovative areas with exciting challenges for all workers in this field (Werner, 2010 and Agarwal & Kumar, 2011).

Regarding psychological needs, as reported by three quarters of the patients the needs were: answers their queries, receive information about the changes after surgery and about effect of psychological factors on the surgery. This finding could be attributed to the reports of nurses about short stay of the patients in the hospital on day case surgery; also the researchers observed that there is no psychological preparation by the nurses. Lansingh and Carter (2009) listed that, psychological preparation play a vital role in the successful outcome of the surger and psychological assessment should be made to assist in alleviating any worries the patients may have.

As regards social needs, more than half of the patients in this study reported no relation with health care team. *Sach et al. (2010)* recognized that, building up relationships with patients will allow discussing the problems confidentially. So it is essential to use an efficient interaction with patients stayed for a short period in the hospital. Considering educational needs, patients had a higher need before the surgery. Instructions about: Local anesthesia, permissible activities, sleep, food, unusual signs and symptoms, eye irrigation, medications and eye care, ADL and infection control. These findings may be due to, lack of nurses' knowledge and practices, added to the dependence on the ophthalmologist to give surgical instructions. The previous findings were supported by *Belal et al. (2004)* who reported that, patients' families are now responsible for almost all post-operative care, so written and verbal instructions before discharge are imperative. *Fedorowicz et al. (2006)* reported that, in day surgery because patients' journey is a short one, therefore post discharge advise, *as well as,* teaching patients and the families dispelling misconceptions and provide with the factual information. In addition, patients should be informed that the most common problems following Local anesthesia post cataract surgery are eye redness and temporary double vision.

According to Hickman *et al.* (2010) patients should be informed that at 4 weeks the following complaints should be absent: pain, photophobia, redness (except remaining sub – conjunctival hemorrhage) and cells in the anterior chamber. All mobility permissive patients should be taught to take extreme care of the eyes at home. In the same line, postoperative examination are crucial**,** so it is customary that the patients be seen the next day, however writing detailed note about what is normal and what complications on the first day is unacceptable. It is imperative that a 24 hour contact number be provided to all patients (Hegazy *et al.,* 2008 and Potter & Perry, 2011). In addition, the patients should be educated about the risks and benefits of cataract surgery and alternatives to the treatment. Moreover, determine if the expected improvement of the disability outweighs the potential risk, cost and inconvenience of the surgery (Rengaraj *et al.,* 2012).

Concerning psychometric assessment the current study indicated that, more than half of the patients had severe anxiety. This result may be due to lack of psychological preparation and other cases had surgical complications. The previous findings confirmed by *Hegazy (2004)* and Timby & Smith (2008) who listed that majority of the nurses perform psychological preparation incorrectly and stressed on the value of the preoperative preparations in reducing anxiety. He also adds that, anxiety results when patients are unable fully to comprehend the world around as regards the surgery.

Conclusion:

In the light of the current study it can be concluded that, there were statistically significant differences between studied patients needs(ychological, social, spiritual and educational) pre / post surgery, whereas significant improvement was indicated in these needs post surgery. Meanwhile, insignificant difference as regards the physical needs. Moreover, on day of the surgery, mean number of unsatisfactory patients care was higher than satisfactory. Furthermore, significant elevation was observed on anxiety level pre - surgery among the studied patients.

Recommendations:

Based on the results of the present study, it can be recommended that:

- The proposed protocol of patients' needs management that's evidence – based should be implemented and evaluated in relation to the incidence of cataract surgery complications.
- Further research study should be done to implement and investigate the effect of the proposed protocol for cataract extraction surgery on decreasing the incidence of complications after the surgical technique.
- An orientation program should be prepared for the patients undergoing cataract surgery.
- Patients are in need to a simplified illustrated and comprehensive Arabic booklet including information about cataract surgery.

Based on findings of the present study, health needs management protocol has been proposed (Appendix I).

Appendix I

A developed health needs management protocol for patients undergoing day case cataract surgery.

Campbell *et al.* (2009), Hickman *et al.* (2010), Casparis *et al.* (2012) and Vincent & Patalano (2012).

Purpose: To outline nursing responsibilities on needs management pre / post cataract extraction surgery

Expected patient outcomes:

•Regain sufficient visual acuity to maintain ADLs, including reading and watching television for enjoyment

•Patient will experience reducing level of anxiety.

• Patient will follow prescribed postoperative care and safety precautions.

Clinical assessment:

- Check physical assessment sheet (neurologic, respiratory, cardiovascular, and abdominal assessments are essentially normal).
- Check patients' eye assessment sheet (pupils are round and equal, and react briskly to light and accommodation. conjunctivae is pink, sclera and corneas are clear).
- Assist doctor on examining patients by the ophthalmoscope or Fundoscope.
- Check the intraocular pressures measurement.
- Assure that no disease of the blood vessels, retina, macula or disc is found.
- Reviews operative procedure with the patients, answering their questions and telling them what to expect after the surgery.
- Follow preoperative protocols in preparing and transporting patients to surgical room.

Implementation:

****Morning of the surgery**

Assess patients for the following:

- Take the medications on morning of the surgery and do not take insulin unless told to do so, added to Bring inhalers to the hospital.
- Wear short-sleeved button front shirt or blouse and no undershirts, pantyhose or girdles.
- Leave all jewellery and valuables at home.
- Do not use scented products (perfume, aftershave, powder, spray) and eye or face make-up.
- Bring their glasses or sunglasses with them.
- Have a responsible adult who arrive them to home.

****Immediately before the surgery**

- Report family member to stay in the waiting room
- Check on results of the Blood tests which done before the surgery.
- Measuring the vital signs.
- Put the prescribed eye drops or gel into the eye.
- Connect patients to an intravenous if ordered.
- Reviewing the post-operative instructions with the patients.
- Sure that the patients are ready for the surgery.
- Ambulate patients to the operating room.
- Check on no solid food for 6 hours prior to the surgery.
- Check on no thick liquids after midnight (milk, cream, orange juice, prune juice.).
- Allow patients Up to 2 hours before the surgery, to drink clear liquids (coffee, tea, apple juice, and water, soft drinks or meat broth.). Sugar in coffee and tea is okay, but no milk products.

****Immediately after the surgery**

- Check the blood sugar and if patients are diabetic, medication should be given if needed.
- Measuring the vital signs.
- Ensure that Patients' family stay with them night of the surgery.
- Told patients to Leave eye shield on or wear glasses to protect the eye.
- Inform Patients that there will be some mucous and tears from the eye, added to the eye may feel irritated or scratchy.
- Given the prescribed medications.
- Allow oral light fluid.
- Assess patients level of pain and irritability.

****Discharge instructions following the surgery**

Do

- Resume prescribed eye drops and medications as instructed.
- Spend rest of the day quietly.
- Wear glasses or eye shield at all times to protect eye from injury, at least for 1-2 weeks.
- Wash the eyelid gently with a cotton makeup pad or clean facecloth with Luke-warm tap water. Wipe from the inside corner outward.
- Wear eye shield when showering, bathing or hair washing for one week. Let the water hit the back not the face.
- Gradually increase daily physical activities.
- Sit in a chair to put on shoes.
- Sleep on back or unoperated side for 1 week. Place a pillow between the knees to help avoid turning over during sleep.
- Walking is fine.
- Television or reading will not hurt your eyes, but be sure to stop when feeling tired.
- Combing or brushing the hair gently is permitted.
- Resume the usual diet slowly

- **Contact the surgeon if:**

* The operated eye becomes very painful or swollen.
* Mucous discharge from the eye becomes more.
* Vision reduction occur in the operated eye.

Don't

- Rub or bump the operated eye.
- Get water in the eye.
- Getting soap or shampoo in the eyes.
- Drive until permitted from the surgeon.
- Put the head down below the waist for 2-3 days when bending over.
- Strain or lift anything heavier than 20 lbs. for the first week after the surgery.
- Get constipated.
- Swim, golf, or play contact sports until permitted.
- Make any important decisions or sign important papers for 24 hours.
- Drive or operate mechanical equipment for 24 hours.

Side – effects

- An itching, sticking and blurring vision in the eye.
- Aching of the eye.
- Bruising of the eyelid or eye.

Eye drops instillation

- Wash hands before and after putting in eye drops.
- Pull the lower eyelid down.
- Put the drop into the space between the lower eyelid and the eyeball.
- If using more than one type of drop, wait 5 minutes between each bottle.
- Close the eye after putting the drops.
- Gently wipe away any drops or tears with Kleenex from inside to outside corner of the eye.

Evaluation

Patients is visibly relieved when the eye patch is removed because, the vision in the operated eye is better than before the surgery even without the glasses. Patients are able to relate the recommended activities restrictions and implementing of the prescribed postoperative instructions. In addition, absence of the complications posts – surgery.

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