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Cover Page, Introduction, Contents, Call for Papers, All papers in one file

Contents

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$^{87}Sr/^{86}Sr$ Isotope Evolution Trend of Groundwater Resources in the Hebei Plain, Northern China

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Abstract: In Hebei plain, the most important source of water supply is groundwater, which as system, can be divided into 7 aquifers. In this study, major ion hydrochemistry and $^{87}sr/^{86}sr$ ratios data were analyzed in order to understand strontium isotope evolution mechanism of groundwater in the Hebei plain. Based on integrated analysis, it is considered that the radiogenic Sr in groundwater from quaternary sediments (Q_4-Q_1) comes from the weathering of silicate mineral rich in Na and Rb. The $^{87}Sr/^{86}Sr$ ratios increase systematically with the increasing age and depth of groundwater, and this reflects "the accumulative effect of time" to water-rock interaction. Although the low $87Sr/^{86}Sr$ ratio of groundwater is associated with the low content of Rb in the rocks, further research is required to reveal its evolution mechanism. [The Journal Of American Science. 2007;3(4):1-6]. (ISSN: 1545-1003).

Keywords: $^{87}Sr/^{86}Sr$ Isotope; Evolution Trend; Hebei Plain; China

Introduction

As natural tracers of groundwater flow, Strontium isotopes have been extensively used (Bullen et al. 1996; Frost et al. 2002) due to its geochemical characteristics. During recharge, groundwater acquires Sr and as it moves along its flow path it interacts with Sr-bearing minerals within geologic units. Therefore the chemical evolution of groundwater is a function of a variety of factors that include residence time, initial water composition, and differences in the distribution and reactivity of individual minerals. According to Ma and Liu (1999), the strontium isotope composition of groundwater is affected by recharge water chemistry, host rock geochemistry and water-rock interaction (WRI) in groundwater systems, the residence time of groundwater in the aquifer and mixing of different groundwaters. Since WRI has an important control on strontium enrichment, strontium isotope ratios have been widely used for investigating the WRI occurring in groundwater systems (Oetting et al. 1996; Armstrong et al. 1998; Woods et al. 2000). In addition, strontium isotope ratios frequently serve as tracers for delineating recharge sources and mixing processes in groundwater system; representative studies including Lyons et al. (1995), Katz and Bullen (1995), Gosselin et al (2004), and Wang et al (2006).

For Hebei Plain, deep groundwater is the most important source of water supply in the region. In this study, twenty three groundwater samples were collected in Hebei Plain, and strontium isotopes and major ion hydrochemistry used to identify hydrochemical processes and water-rock interaction in water system. The differences of Sr isotope composition reflect regional geologic control on water flow in the Plain, and the distribution characteristics for Sr isotope in groundwater system were discussed.

Regional Hydrogeology

The Hebei Plain is one part of the great North China Plain located in the eastern part of the People's Republic of China. Hebei plain covers an area of 6.19×10^4 km^2 (lat.36°06'N - 39°35'N , long.114°26' E - 117°50'E) and is bordered on the north by theYanshan Mountains, on the east by Bohai Sea, on the south by the yellow river and on the west by the Taihang Mountains. The plain has a relatively low and gentle topography of no more than 100 meters above sea level in some places and less than 50 meters in most sections. However, it has many depressions, totaling about 1,000 km^2. In the northern part of the plain there is a depression area, which lies between Baoding and Dagu. Some well-known depressions, such as the Baiyangdian, Wenanwa and Dawa, are located in this area.

The Hubei plain lies in the temperate zone, with a continental monsoon climate. It is windy in the springs, hot and rainy in summers, and cold and dry in winters. The annual mean air temperature of the plain is 12˚C to 13˚C; with the annual average rainfall of 400-800mm. The annual mean water surface evaporation is 1100-1800mm. The groundwater system of Hebei Plain is mainly from quaternary aquifers which can be divided into 7 groups, depending on the various depths as follows: (1) aquifer (Q_4), 20-50m deep; (2) aquifer (Q_3) ,100-150m deep; (3) aquifer (Q_2), 200-300m deep; (4) aquifer (Q_1) , 350-500m deep; (5) aquifer (N) ,500-1000m deep; (6) aquifer (E), 1000-4000m deep; (7) aquifer (Є and Z) , also called "the old buried-ills groundwater", and up to 4000m deep.

Materials and Methods

Sampling: A total of 23 groundwater samples from the wells were collected over the Hebei plain. These wells are either active municipal wells, domestic or agricultural wells equipped with sub-mersible pumps or windmills that penetrate different lithological units. The groundwater sampling locations are identified by the inventory numbers as shown in table1. Just prior to sampling, the wells were pumped until at least three casing volumes were drawn away. At each location, samples filtered through 0.45μm membranes were obtained in three new350 ml low- density polyethylene bottles well rinsed with deionised water before sampling. One of these three samples for the determination of metallic elements, was acidified with HNO_3 until its pH was around 1.0; sufficient to stabilize trace metals. The second and third samples were unacidified and collected for the anions and strontium analyses respectively.

Test method: Temperature and pH values of the samples were determined in situ using portable Hanna pH meter which had been calibrated before use. Alkalinity was measured on the sampling day using the Gran titration method. Major anions and cations were determined using ion chromatography and ICP-AES with the precision of 0.01 mg/L. Strontium concentration measurements and $87Sr/^{86}Sr$ ratio analyses were completed at Yichang Institute of Geology and Mineral Resources. Dissolved Sr^{2+} was determined using a standard flame atomic absorption method. Strontium was isolated from each sample solution using conventional anion exchange chromatography (Dowex AG50- X8 resin). Strontium isotope ratios were measured on a Finnigan MAT 261 Thermal Ionisation Mass Spectrometer. Following internal normalization, results were corrected for fractionation against a monitored value of the NBS 987 standard of 0.710221 ± 0.000015 (2σ, n = 8).

Table 1. Hydrochemical Properties, Major Ions and Strontium Isotope Composition of Water Samples from Hebei Plain (in mg/L except pH and $^{87}Sr/^{86}Sr$)

No.	Depth (m)	pH	HCO_3^-	SO_4^{2-}	Cl^-	Ca^{2+}	Mg^{2+}	K^++Na^+	Sr^{2+}	$^{87}Sr/^{86}Sr$
Hb02	1600-1700	7.05	204.65	95.43	2236.57	105.36	15.28	1875.39	6.87	0.70864
Hb03	241.5-280	6.62	471.56	21.09	372.23	5.11	3.59	452.93	0.24	0.71220
Hb04	380	6.53	412.62	93.95	292.46	9.20	9.01	394.20	0.29	0.71268
Hb05	680	6.82	471.56	89.10	638.10	11.24	7.16	660.83	0.30	0.71077
Hb06	1150-1599	7.01	176.84	91.93	2539.11	95.05	13.45	1764.68	6.34	0.71075
Hb07	400	7.04	412.62	94.28	345.64	6.13	6.61	440.63	0.24	0.71340
Hb08	400	6.82	412.62	70.32	110.78	3.07	1.79	276.28	0.05	0.71393
Hb09	370	6.91	324.20	52.35	400.08	9.20	8.40	400.08	0.21	0.71409
Hb10	350	6.93	294.73	101.92	664.69	25.55	22.18	565.05	0.82	0.71288
Hb11	＞300	6.84	442.09	84.92	141.80	3.07	3.00	315.33	0.08	0.71319
Hb12	＞300	7.52	442.09	86.79	137.37	6.13	8.43	298.18	0.24	0.71234
Hb13	360	7.52	265.25	47.89	159.53	5.11	5.41	228.60	0.09	0.71324
Hb14	360	7.55	265.25	45.77	66.47	4.09	1.77	170.63	0.17	0.71527
Hb15	350	7.06	147.36	55.52	132.94	5.11	1.76	173.03	0.10	0.71221
Hb16	500	6.51	501.04	8.50	447.56	8.18	5.98	502.83	0.28	0.71251
Hb17	380	6.52	235.78	43.32	93.06	3.07	2.39	176.03	0.09	0.71452
Hb18	2571-2694	7.05	1098.22	24.36	3024.21	284.26	30.25	2145.89	7.04	0.71114
Hb19	370	6.58	206.31	84.68	177.25	7.15	3.56	237.35	0.20	0.71260
Hb20	300	6.59	176.84	60.42	110.78	5.11	1.76	172.03	0.20	0.71251
Hb21	1100	7.03	1178.91	21.95	3500.69	295.37	33.55	2525.68	11.36	0.70902
HB27	240	7.03	383.14	136.12	48.74	62.34	1.08	182.20	0.36	0.71153
Hb28	350	7.01	147.36	78.19	101.92	26.57	11.84	115.45	1.17	0.71156
Hb29	370	7.05	442.09	1.63	159.53	3.07	3.00	284.48	0.14	0.70912

Results and Discussion

Strontium and major ion chemistry: The positive correlation between $[Sr^{2+}]$ and $[Cl^-]$ in groundwater samples indicates that subsequent evaporation causes the increase in $[Cl^-]$ and an approximately proportionate increase in $[Sr^{2+}]$ (Fig.1). However, the $[Sr^{2+}]/[Cl^-]$ ratio of each groundwater sample is significantly higher than that of seawater (Fig.2), and this is particularly noticeable at the dilute end of the spectrum. Since there are no chloride-bearing minerals in the plain, dissolved Cl^- is thought of to be conservative and controlled only by evapo-transpiration. Therefore, the $[Sr^{2+}]/[Cl^-]$ ratio reflects an excess of $[Sr^{2+}]$ in the groundwater. The $[Sr^{2+}]/[Na^+]$ ratio of the most dilute groundwater samples show no obvious correlation with increasing $[Cl^-]$ (Fig.3). Because Sr^{2+} can be derived from weathering of carbonate, sulphate and silicate minerals , and since Na^+ predominantly comes from silicates; variations in the $[Sr^{2+}]/[Na^+]$ ratio may be indicative of the relative importance of these reactions in determining solute compositions. This low $[Sr^{2+}]/[Na^+]$ tends to be associated with Na-silicate minerals, whereas high $[Sr^{2+}]/[Na^+]$ are associated with carbonate dissolution.

Strontium and $^{87}Sr/^{86}Sr$ ratio: The relation between $^{87}Sr/^{86}Sr$ and $[Sr^{2+}]$ is shown in Fig.4, and the groundwater samples can be divided into three groups:

(1) Middle Sr^{2+}, high $^{87}Sr/^{86}Sr$ ratio groundwater (group I).This group of groundwater can also be divided into two types based on the difference of strontium concentration. The groundwater samples with high Sr^{2+} (type A) mostly distribute in piedmont area, which is the recharge area of the plain groundwater

system. Lateral recharge is an important recharge source to above groundwater (type A), and the recharge water possibly flows across the metamorphic rocks regions and igneous rocks region with high Rb content. The $^{87}Sr/^{86}Sr$ ratios of groundwater samples with low Sr^{2+} (type B) are related to the water's age. The $^{87}Sr/^{86}Sr$ ratios show a systematic increase with the increasing age and depth of groundwater, and this reflects "the accumulative effect of time" to water-rock interaction.

(2) Low Sr^{2+}, high $^{87}Sr/^{86}Sr$ ratio groundwater (group II). This group of groundwater is distributed in the center of Hebei Plain. The low Sr^{2+} is relative to the rocks with low Sr content, and the high $^{87}Sr/^{86}Sr$ ratio is relative to the groundwater with old age.

(3) High $[Sr^{2+}]$, low $^{87}Sr/^{86}Sr$ ratio groundwater (group III). The host lithology for this type of water is limestone of Ordovician age since the high Sr content and low $^{87}Sr/^{86}Sr$ ratios of these waters is a direct reflection of those characteristics in the limestone. The high Sr content for this water is due to high temperature, which increased the solubility of Sr. however, it is difficult to explain the low $^{87}Sr/^{86}Sr$ ratio in these samples.

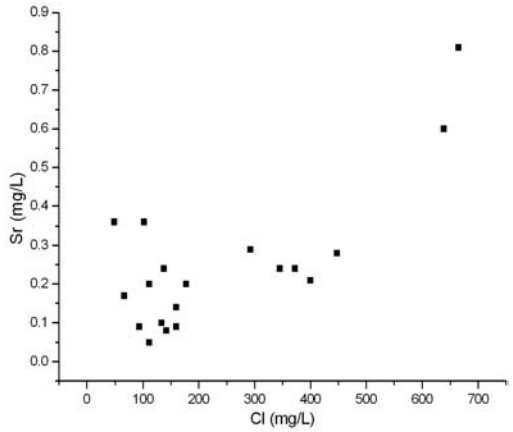

Figure 1. Relationship between Cl^- and Sr^{2+}

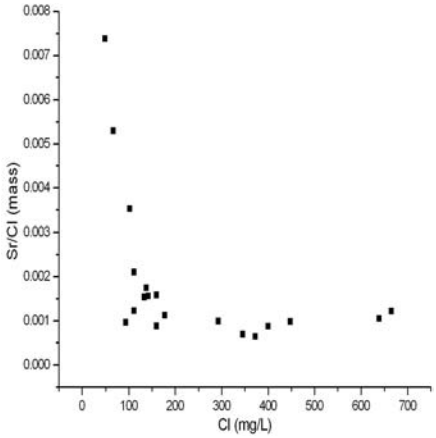

Figure 2. Relationship between Cl^- and $[Sr^{2+}]/[Cl^-]$

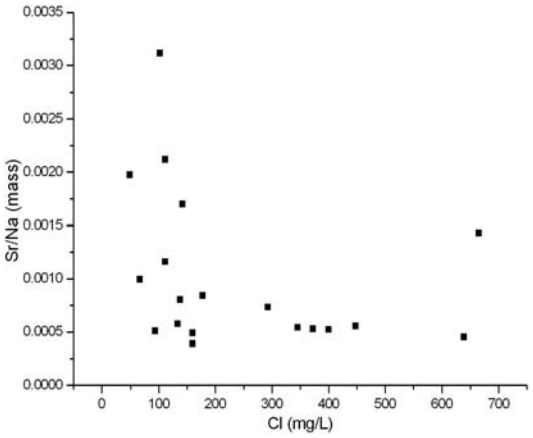

Figure 3. Relationship between Cl^- and $[Sr^{2+}]/[Na^+]$

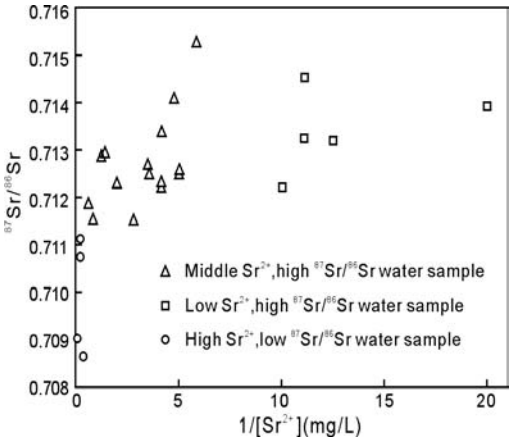

Figure 4. Relationship between $[Sr^{2+}]$ and $^{87}Sr/^{86}Sr$

Conclusion

$^{87}Sr/^{86}Sr$ compositions of Hebei Plain groundwater samples are higher than the values of seawater (0.709073±0.000003). These data all reflect groundwater interactions with marine carbonates and/or silicate minerals with slightly higher $^{87}Sr/^{86}Sr$ ratios than the carbonates. These $^{87}Sr/^{86}Sr$ ratios therefore provide compelling evidence that weathering of old silicate minerals (which have high $^{87}Sr/^{86}Sr$ ratios) in a sedimentary environment is an important source of major ions (especially Na^+ and HCO_3^-) and dissolved silica in this groundwater system. However, no explanation is available for waters with low $^{87}Sr/^{86}Sr$ ratios; hence further research is needed to reveal the formation mechanism of these waters. Above all, there are three possible sources of $[Sr^{2+}]$ to groundwater in the Hebei Plain:

(1) $[Sr^{2+}]$ delivered via atmospheric aerosols dissolved in rainfall;

(2) $[Sr^{2+}]$ derived from dry fallout associated with salts, carbonates and silicates from outside the plain; and

(3) $[Sr^{2+}]$ released from minerals during weathering within the soil zone or aquifer. These processes are believed to have occurred at the initial stage of groundwater chemical evolution.

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Delineation Of The Aquifer In The South-Western Part Of The Nupe Basin, Kwara State, Nigeria

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Abstract: A geophysical study using the Vertical Electrical Soundings (VES) techniques has been used to investigate the sub-surface layering in the southwestern part of the Niger Basin in order to determine the nature, characteristics and spatial extent of the components of the aquifer underlying the region. The results of the interpreted VES data suggest that the layering in the region range from three to five layers. The geologic sections derived from the analyzed geoelectric section suggest that the alluvial deposits of sand, sandy clay, medium to coarse sandstones, as well as the weathered and fractured basement constitute the aquifer found in this sedimentary region. Furthermore the results of the interpretation of the VES data indicate that the thickness values of the aquifer vary from 6.01 m to 58.60 m. The geoelectric section generated also suggest that the resistivity values of the aquifer components range from 4.2 Ω-m to 106.7 Ω-m for the alluvial deposits; 33.7 Ω-m to 108.6 Ω-m (weathered basement); and 345.7 Ω-m to 564.0 Ω-m for the fractured basement rocks. [The Journal Of American Science. 2007;3(4):7-14]. (ISSN: 1545-1003).

Keywords: Delineation, Aquifer, Vertical Electrical Soundings (VES), Nupe Basin

Introduction

Water supply problems are very common in most localities in Kwara State just like in most tropical areas; the situation is really disturbing in tropical areas like in the study area of this work. In most cases, water required for domestic and agricultural uses are obtained from rivers, streams and shallow hand-dug well. Moreover, most of these rivers and streams are often situated at great distances from the villages they serve. The surface water sources are usually ephemeral/seasonal and prone to contamination by human beings and animals. The consequences of these pathetic situations on the water supply systems of the people in this region are the prevalence of such water-borne diseases like guinea worm, cholera and typhoid fever. According to United Nations International Children Education Fund's Rural Water Sanitation, UNICEF-RUWATSAN Project (1988), more than one million people are yearly affected by guinea worm. A prevention of the scourge of such water-borne diseases could have saved a lot of scarce resources spent on health care facilities. There is no gainsaying the fact that substantial losses in man-hours required for productive ventures associated with the sick and their relatives who care for them could have been saved and channeled to other productive sources. In view of this scenario, the provision of sustainable potable water for the people should be the main priority of any government which is serious in eradicating poverty and enhancing the socio-economic status of its people. Moreover, according to the United Nations, one of the cardinal programmes of the Millennium Development Goals (MDG) is the provision of potable water to every community so that the impoverishment of the rural folks in most especially, the tropics and the least developed countries (LDCs) can be wiped out from the global road map of economic development. As a contribution to the improvement and development of the water resources in this region, this work was aimed at identifying the nature, extent and spatial distribution of the components of the aquifer in the southwestern/south-central part of the Lower Niger Basin. It is hoped that the results of this study could also be used to determine the groundwater potentials of the study area.

Physiography, Geomorphology, Geology And Hydrogeology

Physiography and Geomorphology

The project area lies within the Cretaceous-to-Upper Maestrichtian Nupe (Bida or Niger) Basin, Niger trough or better still Middle Niger Valley just south of the River Niger between longitude $4^0$45'E and $6^0$10'E and latitudes $9^0$10'N and $8^0$47'N (Figure 1). It covers an area of about 4870 km^2. According to Idachaba (1982), this study area which is part of the former Edu Local Government Area (LGA) of Kwara State and now split into Edu and Pategi LGAs is a sparsely populated region with an estimated population density of about 34 persons per square kilometer, and is mainly dependent on subsistence agriculture and

fishery. The dominant topographic feature is the peneplain of the Niger River Trough which stretches from Jebba to Eggan in such a way that the topography is relatively flat-lying near the River Niger, and rises to the crystalline uplands in the south to an elevation of less than 150m above mean sea level (msl). Vegetation is of the Guinea Savannah type, which according to Udo (1982) has a characteristic mean annual temperature or 29°C and mean annual rainfall of 300mm. The River Niger and its tributaries control the course of most of the rivers in this area. Such rivers like Oro, Oyi, Oyun, Ebba existing in this region have a North-Northeasterly flow towards the River Niger.

Geology and Hydrogeology

This Basin is an approximately NW-SE trending trough filled with mainly Santonia to Maestrichtian sediments of sandstones, siltstones and superficial alluvial deposits (Adeleye, 1976, Ajibade, 1980). Borehole log reports from UNICEF-RUWATSAN project (1988) show that primary porous and permeable formations of the Nupe Sandstones Group predominate the northern and central parts of the Edu and Pategi LGA. The lithology of these formations, according to Idornighie and Olorunfemi (1992) and, Mallam and Ajayi (2000), are alluvium, weathered laterite, sandy clay and clayey sand. The southern part of the study area is characterized by formations with secondary permeabilities with the following lithologies: weathered laterite, sandy clay/ clayey sand, fractured basement and fresh basement rocks. Generally the rock units in this region are suggested to be highly characterized by intercalations of claystone, siltstone, silt, clay and weathered bedrock (Biwater Shellabear, 1985; UNICEF-RUWATSAN Project, 1988). These geological materials are usually liable to form aquitard and permeable zones to the bedrocks in both the sedimentary terrain and the crystalline basement complex existing in this area. In areas underlain by crystalline rocks, presence of structures like fractures, fissures, veins, joints and such other structural deformations of the basement complex control the flow of groundwater and also influence the rate of recharge and discharge of the main aquiferous units (Biwater Shellabear, 1985). Fig. 2 shows the existence of minor fractures with approximate NW-SE trends. These structures intrude the basement complex rock in the southern and eastern part of Edu LGA and create relatively thick highly weathered overburdens.

Ves Data Collection And Interpretation

Collection of VES data

Twenty-two (22) Vertical Electrical Soundings (VES) data using Schlumberger array were carried out by UNICEF-RUWATSAN Project team based in Ilorin, Kwara state at twenty-two (22) communities in Edu and Pategi LGAs. The VES data were collected and their corresponding borehole logs were collected for quantitative analyses in order to basically determine the subsurface layering, thickness of the surface layers, thickness of the overburden and thickness of the aquiferous or saturated ground water layer beneath each of the studied VES sites.

Interpreting the VES data

The sets of VES data collected at each VES site were plotted to obtain the apparent resistivity, ρ_i against half the current electrode spacing, AB/2 on a bi-logarithmic graph sheet, as resistivity, ρ_a field curve. The field curves were interpreted using the conventional curve matching technique (Keller and Frischnecht, 1966) and empirical method (Van Nostrand and Cook, 1966; Shiftan, 1970) as well as computer modelling which make use of interactive program written and published by Mooney (1980). Fig. 3 shows the results of the interpretation of the VES data collected for a VES site and a drilled borehole in Shonga Village. The shape of the curve suggests that three or four geoelectric layers of various lithologies were sampled at the VES site. As can be seen in the figure, the third layer has a moderate resistivity of 60. Ωm and the highest estimated thickness value of 30.7 m the third layer is thus taken to comprise or the saturated groundwater zone at this VES site. The procedure described above for determining the aquiferous zone was repeated to analyze the nature and characteristics of the aquifer beneath the 22 VES data collected for the various VES sites in the study area of this work.

Results And Discussions

The results of the interpretation of the VES data and information obtained from the borehole logs collected for this work have been used to produce the geoelectric sections and geologic sections associated with each of the VES sites investigated.

Geoelectric and Geologic Sections

These sections were derived in order to know and understand the geological components of the aquifers beneath each VES site and determine the possible hydrogeophysical/hydrogeological parameters of thickness and resistivity characterizing each aquifer. Interestingly, in this study, a good knowledge and understanding of the geological formations below each VES point studied, were obtained from the borehole logs even before the set of VES data were interpreted. These are unlike what obtains in most previous related works reported by for example, Worthington (1977), Van Overmeeren (1989), Olayinka (1990), Ajayi and Hassan (1990), Idornighie and Olorunfemi (1992), and Shemang (1993). In all these geophysical studies, electrical resistivity data were interpreted using generalized geological information obtained for the areas studied and/or using geological data from areas similar to these study areas. This means, such interpreted VES data gives generalized geological information from their interpreted geoelectric data. However, in this study, we have available borehole logs to serve as control on the interpretation of the corresponding VES data for each site.

Figures 4 (a) - (d) show typical geoelectric sections obtained from the interpreted VES data and the geologic sections derived from the borehole logs collected for some of the investigated VES sites. The analyses of the 22 pairs of the sections obtained for this work suggest that the storage elements for ground water in the south western part of the Middle Niger Basin are mostly the Recent to Tertiary alluvial deposits of sandy clay/clayey sand, lateritic clay and gravelly clay; Nupe Sandstone Group consisting of quartzose gravel, conglomerates, brecciated conglomerate, clayey sandstones, as well as clayey weathered basement and fractured basement rocks of the Precambriam Basement complex. The result of this work revealed that the components of the aquiferous zone existing in the northern and central part of the study area are mainly the Nupe Sandstone Group formations. On the other hand, the weathered basement and fractured basement rocks constitute the aquifers in the southern part of the study area. Specifically, fractured basement rocks predominate the areas around Gbagota, Macha, Bishewa and Ndanaku. The results of the interpretation of the VES data studied suggest that this sedimentary region is underlain by three to five geoelectric layers. Furthermore, the information obtained from the borehole logs and the interpreted VES data results suggest that the thickness of the aquifers varies from 6.10m to 75.10m. The geoelectrically interpreted VES data result indicate that the value of the resistivity of the aquifer range from 4.2 Ω-m to 564.0 Ω-m. The result of this investigation also suggest that the alluvial deposit in the northern part of the study area comprise of aquifers which are associated with low to medium resistivity values of 4.2 Ω-m to 106.72 Ω-m. On the other hand in the southern part of the area studied, it was found that the weathered basement aquifers are characterized by resistivity values in the range of 33.7 Ω-m to 180.6 Ω-m, while resistivity values of 345.7Ω-m to 564.0Ω-m are associated with the aquifers of the fractured basement rocks. The average specific yield capacity value for the aquifer within the sedimentary terrain of the study area was 0.2158 l/s/m while the corresponding value estimated for the basement area aquifers was found to be 0.1799 l/s/m. Hence in agreement with the report of Idornighie and Olorunfemi (1992), this study shows that the aquifers derived from the Nupe Sandstone Group are more productive than the weathered basement aquifers which exist within the southern fringe of the Niger Basin.

In order to have an insight to the groundwater potentials of the study area, a preliminary aquifer resistivity map (fig. 5) was produced from the interpreted VES data results of this work. The map is considered preliminary as a result of the fact that the VES stations used in this work are not uniformly spread across the two LGAs studied. The map is therefore, produced for preliminary deductions pending future detailed work in the project area. The resistivity value of the aquifers at each VES site location was plotted and contoured al 25 Ω-m to give the preliminary resistivity of the aquifer map for the study area of Edu and Patagi LGA (fig. 5). The map was produced in order to delineate the fresh water–bearing areas and the saline water–bearing zone. As shown on the map, the resistivity value of the aquifer is highest (about 225 Ω-m) in the north central part (around Sakpata and Dumaji) and southern part (around Wariku) of the study area. The high resistivity value associated with the aquifers in the northern part is possibly due to the presence of loose sand and sandy formations which corresponds to the local geology of the area (Biwater

Shellabear, 1985). The aquifer thickness values in the northern part have also been determined from the interpreted geoelectric and geologic sections to be quite high. These therefore, suggest that the Nupe Sandstone Group aquifer component around Shonga and Sakpata as well as the weathered/fractured basement aquifers around Wariku and Macha are probably saturated with freshwater-bearing horizons. On the other hand, the less than 25 Ω-m associated with the aquifer in the entire west end of the study area is probably due to the presence of highly weathered and/or clayey nature of the aquifer. It is also most likely that the groundwater in the western part of the Edu and Pategi LGAs is salty (saline) or it contains high content of ferruginised particles derived from the thick laterite cappings which, according to Wigwe (1974) and Offodile (1992), predominate the area. However, information obtained from the borehole logs collected for the well site drilled in the southwestern part of Edu LOA suggest the existence of thick aquifers in these areas and the aquifers possibly lie at moderate depth below the ground. The low resistivity value of the aquifers in the western part of Edu LGA is also most probably due to the high level of saturation of the aquifer in this part of the area studied.

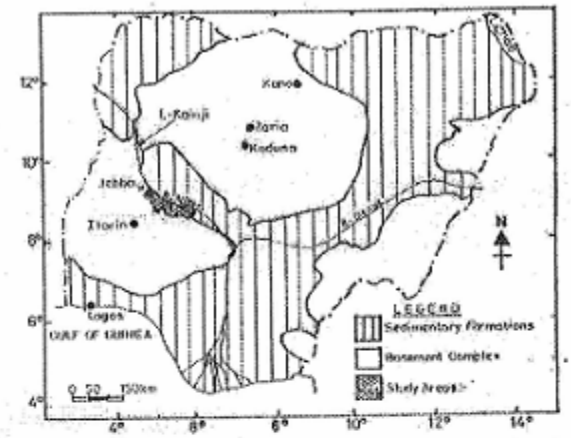


FIG-1: SIMPLIFIED GEOLOGICAL MAP OF NIGERIA SHOWING THE TWO LOCAL GOVERNMENT AREAS STUDIED (Courtesy of GSN)

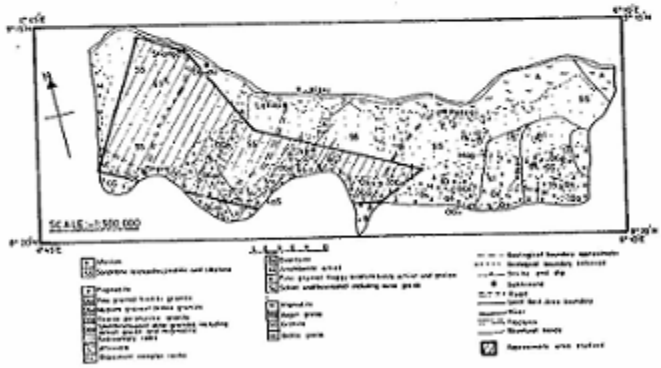


FIGURE 2: GEOLOGICAL MAP OF EDU AND PATEGI LOCAL GOVERRNMENT AREAS OF KWARA STATE (COURTESY G.S.N, ILORIN)

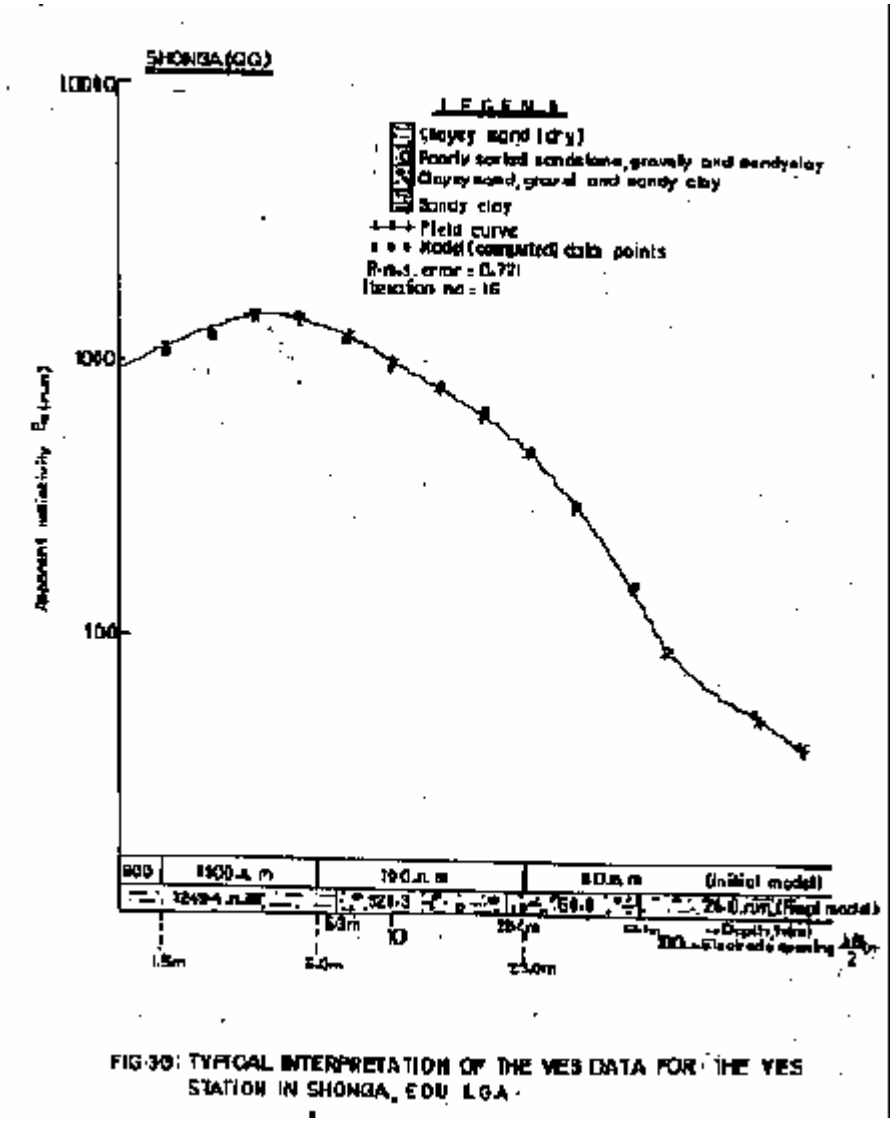


FIG 3B: TYPICAL INTERPRETATION OF THE VES DATA FOR THE VES STATION IN SHONGA, EDU LGA.

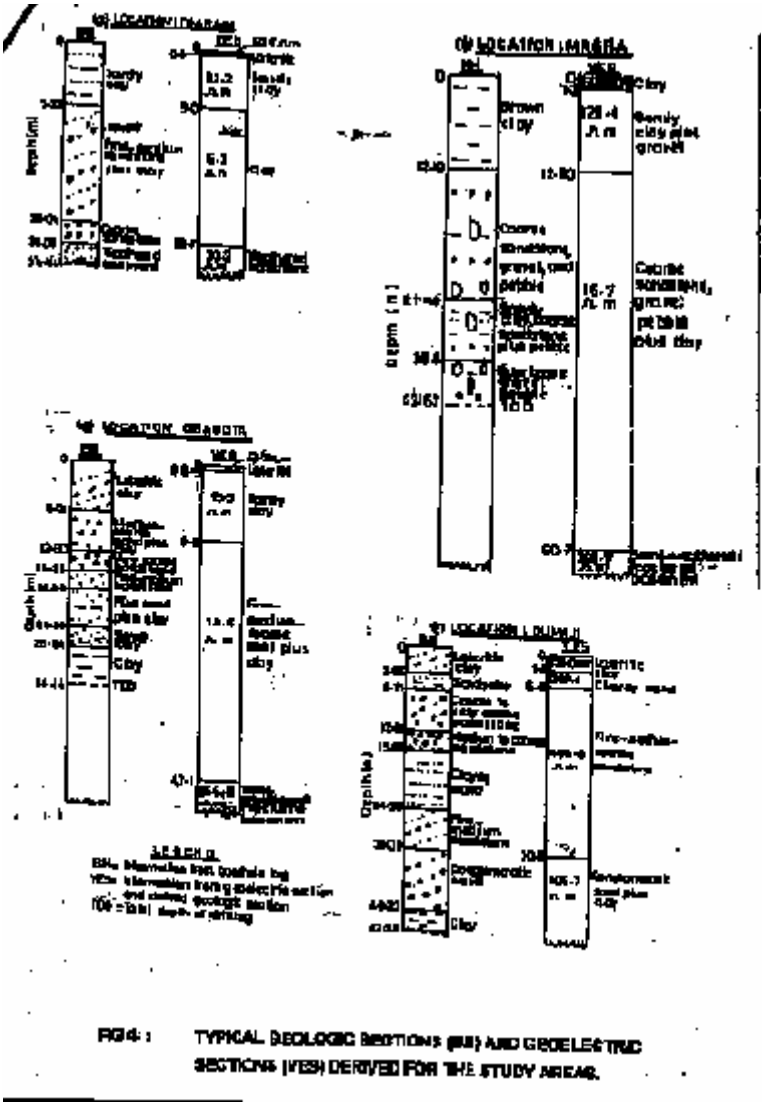
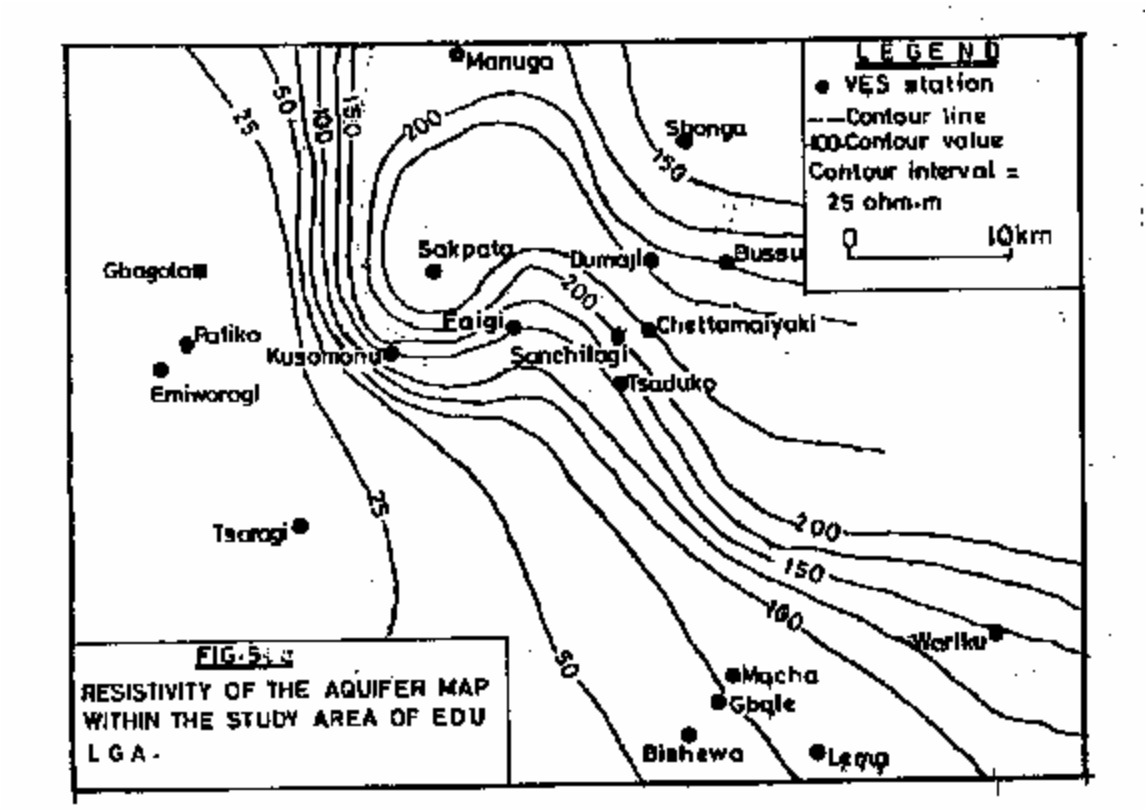


FIG 4 : TYPICAL GEOLOGIC SECTIONS (GS) AND GEOELECTRIC SECTIONS (VES) DERIVED FOR THE STUDY AREAS.



Conclusion

From the interpreted VES data results and analyses of the borehole logs collected for each VES site drilled for productive water wells, the following conclusions could be drawn:

I. For the studied area within the southwestern end of the Niger Basin in Edu and Pategi LGA, the alluvial deposits of sand, sandy clay, clayey sand, clay and gravel as well as the medium-to-coarse sandstones of the Nupe Sandstone Group, and the weathered/fractured basement rocks constitute the main aquifer component in this sedimentary region.

2. The thickness values of the aquifers vary from 6.10m to 58.6m. The aquifer resistivity values range from 4.2Ω-m to 282.5Ω-m for the Nupe Sandstone Group aquifer. The resistivity value of the aquifer derived from the weathered basement rocks vary from 10.3Ω-m to 345.7Ω-m while the resistivity values of the fractured basement aquifers range from 345.7Ω-m to 566.0Ω-m.

3. The preliminary deductions from the resistivity of the aquifer map produced for the study area suggest that the north-central part of Edu LGA and the south-eastern part of Edu LGA, which coincides with the south-western part of Pategi LGA have the best prospects for groundwater production in this region. The area around the western part of the study area, that is, Gbagota, Patiko and Emiworogi have the least groundwater potentials in this sedimentary region.

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Development of Magnetic Hollow Cold Cathode for Ion Source

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Abstract: The research presented in this work focuses on the development of ion source with hollow cold cathodes which supplies low –power and high ion-current density applications. The theoretical and experimental results were used to design a second-generation laboratory model, low-current hollow cathode. Our experiment is to design a hollow cold cathode with two application possibilities. [The Journal Of American Science. 2007;3(4):15-19]. (ISSN: 1545-1003).

Key words: hollow cold cathode, magnetic hollow cathode, ion source, plasma.

1. Introduction

The magnetic hollow cathode is an element for the construction of discharge arrangements for different purposes, which are still functional with relatively low gas pressures.

It can be widely used for the ionization of gas flows, the production of high plasma densities, Magnetron arrangements for thin films deposition (Pessoa at al., 2006), spectra light sources, low-vacuum electron beam sources and gas lasers.

The construction by plasma beams for the material denudation or to the building by cold cathode ion source.

Hollow cathodes discharge, in their various forms, are very suitable in a large variety of applications where high current densities, low cathode-fall voltages, and extended lifetimes are required (Rawlin, at al. 1968, Kirkici, at al. 1995, Fradkin, at al.1970, Ferreira, at al. 1978). They are capable of operation at a fraction of an Ampere up to several hundred Amperes, all in arc discharge.

Although there could be various technical applications of the magnetic hollow cathode, essentially two application possibilities were tested in this work:

- Sputtering plant with magnetic hollow cathode for layer demolition.
- Cold cathode ion source with magnetic hollow cathode.

2. Experiment

Two differently ion source types were developed and their characteristics are examined (Boubetra, 2007). The description of the sources is represented in figures 1 and 2.

COLD CATHODE SOURCE IN CATHODE CIRCUIT

The ion source is similar to a Penning ion source in its structure (Kerkow, at al. 1992). But, the cathode is replaced by the magnetic hollow cathode as shown in Fig.1. A high magnetic field with similar characteristics to that used in Penning ion source is applied to the anode region.

At low pressure the gas enhancement in the hollow cathode is not sufficient for the maintenance of discharge.

In this case, only the pendulum effect of electrons ensures low discharge current of about 1 mA.

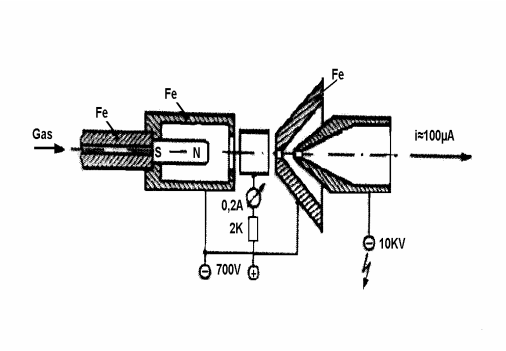

Figure 1: The cold cathode Ion source with magnetic hollow cathode in cathode circuit.

Increasing the pressure to around 0,1Pa leads to the enhancement of gas, and discharge current with the magnitude of about 2 within the range of some 100mA.

Under these pressure conditions, the discharge is better collimated as that observed in Penning-arrangement where discharge is distributed over the entire cathode surface. According to higher unloading pressure the current density is therefore higher in a discharge with magnetic hollow cathode than in the Penning ion source.

Bethge and Baumann (1974) tried to limit discharge by the reduction of the cross sections and by drillings in the magnetic pole with small surface ranges. However the volume of the small hollow cathode is not sufficient to ensure the necessary Gas reinforcement for discharge. The importance of the developed ion source is not only apparent in the production of ions using the oscillating electrons but it raises in directing plasma through an inhomogeneous magnetic field towards the counter cathode so that the appearance of ions opening takes place .

With following reflection the electrons and the plasma become in opposite directions by the magnetic field absent-minded. The pendulum effect of the electrons is small thereby. The ion source reacts very flexibly to pressure changes, i.e. the discharge current can be adjusted over a pressure control between the smallest and the greatest possible river continuously. A substantial advantage is that discharge in the Penning arrangement themselves with vacuums of 0,01Pa still burns.

A special execution of the source for a 350kV-Ion accelerator was tested. Typical ion stream, which were based on the target system of the 350kV-Ion accelerator, are arranged in table.1 for different feed gases.

Table1. Ion current in μA with corresponding gas

Ion Gas	H^+	H_2^+	H_3^+	He^+	C^+	N^+	O^+	H_2O^+	F^+	N_2/CO^+	NO^+	O_2^+	Ar^+
H_2	17	65	22	_	_	_	1	8	16	_	_	_	_
He	0,3	0,4	_	65	_	15	2,5	_	_	16	4,5	_	_
N_2	0,2	0,2	_	_	0,4	15	2,5	2	_	55	20	1,7	2,5
O_2	0,3	_	_	_	0,3	0,4	20	2,5	_	3	2,5	55	0,8
Ar	-	1,7	_	_	_	_	_	_	_	_	_	_	75

The ion current were measured with a hole diameter of about 0,7mm at the output slit of the ion source.

More than 100 μA can be reached for all permanent gases according to the surface increase and enlargement of the output slit.

The use of another design for the magnetic hollow cathode, e.g. as shown in Fig**.** 2, permits to obtain very handy and productively ion source with small dimensions.

This type of particularly ion source should be suitable to produce ions by atomization of difficulty evaporable materials.

By the conversion achievement in the ion source the parts for the magnetic field generation must separate then from the ion source interior. In this case the material in the hollow cathode is sputtered or evaporated by diffusion process into the anode region.

For geometry reasons, the largest part of the steam cloud is condensed on the cathode. While this electrode is also met by ions a sputtering process take place. On the other hand it contributes to the increase of the density within the plasma range. A substantial disadvantage of the ion source is that the ion emission current density is determined by the internal field gradient between plasma and output. It determines the number of the emitted ions. Since the magnetic field hardly affects the ion movement, one must steer the ion emission from the plasma by suitable deformation of the output. In addition, the atomization of the screen material at the output limits the life span of the ion source.

Cold Cathode Ion Source in Anode Circuit

In anode circuit the face with the output of the ions is on anode potential in accordance with Figure 2.

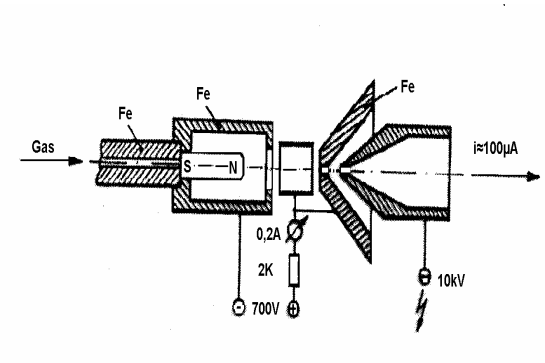

Figure 2. A cold cathode Ion source with magnetic hollow cathode in anode circuit. Held together of the magnetic field in the anode region. Only the anode drop as characteristic of the anode-lateral polarization of the plasma separate the ions at the output slit.

The output slit serves as gas throttle for the neutral gas atom. When it stays before the magnetic disk likewise anode potential, then a part of the plasma is transferred of the ion source by the magnetic field into the outside space.

The ions were aspired and by the electrode and the emission amperage of ion source depends then on the plasma volume, which is seized by the suction electrode, and on the charge carrier density.

Therefore the arrangement of electrode geometry is crucial with this switching type for the ion yield of the ion source. Technically these criteria could be considered with the testing of the ion source in anode circuit only insufficiently, because the cover plate for constructional reasons at cathode potential and be switched only one sheet metal screen with the output had to be put as anode could.

Nevertheless ion current could be obtained, which were comparable with the source in cathode circuit. The advantage of the ion source in anode circuit consists above all of the fact that one can vary the suction conditions independently of the fuel conditions. During the cathode circuit the plasma is separate by an

outer zone from the output. The thickness of this outer zone depends on the plasma density, i.e. on the discharge current and on the magnetic focusing conditions. Also still another small portion of a cathode-falls becomes effective; however the source of discharge is the magnetic hollow cathode and not the back plate electrode as with a Penning source. Since the potential difference between plasma and back plate electrode amounts to for instance 300V (Burning tension), the distance between plasma and back plate electrode 1 to 2mm amounts to, field strengths of 10^3V/cm arise, which affects however diverging the emission direction of the ions. In the anode circuit geometry of the suction field is determined by the shape of the suction electrode and the output and can contribute to the focusing of the ion beam. Since the energy distribution of the ions lies within the range of 10eV, a better jet forming should be possible in anode circuit. A disadvantage of the ion source in anode circuit is however that the ion source must be operated with higher gas pressure than in cathode circuit and the combustion behaviour of the ignition characteristic of the magnetic hollow cathode is determined. This disadvantage becomes balanced by the fact that the atomization is small only in the range of the output and can only occur, if negative ions are present in discharge.

Conclusion

We have proposed a simple technological construction of cold cathode in order to compare it with glow cathode or HF-ion sources. The disadvantage is however the high gas pressure for an discharge and the smaller ion yield. By using a magnetic hollow cathode it is well possible to realize an homogeneous transfer of discharge by inhomogeneous magnetic fields within the plasma range to the anode. The plasma follows the magnetic field lines in its density and direction.

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Fertility Status Under Land use Types on Soils of Similar Lithology

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Abstract: Fertility status under six landuse types (fallow, cassava, plantain, cocoyam, bamboo, and oil palm) in Mbaitoli and (Oil palm, Industrial site, cocoyam, bamboo, plantain and cassava) in Ikeduru were evaluated. Triplicate soil samples (0 -15cm) collected along a transverse on each landuse were characterized. Soils were predominantly sandy. Except fallow in Mbaitoli and oil palm, plantain and cocoyam in Ikeduru, silt/clay ratios of all landuses were below unity indicating high degree of weatherability. In both locations (Mbaitoli and ikeduru), bulk density was significantly lower in bamboo than others due to high organic matter and low soil disturbance. Wide variability in soil pH, organic matter, P, N, C/N ratio, CEC, Fe and Zn occurred under various landuses, with performance in cocoyam being better than others for both Mbaitoli and Ikeduru. Generally, soil properties were better in Ikeduru than Mbaitoli indicating that management requirement for sustained crop production in the later will be lower than the former. [The Journal Of American Science. 2007;3(4):20-29]. (ISSN: 1545-1003).

Introduction

Nigeria has an estimated land mass of about 923 768 sq km of which 75 488 square kilometers are in Southeastern Nigeria (Enwezor, et al: 1990, Esu, 2005, Anon, 2005). Several activities take place on the land. The purpose for which a tract of land is used constitutes its landuse (Anon, 2005). According to Vink (1975), landuse is any permanent or cyclic human interactions to satisfy human needs from complex natural and artificial resources which constitute land. It is a resultant interplay of available land resources with cultural, social and economic conditions of the past and present development when two or more landuse types occur on the same soil (Akamigbo and Asadu 2001).

Landuse has been categorized into major kind; as rain fed agriculture, grassland, fishpond, forestry, grazing and tourism and into primary or compound kinds in which more than one kind of landuse is practiced within an area (FAO, 2002). Of an estimated agricultural land area totaling 700,000sq km in Nigeria, about 300,000, 27,000, 40,000 and 150,000 sq kms are currently used for annual crops, perennial crops, permanent pasture and forestry respectively (Anon 2005).

Landuse affects soil fertility and productivity. These manifests as changes in soil properties such as nutrient content (N, P, K, Ca, Mg, S etc), pH, organic matter, CEC, structure etc (Aluko and Fagbenro 2000, Akinrinde and Obigbesan 2000, Akamigbo and Asadu 2001). For instance Aluko and Fagbenro (2000) observed increased pH and organic matter for soils under Gmelina aborea than those under Pinus canaborea, Treculia Africana, agro forestry and fallow. They also observed increased P in fallow compared to other land uses. Furthermore, Akamigbo and Asadu (2001) reported marked changes in morphological, physical and chemical properties which resulted to accelerated pedogenic processes and a decline in fertility of soil under traditional than forest landuse. It has been observed that as the fertility of the soil declines, soil structure weakens and the soil becomes susceptible to erosion (Adetunji, 2005).

Agricultural sustainability requires a periodic evaluation of soil fertility status. This is important in understanding factors which impose serious constraints to increased crop production under different landuse types and for adoption of suitable land management practices. Information so generated could also be useful in adjusting present landuse types or in the development of appropriate landuse policy for a given area. This is particularly important in southeastern Nigeria, where demographic factors, poor land management and inherent low soil fertility (Enwezor, et al; 1990) under different landuses often result in poor crop yields. The main objective of this study was therefore to evaluate the fertility statues under landuse types of soils over similar lithology in Imo state.

Materials and Methods

Soil Sampling and Site Description

Triphcate surface (0 – 15 cm) soil samples were collected from different landuse types (Table 1) at two locations (Mbaitoli and Ikeduru local government areas of Imo State Nigeria) in the rainforest agro ecological zone of southeastern

Nigeria. Mbaitoli LGA lies between latitude 5^{o} $29^{/}$ N and longitude 7^{o} $07^{/}$ E while Ikeduru is located between latitude 5^{o} $27^{/}$ N and longitude 7^{o} $07^{/}$ E. The two locations are characterized by a bimodal rainfall pattern that peaks in the months of June and September with a short dry spell, the August break in the month of August. Mean annual rainfall ranges between 1750 -2000 mm. Mean annual temperature of 26.5 - 27.5^{oc} and a mean relative humidity that varies between 71.6 – 85.6%. Soil types are UItisol (USDA soil classification) derived from costal plain sand (Enwezor, et al: 1990).

Sample Preparation and Laboratory Analysis.

Soil samples were air derived under laboratory conditions for seven days, sieved using 2mm diameter sieve and the fine earth fractions analyzed for selected physical and chemical properties. Physical properties of soils from Mbaitoli and Ikeduru are presented in Tables 2 and 3 respectively while the chemical properties of soils from the locations are shown in Tables 4 and 5 respectively. Particle size (Gee and Bauder 1986), pH in 1:2.5 (solvent/solute) ratio (Maclean1982), available P using Bray II extractant (Olsen and Sommers 1982), exchangeable bases using NH_4 OAC extractant (Thomas 1982), exchangeable acidity in 1N KCL solution (IITA 1979); CEC obtained as a summation of exchangeable bases and acidity, total nitrogen (Bremner and Malveney 1982); organic carbon (Nelson and Sommers 1982), organic matter by multiplication of organic carbon with a factor of 1.72, Fe and Zn by AAS (Page et al 1882) and bulk density in undisturbed cone (Blake and Hartage 1986).

Table 1. Landuse Types in Mbaitoli and Ikeduru LGA

Sample No.	Town	Landuse type	Remarks
A. Mbaitoli			
M_1	Ubomiri	Fallow	$1^{1}/2$ year fallow
M_2	Ogwa	Cassava	8 month's old cassava farm
M3	Mbieri	Oil Palm	Over 40yrs oil palm plantation interspersed with cassava
M4	Mbieri	Plantain	5yrs with kitchen waste as nutrient source
M5	Orodo	Cocoyam	9 months mulched with palm fronds
M6	Ogbaku	Bamboo	10yrs old and harvested every 2yrs.
B. Ikeduru			
IK 1	Inyishi	Industrial	Aluminium extrusion site
IK2	Iho	Oil palm	Over 20yrs old interspersed with Cocoyam and cassava
IK3	Akabo	Plantain	5 yrs old with household waste as nutrient source
IK4	Atta	Cassava	A year old with NPK fertilization
IK5	Iho	Cocoyam	7 months and mulched with palm fronds
IK6	Iho	Bamboo	Recently harvested for roofing.

Table 2. Physical properties of soils under varying landuse in Mbaitoli

Sample No.	Landuse Type	Sand	Silt	Clay	Silt/Clay Ratio	Bulk density g/cc	Textural Class
		--------- g/kg ------					
M1	Fallow	882	84	34	2.5	1.54	Sandy loam
M2	Cassava	762	80	158	0.5	1.35	Sandy loam
M3	Oil palm	782	92	126	0.7	1.24	Loamy sand
M4	Plantain	902	40	58	0.7	1.47	Sand
M5	Cocoyam	802	93	105	0.9	1.42	Loamy sand
M6	Bamboo	842	60	98	0.6	0.86	Sand
L.s.d (0.05)			9.60	8.4	7.37	4.32	0.06
CV			0.6	3.2	4.2	3.4	2.6

Table 3. Physical Properties of Soils under varying landuse in Ikeduru

Sample No.	Landuse Type	Sand	Silt	Clay	Silt/Clay Ratio	Bulk density g/cc	Textural Class
		--------- g/kg --------					
Ik1	Industrial	822	33	45	0.3	1.59	Loamy sand
Ik2	Oil Palm	900	70	30	2.3	1.35	Sand
Ik3	Plantain	840	100	60	1.7	1.41	Sandy loam
Ik4	Cassava	760	40	200	0.2	1.31	Sandy loam
Ik5	Cocoyam	772	123	105	1.2	1.45	Loamy sand
M6	Bamboo	802	60	158	0.3	1.16	Sandy loam
L.s.d (0.05)			31.71	7.63	11.25	0.20	0.10
CV			2.1	0.2	5.3	11.02	3.9

Table 4. Chemical Properties of Soils under varying Landuse Types in Mbaitoli

Landuse Type	pH H_20 1 N KCL	pH	OM gkg^{-1}	TN %	C/N	P mg g^{-1}	Ca	Mg	K	H	CEC cmol kg^{-1}	Fe	Zn m g kg^{-1}
Fallow	6.50	6.48	27.9	0.09	18.0	9.34	0.34	0.38	0.02	0.38	1.43	6.61	6.53
Cassava	4.48	4.60	21.2	0.08	15.3	2.87	0.15	0.15	0.02	1.05	2.25	6.82	3.37
Oil palm	5.20	4.80	26.1	0.09	16.9	5.89	0.25	0.80	0.01	0.95	2.81	7. 66	3.41
Plantain	7.79	4.60	33.2	0.13	14.9	4.97	0.67	0.83	0.02	0.28	2.05	6.83	14.0
Cocoyam	8.31	7.36	23.0	0.09	14.9	5.89	1.33	1.32	0.02	0.40	3.42	7.25	11.4
Bamboo	8.31	4.74	18.6	0.07	15.4	1.08	0.18	0.22	0.02	0.58	1.54	7.51	2.46
L.s.d	0.16	0.16	0.67	0.32	1.51	0.17	0.04	0.04	0.01	0.14	0.25	4.32	0.32
CV	1.4	1.6	1.5	19.0	5.2	1.9	5.1	4.4	34.5	12.4	6.2	3.4	19.0

OM = Organic matter, TN = Total nitrogen, C/N = Carbon/ nitrogen, CEC = Cation exchange capacity.

Table 5. Chemical Properties of Soils under varying landuse in Ikeduru

Landuse Type	pH H_2O	pH 1NKCL	OM gkg^{-1}	TN %	C/N	P $mg\ g^{-1}$	Ca ----	Mg cmol	K kg^{-1}---	H ------	CEC	Fe --	Zn mg kg^{-1} --
Industrial	5.31	4.90	28.96	0.1	16.42	4.55	0.21	0.17	0.03	0.33	0.98	6.82	3.8
Oil Palm	5.4	5.37	16.55	0.06	15.46	5.62	0.23	0.26	0.02	0.38	1.19	6.80	2.56
Plantain	7.48	6.94	31.65	0.12	15.33	2.66	0.19	0.72	0.02	0.33	2.14	7.20	13.19
Cassava	6.80	5.51	14.48	0.06	13.66	3.11	0.27	0.31	0.03	0.26	1.31	7.42	2.90
Cocoyam	8.50	7.90	31.72	0.11	15.55	3.18	1.99	1.97	0.03	0.43	4.76	6.83	12.19
Bamboo	5.50	4.61	14.83	0.05	16.80	6.30	0.15	0.21	0.03	0.73	1.81	7.05	3.89
L.s.d(0.05)	0.03	0.03	0.91	0.29	0.05	0.03	0.02	0.02	0.02	0.02	0.02	0.23	0.18
CV	0.3	0.3	5.3	19.7	0.2	0.4	1.8	2.3	38.1	4.0	0.7	2.0	1.6

OM = Organic matter, TN = Total nitrogen, C/N = Carbon/ nitrogen, CEC = Cation exchange capacity.

Results and Decisions

Soil texture, Silt/clay ratio and Bulk density

Soils were predominantly sandy with about 75% of the land use being sandy loam in both Mbaitoli (Table 2) and Ikeduru (Table 3). The sandy nature of the soils reflects the parent materials from which they were formed which is coastal plain sand (Enwezor, et al: 1990, Uzoho 2005). Silt/clay ratio varied between 0.6 – 2.5 (CV =3.4%) with fallow land use being significantly higher than others in Mbaitoli (Table 2). The range was 0.20 – 2.3 (CV = 11.02%) being significantly lower in oil palm, bamboo and industrial site than the other landuses in Ikeduru. (Table 3). Averaged over locations, values for Mbaitoli (1.0) and Ikeduru (1.02) were similar (Table 6) probably due to the fact that the soils were of similar origin. It has been reported that Silt/clay ratios less than unity indicate low values, signifying that the soils are pedogenically ferraltic in nature (Essoka and Esu 2000). Comparing soils under the various land uses, one would conclude that the soils are highly weathered and pedologically mature due to the low silt content (Ahn 1993), and with the degree of weathering being higher in Mbaitoli than Ikeduru. Nwaka and Kwari (2000) observed that in sandy soils, high silt/clay ratio
may be related to the coarse texture or resistant skeletal composition of the parent material and youthfulness of the profile. Values of silt/clay ratio reported were low, confirming high weatherability of the soils and increased pedegenesis under land uses with silt/clay ratio less than unity

Bulk densities ranged between 0.86 - 1.54 g/cc (CV =2.6%).and 1.16 – 1.59 g/cc (CV = 3.9%) in Mbaitoli and Ikeduru respectively. In Mbaitoli fallow landuse was significantly higher than others while in Ikeduru the value was higher in industrial site than others. Averaged across locations, bulk density was higher in Ikeduru than Mbaitoli (Table 6). High bulk densities in fallow (Mbaitoli) and industrial site (Ikeduru) could be due to poor vegetal cover and soil compaction due to raindrop impact (Lal 1990, Brady and Weil 1990, Ogban et al., 2000, Babalola et al; 2000, Isirimah et al; 2003).

pH, Organic matter, N and C/N ratio

Soil pH varied as 6.50, 4.48, 5.20, 7.79, 8.31and 8.31 for fallow, cassava, oil palm, plantain, cocoyam and bamboo respectively in Mbaitoli (Table 4) and 5.31, 5.40, 7.48, 6.80, 8.50 and 5.50 for

industrial site, oil palm, plantain, cassava, cocoyam and bamboo respectively in Ikeduru (Table 5) respectively. This showed that soils under cassava and oil palm in Mbaitoli and those under Industrial site, oil palm and bamboo in Ikeduru .were acidic. Under these landuses, crop production could be limited due to aluminium toxicity. It has been reported that aluminium toxicity occur in soils with pH value of about 5.5 and increases in intensity as pH decreases (Opara – Nadi 1988, Ernani et al; 2002.

Table 6. Means of Selected Properties of Landuse Types in Mbaitoli and Ikeduru

Parameters	MBAITOLI	IKEDURU
Texture	Sandy loam	Sandy loam
pH (H_20)	5.91	6.50
Silt / Clay ratio	1.0	1.0
Bulk density (g/cc)	1.31	1.36
Total Nitrogen (gkg^{-1})	0.09	0.08
Organic Matter (gkg^{-1})	24.98	23.03
Carbon/Nitrogen ratio	15.89	11.54
Available P (mg kg-1)	5.01	3.40
CEC (cmol kg^{-1})	2.25	1.34
Fe (g kg^{-1})	7.03	7.02
Zn (g kg^{-1})	8.45	2.50

However, being tolerant of soil acidity up to pH of 5.59, cassava and oil palm production may not be seriously affected (FMANR 1990). Generally, more than 60% of landuse types in both Mbaitoli and Ikeduru had pH values higher than 6.0 and attributed to complexion of soluble aluminum from organic matter decomposition (Aluko and Fagbenro 2000, Lillie 1999, Brady and Weil 1999). Averaged over locations, soil pH was lower in Mbaitoli than Ikeduru (Table 6). Optimum pH for most agricultural crops fall between 6.0 and 7.0 because nutrients are more available at pH about 6.5 (Wong, et al; 2001). It follows that, for the more acidic Mbaitoli soils, oil palm and cassava production are well suited while liming could be effective in reducing acidity for the production of other crops (Ernani et al; 2002, White et al; 2006).

Values of soil organic matter in Mbaitoli were 33.2, 27.9, 26.1, 23.0, 21.2 and 18.6 g/kg being a decreasing order of Plantain > fallow > oil palm > cocoyam > cassava > bamboo and a variability of 1.5%. Differences amongst the various land uses were significant. Values for Ikeduru were 31.72, 31.65, 28.96, 16.55, 14.83 and 14.48 g/kg and a decreasing trend of Cocoyam > plantain > industrial > oil palm > bamboo > cassava with a variability of 5.3% .Differences between cocoyam and plantain and between bamboo and cassava were not significant but differed significantly with the other landuses. Averaged over locations, organic matter was higher in Mbaitoli than Ikeduru (Table 6). When compared with critical values of 15 -20 g/kg (1.5 -2.0%) for tropical soils (Enwezor, et al; 1990), only soil under bamboo in Mbaitoli and oil palm, bamboo and cassava in Ikeduru fell below critical levels. Mean organic matter varied between 23.03 - 24.98g/kg in Mbaitoli and Ikeduru (Table 6). This is low when compared with critical values of 25.0, 30.0 and 35.5g/kg for West, North and Eastern Nigeria respectively (Akinrinde and Obigbesan 2000). It is also low when compared with a value of 30.0g/kg suggested as level to which

response to N fertilization is not expected (Agboola 1973). The general low levels could be attributed to management practices involving high burning and to continuous farming as well as a reduction in the fallow period (Akinrinde and Obigbesan 2000).

Total N followed a similar trend as soil organic matter since organic nitrogen constitute the bulk of total N for tropical soils (Noma et al; 2005). It has reported that fertility of tropical soils depends on their organic matter content (Enwezor et al; 1990). Total N for all land uses in both Mbaitoli and Ikeduru were below 0.15%, the critical value for tropical soils (Enwezor, et al; 1990), and indicates high N deficiencies. It has been observed that the main cause of N deficiency in tropical soils is intense leaching and erosion due to the high tropical rainfall (White and Reddy 1999, Isirimah et al; 2003). This low N level signifies responses to N fertilization.

Except oil palm, C/N ratio was significantly higher in Fallow than other land uses in Mbaitoli (Table 4). Variation in Ikeduru was significant amongst landuse types being higher in Bamboo than others. C/N ratio relates to soil organic matter decomposition and nitrogen mineralization. It has been reported that net N mineralization occurs at C/N ratio below 30:1 (Catherine et al 1992). However, Ma et al; (1999) has observed, N mineralization in soils with higher C/N ratios and thus concluded a no relationship between C/N ratio and N mineralization. Averaged over location, C/N ratio did not significantly vary between Mbaitoli and Ikeduru.

Exchangeable Cations (Ca, Mg, K and H) and CEC

Ranges and variability in exchangeable Ca, Mg, K and H were 0.13 – 1.33 (5.1%), 0.15 – 1.32 (4.4%), 0.1 – 0.2 (34.5%) and 0.28 – 1.5 Cmol/Kg (12.4%) respectively in Mbaitoli (Table 4) and 0.19 – 1.99 (1.8%), 0.17 – 1.97 (2.3%), 0.02 – 0.03 (38.1%) and 0.26 – 0.73 Cmol/Kg (4.0%) respectively in Ikeduru (Table 5). Variation amongst landuse types was significant for exchangeable Mg and H, non significant for exchangeable K and non significant between bamboo and cassava for exchangeable Ca in Mbaitoli. In Ikeduru variations in Mg and K were similar amongst landuse as in Mbaitoli but non significant between Industrial, oil palm and plantain for Ca and between industrial and plantain for H. Critical values of soil nutrients have been reported by various workers. For instance Adeoye and Agboola (1984) have reported critical values of 2.0, 0.4 and 0.20 cmol/kg for Ca, Mg and K respectively. When compared with values obtained for landuse types in Mbaitoli and Ikeduru, shows low values and thus deficiencies of the nutrients in the soils studied. Compared with critical k values of 0.16 and 0.20 cmol/kg ((White and Reddy 1999, Isirimah et al; 2003). 0.10 cmol/kg (Ekpete 1972) and 0.18 - 0.20 cmol/kg soil (Obigbesan and Agboola 1974), all the soils studied still showed great deficiencies. Also using critical Mg value of 1.04 cmol/kg proposed for western Nigerian soils (Agboola and Corey 1976), more than 83% of soils under various landuse in both Mbaitoli and Ikeduru (exception being soil under cocoyam) showed deficiencies and will respond to Mg fertilization. Furthermore, when compared with 0.15-0.42 cmol/kg critical Mg values suggested by Lombin (1974), Mg deficiency may not be a problem for the soils studied. Low values of Ca, Mg and K, have however been reported for most Nigerian soils (Akinirinde and Obigbesan 2000) and could be attributed to leaching losses by the high tropical rain fall as well as low content in the parent rock. Exchangeable H varied with soil acidity with cassava and bamboo having highest values in Mbaitoli and Ikeduru respectively.

Variation in soil CEC was an increasing trend of fallow (1.43cmol/kg),bamboo 1.54 (cmol/kg), plantain (2.05cmol/kg), cassava (2.25 cmol/kg), oil palm (2.81 cmol/kg) and cocoyam (3.42cmol/kg) in Mbaitoli and industrial site (0.98 cmol/kg), oil palm (1.19 cmol/kg), cassava (1.31cmol/kg), bamboo (1.81 cmol/kg), plantain (2.14 cmol/kg) and cocoyam (4.76 cmol/kg) in Ikeduru. landuses. Soil CEC has been classified as low (<6cmol/kg-1), medium (6-12cmol/kg-1) and high (>12cmol/kg-1) (Adepetu et al 1979). On the basis of this classification, all the soils studied fell within the low range since their values were below 5 Cmol/kg. It has been reported that low to medium CEC value of tropical soils is due to the dominance of kaolinitic clays in the fine earth fractions (Ojanuga and Awojuola 1981). Most workers have observed that CEC of tropical soils is related to their organic matter content (Aluko and Oguntala 1997, Noma et al; 2005). This is true of the soils studied especially those under different land use type in Ikeduru. Also as the pH increased, CEC also increased

Available P, Fe and Zn

In Tables 4 and 5 are shown values of available P, Fe and Zn in Mbaitoli and Ikeduru respectively. Available P ranged from 1.09 to 9.40 mg kg-1 with the least and largest values being in bamboo and plantain respectively in Mbaitoli while the range was 2.66 to 6.30 mg kg-1 with a reverse least and largest values of plantain and bamboo respectively in Ikeduru. Averaged over locations, the range was 3.40 to 5.01mg kg-1 with Mbaitoli being higher than Ikeduru. A critical P range of 8 -12 mg kg-1 P has been reported for tropical soils (Enwezor et al 1990). This showed that with the exception of fallow soil in Mbaitoli all other land use types in the two locations are P deficient. Several workers (Busari et al 2005, Uzoho and Oti 2005, Jubrin et al; 2000, Bubba et al; 2003, Aluko et al 2000) have reported high P deficiency for tropical soils. Cause of the deficiencies has been attributed to high weatherability of the soils, presence of koalinitic clay as the dominant mineral, leaching by intense rainfall and adsorption reaction by soil constituents (Bubba et al; 2003,). P statues under various landuse types showed an increasing trend with soil organic matter and CEC and a decreasing trend with clay, Fe and Zn. The trend with soil pH was irregular.

Available Fe was significantly higher in oil palm than other landuses with variation being an increasing order of fallow < cassava < plantain < cocoyam < bamboo < oil palm and a coefficient of variability of 3.4% in Mbaitoli. Cassava varied significantly with other landuses, being a decreasing trend of cassava > plantain > bamboo > cocoyam > industrial > oil palm and a coefficient of variability of 2.0% in Ikeduru. Averaged over locations, Mbaitoli was higher than Ikeduru. In both locations, Fe contents under various landuses were beyond critical available level of 4.5 mg kg-1 (Kparmwang et al; 2000) or 2.5 – 5.0 (Sims and Johnson 1991).This indicates that Fe deficiency is not likely a problem as have been reported by others for most acid soils of warm humid zones of Nigeria (Kparmwang and Malgwi 1997, Kparmwang et al; 2000).

Variations in available Zn were 2.46, 3.37, 3.41, 6.53, 11.40 and 14.00 mg kg-1 for bamboo, cassava, oil palm, fallow, cocoyam and plantain respectively in Mbaitoli. It was 2.56, 2.90, 3.80, 3.89, 12.19 and 13.19 mg kg-1 for oil palm, cassava, industrial, bamboo, cocoyam and plantain respectively for Ikeduru. Plantain and cocoyam did not differ significantly but were significantly higher than other land uses in Mbaitoli while plantain was significantly higher than others in Ikeduru. As for Fe, available Zn was better in Mbaitoli than Ikeduru. Using critical available Zn levels of 0.8 mg kg -1 (Kparmwang 2000) or critical range of 0.2 – 2.0 mg kg-1 zinc deficiency did not appear a problem in the soils as have been reported for some sandstone and shale in Benue valley (Kparmwang 2000). However zinc deficiency has been reported for soils of coastal plain sand (Udo and 1979) and on basaltic soils of Northern Guinea savanna (Kparmwang et al 1995).

Conclusion

Landuse had no effect on soil texture being predominantly sandy, a reflection of the parent material (coastal plain sand). Silt/clay ratio in less than 40% of the land use types (Fallow in Mbaitoli, oil palm, plantain and cocoyam in Ikeduru) was more than unity indicating high degree of weathering in more than 60% of the landuses. Bulk density was significantly higher under fallow and industrial site than other landuses in Mbaitoli and Ikeduru respectively, probably due to higher organic matter content and lesser compaction. Soil chemical properties (pH, organic matter, N, C/N ratio, exchangeable Cations (Ca, Mg, K and H), CEC available P, Fe and Zn) varied widely with landuse, being better under cocoyam than other landuses in Mbaitoli and Ikeduru. Generally, landuse in Ikeduru had better properties than Mbaitoli indicating that for sustained crop production lesser inputs will be required in Ikeduru than Mbaitoli.

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The synthesis and properties of Fe_3O_4 / Sodium acetate / CMS ternary nanocomposites as electrorheological fluid

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Abstract: In this paper have been studied a ternary nanocomposite as rheological fluid based on Carboxymethyl starch and sodium acetate with nano metallic oxides. These nano composite was prepared by the two – step composite method .Firstly, the polar sodium acetate compound was directly intercalated into the interlayer of nano ferric oxideand then the intercalated complex was composite with CMS by the solution method. The composites, thus synthesized have been characterized by Fourier transfer infrared (FTIR) spectrophotometer and X-ray diffraction. The morphology of these composites was studied by scanning electron microscopy. [The Journal Of American Science. 2007;3(4):30-34]. (ISSN: 1545-1003).

Keywords: electrorheological fluid,ternarysystem , nanocomposite, CMS,

Introduction

Electrorheological materials are typical suspensions composed of micrometer size particles and dielectric liquids[1]. The material parameters of these nanocomposites such as viscosity, yield stress and shear modulus can change obviousely and reversibly upon the application of an external electric field[2]. These characteristics find practical applications in many fields[3,4].

Fe_3O_4 is a mineral as nano particles (10-12 nm).This material is as guest species in sodium acetate(CH_3COONa) layers. Only a limited number of polar guest species, such as N-methylformamide (NMF) and dimethylsulfoxide (DMSO), can directly be intercalated[5,6]. Because of small particle size and intercalation properties, Fe_3O_4 /organic nanocomposites exhibit noval hybrid synergetic properties derived from two components[7]. Nanocomposites have become the subject of considerable interests for the design of high performance engineering materials with enhanced stiffness, strength, two dimensional stability, mechanic, magnetic, thermal, electric and optical characteristics. The choice of intercalation Fe_3O_4 with CH_3COONa is aimed at modifying the dielectric and polarization properties of Fe_3O_4, so as to improve its electrorheological activity, reduce cost and attain the high cost performace[8,9]. As opposed to starch, CMS is a cold water-soluble starch that can form a transparent solution in water. CMS, as an anionic polyelectrolyte[10].

In this study, The Fe_3O_4/ CH_3COONa/CMS nanocomposite is fabricated according to the physical and chemical design of the electrorheological material.The polar compound (CH_3COONa) is directly intercalated into the interlayer of ferric oxide,and then the intercalated complex is interacted with CMS by solution method[11]. The dielectric and conductivity properties of ternary nanocomposite are improved enormously, which results in the strength of the particle polarization and a large enhancement of the electrorheological effect[12].

The experiment results show that by the design and control of the molecular chemical structure, the physical design for dielectic properties isachieved and thus the characterization of ternary nanocomposite is optimized[13].

Experimental Materials

Nano particles of Fe_3O_4 were purchased from nanotechnology center of baku state university. The particles have an average of 10-12 nm. The sodium acetate sample employed in this work was obtained from merck chemical company and used to prepare the nanocomposites without further purification. The cornstarch, sodium hydroxide and chloroacetic acid was purchased from Merck company.

Instruments

The images of nanoparticles were investigated using Philips XL30 scanning electron microscope.The Fourier transfer infrared(FTIR, Bruker) spectroscopy was used to identified the polymer on the Fe_3O_4 nano particles surface.Spectra were obtained in the wave number range of 400-4000 cm^{-1}. Spectra of the nanocomposite were recorded from KBr in 1:10 (wt/wt) ratio. Powder X-ray diffraction (XRD) patterns were obtained with a Phillips diffractometer using CuKα radiation with a scanning rate of 2 min^{-1}.

Preparation of carboxymethyl starch (CMS).

Firstly, the 0.5 g cornstarch and 120 ml 2-propanol were placed in a 500 ml vessel and stirred for 2 h. The 5 g sodium hydroxide was added and reacted for 1 h at 78-80˚C. After that, the 10 g chloroacetic acid was added to the vessel and stirred for another 2 h at 50˚C. The product was filtered and washed several times with ethanol, then dried under vacuum. The resulting carboxymethyl starch (CMS) was crused in a mortar [degree of substitution (DS) = 0.49][14].

Preparation of Fe_3O_4 / sodium acetate intercalate

Fe_3O_4 (3 g) was dispersed in 40 ml ethanol and stirred for 3 h . Then 2.25 g sodium acetate (mass ratio Fe_3O_4: CH3COONa is 1: 0.75) were added drop by drop into the Fe_3O_4 suspension. When sodium acetate solution was dropped , the temperature was increased to 50 ˚C for the purpose of evaporation of ethanol . the sample was sealed in a weighting bottle and placed in an oven for 14 h at 80 ˚C and the resulting material was got.

Preparation of Fe_3O_4/ sodium acetate/ carboxymethyl starch nanocomposite.

1.8 g CMS and 50ml distilled water were mixed and stirred for 10 h in a 100 ml vessel , then the appropriate amount of Fe_3O_4/ sodium acetate intercalation was added slowly into the vessel and stirred for 12 h at room temperature . After approximately 180 min, the product was sprayed into a liquid nitrogen bath cooled down to 77° K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze-drying process, the products are dried by a sublimation of the water component in an iced solution. The ternary nanocomposite was crused in a mortar.

Results and discussion

Scanning electron micrography(SEM).

SEM of Fe_3O_4/ CH_3COONa/CMS nanocomposite synthesized by chemical oxidative is shown in Figure 1. Fe_3O_4/ CH_3COONa/CMS nanocomposite is very sensitive to the temperature. Due to the intraction electron and sample. Scanning electron micrography images were obtains from a diluted solution of the nanocomposite particle. The white spots are Fe_3O_4 and CH_3COONa nano particles. The SEM image shows the presence of spherical Fe_3O_4 particles in CMS matrix, which are homogenenously distributed throughout the composites ,which is also confirmed from XRD studies.

X-ray diffraction

The crystallinity of the formed composites was followed with X-Ray diffraction(XRD) as s function of weight percent inorganic component. Figure 2 shows X-ray diffraction pattern of Fe_3O_4/CH_3COONa/CMS nanocomposite.Diffraction of Fe_3O_4/ CH_3COONa/CMS nanocomposite have a very intense, sharp and narrow peak at about $2\Theta = 8.35$ ˚, which is a characteristic peak of nanocomposite . We can observe that the X-ray diffraction pattern of the original Fe_3O_4 modifies dramatically.

Fourier transfer Infrared spectra

Figure 3-a shows the FT-IR spectrum of carboxymethyl starch (CMS), where the % of transmittance is plotted as a function of wave number (cm^{-1}). The wide peak around 3411 cm^{-1} is attributing to the O-H stretching vibrations of CMS.The peaks at 1597 and 1417 cm^{-1} attribute to the COO^- unsymmetrical and symmetrical stretching vibration respectively. The FT-IR spectra of the ternary

nanocomposite in Figure 3-b shows that those peaks associated with intercalation have a small change. But the peaks of the COO^- unsymmetrical and symmetrical stretching vibrations are moved to 1580 and 1424 cm^{-1} respectively ,and the results show that the COO^- groups of CMS have a strong interaction with the group of Fe_3O_4.

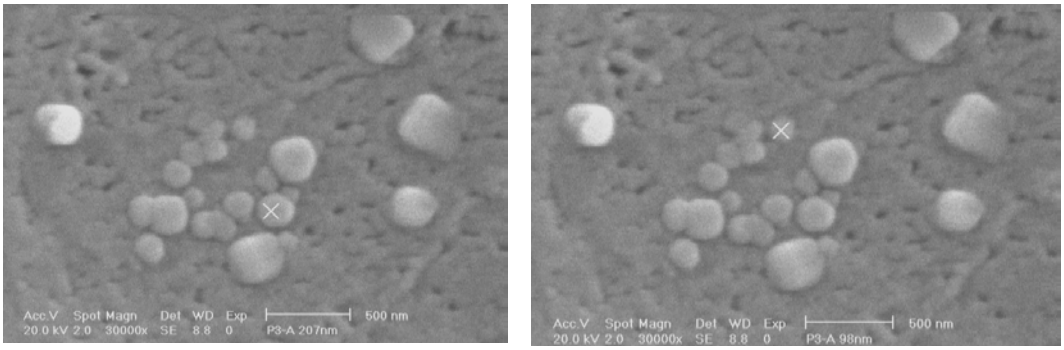

Figure 1. Scanning electron micrograph of Fe_3O_4/ sodium acetate/ carboxymethyl starch ternary nanocomposite

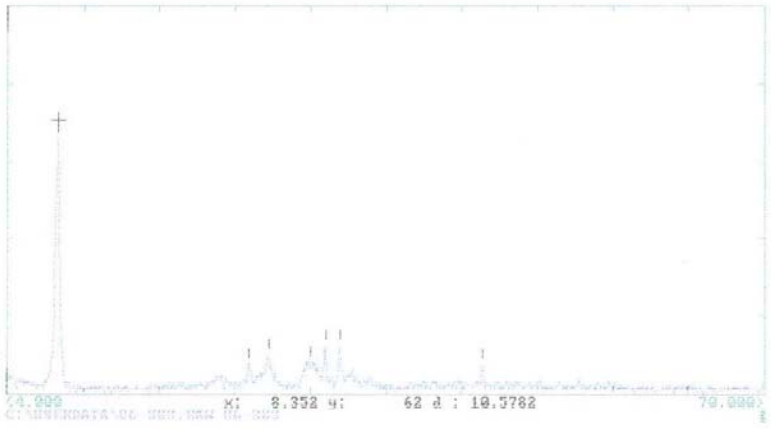

Figure 2. XRD spectra of Fe_3O_4/ sodium acetate/ carboxymethyl starch ternary nanocomposite

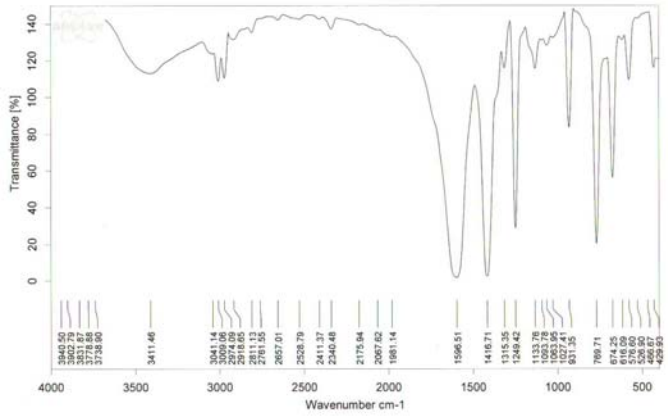

Figure 3-a. FT-IR spectra of pure carboxymethyl starch (CMS)

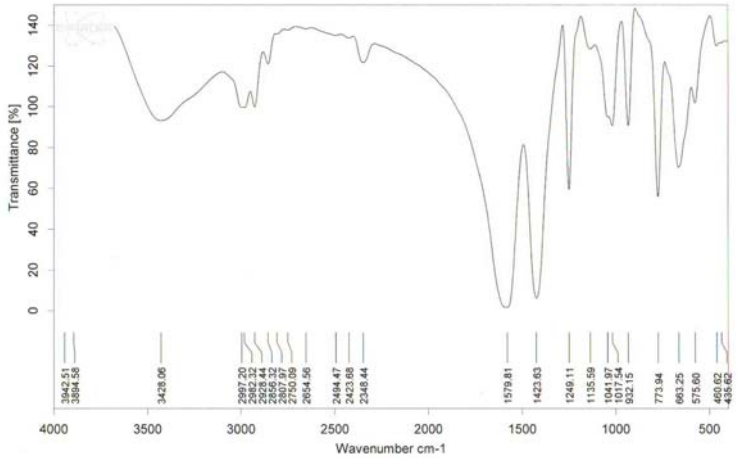

Figure 3-b. FT-IR spectra of Fe_3O_4/ sodium acetate/ carboxymethyl starch ternary nanocomposite

Conclusions

We synthesized the two – step nanocomposite by organic /inorganic materials. The results showed that the polar species such as sodium acetate intercalated in the interlayer of Fe_3O_4 with different sodium acetate content. The Fe_3O_4/ CH_3COONa/CMS nanocomposite is fabricated according to the physical and chemical design of the electrorheological material. The polar compound (CH_3COONa) is directly intercalated into the interlayer of ferric oxide, and then the intercalated complex is interacted with CMS by solution method. The experiment results show that by the design and control of the molecular chemical structure, the physical design for dielectic properties isachieved and thus the characterization of ternary nanocomposite is optimized.

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The Analysis Method of Numbers with the Same Last Digit for Lottery Number Selection

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Abstract: In this paper the analysis method of numbers with the same last digit for the application of the lottery numbers selection has been introduced. A number selection software is developed according to this method. It also discusses the existence, sequence of the numbers with the same last digit and data processing in details. [The Journal Of American Science. 2007;3(4):35-39]. (ISSN: 1545-1003).

Keywords: numbers with the same last digit; lottery mode; permutation and combination; traverse algorithm

1 Introduction

Lottery is a means of generating funds for government projects and providing hope to those less fortunate. Its contributions in education, health and other fields are impressive since its inception. Raising money for charitable organizations by holding a lottery is also popular and usually quite successful[1].

Lottery numbers are randomly drawn. It is a type of game that has the element of chance. There are many scientific number selection skills to choose from[2]. Numbers with the same last digit is one of them. Studying and using it can improve the chances on winning the lottery. It is the method that the lottery player should master[3]. In this paper, numbers with the same last digit analysis method for number selection has been introduced. According to this method, a number selection software with the functions such as data inputting, processing, filtering and printing etc is developed. The purpose of this software is to make full use of the PCs' calculating capacity and assist players to assemble their own play tickets from their selected number.

2 Numbers with the same last digit

In the various lottery games, in addition to analysis the rules of odd/even number, high/low numbers and best sums, numbers with the same last digit is another one that you need to consider[4]. From the statistical report of the previous drawings, the combination of numbers with the same last digit is the characteristics of the winning numbers. It occupies the 95.67% of the winning numbers combination. This means that if you choose the group of numbers without the same last digit, your winning chance will only be 4.33%[5]. Therefore it is important for the players to consider choosing the lottery numbers contain the same last digit.

The range for the number's last digit is from 0 to 9. For example, in a 7/30 game, all the numbers which available for the game are listed, and numbers with the same last digit are putted in a column.

01	02	03	04	05	06	07	08	09	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

In the above list, every column is a group. The numbers in each group are with the same last digit. Thus the first group contains the number 01, 11, 21 and can be represented by '1', the second group contains the number 02, 12, 22 and can be represented by '2' etc[6]. The analysis method of numbers with the same last digit is to analyze the appearances of these numbers in the winning numbers. So you can get the probability of the winning numbers with the same last digit and to improve the chance of winning a lottery.

3 The Application of the Analysis Method and the Implementation of the Software

The data flow chart of the lottery number selection software is shown in fig.1. According to the player's selection, the program will figure out the mode of the game (eg 7/30, 7/26 or 5/23) and generate the tickets combinations depend on the calculating method of the different mode. These number selections which are generated by the program can also be saved in a file. Later the player can view them by opening the file.

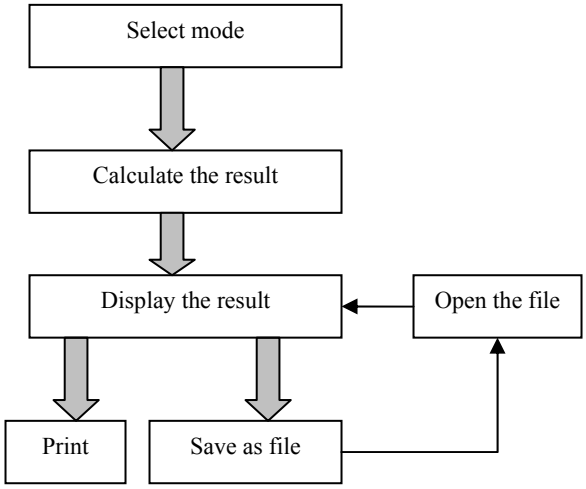

Figure 1. Data flow chart

3.1 Deal with different modes

In this paper, three modes 7/30, 7/36, 5/23 have been discussed.

The program mainly contains two parts: data input and generating ticket selections. In the data input section, the size of the array that is used to generate the ticket is determined by the mode of the user's selection. Meanwhile, in the modes of 5/23 and 7/36, because the amount of the numbers with the same last digit is different, the parameter for the number groups should also be concerned as shown below.

7/30：

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

5/23：

01	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22								

7/36:

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36				

It obviously shows from the above three tables：In the 7/30 mode, there are three lines with equal

amount of the numbers with the same last digit. When the program has the traverse calculation, it only needs to traverse every digit based on the user's selection.

In the 5/23 mode, there are 3 numbers for the digit '1' and '2' arrays, only 2 numbers for the other groups of the same digit array. When the program has the traverse calculation, it needs to consider two cases based on the user's selection. For the digit '1' and '2', the variable needs to traverse from 1 to 3; for the other digit arrays, the variable needs to traverse from 1 to 2.

Also for the 7/36 mode, there are 4 numbers for the digit '1' to '6' array, and 3 numbers for the rest of the digit arrays. For the traverse calculation, it needs to consider two cases based on the user's selection. For the digit '1' to '6', the variable needs to traverse from 1 to 4; for the other digit arrays, the variable needs to traverse from 1 to 3.

3.2 The resolution for the amount of the numbers of the user's selection

When the users come to choose the lottery numbers, sometimes the amount of numbers that the user selected is not equal to the required amount of the numbers. For this problem, the program provides the following resolution. We use the 7/30 game as a example.

(1) If the amount of the user's selection is less than 7: In this case, the missed number can be replaced by 01-30. First we need to permute and combine the numbers that the users selected, then add the permutation and combination operations of the 01-30 for the missed numbers. For example: if the user chooses 6 numbers with the same last digit '5' and '6', that is 5(3), 6(3), then the number for the ticket is: 05 15 25 06 16 26 C_n^r (n=30, r=1, where n is the amount of numbers that the user can selected, r is amount of number that the user missed).

(2) If the amount of the user's selection is 7: In this case, the program will list the permutation and combination operations for the 7 numbers directly.

(3) If the amount of the user's selection is more than 7: In this case the program will list all the possible combinations of the 7 numbers from the user's selection. This means it first need to determine the combinations of the 7 numbers from the user's selection, then have the permutation and combination operations for each combination. The following resolutions are provided for the computer's operation.

For example, if the user chooses the numbers with the last digit '5', '6' and '8', as stated above, for 7/30 mode, each digit group contains 3 numbers. Assuming the user chooses all the 3 numbers in each group, this means that the user chooses 9 numbers in total, that is 5(3), 6(3), 8(3).

Then the combination of the 7 numbers could be:

331 313 133 322 232 223

Where the number in the first, second and third position represents the amount of numbers contains the same last digit '5', '6' and '8' respectively.

In order to use the calculating capability of the computer, we have the total number combinations subtract the possible combinations of the 7 numbers.

333-331=002
333-313=020
333-133=200
333-322=011
333-232=101
333-223=110

Here we get some numbers contain 3 digits in sequence.

001 332

002	331
010	323
011	322
012	321
……	……

For the numbers such as 331, 332, there is no appropriate algorithm for computer program to calculate. Therefore we need to convert this kind of numbers 331, 332 into numbers 001, 002 which are suitable for computer to calculate. In this way, the computer can calculate the result by starting from the number 000, then 001 and so on. The results are checked one by one, the expected results will be kept and unexpected results will be dropped.

3.3 The appearance orders of the numbers with the same last digit

Here, we use an example to illustrate this problem. For example, in a 7/30 game, the user chooses 3 numbers with the same last digit '4'; 2 numbers with the same last digit '5'; and 2 numbers with the same last digit '6', that is 4(3), 5(2), 6(2). For this choosing, there can only be two numbers for 5,15, 25 group. The possible combination could be 5 15, 5 25, 15 25. Actually, these combinations are determined by the human. For the computer, the combinations could be in the order of 15 5, this is recognized as two different combinations for 5 15.

In order to avoid the above repetition, a low to high value traverse method has been used to control the appearance sequence of the numbers with the same last digit. The possible combination is as following:

	05 15	06 16 06 26 16 26
04 14 24	05 25	06 16 06 26 16 26
	15 25	06 16 06 26 16 26

The order of the numbers combination for the tickets is produced based on the user's selection. That is the numbers with the same last digit is arranged in the order of the low to high value. Then the traverse operations can be applied to these numbers. For the above list, we can get the first number combination is 04 14 24 05 15 06 16 and the second number combination is 04 14 24 05 15 06 26, etc. According to the traverse results, we can get all the possible numbers combinations for the tickets.

4 Conclusion

The numbers with the last same digit frequently occur in the winning numbers. It gradually becomes the characteristics of the winning numbers and takes the important part of the player's consideration. The analysis software can effectively help the player to choose the numbers and has certain utilities. It can also improve the chances on winning the lottery.

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Multiple drug resistant Pattern of *Salmonella typhimurium* infections In Osogbo, South Western Nigeria

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Abstract: This study was carried out to find out the extent of multidrug resistance profile of *Salmonella typhimurium* to commonly used antibacterials and its infectious nature in our locality from different samples obtained from Ladoke Akintola University of Technolgy Teaching Hospital, Osogbo. In all, 23 non-duplicate *Salmonella typhimurium* isolates were recovered from different clinical samples of stool (204), urine (48), blood (52), cerebrospinal fluid (20), and high vaginal swab (10). Of all the positive isolates, 17(73.9%), were from patients with symptoms characterised as salmonellosis. High rates of resistance were found in most of the isolates studied. Resistance rates were 91.3%, for amoxicillin and cotrimoxazole, 86.9% for ampicillin, 82.6% for streptomycin and 30.4% for ciprofloxacin, respectively. [The Journal Of American Science. 2007;3(4):40-44]. (ISSN: 1545-1003).

Keywords: Multidrug resistance, symptoms, *Salmonella typhimurium.*

Introduction

Salmonellosis is an infection with Salmonella bacteria, often restricted to the gastro-intestinal tract and is often a self limiting disease. Most individuals infected with *Salmonella typhimurium* experience mild gastrointestinal illness involving diarrhoea, chills, abdominal cramps, fever, head and body aches, nausea, and vomiting (Honish 1999). Infections are usually self-limiting, and antimicrobial treatment is not recommended for uncomplicated illnesses (Aserkoff and Bennet 1969, Gill and Hammer 2001). However, extraintestinal infection can occur, particularly in very young, elderly, and immunocompromised patients (Angulo and swerdlow 1995, Thuluvath and McKendrick 1998). In these cases, effective antimicrobial treatment is essential (Cruchaga et.al., 2001). Every year, approximately 40,000 cases of salmonellosis are reported in the United States. The actual number of infections may be thirty or more times greater. (CDC, 2006). In Nigeria such cases are either not documented or because many milder cases are not diagnosed or reported. Cases, however, of systemic disease due to *Salmonella typhimurium* and other salmonellae have been reported, (Panhot and Agarwal 1982, Varma et.al., 2005). Salmonellosis have been reported to occur more in the winter than summer. Most times it is referred to as gastroenteritis or diarrheoa. Likewise more cases of diarrhoea caused by enterobacteriacea especially *E.coli*, occurring more during wet season than dry season have also been reported. (Olowe et.al., 2003). Children are the most likely to get salmonellosis, however the elderly, and the immunocompromised are the most likely to have

severe infections. It is estimated that approximately 600 persons die each year with acute salmonellosis as reported by Centre for disease control, (CDC 2006).

The present study was undertaken to assess *Salmonella typhimurium* in cases of diarrhoea and to see how often they manifest in a systemic form in disease conditions. It was also intended to study the problem of drug resistance associated with this organism.

Materials and Methods

The study covered a period of nine months; Faeces, blood, cerebrospinal fluid, stool and urine were processed and screened for isolates following standard procedures for proper isolation and identification. (Cowan and Steel, 1970) The isolates of *Salmonella typhimurium* were identified biochemically following standard procedures.

The Antimicrobial susceptibility testing: Isolates were tested by the disk diffusion method on muller Hilton (Hi-Media, Mumbai) following the zone size criteria recommended by the National Committee for Clinical Standards (NCCL, 2000).

From the commercial antibiotics disc used for the studies of susceptibility testing, results were obtained from these: amoxicillin, streptomycin, tetracycline, kanamycin (30µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg) and co-trimoxazole (25 µg), which are commonly used for the treatment of typhoid fever.

Results

Over a period of 9 months, 204 faecal and 52 blood samples, 48 urine samples, 20 CSF samples and 10 high vaginal swab samples were screened for presence of *Salmonella typhimurium* of which 23 isolates were identified to be *Salmonella typhimurium*.

Table 1. shows the distribution pattern of *S.typhimurium* from screened samples; faeces, blood, urine, cerebrospinal fluid, and high vaginal swab.

Table 2. illustrates the isolation frequency of *S. typhimurium* with respect to age.

Table 3. outlines the resistance pattern of the isolated strains to commonly used antibacterial.

Figure 1. outlines the symptoms profile of cases of salmonellosis in the study area. From the findings, salmonellosis occured in six cases that had diarrhoea, followed by two with septicaemia, one case had meningitis and one had urinary tract infection, while none was reported for vaginal infection.

Table 1. Distribution Pattern Of S. Typhimurium From Screened Samples

SAMPLES	**SCREENED**	**POSITIVE**
Stool	204	17
Blood	52	2
Urine	48	3
CSF	20	1
HVS	10	--
TOTAL	334	23

Key:
CSF – Cerebrospinal Fluid
HVS – High Vagina Swab

Table 2. Illustrates The Isolation Frequency Of *S. Typhimurium* With Respect To Age

AGE (yr)	DISTRIBUTION	%
> 1- 5	16	69.6
>5- 12	3	13.4
ADULT	4	17.0
TOTAL	23	100

Table 3. Resistance Pattern Of The Isolated Strains To Eight Commonly Used Antibiotics (N= 23)

ANTIMICROBIALS	**SENSITIVE (%)**	**RESISTANT (%)**
AMOXICILLIN	2(8.7)	21(91.3)
AMPICILLI	3(13.1)	20(86.9)
CHLORAMPHENICOL	5(21.7)	18(78.3)
COTRIMOXAZOLE	2(8.7)	21(91.3)
STREPTOMYCIN	4(17.4)	19(82.6)
TETRACYCLINE	5(21.7)	18978.3)
KANAMYCIN	12(52.2)	11(47.8)
CIPROFLOXACIN	16(69.6)	7(30.4)

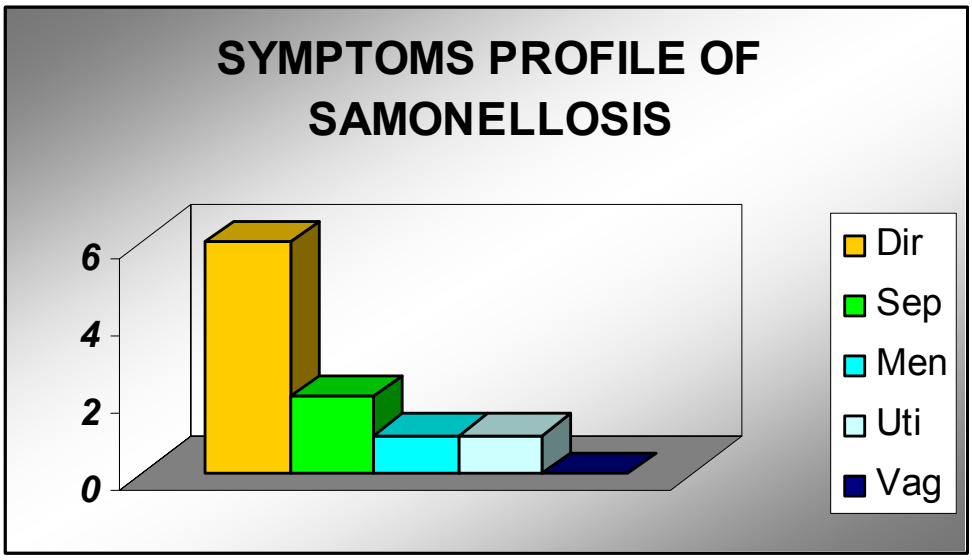

Figure 1.

Key:
Dir - Diarrhoea
Sep - Septicaemia
Men - Meningitis
Uti - Urinary Tract Infection
Vag - Vagina infection

Discussion

The findings showed the occurrence of salmonella typhimurium in the entire samples investigated, except high vaginal swab. The incident rate is not too worrisome since the occurrence of salmonellosis due to *Salmonella typhimurium* is only about 10% of the cases of diarrhoea. More cases are seen in children compared with adult individuals in the study area and it conforms with earlier reports of (Olowe et.al., 2003, NCCLS 2000). Multidrug-resistant (MDR) strains of *Salmonella* are now encountered frequently and the rates of multidrug-resistance have increased considerably in recent years. (CDC 2006). Even worse, some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance. (CDC, 2006). This might be the reason for the high resistance value observed in this present study also. Most of the strains of *Salmonella typhimurium* isolated were resistant to drugs like streptomycin, amoxicilin, tetracycline, ampicillin, kanamycin and chloramphenicol. This data is very alarming since the isolates were already showing high resistance to drugs that are meant as alternate therapy to salmonellosis treatment; especially isolates from blood were resistant to the commonly used antibiotics. Similar drug resistance has been observed by (Verma et al, 2005), in urinary tract infection. Drug-resistant *Salmonella* emerged in response to antimicrobial usage in food animals, which has also contributed or resulted in major outbreaks of salmonellosis was reported by Guardian Unlimited 2006. Selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance, but other factors also need to be taken into consideration. For example, dirty and poor sanitary environment, poor drainage system characterised by majority of our system in this area , even the society as a whole encourage the spread of this organism in our environment. Likewise some *Salmonella* serotypes are more prone to develop resistance than others. Furthermore, major shifts in the occurrence of *Salmonella* serotypes in food animal and humans are regularly seen. A recent example is the global spread of a multidrug-resistant *S. typhimurium* phage type DT104 in animals and humans. While the spread of DT104 may have been facilitated by the use of antimicrobials, international and national trade of infected animals is thought to play a major role in international spread (WHO, 2005).

This work confirmed that salmonella isolates are present in our clinical specimens in this area and are seriously becoming a concern due to their multidrug resistance pattern observed in this study, and urgent steps should be taken to have an evaluation principle and documented analysis of trends of occurrence of this resistant isolates to help guide in administration of less commonly resisted antimicrobials when cases occur.

The high percentage of resistance to the antibiotics studied could be attributed to their prevailing usage and abuse in the area under study. The implication of the high percentage resistance recorded for the antibiotics is that only ciprofloxacin will effectively treat *S. typhimurium* infections, though some strains were resistant. These results call for intensive surveillance programme to monitor microbial trends and antimicrobial resistance patterns in other parts of Nigeria.

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Synthesis and characterization of aromatic polyether dendrimer / Mesalamine (5-ASA) nanocomposite as drug carrier system

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Abstract: Highly branched , functionalized polymers have potential to act as efficient drug carrier system.The aromatic polyether dendrimers are spherical, highly ordered,multibranched,nanometer-sized macromolecules having positively charged ether groups on the suface at physiological conditions.In this study, we synthesized a kind of dendrimer / Mesalamine [5-aminosalicylic acid (5-ASA)] nanocomposite for oral drug delivery. The aromatic polyether dendrimer (generation 2, hyperbranched polyether with -CH_2OH functionality, 3,5-Dihydroxybenzoic acid core) was prepared from generation 2, hyperbranched polyether dendrimer with -$COOCH_3$ form in excellent yield. FTIR and NMR studied suggest that Mesalamine predominantly forms *a complex* with polyether dendrimers because of the ionic interaction between the –OH end groups and the carboxyl group of Mesalamine (Mesalamine contains both amine (basic) and carboxylic acid (acidic) functional groups). [The Journal Of American Science. 2007;3(4):45-51]. (ISSN: 1545-1003).

Keywords: Drug, Mesalamine, nanocomposite, dendrimer, nanocomposite, dendrimer, carrier system

Introduction

The most important characteristic of any drug is efficacy. This characteristic may often reduced because of the inability to deliver the drug to the specific cells or tissues [1,2]. After administration, the drug may pass through different physiologic barriers and / or pathways, decreasing the actual amount drug that reaches the site. In the search for an ideal carrier system, the dendrimers may have significant potential. Dendrimers are synthetic macromolecules with a well-defined globular structure [3]. The need for advanced materials with improved and new properties for a variety of technological applications has created a demand for both new forms of matter and for polymers that have highly controlled molecular architectures [4,5]. The established approach to dendritic macromolecules has traditionally involved a divergent process in which growth is started from a polyfunctional core and continued outwards in a stepwise manner that affords larger and larger macromolecules as the process is continued [6]. The fundamental attribute of the convergent approach is that it begins at what will be the periphery of the molecule, proceeding inwards [7]. It is this feature more than any other that allows for unparalled control over molecular architecture [8]. Figures 1,2 show the structure of the generation 1 and 2 hyperbranched aromatic polyether dendrimer [9,10].

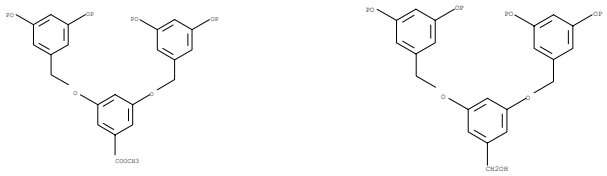


Figure 1. Aromatic polyether dendrimers structure (1 generation)

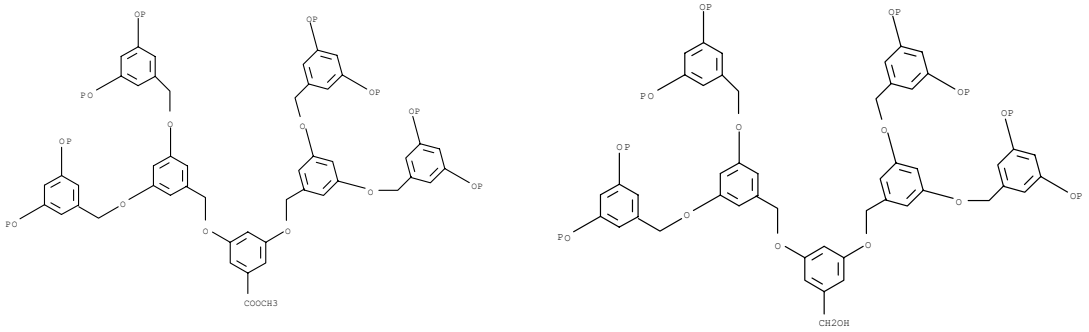


Figure 2. Aromatic polyether dendrimer structures (2 generation)

We investigate the potential of dendrimers and hyperbranched polymers as drug carriers using Mesalamine (5-aminosalicylic acid, 5-ASA) as a model drug, since the methodologies for evaluation of the cellular activity of Mesalamine are well known [11]. Mesalamine contains both amine (basic) and carboxylic acid (acidic) functional groups. Figure 3 shows structure of the Mesalamine [12,13]. In this paper, we explore the interaction of the drug with aromatic polyetheric dendrimer. The nature of the interaction was characterized by FT-IR and ^{1}H-NMR spectroscopy. Figure 3 show schematic synthesis method of polyether dendrimer – mesalamine conjugate [14].

Experimental

Materals

All reactions were performed under an atmosphere pressure. All reagents and solvents, unless otherwise specified, were obtained from Merck Chemical Co. Melting points were obtained on a Mel-Temp melting point apparatus. G2 polyether dendrimer and mesalamine (5-ASA) were obtained from Aldrich chemical company.^{1}H- NMR spectrum were recorded on a 400 MHz spectrometer, but were referenced to tetramethylsilane. Analytical TLCs were run on commericial Merck plates coated with silica gel GF250 (0.25 mm thick). Fourier transfer infrared (FTIR, Bruker) spectroscopy was used to identified the polymer surface. Specta were obtained in the wave number range of 400-4000 cm^{-1}. Spectra of samples were recorded from KBr in 1:10 (wt/wt) ratio.

- *Preparation of 3,5-bis(3,5-bis(3,5-bis(t-butyldimethylsilyloxy) benzyloxy)benzyloxy)benzyl alcohol*

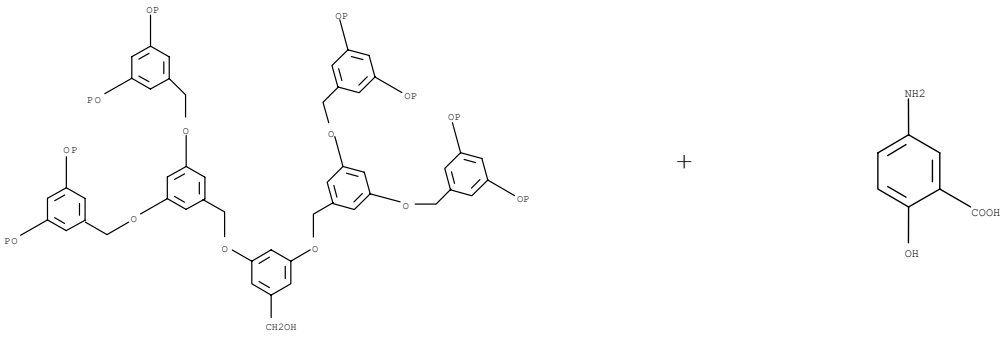
A mixture of the G2-$COOCH_3$ dendrimer (AB8, heptamer) (0.8 g, 0285 mmol) in dry THF (100 ml) and dropwise to a suspension of $LiAlH_4$ (2.5 g, 60 ml) in dry THF (50 ml). After reflux for 1 h, the solution was treated with aqueous NaOH (1 M, 15 ml), filtered and evaporated. The residue was chromatographed on silica gel with dichloromethane as the eluent to give the heptameric alcohol as a colorless glass (0.768 g, 96%).

R_f = 0.91 , ψ_{max}(KBr)/cm^{-1} 3053, 2960, 2932, 2860, 1544; δ_H (CDCl3) 0.1 (s, 48H, CH3), 0.9 (s, 72H, t-Bu), 1.60 (t, 1H, OH), 4.62 (d, 2H, CH2OH), 4.68 (s, 8H, CH2O-), 4.88 (s, 4H, CH2O-), 6.14 (t, 4H, J2, 4H 3nd gen. Ar), 6.21 (t, 2H, J2, 2/4-H 2nd gen. Ar), 6.41 (d, 8H, J2, 2/6-H 3nd gen. Ar), 6.41 (d, 4H, J2, 2/6H 2nd gen. Ar).

Found: C, 76.4%; H, 6.8%, $C_{177}H_{188}O_{15}Si_8$)

- ** preparation of 3,5-bis(3,5-bis(3,5-bis(t-butyldimethylsilyloxy) benzyloxy)benzyloxy)benzyl alcohol dendrimer and 5-ASA conjugate.*

5-aminosalicylic acid (5-ASA) was dissolved in methanol following which the dendrimer was added .The reaction mixture was stirred for 24 h in the dark, then evaporated using rotaevaporator to emove methanol. The traces were dried under vacuum in order to remove methanol completely. To these traces, deionized water was added. This solution was stirred in the dark for 24 h. This was extracted the drug – dendrimer coplex, as dendrimer is soluble in water while 5-ASA is not. The solution was then filtered through PTFE membrane (Millix Millipore) of pore size 200 nm, and then lypophilized to remove water. After approximately 180 min, the sample was sprayed into a liquid nitrogen bath cooled down to 77° K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze-drying process, the products are dried by a sublimation of the water component in an iced solution. The drug – dendrimer complex obtained was in the form of a white powder.



schematic representation polyether dendrimer *Mesalamine (5-ASA)*

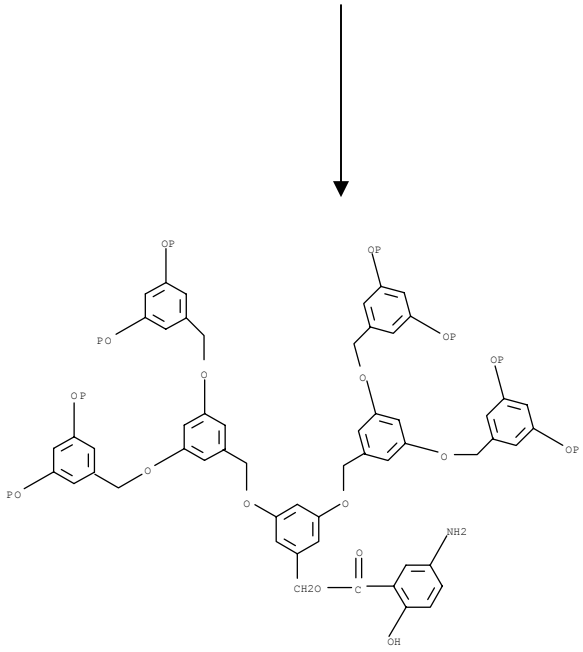


Figure 3. Sschematic synthesis method of mesalamine conjugated polyether dendrimer

Results and discussion

- ***Fourier transfer Infrared spectra***

Figure 1 shows the FT-IR spectrum of the G2-$COOCH_3$ dendrimer (AB8, heptamer) where the % of transmittance is plotted as a function of wave number (cm^{-1}). The characteristic FT-IR peaks at 3078, 3053, 2961, 1718 cm^{-1} are due to the presence of =CH bond stretching vibrations , the aliphatic CH bond in protect groups (t-butyldimethylsilyloxy) and carbonyl (C=O) group ,respectively.

Also, Figure 2 shows the FT-IR spectrum of the G2-CH_2OH dendrimer (AB8, heptamer). The characteristic FT-IR peaks at 3408, 2960, 2932, 2860, 1544 cm^{-1} are due to the presence of OH phenolic group, the aliphatic and aromatic =CH bond in protected methyl groups and C=C bond of aromatic group, respectively.

- ***Scanning electron micrography***

Figure 3 shows the SEM of aromatic polyether dendrimer / Mesalamine (5-ASA) nanocomposite that synthesized by chemical reaction. This nanocomposite is very sensitive to the temperature that due to the intractionelectron and sample. Scanning electron micrography images were obtains from a diluted solution of the nanocomposite particle. The white spots are drug nano particles. The SEM image shows the presence of 5-ASA spherical particles in polyfunctional dendrimeric matrix, which are homogenenously distributed throughout the composites, which is also confirmed from ^{1}H-NMR studies [15].

- ***^{1}H-NMR spectroscopy***

The ability of the dendrimer to form a complex with drugs depends on the core- suface groups of dendrimer, electrostatic interactions between the dendrimer and the drug, and the ability of the drug to form a conjugate with the dendrimer through chemical bonding. Therefore, it is possible to manipulate the incorporation process for a given drug by appropriate selection of the dendrimer and the surface functionality. One might expect that the mesalamine with the carboxylic group may form a complex with surface OH groups of polyether dendrimer.

Figure 4 shows the 400 MHz ^{1}H-NMR spectrum of of [G2] – CH_2OH Dendrimer/Mesalamine conjugate in which three regions can be seen.The resonances for the aromatic protons of the monomer units at dendrimer occur in the region 6.5-6.7 ppm separate resonances are observed in the appropriate ratio for each layer of monomer units ,and at highest field , resonances for the methylene protons occur in the region 4.90-5.00 ppm. The aromatic protons of 5-ASA are observed at 6.9 and 7.8-7.95 ppm, phenolic protons at 8.1 ppm, amidic protons at 8.9 ppm and acidic protons of 5-ASA at 9.3 ppm.

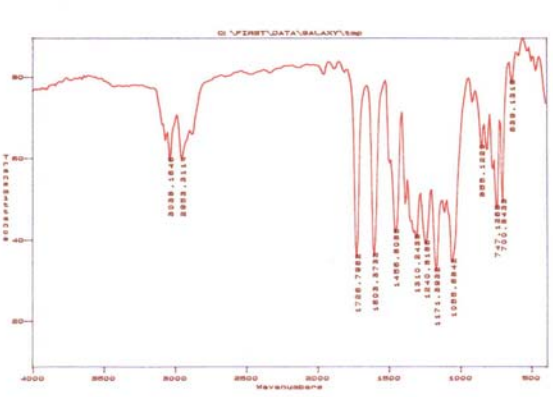


Figure 4. *FT-IR spectrum of [G2] –$COOCH_3$ Dendrimer*

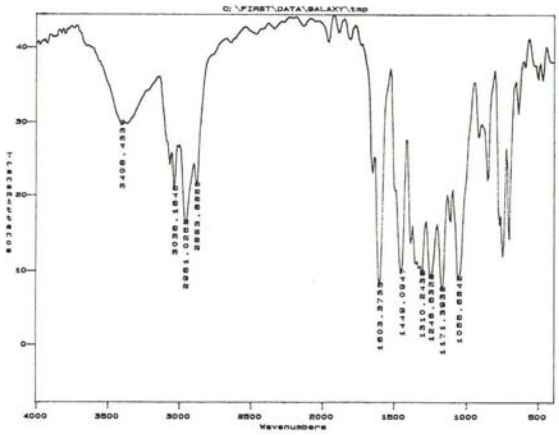


Figure 5. *FT-IR spectrum of [G2] – CH_2OH Dendrimer*

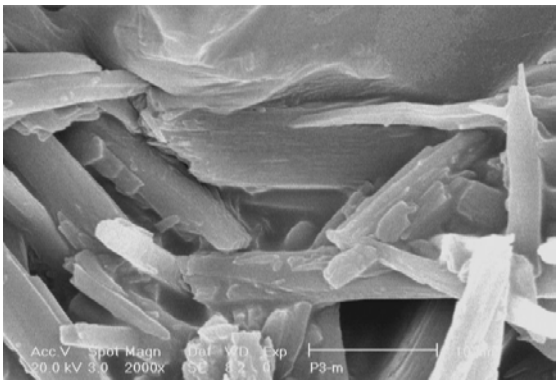


Figure 6. *SEM of [G2] – CH_2OH Dendrimer/Mesalamine(5-ASA) conjugate*

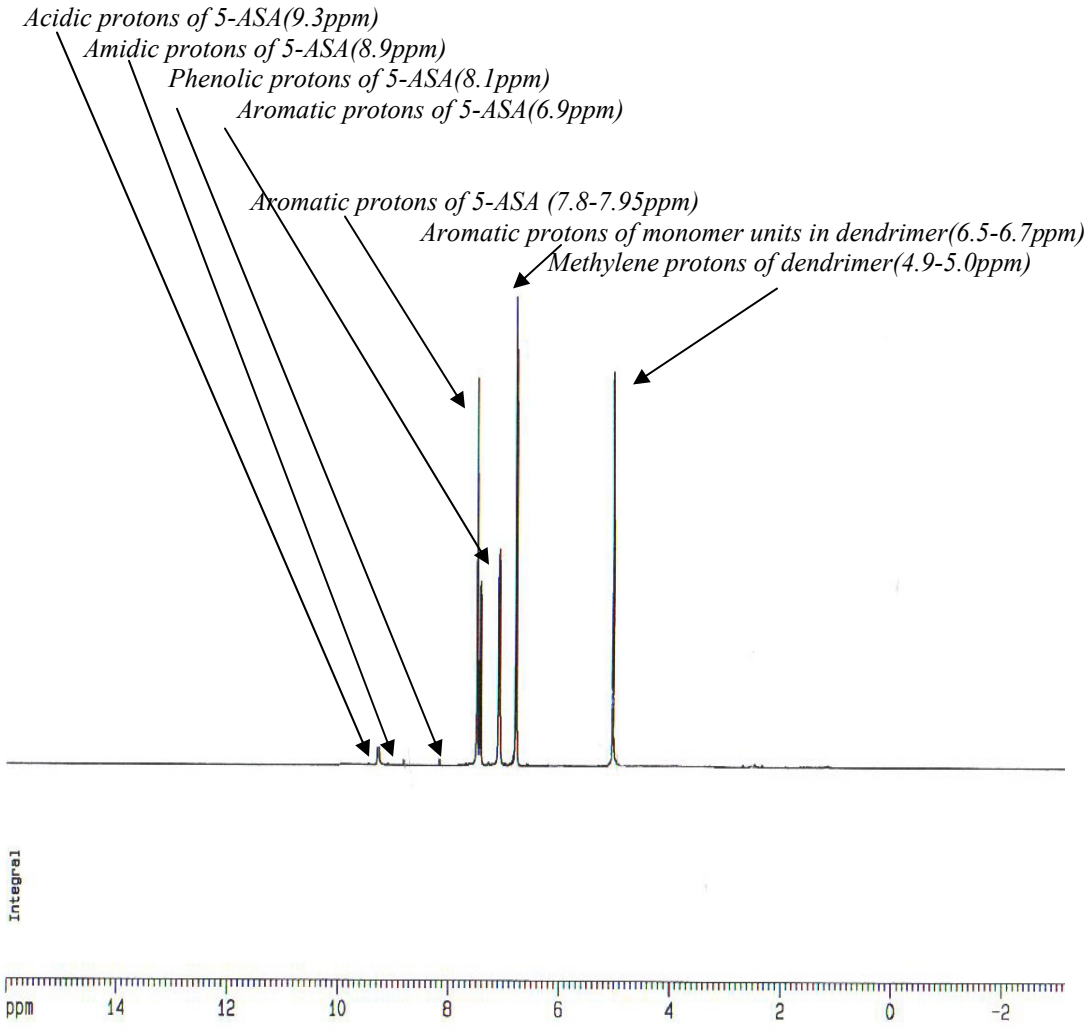


Figure 7. 400MHz ^{1}H-*NMR spectrum of [G2] – CH_2OH Dendrimer/Mesalamine(5-ASA) conjugate.*

Conclusions:

The ability of the dendrimer to form a complex with mesalamine (5-ASA) was explored using aromatic polyether dendrimer as model base polymer.The nature of drug-dendrimer interaction were explored using FT-IR, ^{1}H-NMR and SEM. Our studies suggest that the aromatic polyether dendrimer may predominantly form a complex with the carboxyl group of mesalamine .this complex is stable in deionized water and methanol. Current studies are exploring the complexation/conjugation ability of these dendrimers to a wide variety of drugs.

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Anisotropy of Edaphic Properties in slope soils of a University Farm in Owerri, Southeastern Nigeria

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Abstract: This study investigated variability in properties of soils of three physiographic positions in the Teaching and Research Farm of the Federal University of Technology, Owerri, Southeastern Nigeria. A transect was used to align three profile pits representing three physiographic positions of crest, midslope and foot slope before the rains in 2007. Soil profile pits were dug and samples collected based on degree of horizon differentiation. Collected soil samples were prepared and analyzed in the laboratory using standard techniques. Data generated from various analyses were subjected to analysis of variance to measure anisotropy and correlation analysis was also used to estimate degree of relationship among soil properties in the slope soils. Results showed significant variation (p=0.05) in soil total porosity, clay, pH, organic carbon, total nitrogen, available phosphorus and base saturation. Organic carbon had significant relationship (p=0.01-0.05) with total nitrogen, available phosphorus, cation exchange capacity and base saturation. Detailed studies on these soils will certainly improve reliability and applicability of soil data especially in this era of precision agriculture. [The Journal Of American Science. 2007;3(4):52-61]. (ISSN: 1545-1003).

Key words: Anisotropy, arable farming, soil fertility, pedology, toposequence

Introduction

Topography is a principal factor in soil pedogenesis and variability of soil properties in response to topographic forms is used in predicting rates of ecosystem processes (Schimel *et al.,* 1991). Marked differences due to slope aspect were reported by Birkeland (1999). Hunkler and Schaetzi (1997) observed that slope aspect has its greatest influence in locations between latitudes 40 to 60 ^{o}N. Yet, Esu, (2005) reported pronounced increase in soil temperature for south-facing slopes in the northern hemisphere, possibly due to perpendicularity of such slope soils to sunrise. Soil organic carbon losses are more in shoulder complexes while gain of the same attribute were found in footslope ecomplexes (Pennock *et al.,* 1994). Marked changes in soil moisture content due to topography were also recorded in southwestern Nigerian soils (Ogunkunle and Onasanya 1992; Doer, *et al.,* 2000). In addition to the above, landscape positions influence run off, soil erosion, drainage and distribution of heavy metals such as mercury (Manville *et al.,* 2006; Parizanganeh *et al.,* 2007) Introduction of localized differences in soil properties such as carbon and nitrogen processes was due to toposequential variations (Hobbie, 1996).

Characterization and classification of soils of any given location help in generating soil and soil-related data which are useful in sustained use of soil resource. Non-use of soil survey data has resulted in soil and soil-related environmental problems like nutrient depletion (Onweremadu, 2006) compaction, flooding, and poor yield (Zinck, 1990). These problems are worsened by socio-economic pressures on soils caused by demographic increase (Ruecker, 2003). The indispensability of soil survey information rises as Krall and Lee (2004) reported a widened spectrum of usage of detailed soil information bringing in other soil users, and this becomes critical in a university environment where the resource is put in many and sometimes conflictive land use types. Taking into cognizance that soils differ with physiographic position and that management of each land use varies, it becomes expedient to investigate degree of variability of selected soil properties for sustainable usage. The main aim of this study was to characterize and classify slope soils of Teaching and Research farm of the Federal University of Technology, Owerri, Southeatern Nigeria for agricultural activities and /or any other likely use.

Materials and Methods

Study area: The study was carried out before the onset of rains in 2007 at Teaching and Research farm proximal to the Centre for Agricultural Research (CAR) farm, Federal University of Technology Owerri, Nigeria. Owerri lies between latitudes $5^0$43'34''62'' and longitude $7^0$39'34.49" (Handheld Global Positioning System-GPS) Receiver (Garmin Ltd, Kansas, USA). Soils are derived from Coastal Plain Sands (Benin formation) of the Oligocene-Miocene geologic era and were influenced by Otamiri River. Generally, the study area lies within the lowland geomorphology of southeastern Nigeria. Mean annual rainfall ranges form 2250 to 2500 mm with a mean annual temperature range of 27 to 28^{o}C. The area is dominated by rainforest vegetation whose density is substantially depleted by anthropogenic influence. Varying and conflicting land uses such as farming, sand mining, fishing, waste disposal, recreational facilities and engineering activities are common in the area.

Field Sampling: A transect was drawn from the crest to the footslope of the Otamiri riverslope at the Federal University of Technology, Owerri, southeastern Nigeria Three profile pits were aligned along a transect at an interpedon distance of 200 metres to represent three topographic positions of crest, midslope and footslope. The three pedons (profile pits) were dug and described in line with the procedure as recommended by FAO (1998). Soils are grouped in field mapping units primarily by identifying landscape attributes which are believed to be similar (Hudson, 1992) and this soil-landscape paradigm is a powerful guide in field delineation of soils (Young and Hammer, 2002).

In addition to profile pit sampling, random surface soil sampling was carried out in the study site. Twenty surface soil samples (0-10 cm) were collected from each physiographic position using an auger giving a total of 60 soil surface samples. Core samples were used to obtain samples for bulk density determinations. With the exception of core samples, other soil samples were air-dried and sieved using 2-mm sieve preparatory to laboratory analysis.

Laboratory analysis: Particle size distribution was determined by hydrometer method according to the procedure of Gee and Or (2002) while bulk density was measured by core method (Grossman and Reinsch, 2002). Total porosity was calculated using a mathematical relationship between bulk density and particle density (Foth, 1984). Soil pH was measured potentiometrically in 1: 1 soil–water ratio (Hendershot *et al.,* 1993). Soil organic carbon was estimated by combustion at 840^{o}C (Wang and Anderson, 1998) while total nitrogen was obtained by microkjeldahl method (Bremner, 1996). Cation exchange capacity was measured using ammonium acetate leaching at pH 7.0 (Rhoades, 1982). Available phosphorus was determined by Olsen method (Emteryd, 1989).

Calculations: Ratios of fine sand to coarse sand (FS/CS), silt to clay (SCR), carbon to nitrogen (C/N), and calcium to magnesium (Ca/Mg) were obtained by calculations.

Similarly, total porosity (TP) was computed as follows:

TP = 100% - BD/PD x100)…… 1 (Foth, 1984)
Where TP = total porosity
BD = bulk density (g/cm^3)
PD = Particle density (g/cm^3)

Again, base saturation (BS) was calculated as
BS = (TEB/CEC) 100%
Where BS = base saturation
TEB = total exchangeable basic cations
CEC = cation exchange capacity

Statistic: Means, analysis of variance (ANOVA) and correlation analysis were performed on the soil data. Levels of significance were tested at 1 and 5 % for correlation analysis and at 5% for ANOVA.
Classifications: Soils were classified using the USDA soil Taxonomy (Soil Survey Staff, 2003) and correlated to with FAO/UNESCO soil Map of the World Legend (FAO, 1998)

RESULTS

Physical Properties: Results of various laboratory analyses on some soil physical properties are presented in Table 1. Sand–sized soil particles dominated other fractions of the fine earth material irrespective of geomorphologic setting. Higher values of total sand were obtained at the crest and footslope that is, 80.0% and 78.0%, respectively. Higher values of coarse sand were obtained when compared with fine sand content in all physiographic positions. Generally, fine sand increased downslope at epipedons having 20% (Crest) 23% (midslope) and 28% (footslope). Higher values of fine sand were from surficial horizons unlike results on coarse sand sub-fractions. Unlike fine sand, coarse sand decreased downslope having 57% (Crest) 54% (midslope) and 48% (Footslope). The FS/CS ratio decreased intrapedally with depth. However, there were slight differences between FS/CS values among the studied physiographic positions. Values of FS/CS were consistent with the findings of Oti (2002) in a classification of erosion-degraded lands of the same agroecology.

Percent silt increased downslope but ranged from 5 to 10% in the study site. Values of percent silt were lower than results from a study conducted by Igwe (2003) in a similar agro-environment in southeastern Nigeria. Clay content ranged from 12 to 15 % in studied soils. Generally, clay content decreased towards the Otanuir River southeastern Nigeria. There was a distinct clay bulge in pedons dug on crest and midslope physiographic positions, and this intrapedal trend was not found in footslope soils. Again, values of silt-clay ratio were lower in crest and midslope soils when compared with soils of the footslope (1.01).

At epipedons, values of bulk density decreased downslope having 1.40, 1.39 and 1.36 g/cm^3 for crest, midslope and footslope, respectively. Mean values of bulk density from pedons showed the same trend as in the surficial distribution of bulk density. Generally, bulk density decreased with depth in all the profile pits irrespective of geomorphic setting. These results on bulk density are consistant with the findings of Akamigbo (1999) in soils of the same agroecology. However, values of bulk density were lower than critical limits for root restriction (1.75-180 g/cm^3) (USDA-NRCS, 1996)

Total porosity slightly decreased downslope at epipedal horizons as well as soils of various geomorphic settings. However, total porosity decreased consistently with depth in all the pedons. Total porosity values were similar to the results obtained by Nnaji *et al.* (2002) in soils of Nsukka area of the same Southeastern Nigeria agroecology.

Chemical properties: Table 2 presents results of chemical properties of the studied soils indicating strong acidity and low exchangeable bases, low cation exchange low organic matter content (Organic carbon content), low total nitrogen content, low calcium-magnesium ratio and low available phosphorus in the study site. Soil acidity decreased towards the footslope with mean values of 4.82 (Crest), 4.84 (midslope) and 5.04 (footslope). Values of soil pH were higher in middle horizons of soil profiles irrespective of physiographic position. Exchangeable calcium and magnesium increased towards the footslope and consequently calcium-magnesium ratio. Exchangeable potassium decreased downslope while there was no trend in the distribution of exchangeable sodium. Cation exchange capacity and base saturation increased towards the footslope. The relative abundance of exchangeable bases is in the decreasing order of Ca, Mg, Na and K. Generally, values of exchangeable Ca, Mg, K, and Na were high at surface and middle horizons of all soil of the study site.

Organic carbon values were higher in surface horizons, and showed no regular trend topographically. Organic carbon decreased with depth in all profile pits of the University Farm. Total nitrogen content was generally very low, and the pattern of its distribution closely follows that of organic carbon. Highest values of total nitrogen was obtained in footslope soils (0.064). and available phosphorus increased downslope and as follows: 5.11 ppm (crest), 5.37 ppm (midslope and 8.74 ppm (footshope). Carbon-nitrogen ratio ranged from 9.20 – 16. 42, with higher C/N ratios recorded in lower horizons of the profile pits especially soils of midslope and footslope.

Variability and Relationships: Some properties showed significant (p=0.05) variations in the study site at surface and sub-surface horizons (Table 3). With the exception of soil pH at sub-surface horizons of studied soils all measured attributes varied significantly (p=0.05), and this is consistent with the

findings of Wang et al. (2001) in soils of Da Nangou catchment in China ($36^0$53'N; $109^0$19'E).Oti (2002) reported significant variations (p=0.05) in total nitrogen, bulk density, available phosphorus, cation exchange capacity and a non-significant relationship in soil pH and bass saturation. However, this study considered only two layers namely 0-10 and 10-20 cm.

A correlation matrix showing relationships between soil properties in the study site is shown in Table 4. Total porosity had a significant negative correlation (<0.05) with clay content, organic carbon, total nitrogen and available phosphorus. In all soils of the three physiographic positions,clay content had significant positive relationship (p=0.05) with organic carbon at the crest soil, and with cation exchange capacity in soils of all physiographic positions. Soil pH was highly correlated with available phosphorus (p=0.01) and cation exchange capacity (p=0.05). Organic carbon had a significant positive correlation with total nitrogen (p=0.01), available phosphorus (p=0.05), base saturation (p=0.05) and cation exchange capacity (p=0.01) irrespective of physiographic position. Available phosphorus had strong relationships (p=0.05) with cation exchange capacity and base saturation in the study site.

Classification: Based on the results of field, physical and chemical analyses, soils were classified as Typic Hapludults (crest and midslope soils) and Typic Eutrudepts (Footslope soils). These soils were correlated to FAO/UNESCO legend as Dystric Nitisols (Crest and Midslope soils) and Eutric Fluvisols (Footslope soils).

Table 1: Some physical properties of studied soils

Horizon	Depth (cm)	FS (%)	CS (%)	TS (%)	SL (%)	CL (%)	FS/CS	SCR	BD (g/cm^3)	TP (%)
	Crest									
	0-12	20	57	77	10	13	0.35	0.76	1.40	47
	12-29	30	59	89	5	6	0.50	0.83	1.41	46
	29-65	31	47	78	5	17	0.65	0.09	1.45	45
	65-105	10	60	77	3	20	0.16	0.15	1.48	44
	105-190	11	68	79	2	19	0.16	0.10	1.50	43
	Mean	20.4	58.2	80.0	5.2	14.8	0.36	0.38	1.44	45
	Midslope									
	0-13	23	54	77	9	14	0.42	0.64	1.39	47
	13-32	28	55	82	8	9	0.50	0.88	1.42	46
	32-75	29	45	72	9	19	0.67	0.47	1.46	44
	75-115	12	60	72	8	20	0.20	0.40	1.49	43
	115-196	9	68	77	5	18	0.13	0.27	1.56	41
	Mean	20.2	56.0	76.2	8	15	0.36	0.53	1.46	44
	Footslope									
	0-18	28	48	76	11	16	0.58	0.84	1.36	48
	18-33	26	51	77	13	10	0.51	1.30	1.44	45
	33-70	25	54	79	11	10	0.46	1.10	1.47	44
	70-118	10	68	78	10	10	0.14	0.83	1.54	41
	118-205	7	73	80	10	11	0.09	1.00	1.58	40
	Mean	**19.2**	**58.8**	**78**	**10**	**12**	**0.35**	**1.01**	**1.47**	**43**

FS – fine sand, CS = coarse sand, TS = total sand, Si = silt, Cl= clay, FS/CS = fine sand –coarse and ratio, SCR = silt-clay ratio, BD = bulk density TP = total porosity.

Table 2. Some chemical properties of studies soils

Horizon	Depth (cm)	pH (H_20)	Ca^{2+}	Mg^{2+}	K^+	Na^+	CEC	BG (%)	OC (%)	TN (%)	Av.P (ppm)	Ca/Mg	C/N
	Crest (7-9 % slope)												
	0-12	4.8	0.8	0.3	0.19	0.10	3.86	36	1.22	0.098	8.12	2.66	12.44
	12-29	4.7	0.5	0.2	0.04	0.08	3.72	22	0.92	0.56	6,08	2.50	16.42
	29-65	4.9	0.6	0.2	0.06	0.08	3.96	24	0.63	0.28	4.16	3.00	10.86
	65-105	4.9	0.9	0.3	0.08	0.10	4.02	34	0.39	0.42	4.11	3.00	9.20
	105-190	4.8	0.6	0.2	0.02	0.03	3.36	25	0.22	0.020	3.11	3.00	11.00
	Mean	4.82	0.68	0.24	0.07	0.08	3.78	28.2	0.67	0.040	5.11	2.83	11.78
	Midslope (3-5% slope)												
	0-13	4-9	0.9	0.4	0.09	0.09	0.09	4.42	33	0.068	9.16	2.25	11.47
	13-32	4.9	0.4	0.3	0.05	0.06	0.06	3.62	22	0.026	5.26	1.33	12.69
	32-75	4.9	0.7	0.4	0.06	0.06	0.06	3.86	28	0.022	4.22	1.75	13.63
	75-115	5.0	0.9	0.3	0.05	0.07	0.07	2.98	44	0.020	4.10	3.00	13.50
	115-196	4.8	0.6	0.2	0.02	0.02	0.02	2.56	28	0.019	4.16	3.00	12.63
	Mean	4.84	0.7	0.32	0.05	0.05	0.06	3.49	31	0.031	5.3	2.26	12.78
	Footslope (2-3% slope)												
	0-18	5.0	2.3	0.6	0.10	0.16	5.05	62	1.41	0.150	12.89	3.83	9.40
	18-33	5.0	1.8	0.5	0.07	0.09	4.64	53	0.52	0.080	9.24	3.60	10.00
	33-70	5.2	1.9	0.5	0.06	0.06	4.30	58	0.80	0.048	9.54	3.80	10.83
	70-118	5.1	2.4	0.6	0.06	0.06	4.88	63	0.23	0.18	6.82	4.00	11.15
	118-205	4.9	1.1	0.4	0.04	0.04	3.26	48	0.29	0.026	5.26	2.75	12.77
	Mean	5.04	190	0.52	0.06	0.08	4.42	56.8	0.65	0.064	8.74	3.59	10.83

CEC = cation exchange capacity, BS= base saturation, OC = organic carbon, T. N. = total nitrogen, Av. P = available phosphorus, Ca/Mg = calcium magnesium ration, C/N = carbon – nitrogen ratio.

Table 3. Variability in some physico-chemical properties of studied soils (surface and substance samples)
N = 60

Physiography	TP (%)	Clay (%)	pH (H_2^0)	OC (%)	TN (%)	Av.P (ppm)	CEC (mg/100g)	BS (%)
	Surface soils							
Crest	1.41	12	4.8	1.23	0.089	8.10	3.96	38
Midslope	1.38	15	4.9	0.96	0.071	6.62	4.52	34
Footslope	1.33	11	5.2	1.34	0.073	9.98	5.08	60
LSD (0.05)	0.03	0.2	0.02	0.04	0.007	0.20	0.92	2.60
	Subsurface soils							
Crest	1.47	20	4.9	0.93	0.063	4.30	3.64	28
Midslope	1.49	21	4.9	0.68	0.048	2.96	4.04	29
Footslope	1.52	18	5.0	0.99	0.052	5.49	4.19	53
LSD (0.05)	0.01	0.01	NS	0.03	0.008	0.19	0.87	4.06

OC. Organic carbon, T.N. Total nitrogen, Av. P = available phosphorus, CEC = cation exchange capacity, BS= Base saturation, TP= total porosity.

Table 4. Correlation Matric among physiochemical properties on the three physiographic positions.

		TP %	CLAY (%)	pH (H_20)	OC (%)	TN (%)	Av. P (ppm)	CEC (Meg/100g)	BS (%)
TP (%)	Crest	1.00							
	Midslope	1.00							
	Footslope	1.00							
Clay (%)	Crest	-0.32*	1.00						
	Midslope	-0.44*	1.00						
	Footslope	-0.73*	1.00						
pH (H_20)	Crest	- 0.12	- 0.02	1.00					
	Midslope	0.02	-0.11	1.00					
	Footslope	0.03	-0.11	1.00					
OC(%)	Crest	-0.72**	0.36*	0.23	1.00				
	Midslope	-0.78**	0.27	0.24	1.00				
	Footslope	-0.83**	0.21	0.31	1.00				
TN %	Crest	-0.62*	0.22	0.26	0.91**	1.00			
	Midslope	-0.66**	0.12	0.21	0.98**	1.00			
	Footslope	-0.69*	0.22	0.19	0.79**	1.00			
Av.P (ppm)	Crest	-0.52*	0.29	0.92**	0.88*	0.42*	1.00		
	Midslope	-0.48*	0.31	0.91**	0.82*	0.32*	1.00		
	Footslope	- 0.49	0.21	0.92**	0.92*	0.21	1.00	1.00	
CEC Meg/100g	Crest	-0.52	0.51*	0.43*	0.78**	0.22	1.00	1.00	
	Midslope	-0.41	0.49*	0.53*	0.77**	0.29	0.62*	1.00	
	Footslope	-0.42	0.62*	0.48*	0.76**	0.42	0.64*	1.00	
BS (%)	Crest	- 0.12	0.42*	0.25	0.64*	0.23	0.59*	0.51	1.00
	Midslope	- 0.21	0.48*	0.32	0.58*	0.42	0.53*	0.49	1.00
	Footslope	- 0.26	0.55*	0.29	0.59*	0.29	0.57*	0.53	1.00

TP = total porosity, OC = organic carbon, TN = total nitrogen, Av.P = available phosphorus, CEC = cation exchange capacity, BS = base saturation.

Discussion

Sandiness of these soils are due to a combination of sandy parent material (Coastal Plain Sands), tropical climate and land use. These factors influence pedogenesis and properties of soils (Akamigbo, 1999; Wang e*t al.,* 2001). Although soils of the crest and midslope are highly weathered given low values of silt-clay ratios (0.38-0.53), higher values of coarse sand (56.0-58.2%) were obtained in the study, implying profound influences of this particle sub-fraction in the determination of maeroporosity and moisture retention characteristics of the study site when ccompared with other size fractions and sub-fractions. The consistent increase in per cent silt and clay at the epipedon downslope implies a preferential removal of these fractions and lightness due to their fineness. However, presence of clay bulge in soils of the crest and midslope is indicative of eluviation,argillation and illuviation typical of Ultisols of the tropics (Esu, 2005). Argillation was at its inception in soils of the footslope and data of silt-clay ratio show higher values (1.01) when compared with 0.38 (Crest soils) and 0.53 (midslope soils). These results show that more advanced weathering has taken place in crest soils, followed by midslope soils while those of footslope are at their youthful stage of pedogenesis.

Bulk density increased with depth in all soils irrespective of physiographic position and this could be attributed to overburden effect on deeper horizons as well as declining organic matter content with depth. There was a trend in the distribution of bulk density along the river slope in which values declined downslope possibly due to land use history of similar to a study conducted by the study site (Akamigbo, 1999) although the results of organic matter distribution in the latter did not show similar distribution. Total porosity contrasted with the distribution of bulk density as it a decreased with depth. However, high values of total porosity were recorded at the entire pedosphere, implying greater availability of soil air, soil water, soil aerobes and root abundance, and these have far-reaching beneficial effects on the agronomic suitability of soils of the University farm. These conditions are on the assumption that values of macroporosities of these soils are greater than those of microporosities since soils are textually coarser. However, the influence of porosity on soil moisture availability and uptake is a result of complex interactions among physicochemical properties in the soilsphere (Eynard *et al.,* 2006)

Increase in pH with depth is indicative of illuviation of basic cations translocated after intensive leaching from the surface horizons. Again, increased pH values downslope suggests movement of basic cations along the slope towards the footslope, and this may account for high base saturation, Ca/Mg ratio, total nitrogen, cation exchange capacity and organic carbon. Agronomically, soils of the footslope hold greater potentials for crop production with less fertilizer input while increased extraneous fertilizer sources are needed for enhancing soil fertility in crest and midslope soils. However, increased soil loss from high slope soils may result to sedimentation and burying of better quality soils of the footslope with time hence the call by Opara et al. (2007) to enhance aggregate stability of soils of southeastern agroecology with organic manure including using rabbit waste. Generally, soils of the study site are vulnerable to calcium and phosphorus deficiencies as they show low calcium-magnesium ratios. According to Landon (1984), a decrease of Ca/Mg ratio to a level below 3 results on the unavailability of calcium and phosphorus. Based on the results, soils of the Crest and Midslope positions are very susceptible to Ca and P fertility constraints. Despite the fact that soils are situated in the rainforest agroecology characterized by abundant leaf litter, soils of the study site have low exchangeable calcium. This trend was reported in European forest soils (Thimonier *et al.,* 2001), and could be attributed to enhanced leaching of soils by organic acids derived from decomposed organic debris. Unavailability of sufficient soils calcium in the root zone have been associated with low productivity of some soils of Nigeria (Osemwota *et al.,* 2003).

Other fertility parameters, namely cation exchange capacity, base saturation, organic carbon, total nitrogen and available phosphorus had values below recommended levels for optimal productivity in tropical soil of Nigeria (FDALR, 1985; Enwezor *et al.,* 1990). Of these factors, organic carbon ranks among the principal factors governing fertility of tropical soils of Southeastern Nigeria (Onweremadu *et al.,* 2007); influencing structural aggregate stability (Mbah *et al,* 2007; Opara *et al.,* 2007), phosphorus availability (Dodor and Oya, 2002), exchangeability of cations and buffering capacity of soils. These soil

parameters varied significantly (P = 0.05) in both epipedons and sub-surface horizons irrespective of geomorphic setting, suggesting delineation of soils of the University farm into mapping units for their sustained use for agricultural and/or non-agricultural enterprises. Again, the relationship between organic carbon and some soil properties notably total nitrogen, available phosphorus cation exchange capacity, and base saturation suggests the use of these parameters for modelling which enhances predictiveness in precision agriculture, especially after subjecting the principal factors to multiple regression analysis using organic matter (organic carbon) as dependent variable.

The classification of a riverslope soils Federal University of Technology into Typic Hapludults (Crest and Midslope soils) and Typic Eutrudepts (Footslope soils) was based on a combination of differentiae (criteria for classification) as contained in Soil Survey Staff (2003). Soils of the crest and midslope geomorphic settings are characterized by argillic horizons (Bt) and low silt-clay ratios, indicating advanced weathering and a consistent decrease in organic matter content with depth while soils of the footslope lacked argillic horizons, had high silt –clay ratio and irregular organic matter distribution with depth.

Conclusions

We evaluated anisotropic properties of soils lying on three physiographic positions of a University farm in southeastern Nigeria. The study revealed variability in selected edaphic properties irrespective of physiography and soil depth. Total porosity, clay content, pH, organic fractions, available phosphorus, cation exchange capacity and base saturation showed a significant variation (p=0.05) at epipedal horizon while only soil pH was non-significant in sub-surface soil horizons. A correlation matrix of attributes in the study showed that organic carbon had pronounced influences on total nitrogen,available phosphorus, cation exchange capacity and phosphorus. This study suggests more intensive sampling as well as detailed investigation of soil and soil -related parameters in the university farm occupying over 200 hectares of cultivable arable land. Data generated from such detailed studies would be beneficial especially when subjected to multivariate statistical techniques for the purpose of increasing accuracy of predictions in present and future land uses.

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Photoluminescence in the polymer nanocomposites on the basis of PP + CdS

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Have been stated the results of research of photoluminescent properties of nanocomposites based on polypropylene (PP) treated by discharge in air quality, which is higher than breakdown strength of air and filling compound CdS in wavelength interval λ= 300-1000 nm. Have been studied by the atomic-force microscope (AFM) the structures of nanocomposites PP + CdS samples, prepared from PP powder, treated and untreated in various intervals of time. AFM research and study of photoluminescent spectra revealed that the dimensions of CdS nanoparticles in PP did not depend on discharge treatment duration, but the concentration of CdS nanoparticles in polymeric matrix PP depended on discharge treatment duration. Has been shown the changes of CdS nanoparticles concentration in PP subject to discharge treatment duration was correlated very well with experimental data, obtained from photoluminescent spectra. It supposed that change of nanoparticles concentration subject to discharge treatment duration is connected with formation of oxidative centers in polymers, which play the role of nucleation centers for CdS. [The Journal Of American Science. 2007;3(4):62-67]. (ISSN: 1545-1003).

Key words: nanocomposite, photoluminescent, polypropylene, atomic-force microscope, nanoparticle

1. Introduction

The search and preparation new photoluminescent polymeric nanocomposites (PNC) is a question of great scientific and practical importance for understanding of energy transfer mechanism, transport carriers in multiphase polymeric systems. Above-mentioned problems are important in terms of preparation luminescent screens, transducers, sensors and other facilities with improved physical-chemical characteristics and broadened intervals of phosphorescence in visible region of spectra based on such multiphase polymeric systems. Development and preparation of new photoactive nanocomposites is closely connected with understanding of interrelation "structure-technology-properties-application" of these materials. Polymeric nanocomposites, composed of two or more phases, challenge the developments of physical and chemical methods of preparation of new active elements by modification of their structures and properties [1-4]. These materials include the positive properties of polymeric matrix (flexibility, possibility to obtain the elements of any configuration and others) as well as active filler (sensitivity) and at the same time possess sufficient photoluminescent properties in combination with physical and chemical characteristics. It should be noted that by variation of components properties it is possible to change the properties of nanocomposites, to study the interface effects, to research intermolecular transfer processes and migration of electron excitation energy in polymeric medium, the influence of interface interactions on photoluminescent properties of filler [5-7]. The research of polymeric nanocomposites structures with inorganic semi-conductors (sulfides, transition metals oxides) is of interest in terms of inherent to them new electrochemical, photochemical, magnetic and other characteristics [8-10]. Structure and properties of these composites associate to each other. The data of structure (dimension and character of distribution of the particles of dispersed phase in the matrix of polymer) allow forecasting the properties of this system and vice versa the study of the properties allow forecasting the structure of nanocomposites.

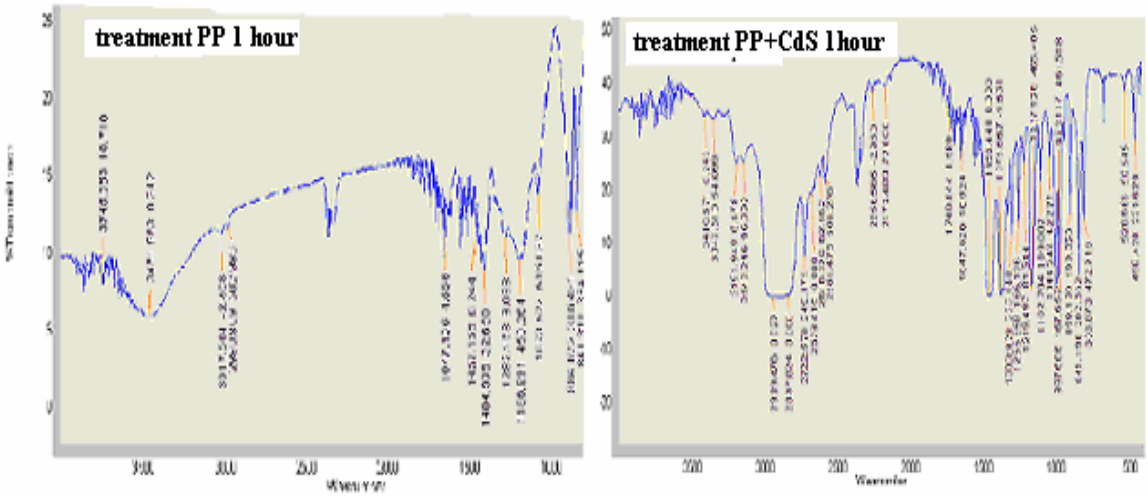
2. The Samples and experiment method

In this work is given the results of research of photoluminescent properties of nanocomposites on the basis of polypropylene (PP) and filler CdS treated in wavelength interval λ= 300-1000 nm. With aim to find out the influence of type of polymeric matrix on structure and photoluminescent properties of nanocomposites have been used the matrix differ in character of partial discharge interaction and in formation of oxidizing groups. The polymeric powder (size

of particles 0, 5-1, 0 mkm) with aim to increase the reactivity towards the transition metal ions Cd^{2+} was treated by electrical discharge in air quality, which is higher than breakdown strength of air in various intervals of time. The nanocomposite polymer + CdS was prepared by treatment of samples of powder of PP in 0,1 M solution of $CdCl_2$ and 0,1 M of Na_2S. The definite proportion of treated polymeric powders of PP first was mixing by magnetic mixer with 0,1 M solution of $CdCl_2$ during 20-25 minutes. Then filtered powder, containing Cd^{2+} ions was cleansed by water in order to remove the weakly bounded Cd^{2+} ions, and then was treated by 0,1 M solution of Na_2S. After that, the powder was getting dry in 24 hours. Further from that powder were prepared the samples of nanocomposites PP + CdS by hot-pressing method at the melting point of PP. The structural modifications of nanocomposites PP + CdS, obtained by hot-pressing method at the melting point of PP and treated in various time intervals, were studied by IR spectroscopy method. Has been studied the relief of nanocomposites samples, treated in various time intervals by electrical discharge AFM. Photoluminescent spectra have been studied on spectrofluorimeter Cary Eclipse in wavelength interval 300-1000 nm.

3. The results of experiments

The spectra, presented in Pic.1 are IR spectra of PP and nanocomposite PP + CdS samples, treated in an hour by electric discharge. It is clear from the pic.1 that there is strong change in IR spectra especially in wavelength region 2846 sm^{-1}, 1456-1186 sm^{-1}. Depending on duration of treatment, was observed the increasing of absorption band strength in wavelength region 2950 sm^{-1} and 2846 sm^{-1}, to result from the activation of CH valence vibrations in spectra of polypropylene. It is also shown in IR spectra of nanocomposite PP + CdS samples, treated by electric discharge, the activation of absorption band strength of CH valence, deformation vibrations and vibrations mutual influence of CH and CH_2 groups were observed. It is known that interaction strength of these groups depends the degree of stereo regularity of macromolecule and rotation about C—C bonds of main chain.

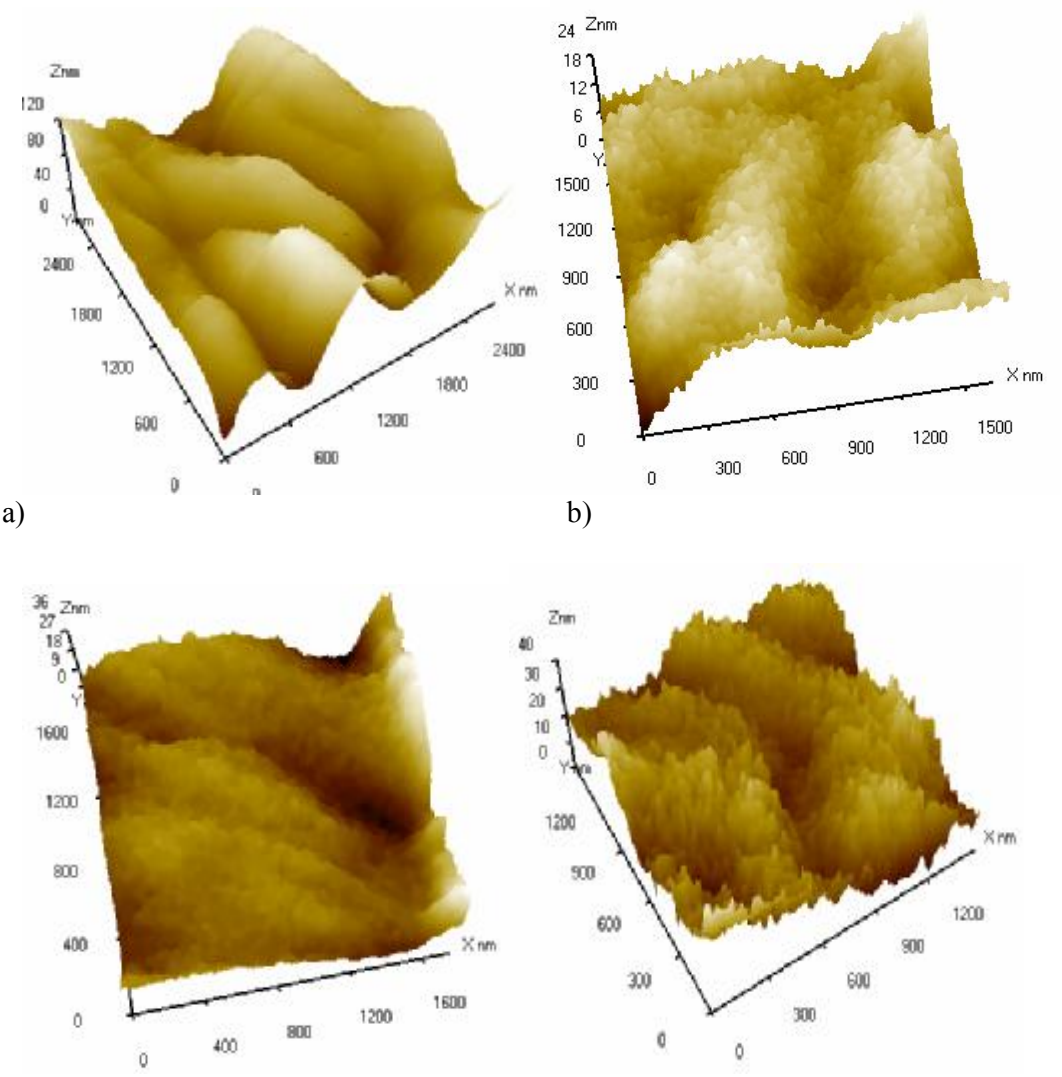


Pic.1 IR spectra of PP and nanocomposite samples, treated by electric discharge

Have been studied by the atomic-force microscope (AFM) the relief of nanocomposites PP + CdS samples, obtained from PP powder, treated and untreated by electrical discharge in air quality in 0,5 h, 1h and 3 hours (Pic.2). As it is seen in the pic.2 the relief of treated samples in various intervals of time becomes rough. The increasing of exposure time leads to increasing of concentration of CdS particles in polymeric matrix to certain extend. The formation of nanophase CdS is the result of interaction of Cd^{2+} and S^{2-} ions in the immediate volume of the polymeric matrix; they become the oxidation centers due to the discharge treatment in air quality. Cd^{2+} and S^{2-} ions move towards each other: the ions Cd^{2+} from the solution towards the oxidation groups in polymer; ions S^{2-} from solution in polymeric matrix towards the Cd^{2+} ions. To vary the concentrations of $CdCl_2$ and Na_2S it is possible to get the uniform distribution of

nanoparticles CdS in polymeric matrix. The usage of very low concentration of Na_2S leads to emission of Cd^{2+} ions in solution and forming CdS phase. One of the principal parameter, influencing the character of dispersed phase distribution in sample volume is the ability of polymeric matrix to form complex with transition metal ions. The ability of polymeric matrix to form complex increase with discharge treatment, i.e. the majority of dispersed component forms around the oxidation centers in polymer. The AFM-scanning of PP + CdS samples relief shows the increasing the CdS nanoparticles on the samples surface.

The dimensions of photoluminiscent particles in PP matrix have been studied by scanning atom force microscope and given in pic.3. It is seen in pic.3 that the CdS nanoparticles dimension is 20-27 nm. The AFM-scanning shows the dimensions of nanoparticles do not depend the time of discharge treatment, and the concentration of CdS nanoparticles in polymeric matrix depends the duration of discharge treatment. The increasing of discharge treatment duration evidently leads to structural damage of polymer. The concentration change of CdS in PP with duration of discharge treatment seemingly is correlated with forming of oxidizing centers in polymer, which are the nuclease center for CdS.

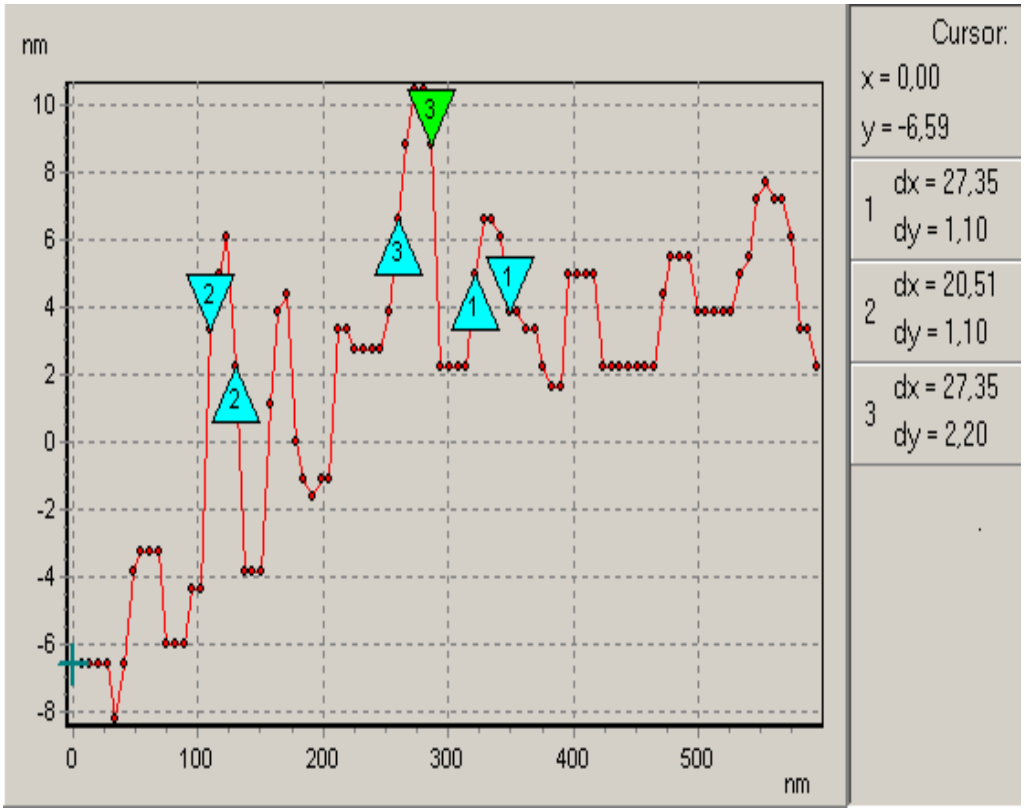


a) b)

c) d)

Pic.2 3D image observed by AFM of nanocomposite PP + CdS

a) untreated powder of PP
b) treatment duration of PP powder 30 minutes
c) treatment duration of PP powder 1 hour
d) treatment duration of PP powder 3 hours



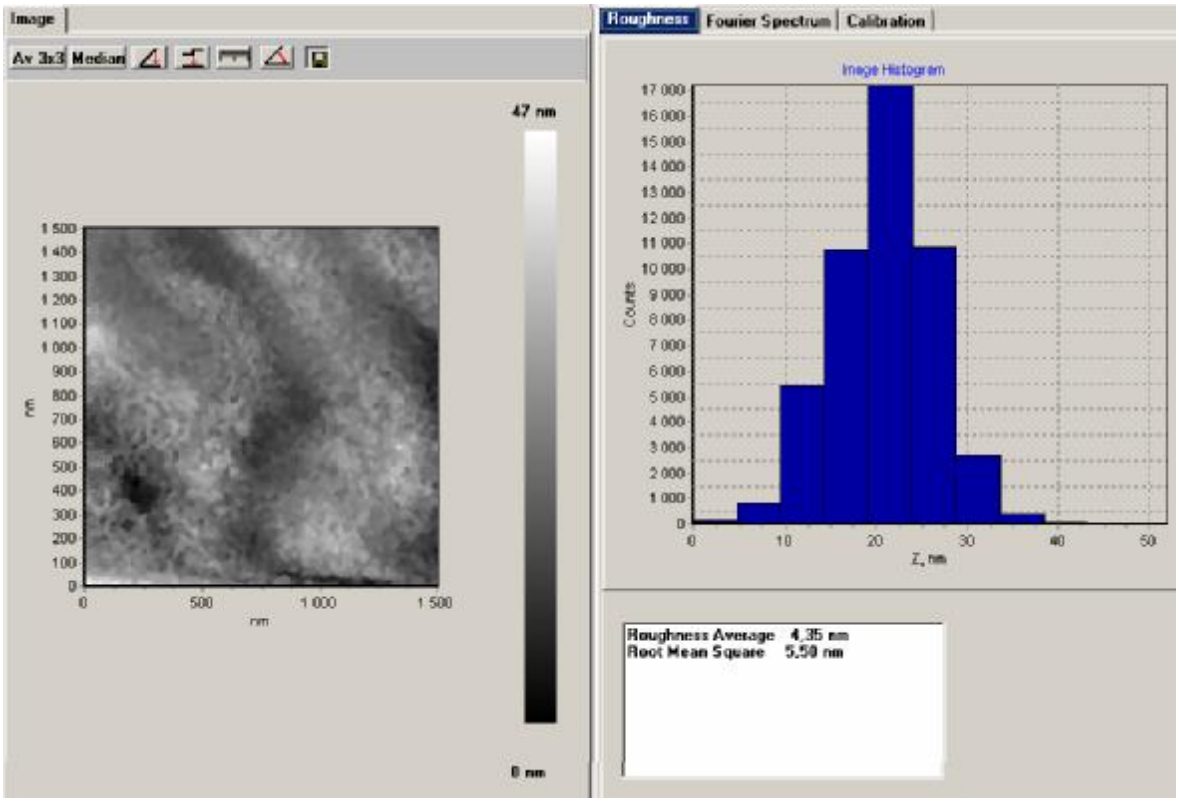
Pic.3 CdS nanoparticles dimensions in polymeric matrix

The analysis of the properties of nanocomposite PP + CdS surface and histogram of image element values is shown in pic.4. It is seen in pic.4 that root-mean-square roughness of nanocomposite surface makes 15-30 nm. Fourier analysis distribution shows that CdS nanoparticles are equally spaced in polymeric matrix.

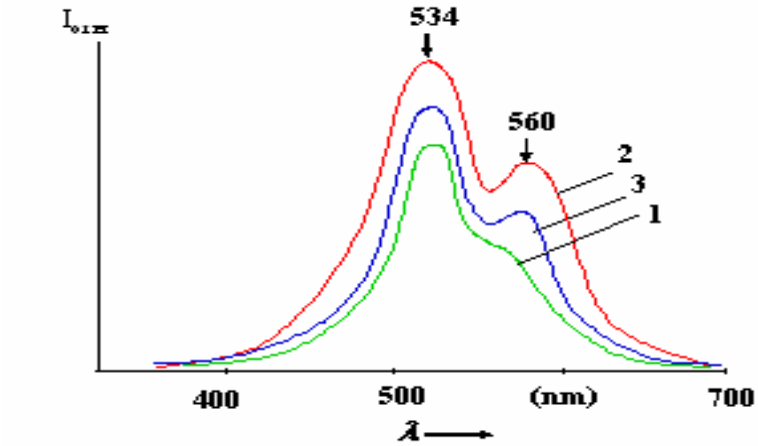
There are luminescent spectra of nanocomposite PP + CdS, prepared from polymer, treated and untreated by electrical discharge in various intervals of time in the pic.5. It is clear in pic.5 there are two maximums in wavelength interval 534 nm and 560 nm and its broadband changes with duration of treatment.

In addition, it is shown that intensity of photo fluorescence increase with discharge treatment duration of powder until 30 minutes and after that decrease.

Thus photoluminescent spectra and AFM-research reveal that scales of CdS nanoparticles in PP do not change with discharge treatment duration, and the concentration of CdS nanoparticles in polymeric matrix changes with duration of discharge treatment. The further increasing of discharge treatment duration evidently leads to damage of chemical structure of polymer. The concentration change of CdS in PP with duration of discharge treatment is correlated with experimental results, getting from photoluminescent spectra and evidently is connected with forming of oxidizing centers in polymer, which are the nuclease center for CdS formation.



Pic.4 The analysis of the properties of nanocomposite PP + CdS surface and histogram of values of element images



Pic.5 Photoluminescent spectra of nanocomposite PP + CdS, prepared from PP, treated and untreated by electrical discharge in various intervals of time.

1. ***t = 0;*** **2.** ***t = 30 min.;*** **3.** ***t = 3 hour***

It is assumed, that discharge treatment of polypropylene increases the active centers in polymeric matrix, so form the traps for ions, and as result Cd^{2+} ions from solution move towards the trap, S^{2-} ions from solution move PP matrix towards the Cd^{2+} ions. The discharge treatment increases complexation ability of PP, creates optimal conditions for CdS nanoparticles formation in free volume of polymeric matrix.

In conclusion it should be noted, that mechanism of formation of nanoparticles and structure of nanocomposite with CdS in oxidized polymeric matrix along with general

characteristic has essential distinctions, caused by thermodynamical characteristics of dispersed phase and whole composition, such as heat of formation of inorganic component and surface free energy of interface of polymer-particle.

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Can Relativity Be Considered Consistent?

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Abstract: The aim of this article is to localize nonlocality within the theory of relativity by applying Gödel's incompleteness theorem to it. The technique applied in this article is based on cultivating consistency within the normal language. When this is achieved this consistent language can then be used to describe the logical system of relativity. According to Gödel's incompleteness theorem it will be possible to reveal the incompleteness of relativity by creating a statement, which is impossible to either prove or disprove e.g. a paradox. A more profound (meta-) language needs to be constructed in order to conceive the truth of this paradoxical statement. By understanding the deeper truth of this paradoxical statement nonlocality becomes localized. [The Journal Of American Science. 2007;3(4):68-71]. (ISSN: 1545-1003).

Key words: nonlocality; Gödel; paradox; meta-language; meta-physics; universal truth;

Introduction: As science has entered the twenty-first century, nonlocality has become more or less accepted and Einstein's accusing finger pointing at quantum mechanics slowly starts making a turnaround by questioning the theory of relativity. Nicolas Gisin reflects on this in his article "Can relativity be considered complete?" as he writes in the conclusion: "Well, if nonlocality is really real, as widely supported by the accounts summaries [sic] in this article, then all complete theories should have a place for it. Hence, the question is: "Does relativity hold a place for non-signalling nonlocal correlations?""[1] This was strangely enough already demonstrated in 1918 by Albert Einstein. In his writing "dialog about objections against the theory of relativity"[2] one can read how Einstein uses the aid of an appearing and disappearing homogenous gravitational field to describe the proceedings related to an accelerated coordinate system K' (below the diagrams, column "K' is the reference frame" in section 1, 3 and 5). Einstein leaves it unmentioned that these gravitational fields have to appear and disappear everywhere at the same time, but this was clarified later by others. Geoffrey Builder (1957) writes: "The concept of such a field is completely incompatible with the limited value c for all velocities [...], so that the specified field would have to be created simultaneously at all points in S' and be destroyed simultaneously at all points in S0. Thus the principle of equivalence can contribute nothing of physical significance to the analysis."[3] This can now be seen in a different light as we realise that the appearing effect of such a gravitational field fits the description of a non-signalling nonlocal correlation and the whole analysis needs a more profound examination instead of being discarded on the grounds of a limited value c.

Albert Einstein must have been aware of the dilemma that was hiding behind his explanation as he writes further on in his dialog: "Critic: […] How are we supposed to believe that a merely fictitious field could have such an influence on the pace of a clock? Relativist: […] This consideration makes it clear that a complete clarification of the questions you have raised can only be attained if one envisions for the geometric-mechanical constitution of the Universe a representation that complies with the theory. I have attempted to do so last year, and I have reached a conception that - to my mind - is completely satisfactory; going into this would however take us too far." Einstein's completely satisfying conception has (as far as can be retraced) failed to appear and after Einstein passed away the case became more or less closed by Builder's article when nonlocality was still unaccepted.

This article goes back to the year 1918 when Einstein was at the peak of his career after having made major contributions to the theory of relativity and science in general. His profound understanding of relativity (reflected in his written dialog) will be combined with Kurt Gödel's expertise on mathematical logic and the quantum revelation of nonlocality. The results of the three most fundamental scientific discoveries of the twentieth century (relativity theory, quantum mechanics and incompleteness theorem) will be synthesized in order to see if a more profound understanding of the Universe can be achieved.

Methodology: A consistent (superficial) language will be constructed in order to be able to describe the theory of relativity. This superficial language is the normal language though it is disciplined with the purpose of maintaining consistency. One of the disciplines is to avoid the use of the word "not" and its derivatives like "nothing", "nowhere", "never", etc. This is necessary because the superficial language is incomplete and this way it remains consistent. Instead of using the word "not" the prefixes "un-", "in-", "il-", "im-", etc. are used to define the opposite or reverse, for example, unsimultaneous.

The incompleteness of the superficial language will be described by a meta-language. This meta-language only consists of words starting with "non~" for example, non~selfishness. It is impossible to define the meaning of this word within the superficial language and its meaning can only be indicated by creating a paradox. Examples of such paradoxes are: selfishness without selfishness, unselfishness without unselfishness, unselfish selfishness and selfish unselfishness. All these descriptions indicate a more profound truth which can be realised if one pictures a mother feeding her young, making all four paradoxes understandable. This example is completely unscientific but it uses awareness of a more profound truth to illustrate how a paradox in the superficial language can actually point out to this deeper truth, which otherwise would be impossible to signify with either "selfishness" or "unselfishness". If one is unaware of this more profound truth indicated by the paradox, all that will appear is a contradiction making "nonsense". One must therefore realise that the paradox created in the superficial language only values as an indicator and refrains from giving a true description of non~selfishness within the superficial language. In the same manner it can be realised that all understanding of non~locality is based on thoughts shaped by the superficial language and is therefore science fiction. One has to take a step backwards and avoid being sidetracked by the fantasy of understanding non~locality, because the thoughts that found this understanding are based on inconsistent reasoning. The discipline of unthinking these thoughts is necessary to maintain the strictness of consistent reasoning within the superficial language. Whether awareness can ultimately conceive non~locality remains undiscussed within this article. The word "non" is chosen to formulate this meta-language because of its meaning: "absence of something rather than the opposite or reverse of it." This aspect is demonstrated in the case of the word "non-logical" which differs from "illogical". The "~" is used to make it extra clear that the word refers to the meta-language instead of the superficial language.

This way the words local, unlocal and non~local become logically consistent and the current ambiguity that lies within the word "nonlocal" is avoided in an intelligent way; signalling nonlocal correlations are unlocal correlations and non-signalling nonlocal correlations are non~local correlations. The word "nonlocal" is now made redundant.

Discussion: The EPR paradox and the twin paradox are both paradoxes in which Albert Einstein played an important role. The twin paradox challenged the theory of relativity and Einstein was left explaining how it is possible for two identical clocks to show different elapsed time when taking different paths. Einstein explains (in his 1918 written dialog) that a homogenous gravitational field appears for the "spaceship twin" when accelerating backwards. The effect of this gravitational field compensates exactly enough to clear up the paradox. If one calls the spaceship twin Alice and the Earth twin Bob the following occurs; as soon as Alice pushes the button to activate the rockets for her return journey Bob's clock will go relatively faster compared to Alice's clock. When Alice pushes the button it affects Bob's clock in the instant moment. It is impossible for Bob though to notice anything of this effect in that instant moment.

In quantum mechanics exactly the same kind of event occurs as pointed out by the EPR trio. When Alice "pushes the button" (to make a measurement on her particle) it affects Bob's particle in the instant moment. It is impossible for Bob though to notice anything of this effect in that instant moment. This is what Einstein called "spooky action at a distance".

It is strange to realise that the man who criticised quantum mechanics for all of his life, partly because of the spooky action at a distance, was in fact also the first person to come up with the spooky action. Niels Bohr missed a beautiful opportunity here. Instead of defending quantum mechanics against the EPR paradox he could have mirrored the paradox upon the theory of relativity. Einstein would have been left with the following dichotomy: Either

1. The result of an acceleration performed locally at part **A** by an object has an unlocal effect on the physical reality of a distant object at part **B**, in the sense that the theory of relativity can completely predict the outcomes of this unlocal effect acting on **B**, or
2. The theory of relativity is incomplete in the sense that some element of the physical reality corresponding to **B** is unaccounted for by the theory of relativity (that is, some extra variable is needed to account for it.)

This mirrored EPR paradox can be solved with the aid of Gödel's incompleteness theorem. Kurt Gödel proved his incompleteness theorem for a particular logical system, but commented in the introduction to his proof that it could be used for almost any logical system. Gödel's incompleteness theorem shows that within a logical system, there exist certain clear-cut statements which are impossible to prove or disprove. The truth of such a statement reveals the incompleteness of the logical system and is often referred to as "the Gödel sentence". [4][5][6]

The theory of relativity can also be seen as a logical system which is "embedded" in the Universe. One can try to understand the Universe in terms of relative truths that are described by the theory, but even a complete understanding of this will still be incomplete. In order to attain a more profound understanding of the Universe one can apply Gödel's incompleteness theorem to the theory of relativity. This is done by constructing a Gödel sentence that will reveal a truth which can only be realised from a universal viewpoint.

The Gödel sentence for the theory of relativity is: "The Principle of local action [7] acts unlocally." It is impossible for the theory of relativity to either prove or disprove this statement, because the truth of this statement is indefinable within relativity. The way of the Universe is therefore more profound than the rules and axioms of the theory of relativity. This paradox reveals the incompleteness of the system and indicates a more profound truth called non~locality. This non~locality is of course the same kind as the "nonlocality" revealed by quantum mechanics. It can now be understood that there exists a universal truth called non~locality.

The mirrored EPR paradox observes the theory of relativity from a universal viewpoint and reveals locally an unlocal effect created by a homogeneous gravitational field. This locally appearing unlocal effect is only realised if one possesses a "birds-eye view" or in this case a "universal-eye view" while zapping between different coordinate systems. One can now understand that this effect is a consequence of a universal truth called non~locality, which can be revealed by applying the incompleteness theorem to the theory of relativity. The theory of relativity gives in this respect a complete prediction of the outcomes and an incomplete understanding of the Universe, while non~locality being a universal truth.

There are more Gödel sentences for the theory of relativity, each one indicating a universal truth with its particular paradox. Examples that have already been pointed out are: "simultaneous occurrences occur unsimultaneously" revealing non~simultaneity and "coordinate systems coordinate unsystematically" revealing non~systematisation also known as "non-aether" (non~aether). These two examples have already been integrated with relativity while non~locality lies patiently awaiting to be welcomed.

There are more ways to demonstrate that something spooky is going on within relativity. One of them is by analysing the symmetry of framework on which special- and general relativity are based. Both of them use the principle of equivalence which leads in both cases to a paradox. Herbert Dingle challenged this paradox in the case of special relativity, but his argument was overthrown by the consistency of the calculations. [8][9] Geoffrey Builder pointed out the paradox within general relativity, but in his case the consistency of the calculations are seen as unreal and the argument is accepted, while this paradox is encountered as a real contradiction. Special relativity enhances the non~aether with non~simultaneity, while general relativity is obstructed in doing any "non~" contribution and is conceived as an empty shell with a contradiction attached. Occam's razor is welcome to chop the special layer, but is still prevented from slashing the general shield.

It is a clear-cut case and science has a lot to answer for. Therefore the question: "Can relativity be considered consistent?" has to wait and the following question needs answering first: "When will relativity be considered with consistent reasoning?"

Conclusion: The whole nature of the theory of relativity is consistent reasoning. Kurt Gödel demonstrated that consistent reasoning within a logical system unfolds a paradoxical statement for this system which indicates a more profound truth. If the Universe is ruled by the principle of local action then this principle must rule everywhere simultaneously and therefore this principle must be acting unlocally. It may thus be concluded that the principle of local action acts unlocally. Although this statement appears to be "nonsense" it is "important nonsense" because it is non~sense. Whether consistent reasoning can ultimately conceive the way of the Universe remains to be seen. One thing though is sure; a paradox can be a gateway towards a more profound truth.

Disclaimer: This article disclaims describing the truthful truth. The purpose of this article is to demonstrate through consistent reasoning within a logical system (relativity theory) the existence of nonlocality (non~locality) by creating a meta-language. This meta-language is in itself again either inconsistent or incomplete. The article chooses for completeness in order to give a clear presentation of non~locality and therefore creates inconsistency. This is demonstrated for example in the following sentence: "It can now be understood that there exists a universal truth called non~locality." As soon as non~locality is understood it is added to the logical system as truth (universal but true) and existing, creating a bigger logical system. When non~locality is localized, the meta-language becomes contained by complete understanding while consistency twists and collapses.

If one decides to maintain consistency instead, this sentence would be as follows: "It can now be understood that there non~exists a non~truth called non~locality." With consistent reasoning the following Gödel sentence can then be constructed within the meta-language: "Non~local action acts un-non~locally." revealing that the way of the Universe is even more profound than described by the meta-language. This paradox indicates a truth called non~non~locality which demands an even more profound meta-meta-language. This meta-meta-language is in itself again either inconsistent or incomplete. This shows that consistent reasoning gets twisted by incompleteness and that each step forward is warped into a loop, while the problem persists. The universal truth keeps on slipping away from truth into non~truth and then into non~non~truth etc. being always one step ahead of its seeker.

This dilemma is clearly demonstrated by Gödel's incompleteness theorem, which can also be stated as follows: "The realisation that suits understanding is inconsistent or incomplete." The ultimate question therefore remains untouched: "How can the seeker of truth bypass this twisting contraption?"

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The Number of Channels during Laser Transmyocardial Revascularization Can Alter Myocardial Function in the Isolated Rat Heart

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Abstract: Background and Objectives: Heart failure is a major potential complication in transmyocardial revascularization (TMR). The objective of this study was to assess the effect of TMR on myocardial function. **Study Design/Materials and Methods:** Two experiments were performed in normal rat myocardium, one using myocardial muscle strips, the other using a whole heart preparation. TMR was performed using Ho:Yag laser (2100 nm, 3 Hz, 280 mJ/pulse) via 0.6 mm core optical fiber. Myocardial muscle strips (n=14) were paced with 1 Hz/80 mV pulse current at a rate of 60 beats/min. A whole heart Langendorff set up (n=37) was used to perfuse the myocardium via a cannula in the aorta. Also, heat shock proteins (hsp) were measured by Western Blotting and ATP measured by a standard enzymatic method (n=10). **Results:** Using the myocardial strip preparation, Frank-Starling curves demonstrated a decrease in contractility compared to baseline by 42% after 20 TMR channels (0.55 0.26 vs. 0.32 0.12 g/cm^2; $p<0.05$). In the whole heart preparation, myocardial contractility decreased by 21% after 20 TMR channels (5.8 0.9 vs. 4.6 1.5 g/heart; $p<0.05$). After 50 TMR channels/heart, hsp70 decreased by 78% and ATP increased by 32%. **Conclusions:** Lasing 20 channels/heart caused a significant decrease in contractility in normal rat myocardium while ATP levels increased. [The Journal Of American Science. 2007;3(4):72-80]. (ISSN: 1545-1003).

Keywords: channel numbers; heart failure; transmyocardial revascularization

1. Introduction

Coronary artery disease continues to be the leading medical cause of death worldwide. However, the development of newer technologies to treat myocardial ischemia has resulted in increased survival of patients with persistent and chronic ischemic heart disease. Many of these patients have diffuse coronary artery disease that is not amenable to further bypass surgery or angioplasty.

Transmyocardial revascularization (TMR) using laser irradiation is a technique reserved for refractory angina that is not responsive to the traditional therapy (1). The rationale for the procedure was based on the reptilian heart that receives its main blood supply from direct myocardial-ventricular channels. However, more recent studies suggest that most TMR channels had become chronically occluded. An alternative hypothesis to explain improvement of patient symptoms has been attributed to neorevascularization that occurs locally around the scarred TMR channels that may be perfusing ischemic muscle. Despite the controversies about mechanism of action, a major concern with the use of TMR is causing or worsening of heart failure by further loss of myocardial tissue. Thus, the number of channels that need to be placed should be defined in order to optimize the potential clinical benefits.

The aim of this study is to evaluate the relationship between the number of lased TMR channels and myocardial contractility as well as biochemical response of the myocardium to TMR.

2. Materials and Methods

TMR was performed on the rat heart tissues using a Ho:Yag (2100 nm, 3 Hz, 280 mJ/pulse) (Optipulse, Trinedyne, Irvine, CA) laser to create a full thickness channel in the myocardium. Laser irradiation for TMR was performed using a Ho:Yag laser delivered via a 0.6 mm core optical fiber.

2.1 Transmyocardial Revascularization

Sixty-one female Sprague Dawley rats (300-400 grams) were used in this study. Heparin (1000 unit/kg, I.M., Sigma, St. Louis, MO, USA) was administered 1 hr prior to anesthesia to prevent intracardiac thrombus. General anesthesia was induced using pentobarbital (80 mg/kg I.M., Abbott Laboratories, North Chicago, IL) and then the heart was rapidly excised via a mid-line chest incision. The hearts were kept in oxygenated PBS at 0^{o}C until use (2). A total of 14 hearts were used to make myocardial muscle strips and 37 hearts were set up as a Langendorff preparation. An additional 10 hearts were used for biochemical

analysis (3,4).

***Myocardial Strip Preparation*:** The myocardial strip model was made by excising both atria and right ventricles. Then, an incision from the apex of the left ventricle to the base on both sides was made while leaving the apex intact. Each side of the left ventricular muscle strip was then fixed to a post and an electrode was used to stimulate the myocardium. Electrical stimulation was delivered from a square wave pulse generator (1 Hz/80 mV/pulse). The preparation was immersed in PBS and allowed to stabilize for over 1 hr. prior to TMR (Figure 1). Twenty TMR channels were placed in the muscle followed by 10 min rest intervals until myocardial contractility deteriorated, and the channels were evenly distributed along the length of the muscle segment.

***Whole Heart Langendorff Preparation*:** In this set up, 37 hearts were perfused using oxygenated PBS via the coronary circuit after cannulating the ascending aorta as demonstrated in Figure 2. The perfusion pressure was maintained at a mean of 60 mmHg. After 1 hr of perfusion, laser irradiation was performed in 23 hearts with laser salvos (4 channels × 5; and 10 channels × 3) and 10 min rest interval after each salvo. The hearts were perfused for up to 6 hr. Fourteen hearts served as control and were treated in the same fashion without lasing.

Myocardial Contractility Measurement: The Frank-Starling curves were measured to evaluate myocardial contractility. A basic physical property of cardiac muscle stipulates that if cardiac muscle is stretched, it will result in greater force of contractility. This continues to increase with stretching until the muscle fails. This process allows the detection of very early myocardial failure prior to changes that can be noted at resting conditions. Contractility was measured using a transducer attached to a strain gauge sutured to the cardiac apex. The electronic signal was then converted to mechanical displacement on a strip chart recorder. Various preloads (0, 1, 2, 3 g) were used to elicit a Frank-Starling response obtained after each salvo of TMR channels.

2.2 Biochemical Analysis

Heat shock proteins (hsp25, 60, 70, 90) and ATP were measured in ten hearts. Three hearts were used as control-control measurements without perfusion. An additional three hearts were first perfused with the PBS in the Langendorff set up for 6 hr and then homogenized for hsp and ATP measurements. Another three hearts were perfused for 1h and then 50 TMR channels were made followed by 5 hr perfusion prior to homogenization for hsp and ATP measurement. One heart was heated in a waterbath at 42^{o}C for 15 min then kept at room temperature (23^{o}C) for 6 hr prior to hsps and ATP measurement (5, 6).

***Heat Shock Protein Measurement*:** Myocardial tissue was homogenized in 3 × volume (W/V) of buffer extract under ice. The homogenized sample was centrifuged at 10,000 rpm for 10 min at 4^{o}C and the supernatant was collected. Heat shock proteins (hsp25, 60, 70 and 90) from the supernatant were detected by Western Blotting using monoclonal heat shock protein antibodies (hsp25, 60 and 90 antibodies, Sigma, St. Louis, MO; hsp70 antibody, StressGen Biotechnologies Corp, Victoria, BC, Canada). Secondary antibody was measured by the alkaline phosphatase method.

***Measurement of ATP*:** The myocardial ATP content was measured according to Beutler (7). Briefly, homogenized heart tissue was treated as above. A 0.2 ml of the supernatant was mixed with 0.8 ml of sample modified Tris buffer (Tris-HCl 100 mM, EDTA 0.5 mM, $MgCl_2$ 2 mM, NADP 0.4 mM, glucose 1 mM, G-6-PD 0.3 U/ml, pH 8.0). Baseline readings were taken at $OD_{340\ nm}$ and 37^{o}C. A 0.01 ml of hexokinase (400 U/ml) was added and readings were repeated at $OD_{340\ nm}$ at 37^{o}C until a constant value was reached. ATP content in the sample was derived by comparison to a standard ATP curve.

2.3 Statistical Analysis

Data were reported as means ± S.D. and compared by a paired Student's t-test. A $p<0.05$ was considered significant. The SigmaStat statistical software was used to calculate statistical comparison.

3. **Results**

3.1 Myocardial Strip Studies

***Contractility*:** The myocardial strip studies (n=14) had normal Frank-Starling curves at baseline.

Following lasing of 20 channels/strip there was a significant decline in the Frank-Starling response. At a preload of 1.2 g/cm^2 lasing compared to non-lasing, myocardial contractility decreased by an average of 42% after 20 channels (0.55 0.26 vs. 0.32 0.12 g/cm^2; p<0.05). Following 60 channels there was 67% decrease (0.55 0.26 vs. 0.18 0.11g/cm^2; p<0.03) and after 180 channels there was 84% decrease (0.55 0.26 vs. 0.09 0.02 g/cm^2; p<0.01) (Figure 3). The ratios of myocardial contractility at a preload of 1.2 g/cm^2 to non-preload levels at baseline and after 20, 60, 120 and 180 channels are shown in Figure 4. Following the placement of 120 channels, there was almost complete obliteration of the Frank-Starling response. After each lasing, the myocardial contractility decreased immediately but then gradually recovered following 10 min of rest. Although initially these achieved baseline contractility they became attenuated with progressive laser salvos.

3.2 Whole Heart Langendorff Preparation

In the whole heart Langendorff preparation, the Frank-Starling curves were normal at baseline (Figure 5). After each set of TMR channels the force of myocardial contractility decreased following lasing at each of the preload settings (0-3 g) (Figure 6). Maximum contractility of the hearts was at 3 g preload. Compared to baseline, the maximum myocardial contractility after lasing decreased significantly by 21% after 20 channels (5.8 0.9 vs. 4.6 1.5 g/heart; p<0.05), by 22% after 30 channels (5.4 1.2 vs. 4.2 1.3 g/heart; p<0.05), by 27% after 40 channels (5.1 0.6 vs. 3.7 1.1 g/heart; p<0.002), and by 32% after 50 channels (5.0 1.5 vs. 3.4 1.0 g/heart; p<0.02) (Figure 7). However, after 30 min of rest following lasing, myocardial function returned to baseline (4.1 1.4 vs. 3.4 0.9; p=ns). Unlike the muscle strips, the whole heart preparation seemed to hold up for longer periods without deterioration.

3.3 Biochemical Analysis

Heat Shock Proteins: hsp25, 60 and 70 were expressed in the all groups but there were no significant differences in expression of hsp25 and 60 amongst the groups. However, after lasing in group 3 hsp70 expression decreased by 78% (0.87 0.10 vs. 0.19 0.03; p<0.01) and hsp70 expression was highest in Group 4 (Figure 8) (Table 1). There was no expression of hsp90 detected in any of the four groups.

***ATP Content*:** ATP content was 165.6±29.6 nmol/g rat heart (n=5) without lasing and 218.9±35.5 nmol/g rat heart after 50 channels/heart. The laser increased ATP content in rat heart tissue by 32% (p<0.02).

Table 1. Relative Heat Shock Proteins Expression

	Group 1	Group 2	Group 3	Group 4
hsp25	+	+	+	+
hsp60	+	+	+	+
hsp70	+	++	+	++++
hsp90	-	-	-	-

Group 1 = Three normal hearts excised; Group 2 = Three hearts perfused with PBS in the Langendorff set up for 6 hr before hsps measurement. Group 3 = Three hearts lased with 50 channels, perfused for 6 hr and then hsps measured. Group 4 = One heart heated in a waterbath at 42^{o}C for 15 min then kept at room temperature at 23^{o}C for 6 hr prior to hsps measurement.

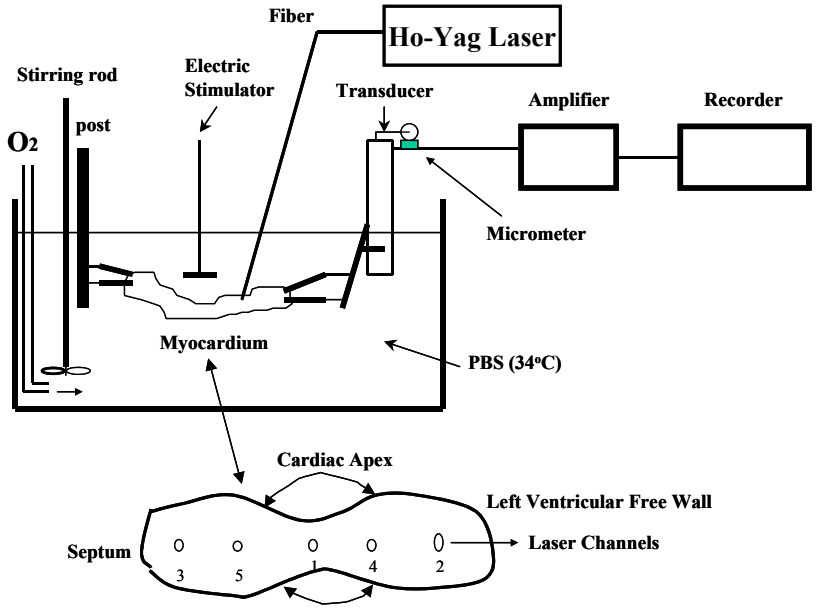

Figure 1. Set up of the myocardial strip model demonstrating the left ventricle dissected but still attached at the apex in the center of the strip preparation with the septum and ventricular free wall opened. Lasing was delivered via an optical fiber and placed in the numbered sequence shown on the muscle strip 1-5 with 1 being the first lased area.

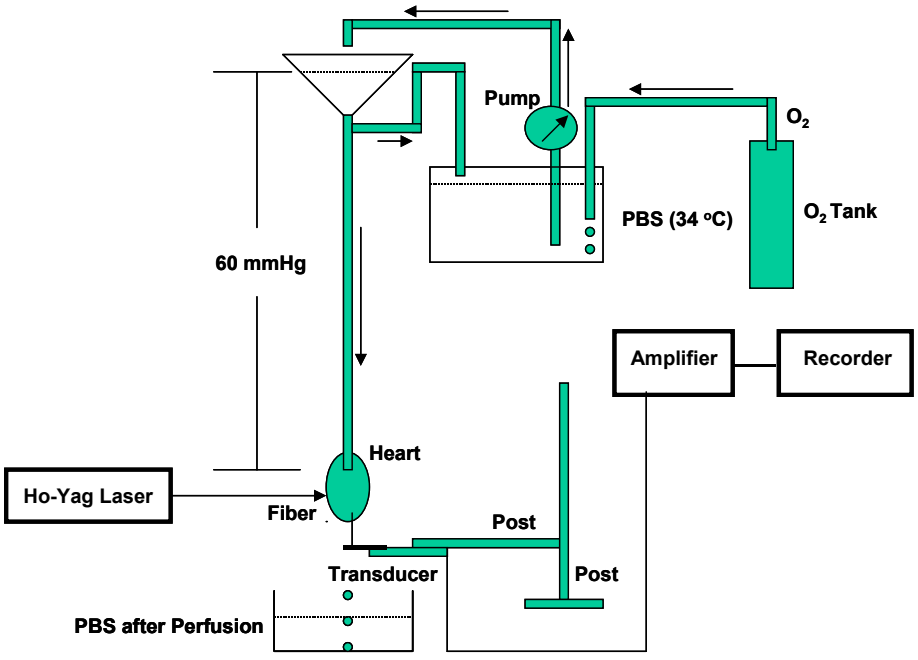

Figure 2. Set up of Langendorff whole heart preparation demonstrating the left ventricle being perfused by oxygenated PBS and the apex attached to a strain gauge to measure contractility of the myocardium with varying tension from 0–3 g.

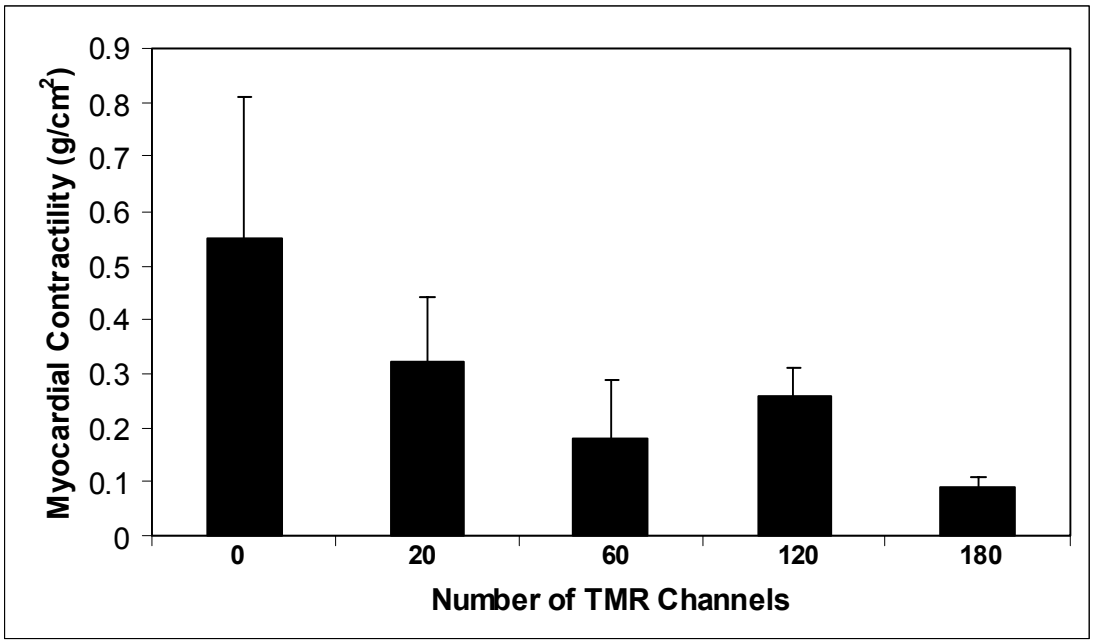

Figure 3. Bar graph demonstrating effect of laser channels on myocardial contractility in the myocardial strip model at a preload of 1.2 g/cm^2. This demonstrates a significant reduction of contractility after 20 channels.

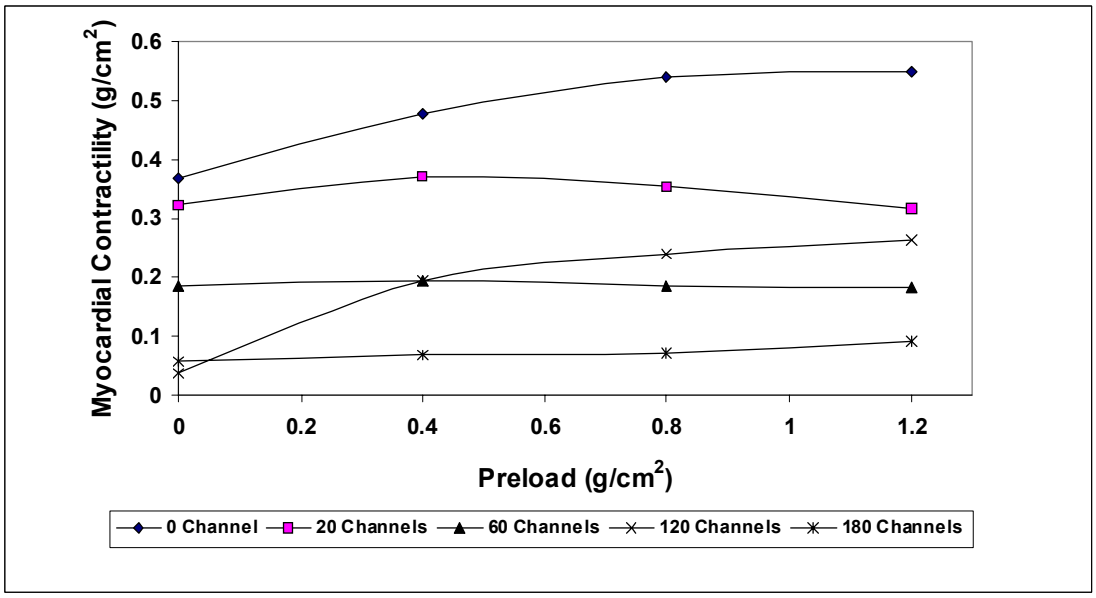

Figure 4. Frank-Starling curves of myocardial contractility at various preloads in the myocardial strip model. The curves represent an average of several Frank-Starling measurements with various TMR channels (0–180). The points on the curves represent an average of 14 experiments at each of the preloads. There is significant drop in contractility with increasing number of channels.

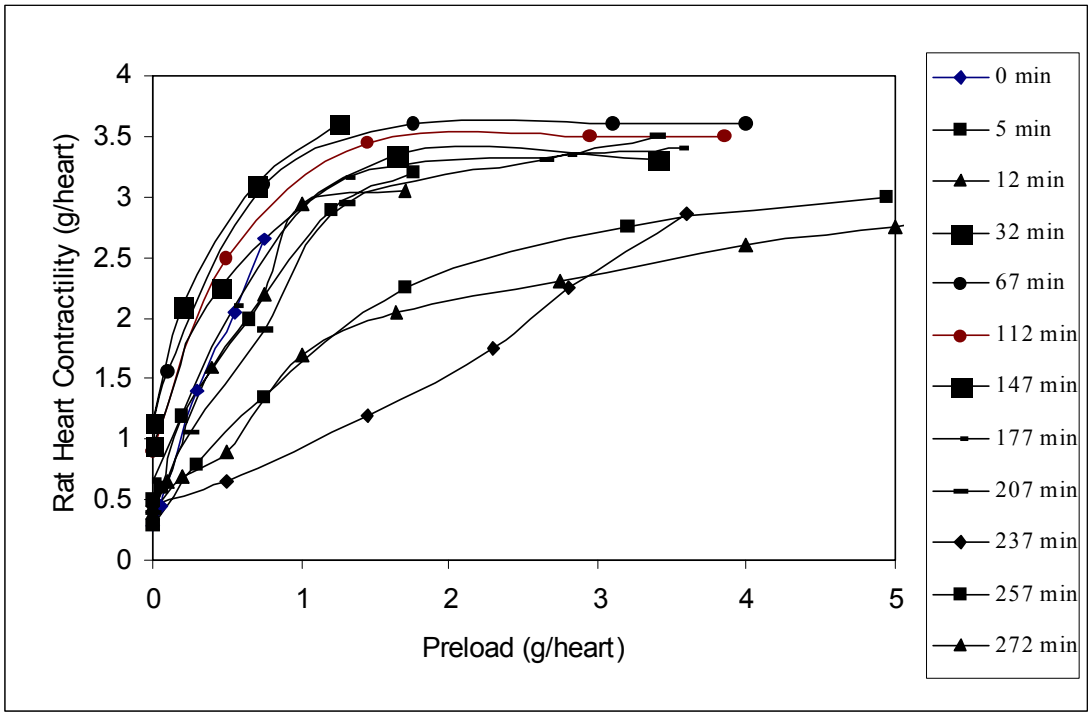

Figure 5. Example of control Frank-Starling curves in a normal non-lased rat heart with varying preloads (0–5 g). The Frank-Starling curves are stable for over 2.5 hr.

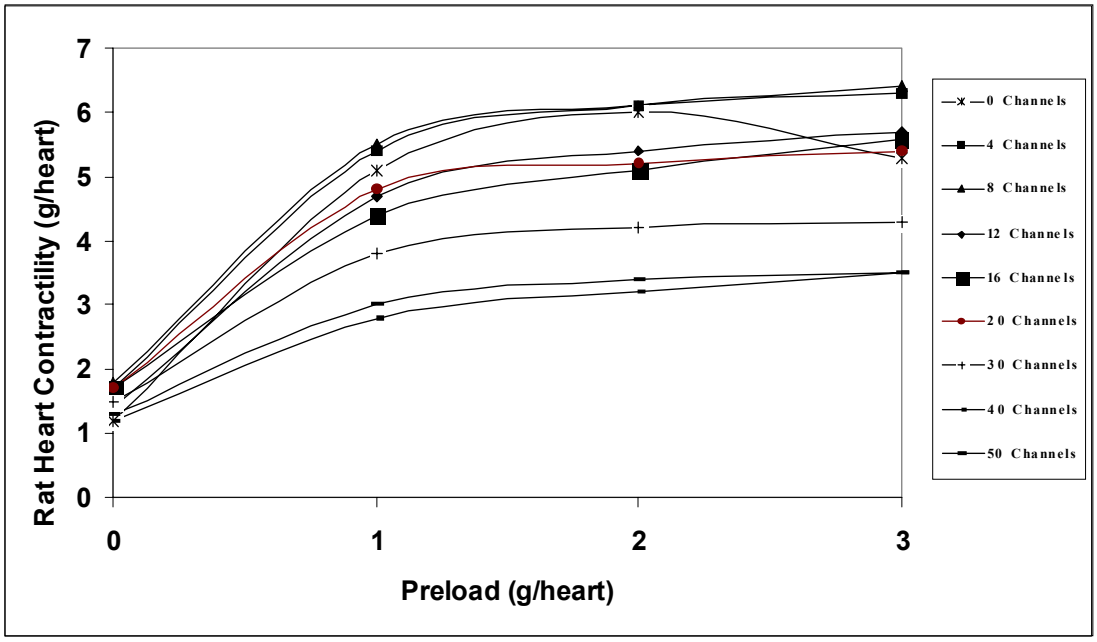

Figure 6. Example of Frank-Starling curves in a normal rat heart after lasing (0–50 TMR channels) at varying preloads (0–3 g). Each curve is representative of different TMR channels made.

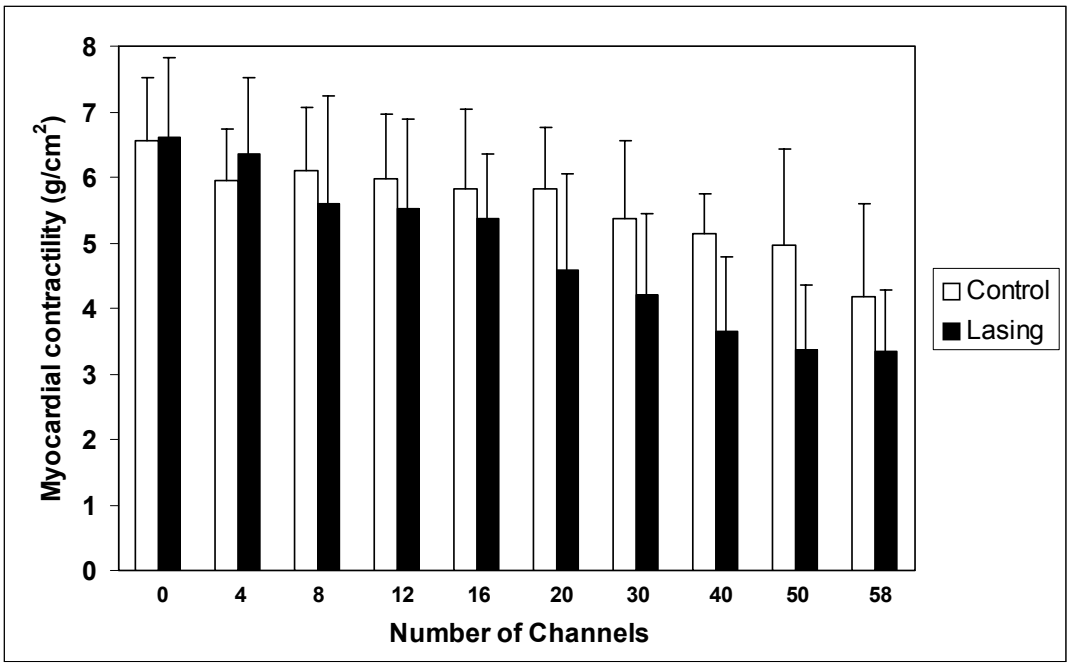

Figure 7. Bar graph of average of myocardial contractility in the Langendorff set up (TMR: n=23; Control: n=14). These demonstrate that there is significant reduction in contractility after 20 channels.

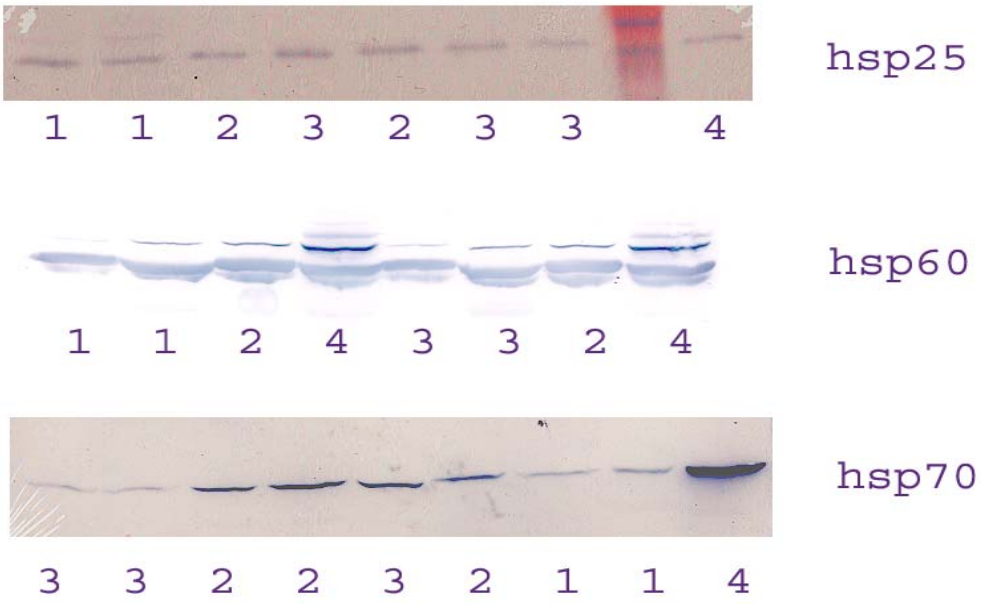

Figure 8. Heat shock proteins expression measured using Western Blotting. 1 = control-control; 2 = control; 3 = TMR (50 channels); 4 = heated heart (42°C). These data demonstrate that hsp 70 is maximally expressed in Group 4 and low in Group 3.

4. Discussion

The basic mechanics that regulate myocardial contractility include four factors: (1) The preload or the Frank-Starling mechanism that sets a passive load which establishes the initial muscle length of the cardiac fibers prior to contraction; (2) The after-load, which is the sum of all the loads against which the myocardial fibers must shorten during systole; (3) The inotropic state of the heart, which is reflected in the velocity of shortening capacity if the myocardium at a given instantaneous load and; (4) The heart rate or frequency of contraction. The above four factors are interrelated in the intact organism. For example, fiber length appears to influence quantitatively the number of active force-generating sites in the myocardium, whereas a change in the contractility is related to a qualitative change in the force generated by the sites i.e., their activations, with or without a change in their number (8).

In ischemic hearts with prior scarring from myocardial infarctions, the basic myocardial properties may be altered or suppressed. Thus, the additional loss of myocytes by TMR could worsen the contractility. So far, TMR has been performed clinically without knowing the exact number of channels needed for the full benefits of the procedure or the number of channels that can lead to heart failure. The results of this research demonstrate that a significant decrease in myocardial contractility occurs with lasing of more than 20 channels in the normal rat heart (13 channels/g wet heart tissue). This suggests that there exists a limit beyond which excessive number of channels will lead to loss of vital cardiac function. Because the average human heart is about 300 g an extrapolated number of channels from this rat heart study would be in excess of 3900 channels/human heart. Although this is entirely a theoretical number most TMR applications have used 4 channels per square cm. Thus, it is suggested that perhaps many more channels are needed for adequate perfusion results. As this was an in vitro study, it could be only a reference to the in vivo application, rather than a real prescription in the clinical application. The studies were done on normal isolated rat hearts, therefore, the results can not really be extrapolated into ischemic model or in vivo hearts, especially for human. The suggestion for clinical study to increase the channel density in ischemic myocardium should be done with caution. It should also state that this was done in normal heart model and that the same result may not be seen in ischemic myocardium. Specifically we were not using a laser with specifications that is seen in the clinical arena.

Cardiac biochemistry greatly influences intrinsic myocardial contractility. Heat and other injuries induce expression of heat shock proteins that are a category of stress proteins. Isolating the rat hearts by extraction from the chest causes severe stressful conditions in the ex vivo set up. Because hsp70 in the lased hearts was lower than in the non-lased control hearts then lasing may have possibly compensated for the stress of the isolated condition. It is possible that the TMR channels provided a route for the oxygenated buffered solution to support the stressed myocytes. However, it is also possible that the laser inhibited hsp70 expression or enhanced its degradation .

The result that lased hearts have higher ATP content supports the above hypothesis that some perfusion could be occurring that enhanced energy metabolism. This data is yet the most important with regards to TMR. This is further supported by the myocardial contractility recovery over time. However, this is in contradiction with the findings of Reuthebuch et al, who demonstrated that there was no beneficial effect on the high phosphate and lactate content after TMR in acute myocardial ischemia in pigs (9). This may be due to the fact that acutely ischemic tissue is inhibited from making adequate ATP.

Overall, this study provides direct evidence that up to 13 laser channels/g of myocardium could be tolerated without significant decrease in myocardial contractility in the normal rat heart. Also, the biochemical data suggest that the number of channels created enhanced ATP production. As currently applied in the clinical setting, perhaps not enough TMR channels are being created to achieve consistent benefit to patients. This may in part explain some of the conflicting observations noted in the clinical literature regarding the efficacy of TMR (10-14). Therefore it would be important to maximize the number of laser channels to make TMR a more effective procedure. Further clinical studies on ischemic myocardium with a much higher channel density may be desirable to evaluate TMR benefits.

The total quantity of ATP in the human body is about 0.1 mole. The majority of ATP is not usually synthesized *de novo*, but is generated from ADP by the aforementioned processes. Thus, at any given time, the total amount of ATP + ADP remains fairly constant. The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily which is around 50 to 75 kg. Typically, a human will use up their body weight of ATP over the course of the day (15). This means that each ATP molecule is recycled 1000 to 1500 times during a single day (100 / 0.1 = 1000). ATP cannot be stored, hence its consumption being followed closely by its synthesis (16). From the dynamic consideration of ATP synthesis there could be physiological effect for the increasing of ATP after TMR (32% increasing).

However, some reports showed that several days are required for de novo ATP synthesis. For example, several papers by Reimer & Jennings describe the time-course of ATP-changes and even 4 days after a 15 minute occlusion in dogs ATP content was still decreased at 88% of control (17). The confliction should be researched further.

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Occurrence of ESBL and MBL in Clinical Isolates of *Pseudomonas aeruginosa* From Lagos, Nigeria

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ABSTRACT: Background: Widespread use and misuse of anti-infective agents have resulted in problems of drug resistance linked to treatment of infectious diseases caused by worrisome pathogens such as *Pseudomonas aeruginosa*. Infections caused by *Pseudomonas aeruginosa* with resistance to extended spectrum β-lactams and the carbapenems is emerging and requires the identification of effective alternative antimicrobial therapy and improved surveillance of the emergence of such resistance. A total of non-duplicate 97 strains of *P. aeruginosa* recovered from various clinical specimens and collected between March and August, 2006 were analysed for their resistance pattern. **Methods:** Antimicrobial susceptibility, Extended-Spectrum β-Lactamase (ESBL)- and Metallo- β-Lactamase-Production (MBL) were determined using disk diffusion method, double disk synergy test and combined disk test respectively. **Results:** The Carbapenems had the highest activity against the strains tested (95.9%). This was followed by ceftazidime (79.4%). Amongst the 20 ceftazidime-resistant strains, 9 were found to be ESBL-producers. While the 4 strains resistant to the carbapenems were detected to be MBL-producers and were observed to be multi-resistant. Amikacin was the most potent amongst the aminoglycosides (78.4%) while there was high resistance to the quinolones. **Discussion:** This study highlights needs to establish antimicrobial resistance surveillance networks for *P. aeruginosa* to determine the appropriate empirical treatment regimen. Bacterial strains resistant to most classes of antibiotics will continue to emerge unless inappropriate uses of drugs are curtailed and continuous education of infection control practices maintained. [The Journal Of American Science. 2007;3(4):81-85]. (ISSN: 1545-1003).

Keywords: Emergence, *Pseudomonas aeruginosa,* ESBL, MBL, Multidrug-resistance

Introduction

Resistance to anti-infective agents is worldwide, both in developed and developing countries. Antimicrobials have been used successfully for over 6 decades, but genes expressing resistance to them have emerged in strains of bacteria and have disseminated through the global ecosystem to reach infecting microorganisms, produce disease, and seriously interfere with therapy, allowing infections to progress and kill, despite antibiotic administration. (Isturiz and Carbon, 2000).

Pseudomonas aeruginosa has been reported to be an opportunistic and a worrisome nosocomial pathogen (Gales *et al*., 2001). It is reported to be a leading cause of nosocomial infections, including pneumonia, urinary tract infections, burn infection, meningitis and bacteremia.(Pollack, 2000). Its ability to survive on inert materials, live on minimal nutritional requirement, with its tolerance to a wide variety of physical conditions and antiseptics; has contributed enormously to its ecological success and its role as an effective opportunistic pathogen (Gales *et al*., 2001). It is known to exhibit intrinsic resistance to several antimicrobial agents.

With the occurrence of ESBLs and MBL-producing *Pseudomonas aeruginosa* being increasingly reported worldwide (Lee *et al*., 2005, Walsh *et al*., 2005, Pagani *et al*., 2004, Gibb *et al*., 2002, Toleman *et al*., 2002), this study evaluated the current resistance pattern of *Pseudomonas aeruginosa* strains from 2 tertiary hospitals in Lagos, Nigeria.

MATERIALS AND METHODS

Bacterial Isolates

A total of non-duplicate 97 isolates of *P. aeruginosa* recovered from various clinical specimens of patients treated at the Lagos University Teaching Hospital (LUTH), and the National Orthopaedic Hospital Igbobi (NOHI) Lagos were collected between March and August, 2006.

Isolation and Identification

Pseudomonas aeruginosa strains were previously isolated and identified from the various specimens at the microbiology laboratory of these 2 tertiary hospitals. The isolates were obtained from these laboratories and their identity reconfirmed using a combination of colonial morphology, positive oxidase test, pigment

formation, growth at 42^0C on nutrient agar, Gram's reaction, motility, growth on *Pseudomonas* agar base medium (Oxoid, UK) to which C- N supplement SR – 102 (Oxoid, UK) has been added using *Proteus vulgaris* ATCC 13315 and *Pseudomonas aeruginosa* ATCC 27853 as controls. The isolates were maintained on nutrient agar slant. (Oxoid, UK).

Antibiotic Susceptibility Testing

Antibiotic susceptibilities were determined on Mueller-Hinton agar (Oxoid, UK) by standard disk diffusion procedures of Bauer *et al* (1966) which conforms to the recommended standard of National Committee for Clinical Laboratory Standards now known as CLSI (NCCLS, 2000). The following antibiotics were used: Levofloxacin (5µg), Gentamicin (30µg), Aztreonam (30µg), Meropenem (10µg), Amikacin (30µg), Tobramycin (30µg), Piperacillin (30µg), Imipenem (10µg), Ceftazidime (30µg), Piperacillin/Tazobactam (110µg) [Oxoid, UK];:Ciprofloxacin (5µg), Ofloxacin (5µg), (Ranbaxy, Nigeria) and Pefloxacin (5µg) (May and Baker, Nigeria). *Pseudomonas aeruginosa* ATCC 27853 was run simultaneously with the test organisms. Results were interpreted according to the NCCLS/CLSI standard (NCCLS, 2000).

Beta-Lactamase Detection

(a) **Nitrocefin Test**

All the isolates were tested for the production of beta-lactamase with the nitrocefin beta-lactamase test strip (Oxoid, UK).

(b) Extended Spectrum Beta-Lactamase Detection

Double Disk Synergy Test (DDST) were performed on ceftazidime resistant strains by placing disks of ceftazidime and aztreonam (30µg each) at a distance of 20 mm (center to center) from a disk containing augmentin (amoxicillin, 20µg, and clavulanic acid, 10µg) (Pagani *et al.*, 2004)). ESBL production was inferred when the cephalosporin and monobactam zones were expanded by the clavulanate.

© Detection Of Metallo – Beta-Lactamase (MBL) Producing Strains

Meropenem and Imipenem- non susceptible isolates were tested for MBL production according to Pitout *et al*'s method (2005). This method recommended the use of both imipenem and meropenem in testing for MBL. In this method, an increase of ≥7mm in zone diameter in the presence of 930µg EDTA compared to those with both IPM and MEM tested alone was considered to be a positive test for the presence of an MBL .

RESULT

A total of 97 *Pseudomonas aeruginosa* strains from different clinical specimens were tested for their antimicrobial sensitivity pattern between March and August 2006.

Antimicrobial Susceptibility Testing

Table 1 shows the distribution of *Pseudomonas aeruginosa* strains by site of isolation and table 2 shows the antimicrobial susceptibility pattern of *P. aeruginosa* strains.

Meropenem and Imipenem were the most active of all the antimicrobial agents used with only four (4.1%) of the 97 strains of *Pseudomonas aeruginosa* resistant to these carbapenems. This was followed by ceftazidime with the strains showing 79.4% susceptibility. Aztreonam, a monobactam, had activity against 63.9% of the strains. Only 30.9% were susceptible to piperacillin while combination of piperacillin with tazobactam (a beta-lactam/beta-lactamase inhibitor) was highly active with 76.3% susceptible to it when compared to piperacillin alone. Activity of the quinolones against the *Pseudomonas aeruginosa* strains in this study was poor. The most active was ofloxacin with 42.3% susceptibility. This was closely followed by ciprofloxacin (40.2%) and then Levofloxacin (30.9%). Imipenem, meropenem and Ceftazidime had good activity against all the quinolone resistant strains. While for the aminoglycosides, susceptibility was highest in amikacin (78.4%), followed by tobramycin (46.4%) and Gentamicin (42.3%).

Beta-Lactamase Production

Beta-lactamase production by the nitrocephin test was observed in 32 (33%) of the *Pseudomonas aeruginosa* strains. All the ESBL and MBL producers were among those positive for beta-lactamase production by the nitrocephin test.

ESBL Production

Of the 20 strains resistant to ceftazidime, 9 were found to be ESBL-producers. Six of the ESBL-producers were from LUTH while 3 were from NOHI. The ESBL-producers were spread across the various sites of isolation. Five were from wound swabs, 3 from Catheter-tips and 1 was from the pus of an abcess. Susceptibility to piperacillin tazobactam amongst the ESBL-producers varied. All the ESBL-producers were found to be highly susceptible to imipenem, meropenem and amikacin Two (22.2%) of the ESBL-producers sensitive to amikacin and gentamicin were found to be susceptible to ofloxacin. There was resistance to the other quinolones by the ESBL-producers.

Detection of MBL

Of the 4 carbapenem resistant strains of *Pseudomonas aeruginosa*, the Pitout *et al*. (2005) method detected all as MBL-producers with meropenem while Imipenem plus EDTA detected 2 as MBL-producer. The 4 carbapenem resistant strains were observed to be multi-resistant to all the antimicrobials tested in this study The four strains were recovered from LUTH. and were obtained from wound sites. ESBL was not detected in any of the 4 carbapenem resistant strains phenotypically.

Table 1. Distribution of *Pseudomonas aeruginosa* by site of Isolation

Source/Sites	**No of Isolates**	**Percentage (%)**
Wounds	65	67
Semen	9	9.3
Gastric Aspirate	7	7.2
Eye Swab	4	4.1
Ear Swab	4	4.1
Pus from Abcess	3	3.1
Catheter-tip	3	3.1
High Vaginal Swab	2	2.1
Total	**97**	**100**

Table 2. Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa* Strains

Antimicrobials	**No**	**(%) S**	**No**	**(%) R**
Gentamicin	41	(42.3)	56	(57.7)
Amikacin	76	(78.4)	21	(21.6)
Tobramycin	45	(46.4)	52	(53.6)
Ofloxacin	41	(42.3)	56	(57.7)
Ciprofloxacin	39	(40.2)	58	(59.8)
Pefloxacin	7	(7.2)	90	(92.8)
Levofloxacin	30	(30.9)	67	(69.1)
Ceftazidime	77	(79.4)	20	(20.6)
Imipenem	93	(95.9)	4	(4.1)
Meropenem	93	(95.9)	4	(4.1)
Aztreonam	62	(63.9)	35	(36.1)
Piperacillin/Tazobactam	74	(76.3)	23	(23.7)
Piperacillin	30	(30.9)	67	(69.1)

Key: S=sensitive; R=Resistant; No=Number

DISCUSSION

Pseudomonas aeruginosa is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen (Todar, 2002). Its' infections are a frequent cause of morbidity and mortality in hospitalized patients. Treatment of these infections is further complicated by the intrinsic and acquired resistance of this bacterium to many commonly used antimicrobial agents (Hauser and Sriram, 2005).

The result of this study showed that there is an increase in resistance to the quinolones by strains of *Pseudomonas aeruginosa*. In previous studies by Oduyebo *et al* (1997) and Kesah *et al*.(1999) in this environment, *Pseudomonas aeruginosa* strains were found to be highly susceptible to the quinolones (96%); but from this study, our data shows increase in resistance to this family of antibacterial agents by the current clinical strains of *Pseudomonas aeruginosa*. Ofloxacin was observed to have the highest activity (41%) and was closely followed by ciprofloxacin (39%). This result implies that quinolones alone, cannot be depended upon as an antipseudomonal antimicrobial in this environment. They will have to be used in combination or replaced with another antimicrobial preferably the broad-spectrum beta-lactams/penicillins. The activities of carbapenems, ceftazidime, piperacillin/tazobactam and amikacin against *Pseudomonas aeruginosa* strains is still high and they can be considered as therapeutic options available for *Pseudomonas aeruginosa* infection treatment in this region.

Of the 20 strains resistant to ceftazidime, 9 were found to be ESBL-producers by the double-disk diffusion test. Amongst the ESBL-producers, there was high resistance to the quinolones. This suggests a possible existence of co-resistance to the quinolones on the gene responsible for ESBL-production (Thomson *et al*., 1999). The 4 strains resistant to the carbapenems were also detected phenotypically to be MBL-producers. These MBL-producers were resistant to all other antibiotics including the aminoglycosides and the quinolones used in this study. In a report by Hauser and Sriram (2005), it was stated that *P aeruginosa* isolates that are resistant to multiple antibiotics are of particular concern and pose a significant clinical challenge.

Multidrug-resistant isolates of *Pseudomonas* spp (defined as being resistant to piperacillin, ceftazidime, imipenem, and gentamicin or ciprofloxacin) have become an increasingly frequent problem, especially in Europe and Latin America, and now constitute from 3.6% to 7.7% of all *P aeruginosa* isolates (Gales *et al*., 2001, Harris *et al*., 1999). As a consequence, strains are now being identified that are resistant to all commonly used antibiotics (Stein *et al*., 2002). Few therapeutic options are hence available for the treatment of patients infected with these strains.

The 4 MBL-producing strains were from LUTH. This is a tertiary hospital where various types of patients who have moved from one hospital to the other both locally and internationally are referred, for management or continuation of therapy. The implication of this is that most of the patient would have been on one form of treatment or the other before getting to this hospital, thus the selective pressure of use, overuse and misuse of antibiotics cannot be ignored in the emergence of these resistance phenotypes in this health-care centre. Not to be underestimated also is the likelihood of importation of resistance genes from other health-care centers around the world.

Data on the detection of ESBL- and MBL- producing *Pseudomonas aeruginosa* strains from clinical samples in Lagos, is scarce and the result of this work shows the presence of these enzymes in the already notorious pathogen, *Pseudomona aeruginosa,* in this environment. Thus, the emergence and existence of metallo-beta-lactamase and extended-spectrum beta-lactamase-enzyme production with multidrug resistance, in strains of *Pseudomonas aeruginosa* in this environment, will greatly complicate the clinical management of patients infected with such strains if utmost care is not taken. Further molecular studies need to be carried out to confirm the MBL- and ESBL- type present in *Pseudomonas aeruginosa* strains in Lagos, Nigeria and their association with resistance to other classes of antibiotics.

In conclusion, this study highlights the need to establish an antimicrobial resistance surveillance network for *P. aeruginosa* to monitor the trends and new types of resistance mechanism emerging. Resistance to different kinds of antimicrobial agents among *P. aeruginosa* is clearly on the increase. Results from this study with regards to high quinolone resistance, ESBL and MBL production suggests that resistance gene carried on an epidemic plasmid which has the ability to travel freely may be present in this environment. Thus optimum caution must be taken; good antibiotic prescription policies and infection control practices must be put in place to prevent spread of the gene, responsible for these resistances, which could result in return to the pre-antibiotic era.

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Plasmids: A Vehicle for Rapid Transfer of Antibiotic Resistance Markers of *Salmonella* Species in Animals

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ABSTRACT: This study was carried out to determine the prevalence of *Salmonella* transferable plasmids mediated antibiotics resistance in broiler chickens and diarrhea patients in Benin City, Edo State, Nigeria. A total of 183 *Salmonella* species were isolated from 1320 fecal samples of diarrhea patients and poultry chickens and tested for antibiotics resistance transferable ability. The rapid alkaline DNA extraction procedure was used to screen for the plasmids resistance markers. Seventy eight (42.6%) of the isolates were resistant to three or more antibiotics. The isolates were highly resistant to ampicillin, chloramphenicol, gentamicin, trimethoprim, tetracycline and sulfamethoxazole, and to a lesser extent, resistant to ciprofloxacin, ofloxacin, ceftriaxone and cefuroxime. Thirty two (17.5%) of the isolates had plasmids of varying sizes ranging from 2.5kb to 5.0kb while 151 (82.5%) appear to have no plasmids. These plasmids were highly transferable at a frequency of 2×10^{-2} to 4×10^{-4} per donor cell by conjugation. The exchange of plasmid(s) between bacterial cells and the integration of resistance genes into specialized genetic elements play a major role in acquisition and dissemination of antibiotic resistance genes among the *Salmonella* species. The results also demonstrated how antibacterial treatment targeted at a pathogenic organism in a host may affect the endogenous flora of another host in a population. The transfer of antibiotic resistant genes among the species is an increased risk considering the fact that all strains of this organism are potential pathogens. [The Journal Of American Science. 2007;3(4):86-92]. (ISSN: 1545-1003).

Keywords: *Salmonella*, Plasmids Resistance Transfer Markers.

INTRODUCTION

Salmonella infections are the most common widespread cause of salmonellosis among humans and animals worldwide, causing an estimated 1.41 million cases of infections and 500 human deaths annually in the United States of America (Blaser, 1996; Mead *et al.,* 1999). Animals used for food production often carry *Salmonella*, thus contaminating meat, dairy products, and eggs (Baird-Parker, 1990; Osterom, 1999). It is however difficult to evaluate the extent of infections in developing countries because of the very limited scope of studies and lack of coordinated epidemiological surveillance systems (Santos *et al.,* 2003).

Selective pressure imposed by the use of antimicrobials in both human and veterinary medicine promotes the spread of multiple antimicrobial resistances resulting in the growing problem of infections that are difficult to treat (Carattoli, 2003). Resistance to some ß-lactam antibiotics, tetracyclines, chloramphenicol, or trimethoprim is reported with increasing frequency (Gallardo *et al.,* 1999 and Velonakis *et al.,* 2001). The adverse effect of antimicrobial resistance has typically been recognized as treatment failure; the disease caused by the pathogen can significantly worsen because of the antimicrobial drug used. Evolution of plasmids through the acquisition of resistant genes had been reported, describing novel mechanisms for short-term accumulation of resistance determinants in plasmids circulating in *Salmonella* (Threlfall, 2002; Carattoli, 2003). There is no documented study on the antibiotic transferability pattern among *Salmonella* species in broiler chickens and patients in Nigeria. This study was therefore designed to investigate the frequency of transfer of plasmid-mediated resistance to antibiotics, widely used for treatment, among *Salmonella* species of human and poultry origin in Nigeria.

MATERIALS AND METHODS

Sample collection and Isolation of bacteria:

Samples were collected from feces of diarrhea patients and feces from 6 reputable broiler poultry farms. A total of 121 *Salmonella* species were obtained from 920 fecal samples of diarrhea patients from Central Hospital Benin City while 62 *Salmonella* species were obtained 400 fecal samples of broiler poultry chickens in Benin City. All the samples were cultured into selenite F broth in screw-capped bottles and incubated at 37 ^{0}C for 3-5 days. Subcultures were then made into plates of deoxychocolate citrate agar, and incubated for another 24 hours. Colonies with black centers were sub-cultured onto nutrient agar and incubated for another 24 hours. The cultures on nutrient agar plates were subjected to Gram-staining, motility, urease production, hydrogen sulfide production and citrate utilization tests. All Gram-negative, rod-shaped, motile, urease-negative isolates that produced acid on triple sugar Iron agar slants and able to utilize citrate as sole carbon source were identified as species of the genus *Salmonella*.

Antibiotic Susceptibility Testing:

The E-test method (AB Biodisk) was used to screen for the antibiotic susceptibility patterns. The minimum inhibitory concentration (MIC) susceptibility test was determined in accordance with the manufacturer's guidelines (AB Biodisk, Sweden). The 0.5 McFarland standards isolates were inoculated onto Mueller Hinton agar plates by swabbing evenly in three directions. The E-test strip (obtained from the refrigerator at 4^{o}C) was applied to each plate with sterile forceps with lowest concentration toward the center of the agar plate. The plates were then incubated at 30 to 35 °C for 24 hours. The E-test MIC values were read directly from the E-test strip MIC scale. The following antibacterial agents: ofloxacin (Ofl), ciprofloxacin (Cip), cefuroxime (Cef), ceftriaxone (Ce), gentamicin (Gn), trimethoprim-sulfamethoxazole (Txm-Sal), ampicillin (Am), chloramphenicol (Chl) and tetracycline (Te) were used. The concentration gradient of each antimicrobial agent on the E-test strips was 0.016 to 256µg/ml with the exception of ciprofloxacin and ofloxacin for which the gradient ranged from 0.002 to 32µg/ml. The susceptibility range as defined by AB Biodisk Sweden were: ofloxacin (S $\leq$2, I = 4 and R $\geq$ 8), ciprofloxacin (S $\leq$1, I = 2 and R $\geq$ 4), cefuroxime (S $\leq$8, I = 16 and R $\geq$ 32), gentamicin (S $\leq$4, I = 8 and R $\geq$ 16), trimethoprim-sulfamethoxazole (S $\leq$2 and R $\geq$ 4), ampicillin (S $\leq$8, I = 16 and R $\geq$ 32), chloramphenicol (S $\leq$8, I = 16 and R $\geq$ 32), ceftriaxone (S $\leq$8, I = 16 and R $\geq$ 32) and tetracycline (S $\leq$4, I = 8 and R $\geq$ 8) where S = sensitivity, I = intermediate and R = resistance.

Conjugation and Plasmids profiles:

Conjugation experiments were performed as described by Yukata *et al.* (2004) and Wang *et al.* (2004) using *E coli* strains obtained from Nigerian Institute for Medical Research (NIMR), Lagos, as recipient. The donors and recipients-plasmid -free - rifampicin resistant strains were incubated both on Mueller Hinton broth culture (Difco Laboratories Detroit, Mich USA) and on Oxoid Mueller Hinton agar (Difco Laboratories Detroit, Mich USA) at 37^{0}C for 18 hours. The transconjugants were selected on Mueller Hinton agar medium supplemented with 200µg/ml rifampicin (Daiichi Pharm. Co. Ltd, Japan) to inhibit the growth of the donor and recipient respectively. The frequency of transfer of the plasmids were determined by dividing the number of transconjugants by the number of donor cells according to Wang *et al.* (2004) and Yukata *et al.* (2004). The transconjugants were re-streaked onto fresh selective culture plates and their identities were re-confirmed on the basis of their biochemical methods and their antibiotics resistance pattern re-confirmed. The Birnboim and Doly (1979) method was employed for screening plasmids (rapid alkaline extraction) of donors and transconjugants. The plasmids DNA were then electrophoresed on 0.8% agarose gel, stained with 14µl ethidium bromide. The DNA was then photographed with Polaroid camera and viewed using UV trans-illumination. The molecular weights and distances were then determined using standard methods according to Meyers *et al*. (1982) and Birnboim and Doly (1979) with standard DNA molecular weight marker II (0.12-23.1kbp) of bacteriophage lambda HindIII (Roche Diagnostic GmbH).

Statistical analysis:

The MIC values were compared using the Chi-square test and the student two-tailed t test. A difference was considered significant when P-value by the two-tailed was less than 0.05 ($P<0.05$). Results were expressed as mean standard deviation ($\overline{\times} \pm S.D$).The calculated values where then

compared with the critical values at the appropriate degree of freedoms at a significant level of P= 0.05 (21).

RESULTS

The results revealed that 121 and 62 *Salmonella* species respectively were obtained from 920 fecal samples of diarrhea patients and 400 fecal samples of broiler poultry chickens (Table 1). Out of the 183 *Salmonella* species, 78 were resistant to three or more antibiotics. The isolates from both sources were highly resistant to ampicillin, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole and tetracycline. The prevalence of resistance in all the groups was very low to ciprofloxacin, ofloxacin, ceftriaxone and cefuroxime. There was no significant difference ($P<0.05$) between the resistance patterns of the species from patients and broiler chickens. Apart from *Salmonella species*, other bacterial isolates encountered were *E. coli, Shigella, Pseudomonas aeruginosa, Enterococcus, Klebsiella* and *S. aureus*,.

The results of the plasmids analyses show that 32 (17.5%) had plasmids of varying sizes ranging from 2.5kb to 5.0kb while 151 (82.5%) appear to have no plasmids (Table 2). These plasmids were highly transferable at a high frequency of 2×10^{-2} to 4×10^{-4} per donor cell by conjugation.

Table 1: Percentage (%) Occurrence of resistant *Salmonella* species from diarrhea patient's and from broiler feces

	Percentage of resistant isolates to antibiotics								
Source	**Cip**	**Ofl**	**Te**	**Am**	**Txm-sal**	**Chl**	**Gn**	**Cef**	**Ce**
Diarrhea patients n= 121	8(6.6%)	4(3.3%)	67(55.4%)	92(76.0%)	58(47.9%)	103(85.1%)	59(48.8%)	7(5.8%)	8(6.6%)
Broiler chickens n = 62	5(8.1%)	3(4.8%)	49(79.0%)	46(74.2%)	37(59.7%)	32(51.6%)	27(43.6%)	2(3.2%)	3(4.8%)

Key: Ofloxacin (Ofl), ciprofloxacin (Cip), cefuroxime (Cef), ceftriaxone (Ce), gentamicin (Gn), tetracycline (Te), Trimethoprim-sulphamethoxazole (Txm-Sal), ampicillin (Am) and chloramphenicol (Chl)

DISCUSSION

The routine use of antibiotics in medicine and agriculture circles has resulted in widespread antibiotic resistance and in the development of genetic mechanisms efficient for the dissemination of antibiotic genes, especially, within and between species of microorganisms. These uncontrolled uses of antimicrobials in agriculture and for treating human patients contribute to increase multi-drug resistance of *Salmonella* species. The present results showed that 121 *Salmonella* species were obtained from human sources while 62 species were obtained from broiler chickens. This high prevalence of *Salmonella* species in both sources highlights the need to monitor the spread of the microorganism through animal products. The high percentage of broiler chickens contaminated with *Salmonella* was higher than previous reports (Morgan, 1980; Velonakis *et al.,* 2001; Threlfall, 2002; Guncagu, 2004). The contaminating of microorganisms in poultry products are reportedly derived from the poultry manure, poultry workers, equipment, poultry's environment which include faecal, soil and water. Many of the outbreaks reported have been epidemiologically linked to the consumption of raw or undercooked eggs, and to a lesser extent, chicken (Hedberg *et al.,* 1993; Araújo *et al.,* 1995; Bangtrakulnonth *et al.,* 2004). The frequent occurrence of *Salmonella* species in chicken suggests that poultry may be an important reservoir, a finding that is consistent with almost all other studies in other countries. Moreover, *Salmonella* infections have been recognized as a major public health concern both from developed and developing countries (Helms *et al.,* 2004; 2005).

Secondly, *Salmonella* is widespread in nature and can colonize or infect a variety of domesticated and wild animals ranging from mammals to birds and reptiles. According to Winokur *et al.* (2000), most human *Salmonella* infections in the United States are related to ingestion of contaminated food products rather than person-to-person transmission or direct fecal-oral transmission. Many outbreaks have been traced to ingestion of contaminated animal products and in some cases, traced to specific farms, flocks, or herds of animals (Altekruse *et al.,* 1993). According to Matofari *et al.* (2007) healthy camels can be carriers of *Salmonella* species which can be isolated from their faeces and lymph nodes on slaughter of camels. Camels that are chronic carriers of *Salmonella* may present a human health hazard through consumption of camel products like milk (Matofari *et al.,* 2007).

Table 2: Antibiotics Resistant Pattern of *Salmonella* species Transconjugants

Code of Isolates	Source	Resistant Marker Spectrum of Donors	Plasmids Profile of Donors (kb)	Plasmids Profile of ransconjugants (kb)	Resistant Marker Spectrum of transconjugants
DB12	Diarrhea	Am,Chl,Txm-Sal,Gn, Te	2.5, 4.3, 5.0	4.5, 4.3	Am^r,Te^r,Chl^r
BB15	Broiler	Am,Gn,Chl,Txm-Sal, Te,Ofl,	4.5, 4.7	4.5, 4.7	$Am^r$$Gn^r$,$Te^r$, Txm-$Sal^r$
BB16	Broiler	Txm-Sal,Te,Am,Ce	2.5, 4.3, 5.0	4.3, 5.0	Txm-Sal^r, $Te^r$$Am^r$
DB18	Diarrhea	Am,Chl,Gn,Ofl Te	2.5, 4.3, 5.0	4.3, 5.0	Am^r,Chl^r,Gn^r,Te^r
BB01	Diarrhea	Am,Gn, Te,Cef	2.5, 4.3, 5.0	4.3, 5.0	Am^r,Gn^r,Te^r
BB23	Broiler	Am,Txm-Sal, Te,Ofl,	2.5, 4.3, 5.0	4.3, 5.0	Am^r,Txm-Sal^r, Te^r
DB18	Broiler	Am,Chl,Txm-Sal, Te,Cip	2.5, 4.3, 5.0	4.3, 5.0	Am^r,Chl^r, Txm-Sal^r,Te^r
DB213	Diarrhea	Am,Gn,Te,Ofl	2.5, 4.3, 5.0	4.3, 5.0	Am^r,Gn^r,Te^r
BB145	Broiler	Am,Gn,Te,Ofl	2.5,3,4. 7,	2.5, 4.5, 4.7	Am^r,Gn^r,Te^r
DB87	Diarrhea	Am,Chl,Gn,Ofl, Te	2.5,4.3, 4.7, 5.0	4.5, 4.7	Am^r,Chl^r,Gn,Te^r
DB92	Diarrhea	Am,Gn,Chl,Cip	2.5, 4.3, 5.0	4.5, 4.7	Am^r,Gn^r,Chl
DB108	Diarrhea	Am,Chl,Txm-Sal, Te	2.5, 4.3, 5.0	4.5, 4.7,5.0	Am^r,Chl^r, Txm-Sal^r, Te^r
BB211	Broiler	Am,Gn,Te,Txm-Sal, Ofl,	4.5, 4.7 4.3, 5.0	4.5, 4.7,5.0	Am^r,Gn^r, Txm-Sal^r
BB266	Broiler	Am,Txm-Sal, Te,Ofl,	2.5, 4.3, 5.0	4.5, 4.7,5.0	Am^r,Txm-Sal^r, Te^r
DB59	Diarrhea	Am,Chl,Gn,Ofl,Te	2.5, 4.3, 5.0	4.5, 4.7,5.0	Am^r,Chl^r,Gn^r, Te^r
BB178	Broiler	Am,Gn,Te,Cip,Ce	4.5, 4.74.5, 4.7,5.0	4.5, 4.7,5.0	Am^r,Gn^r, Te^r,Cip^r
BB283	Broiler	Gn,Chl,Txm-Sal, Te,Ofl	4.5,4.7,4.5, 4.7,5.0	4.5, 4.7,5.0	Gn^r,Chl^r, Txm-Sal^r, Te^r
DB65	Diarrhea	Am,Txm-Sal,Te, Ofl, Gn	4.5, 4.7, 4.5, 4.7,5.0	4.5, 4.7,5.0	Am^r,Txm-Sal^r, Te^r,Gn^r
DB59	Diarrhea	Chl,Gn,Ofl,Te,Am	2.5, 5.0	5.0	Gn^r,Te^r,Am^r

Key: Ofloxacin (Ofl), Ciprofloxacin (Cip), Cefuroxime (Cef), Ceftriaxone (Ce), Gentamicin (Gn), Tetracycline (Te), Trimethoprim-sulphamethoxazole (Txm-Sal), Ampicillin (Am), Chloramphenicol (Chl), BB = Broiler Chickens Transconjugants and DB = Diarrhea Patients Transconjugants.

The present results raises the concern that there may be a link among antibiotic use in feeds, the development and presence of antibiotic resistance among bacteria in food-producing animals, and antibiotic associated bacterial infection in humans. This is because all the *Salmonella* isolates from both sources used in this study showed resistance to the entire antibiotic tested. Overall, *Salmonella* isolates in this study showed high resistance to a number of antibiotics, ranging from 3.3% in human feces to 85.1% as compared to *Salmonella* isolates in broiler chickens that ranged from 3.2% to 79.0%. There was no significant different ($P>0.05$) between the antibiotics resistance pattern from the two sources. This high antibiotic resistance rates could be due to the widespread use of antibiotics in chickens, particularly in feed, as well as their indiscriminate use. The results showed a high level of *Salmonella* resistance to tetracycline in the birds than the quinolones. There are evidences to indicate that tetracycline survives longer in the environment than do other antibiotics which may be critical in maintaining the level of tetracycline resistance at a high level (Frost, 1991). The low level of resistance to quinolones may be because they are relatively new antibiotics and are also more expensive than the tetracycline, ampicillin and chloramphenicol. There is also a probability that enteric bacilli in chicken intestinal tracts are not necessarily selected by antimicrobial supplementation of the feed, but rather on their common presence in the environments from which they can colonize the intestinal tracts of newly hatched chicks. These resistant enteric bacilli proliferate in the intestine and may transfer their resistance to *Salmonella* and from there to human. Because of this, the committee of the Joint Expert Advisory Committee on Antibiotic Resistance in Australia (JETACAR, 1999), agreed that there was evidence for the emergence of resistant bacteria in human and animal following antibiotic use and the spread of resistant bacteria from animal to

human as well as the transfer of antibiotic-resistant genes from bacteria in animal to human pathogens and that strains of resistant bacteria which are zoonotic can cause disease in human (JETACAR, 1999).
The high antibiotic resistance demonstrated by these isolates correlated with the high level antimicrobial resistance in enterobacteria, in fecal flora as well as in clinical isolates reported by Aarestrup, (1999) and Velonakis *et al.* (2001). The isolates were highly resistant to tetracycline, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole and ampicillin and less resistance to ciprofloxacin, ofloxacin, ceftriaxone and cefuroxime from both sources. This also confirms the idea that uncontrolled sale and use of antimicrobials in agriculture and for treating human patients could contribute to increase multi-drug resistance of *Salmonella* species. There is the potential for antibiotic-resistant *Salmonella* to spread through the food chain from animals treated with antibiotics to humans.

There are several reports on the trend of infection through feces, suggesting that *Salmonella* species and *Escherichia coli* are the main carrier of antimicrobial resistance genes in fecal flora of birds and human (Hedberg *et al.,* 1993; Araújo *et al.,* 1995; Carattoli 2003; Helms *et al*., 2005; Osman *et al.,* 2006). Resistance genes are often located on extra-chromosomal genetic elements or in segments inserted within the chromosome that originates from other genomes (Carattoli, 2003; Yah *et al.,* 2007). The acquisition of a new gene may occur by genetic transformation or through mobilization by conjugative transfer. The latter may occur at high frequency and efficiency, and several resistance genes can be acquired simultaneously (Carattoli, 2003).

The results showed that 32 (17.5%) of the isolates had plasmids of varying sizes ranging from 2.5kb to 5.0kb while 151 (82.5%) appear to have no plasmids. According to Carattoli (2003) and Yah *et al.* (2007), the antibiotic resistance in those isolates which seem not to possess plasmids was associated with chromosome and/or transposons instead of being plasmid-mediated. This therefore implies that there is no consistent relationship between antibiotic resistance pattern and the number of plasmid bands present. Conjugation studies were performed to determine whether the plasmids resistance markers could be transferred to *E. coli*. The results showed that all the transconjugants expressed plasmid DNA that migrated approximately on agarose gels. These plasmids were highly transferable at a frequency of 2×10^{-2} to 4×10^{-4} per donor cell. The transferred plasmids DNA varied among the *Salmonella* species. Transfer was higher among ampicillin (Am^r), tetracycline (Te^r) gentamicin (Gn^r) resistance genes while ofloxacin (Ofl^r), ciprofloxacin (Cip^r), cefuroxime (Cef^r), and ceftriaxone (Ce^r), were not transferred at all. The exchange of plasmid(s) between bacterial cells and the integration of resistance genes into specialized genetic elements play a major role in acquisition and dissemination of antibiotic resistance genes among the *Salmonella* species (Winokur *et al.,* 2000; Carattoli, 2003; Helms *et al.,* 2004; Osman *et al.,* 2006; Yah *et al.,* 2007). We suggested that the multiple drug resistance of *Salmonella* in broiler chickens may be transferred from animal strains to the resident flora of the human gut. Such transfer could occur during transient passage through the digestive tract. The results also, demonstrated how antibacterial treatment targeted at a pathogenic organism in a host may affect the endogenous flora of another host in a population. The transfer of antibiotic resistant genes among the species is an increased risk considering the fact that all strains of this organism are potential pathogens.

CONCLUSIONS

The present study support the hypothesis that the versatility of plasmids together with the usage of antimicrobials in human and birds, may largely contributed to the spread of antimicrobial resistance. Also the presence of antibiotics resistant genes in *Salmonella* can be explained by horizontal and vertical transfer of resistance from bacteria of nosocomial origin. This phenomenon is a disturbing development for public health, since *Salmonella* carriage of such transmissible plasmids may facilitate the spread of a variety of resistance. The transfer of such a multi-resistance gene fragment to other pathogenic bacteria could result in a serious health concern. An understanding of the antibiotic resistance gene arrangements will probably have an appreciable impact on antibiotic use in agriculture and medicine.
Salmonella species is a public health risk. All strains of this organism are potentially pathogens. The transfer of antibiotic resistant genes among the species is an increased risk.

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Study of the Properties of Bis{(benzimidazol-2-yl) Pyridenato} Zinc

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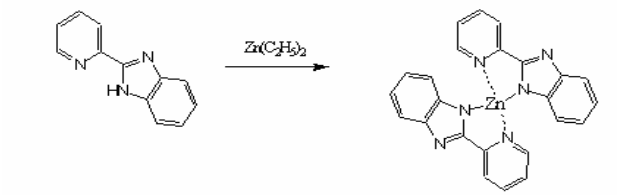
Abstract: A bright blue emission material, bis {(benzimidazol-2-yl) Pyridenato} zinc (**ZnBIP**) used for organic light emitting devices, has been prepared in good yield and characterized by ^{1}H-NMR, elemental analysis, and mass spectrometry. This complex is reasonably stable upon exposure to air and exhibited high thermal stability. [The Journal Of American Science. 2007;3(4):93-94]. (ISSN: 1545-1003).

Keywords: Electroluminescence; white light; OLED

1. Introduction

White organic light emitting diodes have attracted much attention, because their potential applications in the backlights of laptop computers and portable panel light sources. In the literatures, several strategies including multi-layer devices have been developed to realize highly efficient white organic electroluminescence [1-5]. Luminescent chelate complexes have been shown to be particularly useful in electroluminescnt (EL) displays because of their relatively high stability and volatility. The most well-known one of this kind of complexes is $A1q_3$, not only a good emitter but also a highly efficient electron-transporting material, where q is the 8-hydroxyquinolinato ligand [6, 7]. By modifying the structure of ligands of metal complexes, the emission color of which may be tuned, and the other properties, such as thermal stability and carrier mobility, may also be improved. Therefore, in this report, we prepare benzimidazol-2-yl Pyridine (**BIP**) and let it react with diethyl zinc to synthesize a thermally stable complex, bis{benzimidazol-2-yl) Pyridenato} zinc (**ZnBIP**). The thermal stability, an important character for the practical application, of this metal complex was investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).



Scheme 1: Synthesis process for the **ZnBIP** compound

2. Experimental

The synthesis of the title compound was accomplished by following processes, as shown in Scheme 1. The diethyl zinc solution (15wt.% in hexane, 11.34ml, 10mmol) was slowly added to 100 ml of THF solution containing benzimidazol-2-yl pyridine (2.95g, 20mmol) at 0^{o}C under N_2. After the resulting mixture was stirred at room temperature for 6 hours, 5 ml isopropyl alcohol was added to quench the reaction. The solvents were removed under vacuum condition at 5×10^{-3} Torr, and the residual solid was sublimed to purify the final product. Light yellow powder of **ZnBIP** was obtained in 80% yield. ^{1}H NMR and elemental analysis have determined the formula of this compound. The EL spectrum and the Commission Internationale de l'Eclairage (CIE) co-ordinates were measured by Pro-650 Spectroscanner (step size is 1.0 nm and bandpass is 4nm), the current-voltage (I-V) characteristic was measured by Keithley 2400 Source meter. Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer thermogravimeter (Pyris 1) under a dry nitrogen gas flow at the heating rate of 20^{o}C/min. Glass transition

temperature (T_g) and melting point (T_m) of materials were determined by differential scanning calorimetry of the Perkin-Elmer differential scanning calorimeter (DSC-7).

3. Results and discussion

The Photoluminescent (PL) spectra of the **ZnBIP** solutions and neat film, excited with 330 nm laser line, were illustrated in Figure 1.

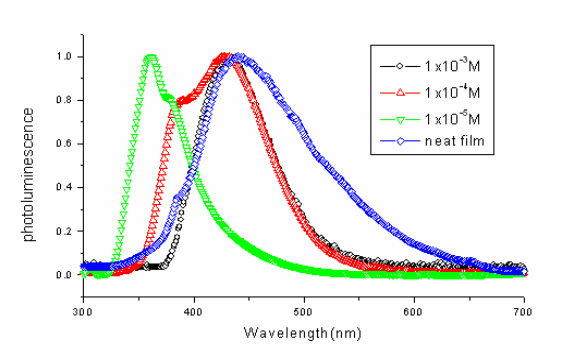


Figure 1: Photoluminescent spectra of the **ZnBIP**.

At low concentration, 1×10^{-5} M in DMF that double emission bands are observed at 357(m) and 385(s) nm, corresponding to the relaxation of **ZnBIP** from the excited state of a single molecule into ground state. Besides the 445 nm band, a new emission band appeared while the concentration of **ZnBIP** increased from 1×10^{-5} to 1×10^{-3} M. This new emission band having a maximum at 450 nm is observed in the spectrum of the **ZnBIP** neat film. A novel metal complex, bis{benzimidazol-2-yl} Pyridenato} zinc (ZnBIP), was successfully prepared by the reaction of benzimidazol-2-yl pyridene and diethylzinc. Because of its high thermal stability and excellent electrical characteristics, ZnBIP suggest a possible application for the use of the organic white light emitting devices.

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A Novel Design of Active Imidazolylquinoline Thin Film Organic Device Antenna

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Abstract: A novel design of the active NIQ/Cu thin film organic device antenna is demonstrated. The purpose of this antenna is applicable to study the promotion of the mobility of the electrons in organic device. [The Journal Of American Science. 2007;3(4):95-98]. (ISSN: 1545-1003).

Keywords: organic active device; mobility; imidazolylquinoline; antenna

I. Introduction

Recently, many designs of characterized organic devices including all types of organic light emission diode (OLED), organic thin film transistor (OTFT) have been reported [1, 2]. Modifying the organic complex and substrate can manoeuvre the characteristics of the device. Applying the technique of the fabrication of the planar organic device and the field measurement of the microstrip patch antenna, NIQ/Cu thin film component on the FR4 substrate can be function as a patch antenna with active organic device (OD) to express special characteristic of the device. Based on the shifting of the operation frequency of the active NIQ/Cu OD antenna in comparison with the micropstrip patch antenna resonated at TM_{11} mode, the dielectric coefficient and the carrier mobility of the carriers in device can be revealed by basic analysis. A simple geometry thin film active NIQ/Cu OD antenna was designed for the S-band at 2.4GHz and presented in this letter. Setting a truncated NIQ/Cu thin film patch OD at 1000/2000 nm (NIQ/Cu) in thickness is for the consideration of circular polarized field transmitted from the patch of NIQ/Cu, which may promote the mobility of the hop electrons at a specific direction provided by NIQ thin film as well as the conduction electrons from the copper [3].

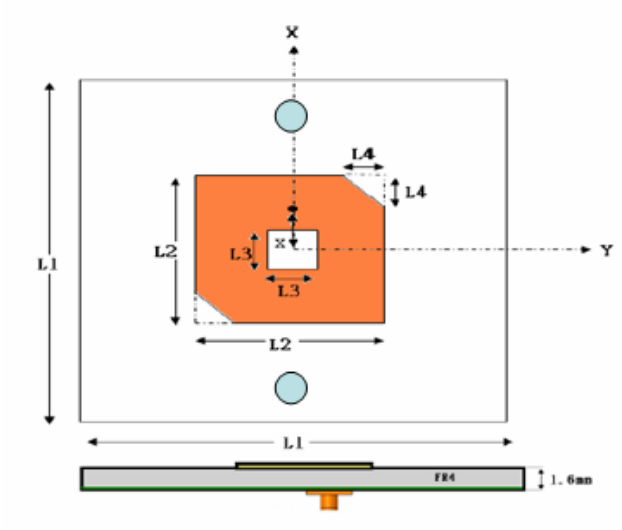
II. Design of the device

An architectural schematic drawing of the truncated 2000 nm thick rectangular cooper patch with depositing 1000nm dielectric organic thin film NIQ is depicted in Figure 1 where the probe-feeding is at distance of 5.1mm to the centre of the patch. Meanwhile, the thickness of the substrate is 1.6mm. SMA connector with characteristic impedance Z_o (50 Ω), loss tangent (tangentδ) and specific ε_r (4.4) for inexpensive FR4 substrate shown in the figure are regarded as major parameters in transmission line model (the size of ground is assumed infinite).

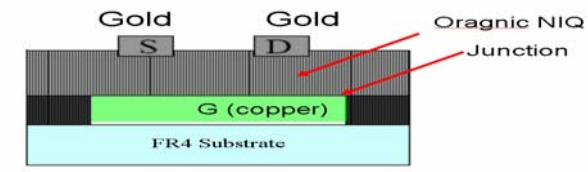
Refer to the reference [3], the mobility of the electrons and the loss tangent of the patch NIQ/Cu OD were calculated and listed in Table 2.

Table 1. Design parameters of the NIQ/Cu patch OD

Substrate	FR4 (1.6 mm)
Patch	NIQ/Cu 1000/2000 nm
Ground	35×35 mm² 10mm margin
L1	45 mm
L2	28 mm
L3	8 mm
L4	4.4mm
X	5.1mm



(a) top and front view



(b) side view

Figure 1. Schematic geometry drawing of the active NIQ/Cu OD antenna

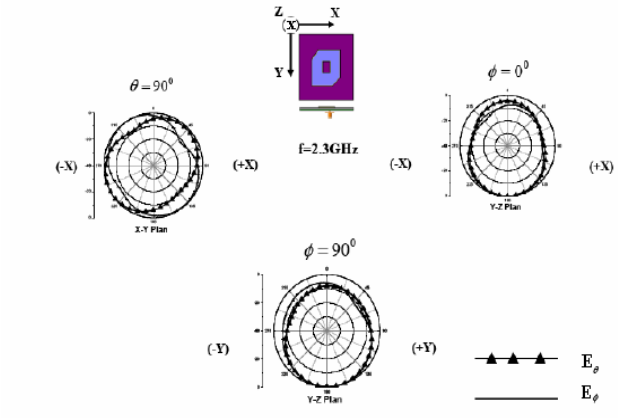


Figure 2. Field measurements of the proposed OD antenna with NIQ/Cu patch on FR4 substrate at thickness of 1.6mm

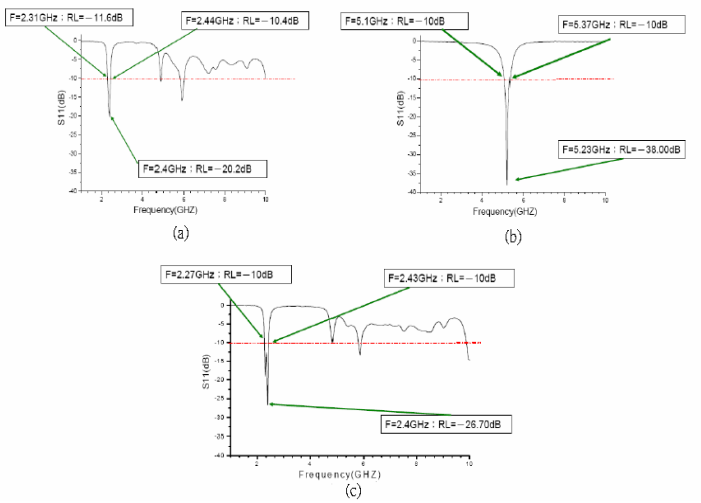


Figure 3. Measurement of the Return Loss of the OD antenna (a) only with patch of cooper (b) with the NIQ (c) with the NIQ/Cu.

Table 2. Results of the Calculation

Effective dielectric constant of NIQ (1000 nm thick)	0.96
Loss tangent of the NIQ/Cu (1000/2000 nm thick)	10^{8}
Loss tangent of the NIQ (1000nm thick)	10^{-4}
Electron Mobility in NIQ (1000nm thick) m^2/Vs	10^{-4}
Electron Mobility in NIQ/Cu (1000/2000 nm thick) m^2/Vs	10^{4}

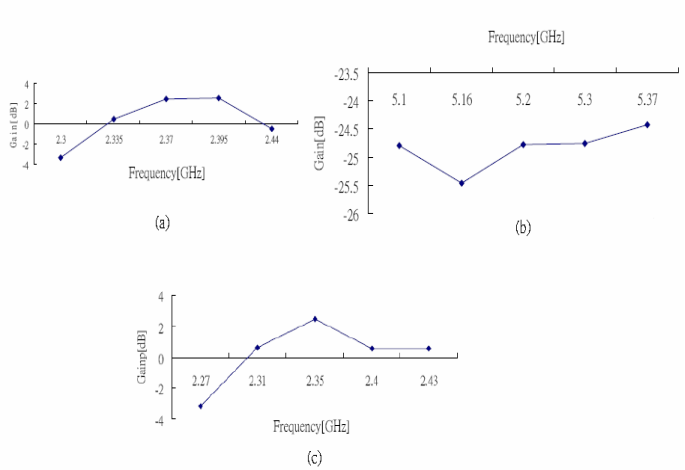


Figure 4. Measurement of the Gain of the OD antenna. (a) only with patch of cooper (b) with the NIQ (c) with the patch of NIQ/Cu.

Figure 4 depicts the measurement of the radiation gain of the active NIQ/Cu OD antenna [4].

II. Calculation Results and Conclusion

An active electronic material, 2-(naphtho[3,4]imidazol-2-yl) quinoline (NIQ), 1, has been synthesized and fully characterized [5]. This compound exhibits field-effect carrier mobility and behaves as a p-type semiconductor (mobility coefficient $\mu = 0.148\times10^{-4}$ m^2/Vs at $V_{DS} = 10$ V). NIQ and its related imidazolylquinoline compounds may have possible applications as active materials in organic thin film active OD antenna.The calculation has shown the loss tangent of NIQ is 10^{-4} and the dielectric coefficient is 0.94 for the NIQ thin film. Those values are patch OD thickness related. In contrast, as we know hop electrons affect the displacement current in OD, since displacement and the conduction current is up to 10^{+8} for the NIQ/Cu active OD antenna, the conduction current is dominated and shall be regarded as the major parameter controlling the characteristics of the OD. Moreover, for the radiation efficiency (gain) is highly related to the loss tangent and the dielectric coefficient, the organic active device can be used as the modulator of the microstrip antenna.

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Isolation and characterization of *Schistosoma mansoni* gene coding for antigenic protein from UV irradiated cercariae cDNA expression Library

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Abstract: The attenuated cercariae model is such a good one for facilitating the identification of antigens capable of evoking the protective host immune response which may be the first step towards the production of an effective anti-schistosomal vaccine. Although it is well known that irradiated cercariae is an effective vaccine against schistosomes in the experimental model, the actual effect of irradiation on DNA, RNA and protein to produce this protection is unknown. In this work in the schistosomiasis research we have try to isolate post-irradiation gene transcripts encoding antigens with immunogenic potentiality. SM21.7 was considered to be such a larval stage antigen and its localization in the tegument and subtegument layers confirmed its importance to be used as a target vaccine candidate in case of hosts challenged with *S. mansoni.* The present study was to clone some gene(s) encoding antigen(s) isolated from UV irradiated *S. mansoni* expression library that might be vaccine candidates. The antigens were recognized by protective antibodies (IgG fraction) separated from rabbits vaccinated with irradiated cercariae. We have screened UV irradiated *S. mansoni* cercariae cDNA expression library constructed in lammda ZAPlI, using IgG fraction taken from rabbits vaccinated with irradiated cercariae. The immunoscreening resulted in eight clones that may code for antigenic proteins. Two highly positive clones were isolated and designated UV-Irradiated *S. mansoni* cercariae 1 and 2 (UVIRSmC1) and UV-IRSmC2. The (UVIRSmC2) clone was determined by restriction enzymes digestion and sequence analysis. The gene has a total size 1088 bp and showed a high degree of identity with differences at 5' end the amino acid level with previously reported *S. mansoni* antigens: SM21.7 and SMS01. The gene encodes a 21.7 kDa antigen with the highest point of hydrophilicity from amino acid 33 to amino acid 38. The significance of the changes that occurred at the noncoding region sequences of the gene compared to the sequences of SM21.7 and SMS01may have a role in the deference of response to normal and UV irradiated cercariae. [The Journal Of American Science. 2007;3(4):99-112]. (ISSN: 1545-1003).

Keywords: *Schistosma mansoni*, Irradiated cercariae, Sm21.7, SMS01, EF-hand, Ca^{2+} binding motif

1. Introduction

Schistosomiasis (also known as Bilharzia), infection with the helminthes parasites in the genus *Schistosoma,* remains an important infection in many tropical areas, especially Africa. More than 200 million people have schistosomiasis, with 20 million exhibiting severe symptoms (Zhang *et al.,* 2007). Recent analyses suggest that the morbidity due to schistosomiasis is grossly underestimated (King *et al.,* 2005), resulting in an estimated 280,000 deaths annually in sub-Saharan Africa alone (Hotez *et al.,* 2006). Since the mid-1980s praziquantel has been the drug of choice for schistosomiasis; effectively it is currently the only choice available. Artemether has shown promise as a new drug for schistosomiasis, targeting larval parasites more effectively than praziquantel, which is primarily effective against adult parasites (Utzinger *et al.,* 2003).

A schistosome vaccine would provide a useful tool for the control of *S. mansoni*. In spite of several decades of research an effective vaccine remains elusive but the successful induction of high levels of protective immunity in laboratory hosts, by radiation-attenuated (RA) cercariae, nevertheless gives hope that it is feasible (Coulson, 1997).

Current advances in post-genomic techniques are providing new avenues to identify the secreted and surface-exposed antigens that mediate protection (Curwen *et al.,* 2004, Dillon *et al.*, 2006) and should eventually lead to replacement of the RA vaccine with recombinant protein formulations. Once their protective potential has been established in laboratory models, human vaccine trials will be required. Such trials will inevitably be undertaken in endemic areas where many people receiving the vaccine will either harbor a schistosome infection or have previously been infected and had curative chemotherapy. Given the fact that, schistosome infections rapidly down modulate from the acute to the chronic stage (King, 2001).

The protective activity induced by vaccination with live attenuated cercariae was found to be directed against early larval stages and associated mainly with IgM and IgG antibodies (Jwo and LoVerde, l989; Mangold and Dean, 1992). The mechanism of protection induced by radiation-attenuated larvae is not completely understood and the molecular effects of radiation or the larval stages that elicit this protection have not yet been recognized. Therefore the aim of the present study was to clone some genes encoding proteins (antigens) isolated from UV irradiated *S. mansoni* expression library that might be vaccine candidates. The antigens were recognized by protective antinodes (IgG fraction) separated from rabbits vaccinated with irradiated cercariae. Also, the effects of UV irradiation, if any, on the genetic material of the parasite and consequently on the released antigens were investigated.

2. Materials and Methods

2.1. Parasites and animals

An Egyptian strain of *S. mansoni* cercariae and the NewZealand rabbits (1-1.2 Kg) were supplied by Schistosome Biological Supply Programm (SBSP) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Fecal screening and serological tests (indirect haemo-agglutination, circum oval precipitin test) showed that all were negative for *S. mansoni* eggs and antibodies.

2.2. UV irradiation of *S. mansoni* cercariae

UV irradiation of *S. mansoni* cercariae was carried out according to Dean *et al.,* (1983). Briefly, the source of UV irradiation was An S-68 Mineral Light Lamp (Ultraviolet. Products, Inc.) , rated deliver 95% of its output at 254 nm. The cercarial suspension was placed in a clear flat-bottomed glass dish; the cercarial suspension depth was adjusted to 1.2 cm. The tubular UV bulb was centered horizontally over the dish position, parallel to the long axis of the dish, at a distance 22.2 cm above the water surface. The UV lamp was suspended from the roof of a dark box inside which the dish was put to avoid the harmful effects of UV radiation on the eyes and skin. The UV intensity at the water surface, as measured by a J-255 short wave UV meter (Ultraviolet, Products, Inc.), was 110 microwatts/cm^2. Cercarial suspensions were exposed to UV radiation for 90 seconds and used in different ways. The first way was for animal infection and the second was for RNA preparation and finally for cercarial antigen preparation.

2.3. Preparation of Sera

Vaccinated and normal rabbit sera were obtained from NewZealand rabbits, immunized by exposure to UV-irradiated *S. mansoni* cercariae. Sera were prepared according to Jwo and LoVerde (1989). In Brief, *S. mansoni* cercariae were irradiated and used within 10-15 min for vaccination 5,000 cercariae / dose / animal (Dean *et al.,* 1983 and Shi *et al.,* 1993). The animals were washed and cleaned with warm water. Then, animals were injected with thiopental and *S. mansoni* irradiated carcariae were applied to the lateral shaved abdominal skin. The vaccination process was carried out at 0, 25 and 51 days. Then, the animals were killed, the blood was collected in

clean sterile tubes, and the serum was isolated for further investigation.

2.4. Isolation and purification of IgG fractions from whole serum

The IgG proteins of normal and vaccinated rabbit sera (NRS and VRS) were purified by protein G agarose affinity chromatography. Readymade protein G agarose minicolumn (GiBCO BRL) was used in this study. The affinity chromatography was done as recommended by the manufacture manual for the purification of IgG fraction. A partial purification step has been carried out by precipitation of immunoglobulins using supersaturated ammonium sulphate solution. The concentration of the IgG was determined using the method of Lowery *et al.,* (1951). The purity of IgG fractions was measured using SDS-PAGE and western blot was used to detect the antigencity. The sera were kept at 4°C until used (Harlow and Lane, 1988).

2.5. Preparation of *S. mansoni* cercarial antigen

UV irradiated *S. mansoni* cercariae (~ half million cercariae), were homogenized with 400µl Tris-buffered saline (TBS) in glass-Teflon homogenizer, placed on ice. Then was transferred into a microcentrifuge tube and centrifuged at 12,000g for 10 minutes at 4°C. Supernatant was transferred to a new tube and protein concentration was estimated by using the method of Lowery *et al*., (1951).

2.6. Western blot analysis

The UV-irradiated cercarial proteins or IgG proteins were separated electrophoretically throughout 10% polyacrylamide gel under reducing conditions according to (Laemmli *et al.,* (*1970*). Then the I gG proteins were stained with Coomassie Blue brilliant stain or transferred onto nitrocellulose membrane (0.45pm pore size) in a Mini Bio-Rad protein transfer unit (Burneete, 1981), using transfer buffer (25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3) for 2 hr at 100 volts.

The nitrocellulose membranes were blocked in 10X TBST (Tris-buffered saline Tween-20) containing 3% BSA, for 1 hr. The membranes were rinsed in TBST, and incubated with antiserum (VRS and NRS) for 1 hr with gentle shaking. Then the blots were washed four times in TBST and incubated with alkaline phosphatase conjugated anti-serum for 1 hr. After incubation, the blots were washed in TBST and soaked in Nitro-blue tetrazolium (NBT) and 5-bromo 4-chloro3-indolyl phosphate (BCIP) **(Sambrook *et al.,* 1989).**

2.7. Construction of cDNA expression library

Total RNA was isolated from the irradiated *S. mansoni* cercariarae by using acid guanidium thiocyanate-phenol-chloroform extraction method (Chomcznski and Sacchi, 1987). The quality of the preparation was evaluated by spectrophotometric reading and agarose gel electrophoresis stained with ethidium bromide. Undegraded total RNA was subsequently to isolate poly A^+ RNA (mRNA) using Oligo-dT cellulose column as described by (Smbrook *et al.,* (1989). A cDNA expression library was constructed using the (λZAPII cDNA cloning system of Stratagene (Short *et al*., 1988), as described by the manufacturer's instruction (Stratagene, La Jolla, CA).

2.8. Immunoscreening of the cDNA Library

The constructed λZAPII cDNA expression library was immunoscreened as described by Huynh *et al.,* (1986)**.** In Brief, plaques were plated onto *E. coli* host strain XL1-Blue at a density of 10^3 plaques per plate and incubated at 42°C for 3 hr. Nitrocellulose filters were presoaked in 10 mM isopropyl-D-thiogalactoside (IPTG) placed onto the plates and incubated at 37°C overnight. The filters were washed in TBST (20 mM Tris-HCl pH 7.5, 150 mM NaCL with 0.05% Tween 20), and blocked in TBS containing 2% bovine serum albumin (BSA). The nitrocellulose filters were probed with the primary antisera VRS and normal rabbit serum (NRS) as a negative control. Then, the filters were washed, incubated in a secondary antibody (BRL, Gaithersburg, MD), diluted in TBS. Bound second antibody was detected using (NBT) and (BCIP).

2.9. Identification and characterization of a positive clones

The clones that remained positive through three rounds of purification were *in vivo* excised, using R408 helper phage (Stratagene, La Jolla, CA). The phagemids were transferred to *XL1-Blue* cells and plated on LB/ampicillin plates. The DNA of the positive clones were extracted and digested with *XhoI* and *EcoRI* to estimate their molecular size. The isolated clones having the most positive signal and the highest molecular size were chosen for further studies. Both strands of the DNA insert of the isolated clones were sequenced by the dideoxy-nucleotide termination method (Sanger *et al.,* 1977) using Sequnase Version 2.0 (Unistated State Biochemical). PC/GENE software (Intelligentsias, Inc.) and Basic Local Alignment Search Tool (BLAST) programs were used to perform sequence analysis and homology comparisons based on the deduced amino acid sequence (Altschul *et al.,* 1990).

3. Results

3.1. Isolation of IgG fractions of vaccinated and normal rabbit sera

The IgG fractions of normal rabbit serum (NRS) and vaccinated rabbit serum (VRS) were partially purified by precipitation of immunoglobulin using ammonium sulphate followed by affinity chromatography using protein G-agarose. To determine the purity of the isolated IgG fraction, the bound fractions (IgG) and unbound were subjected to 7.5% SDS- PAGE. The prepared IgG fractions have shown to be free of other serum proteins (Figure 1).

3.2. Western blot analysis

Western blot analysis was carried out; first to assess the ability of the VRS-IgG to detect the cercarial antigen bound to nitrocellulose membrane to determine the appropriate primary antibody dilution and secondly, to detect the presence of cross reactivity with phage lysate. Finally to check the avidity of the secondary antibody conjugate for the primary antibody for sera before and after treatment using different concentrations of *E. coli* phage lysate (25ng and 100ng) with one dilution of primary antibodies VRS-IgG and NRS-IgG (1:1000). The results indicated a highly background with high positive reaction as an indicator from cross reaction as showed in figure (2) lanes 3 and 4 (100ng) and lanes 5 and 6 (25ng). Therefore the serum was reabsorbed by immobilized on nitrocellulose filter before immunoscreening of the library. The VRS and NRS IgG protein were tested after the treatment with *E. coli* phage lysate by using the same concentration like before treatment and the same dilution of primary antibody (1:1000) as showed in figure (2) lanes 7, 8 (100ng), and 9 and 10 (25ng) of *E. coli* phage lysate. The low concentration of *E. coli* phage lysate antigen did not give visible colour with VRS-IgG protein lane 9 or with NRS- IgG protein lane 10 (Figure 2).

To assess the antibody dilution for immunoscreening and to ensure the efficiency of treatment in removing anti *E. coli* antigen, Western blot was carried out after treatment using 3 different dilutions of primary antibody (1:600; 1:1200 and 1:1800) with 2 different concentration of UV irradiated cercarial antigen 100 µg (figure 3 lanes 4, 6 and 8) and 25 µg (lanes 5, 7 and 9) compared with NRS-IgG (lanes 10 and 11) with the same dilution and concentration.

The results showed that irradiated cercarial proteins were recognized by VRS only. The treatment was efficient in removing anti. *E. coli* antigen, so, there is no need for additional treatment.

3.4. Identification and Characterization of UVIRSmC2

3.4.1. Restriction Enzymes

The immunoscreening of UV irradiated *S. mansoni* cercariae cDNA expression library using of VRS-IgG fraction identified eight positive clones that survived through three rounds of plaque purification. The pattern of DNA digestion of one of the identified positive clones, designated UVIRSmC2, with *EcoR*I and *Xho*I revealed that the DNA of the insert has two internal sites for *EcoRl* and the estimated molecular size around1 kb (Figure 4).

3.4.2. DNA sequences and computer software analysis

The sequence of UVISmC2 insert revealed that the insert has 1088 bp Long, which corresponds to a translation product of 184 amino acids corresponding to a protein of 21.7 kDa, and it revealed an open reading frame of 552 nucleotides starting by the first methionine codon, ATG, at the base pair 376 and ending by the codon TAG at the base 928; two polyadenylation signals were identified (Figure 5).

The use of the BLASTN (for DNA) program to search the similarity between the DNA sequence of the insert of the isolated clone, UVISmC2 and other DNA sequences, using non-redundant Gene Bank + EMBL + DDBJ + PDB sequences database has revealed a high degree with *S. mansoni* Sm21.7 (Francis and Bickle, 1992) and S. *mansoni* antigen (Ahmed *et al.,* 2001). Also, a significant similarity was detected with a score of 420 with S. *japonicum* 22.6 kDa tegumental associated antigen (Jeffs *et al.,* 1991 Waine *et al.,* 1994: Yang *et al.,* 1997) and a score of 360 with *S. mansoni Sm* 22.6 antigen (Stein and David, 1986; Jeffs *et al.,* 1991).

The search with BLASTP (for protein sequence) program to investigate similarity between the predicted amino acid sequence and other protein sequences, using non-redundant Gene Bank CDS translations +PDR+ Swiss port+ PIR database revealed a striking identity with score of 964 with S. *mansoni* 21.7 kDa antigens (Francis and Bickle, 1992: Ahmed *et al.,* 2001). Also, a similarity with a score of 276 was detected with S. *mansoni* 22.6 kDa antigen (Stein and David, 1996; Jeffs *et al.*, 1991) and with a score of 270 with *S. japonicum* 22.6 kDa antigens (Jeffs *et al.,* 1991; Waine *et al*., 1994; Yang *et al*., 1997). Figure (6) represents the alignment between the predicted protein encoded by UVISmC2 insert and 22.6 kDa antigens of S. *mansoni and S. japonicum.* Amino acid composition analysis of the DNA sequence revealed the presence of a high percent of amino acids lysine (9.78%) glutamic acid (9.23%) and serine (7.6%). The isoelectric point was found to be at pH 7.26 and the greatest point of hydrophilicity was located from amino acid 33 to 38 that is always found to be correlated with a known antigenic determinant (Hopp and Woods, 1981). PC/GENE Computer analysis revealed the presence of sites and signatures in the predicted protein sequence as follows: 4 protein kinase C phosphorylation sites, 5 casein kinase II phosphoylation sites, one tyrosine kinase phosphorylation site, one N-myriostylation site, and one EF-hand calcium- binding motif.

The identified EF-hand Ca^{2+} binding motif in 21.7 kDa antigen encoded by UVISmC2 was compared with previously reported EF-hand Ca^{2+} binding motif in 22.6 kDa antigen of S. *mansoni* and S. *japonicum.* In the 21.7 kDa antigen, the domain was located between amino acids 42-69; while for the 22.6 kDa antigen of S. *mansoni* and S. *japonicum,* the domain was located between amino acids 47-74 and 48-75 respectively (Figue 7).

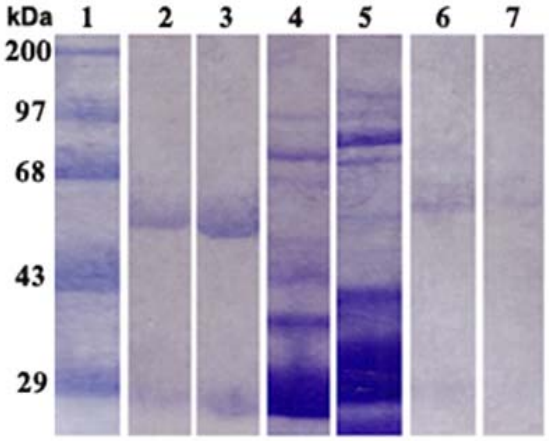

Figure (1): SDS-gel electrophoresis of fractions eluted from agarose affinity column. Lane 1: Low molecular weight protein marker. Lane 2: Unbound fraction of NRS. Lane 3: Unbound fraction of VRS. Lane 4: Bound fraction of VRS. Lane 5: Bound fraction of NRS. Lane 6: Normal rabbit sera (polyvalent). Lane 7: Vaccinated rabbit sera (polyvalent).

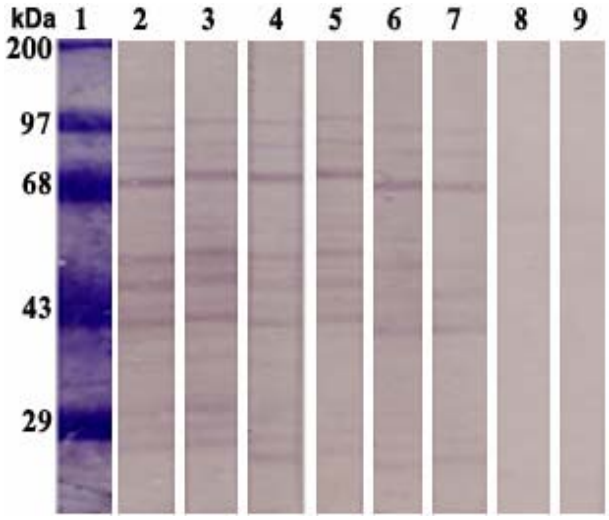

Figure (3): Western blot analysis of VRS and NRS-IgG treated against UV irradiated cercarial antigens. **Lane 1**: Low molecular weight protein marker. **Lane 2** :(150 µg /ml) UV. Irradiated cercarial protein with (1:500) treated VRS IgG. **Lane 3**: (75 µg /ml) UV irradiated cercarial protein with (1: 500) treated VRS IgG. **Lane 4**: (75 µg /ml) UV irradiated cercarial protein with (1:1000) treated VRS IgG. **Lane 5** :(25 µg / ml) UV irradiated cercarial protein with (1:1000) treated VRS IgG. **Lane 6** :(100 µg /ml) UV irradiated cercarial protein with (1:1500) treated VRS IgG. **Lane 7** :(25 µg /ml) UV irradiated cercarial protein with (1: 1500) treated VRS IgG. **Lane 8** :(100 µg /gm) UV irradiated cercarial protein with (1:500) treated NRS IgG. **Lane 9** :(25 µg /ml) UV irradiated cercarial protein with (1:500) treated NRS IgG.

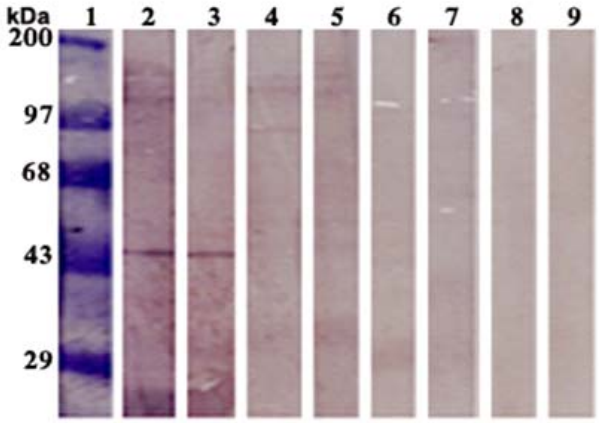

Figure (2): Western blot analysis of VRS and NRS-IgG treated and untreated against *E. coli XL-1-Blue* phage lysate. Lane 1: Low molecular weight standard; Lane 2:Untreated VRS (1:1000) with 150 ng of *E. coli* lysate; Lane 3:Untreated NRS (1: 1000) with 150 ng of *E. coli* lysate; Lane 4:Untreated VRS (1:1000) with 25 ng of *E. coli* lysate; Lane 5:Untreated NRS (l: 1000) with 25 ng of *E .coli* lysate; Lane 6:Treated VRS (1: 1000) with 150 ng of *E. coli* lysate; Lane 7:Treated NRS (l: 1000) with 150 ng of *E. coli* lysate; Lane 8: Treated VRS (1:1000) with 25 ng of *E. coli* lysate; Lane 9:Treated NRS (1:1000) with 25 ng of *E .coli* lysate.

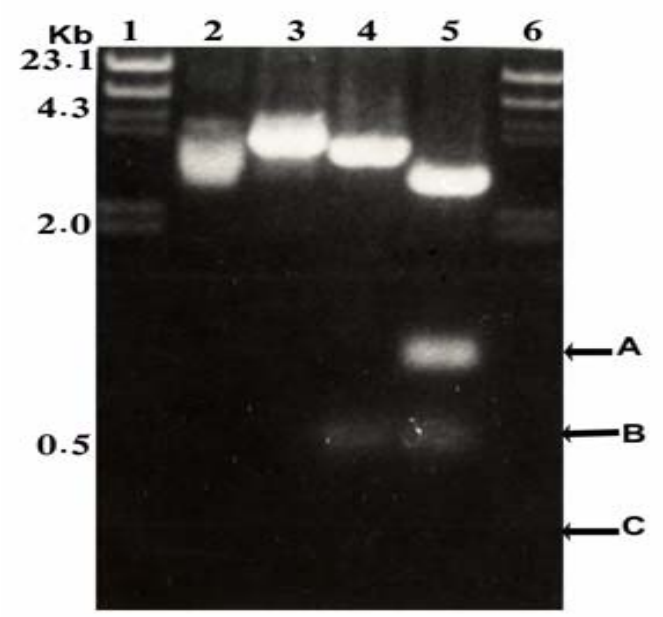

Figure (4): Agarose gel electrophoresis pattern of UVISmC2 phagemid. Lanes 1 and 6 represent Lambda DNA marker digested with *Hind*III (23.1-0.5kb). Lane 2 undigested DNA. Lane 3: *XhoI* digested DNA. Lane 4, *EcoRI* digested DNA. Lane 5 *XhoI* and *EcoRI* digested DNA of UVISmC2, respectively.

Figure (5): cDNA sequence of UVISmC2 clone with the predicted amino acid sequence immediately beneath. Nucleotides are numbered from the end of *Eco*RI adaptors and the amino acids from the initiator methionine; **M**. Two potential polyadenylation sequences are shown underlined.

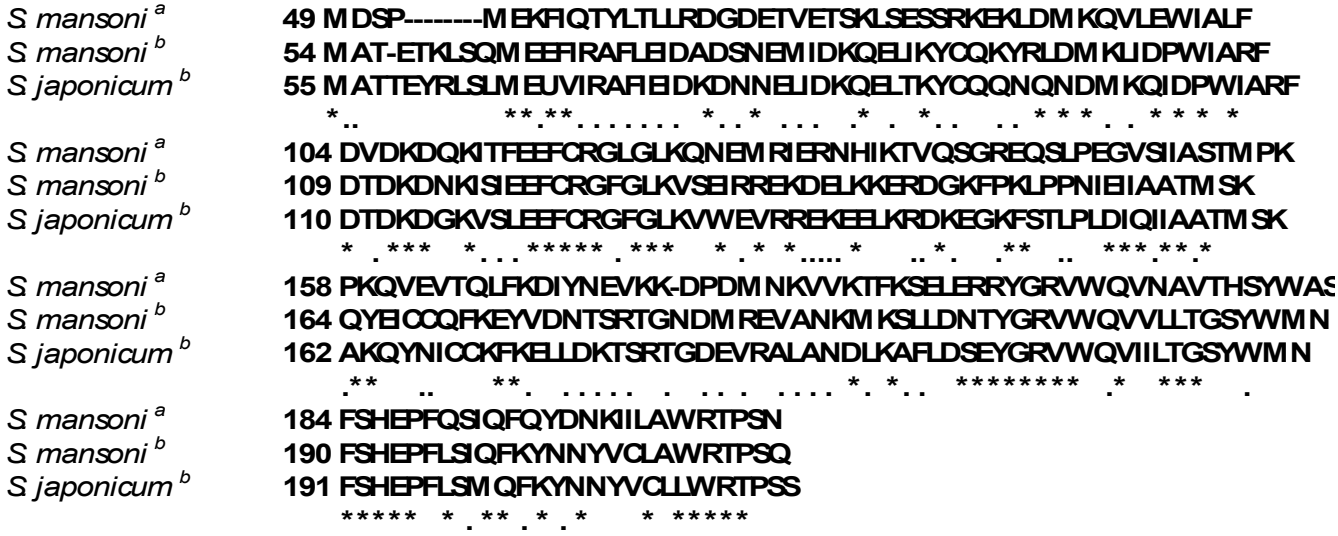

Figure (6): Alignment between the predicted proteins encoded by UVISmC2 insert, *S .mansoni* 21.7 kDa antigens and 22.6kDa antigen of *S. mansoni* and *S. japonicum.*

*a position in the alignment is perfectly conserved a position is well conserved.

[a] The *S .mansoni* 21.7 kDa antigen encoded by UVISmC2 insert is indicated by *S. mansoni*

[b] The *S. mansoni* and *S. japonicum* 22.6 kDa antigen sequences are indicated by *S. mansoni* and *S. japonicum* respectively.

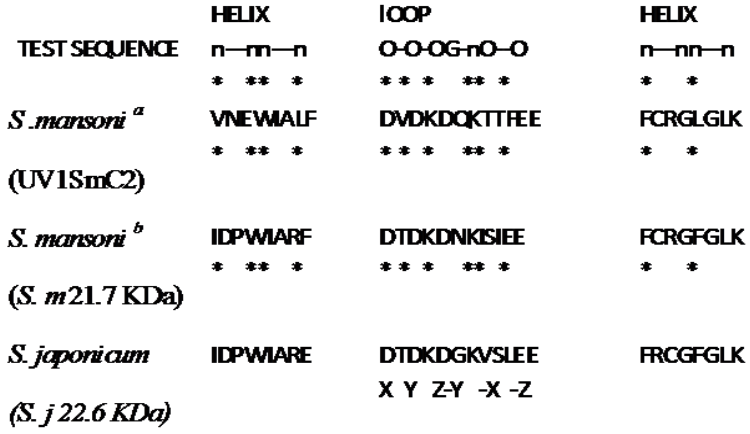

Figure (7): Comparison of the EF hand of test sequence and the deduced protein of UVISmC2 insert *S .mansoni* 21.7 kDa antigens (a) and 22.6 kDa antigens of *S .mansoni* and *S .japonicum.* In the test sequence **(Strynadka and James, 1989)** O denotes residue with side chain oxygen (D, E, N, Q, S, T); n denotes a non-polar residue; G denotes glycine; - denotes any amino acid may appear in this position; * denotes residue matching the test sequence, X, Y, Z, -X,-Y, -Z refer to the six Ca^{2+} ion ligating positions in the loop.

[a] The *S. mansoni* 21.7 kDa antigen encoded by UVISmC2 insert is indicated by *S. mansoni*

[b] The *S. mansoni* and *S .japonicum* 22.6 kDa antigen sequences are indicated by *S. mansoni* and *S. japonicum,* respectively.

5. Discussion

The radiation-attenuated (RA) schistosome vaccine is highly effective under laboratory conditions but, for ethical and practical reasons, cannot be used in humans. Nevertheless, it serves as a compelling model for the development of a recombinant vaccine **(**Coulson, 1997; Wilson and Ivens, 2006). The protective capabilities of the RA vaccine were originally established in rodents (Dean, 1983) and subsequently extended to primates (Eberl *et al.,* 2001, Soisson *et al.,* 1993 and Yole *et al.,* 1996a,b), with up to 86% protection obtained after five vaccinations of the olive baboon (*Papio anubis*) (Kariuki *et al.,* 2004). The baboon has the capacity to harbor a substantial schistosome infection long term, unlike mice, which succumb to egg-induced pathology even with low worm burdens (Warren *et al.,* 1972). This makes it an ideal host to investigate the interaction of infection and vaccination. Another advantage of baboons is that they are closer phylogenetically and in body scale to humans, so that experimental results are likely to have a greater relevance (Nyindo and Farah, 1999).

Irradiated cercariae have been shown to stimulate the host immune system and confer a high level of resistance without causing the pathological symptoms of schistosorniasis (Richter *et al.,* 1995). Thus, in the present study, we tried to clone some genes encoding proteins (antigens) isolated from UV-irradiated *S. mansoni* expression library recognized by protective antibodies (IgG fraction) isolated from rabbits vaccinated with irradiated cercariae. Identified cDNA clone, UVISmC2 encoding an antigen showed 100% identity at the amino acid level with previously identified *S. mansoni* clones, encoding 21.7 kDa antigens, isolated from *S. mansoni* sporocyst and 25 days schistosomula cDNA expression libraries (Francis and Bickle, 1992; Ahmed *et al.,* 2001). However, a lower degree of identity with other previously identified *S. mansoni* and *S. japonicum* clones, encoding 22.6 kDa antigens, isolated from *S. mansoni* and *S. japonicum* adult worm expression library was detected (Stein and David, 1986; Jeffs *et al.,* 1991; Waine *et al.,* 1994: Yang *et al.,* 1997). At the nucleic acid level, the DNA sequence homology search revealed a striking degree of identity with *S. mansoni* antigen, encoding 21 .7 kDa antigen, in the open reading frame (ORF) sequence (Francis and Bickle, 1992; Ahmed et *al.,* 2001).

The UVISmC2 clone, isolated from UV irradiated *S. mansoni* cercariae cDNA library, was characterized by the differences at the level of the nucleotide sequence restricted to the 5' and 3' non-coding sequences. The first 295 nucleotides 5' non-coding sequence was completely different from the previously sequenced 5' non-coding sequence of Sm21.7, and particuar1y SMS01 that nearly encodes the full length sequence of 21.7 kDa antigen and the 3' non-coding sequence that has a truncated non-coding sequence of 107 bp preceding the poly A tail. Also, an ambiguous unmatched T preceding the poly A tail was detected.

The identified EF-hand Ca^{2+}-binding motif in 21.7 kDa antigen encoded by UVISmC2 has been compared with previously reported EF-hand Ca^{2+}-binding motif in 22.6 kDa antigen of *S. mansoni* and S. *japonicum* (Strynadka and James, 1989), which was based on the earlier work of Tufty and Krestinger, (1975). The comparison study revealed divergence in one of the helices flanking the binding loop of 21.7 kDa antigen of UVISmC2 and 22.6 kDa antigens of *S. mansoni* and S. *japonicum.* Previous studies (Marsden *et al.,* 1988; MacManus *et al.,* 1990) indicated that a complete helix-Loop-helix unit is not required for the Ca^{2+}- binding. Hence, it is possible that the loop can bind Ca^{2+} even if the flanking helices do not match the test pattern exactly. This may explain why the Ca^{2+}-binding motif in *S. japonicum* antigen is functional.

Neither S. *mansoni* 21.7 kDa nor 22.6 kDa antigens loops bind calcium. The crucial difference in the Sm22.6 upon which its inability to bind Ca^{2+} was explained is that it lacks the glycine in the Ca $^{2+}$-binding loop (Stein and David, 1986). The same may also hold true for the 21.7 kDa protein encoded by

UVISmC2 in which glycine at position 6 is replaced by glutamic acid. From the previous findings, it is clear that the 21.7 kDa antigen (encoded by UVISmC2) is highly related to 22.6 kDa antigen which was identified in different schistosome species. Francis and Bickle (1992) suggested that the 21.7 kDa and 22.6 kDa antigens may be derived from an ancestral protein that had a functional calcium binding domain.

Sites annotated analysis of the predicted protein encoded by UVISmC2 has shown that it has many kinase phosphorylation sites along the protein. Protein kinases represent critical nodes for the amplification and distribution of signals (Watson *et al.,* 1992). N-myristoylation site also was detected in the predicted protein; it is post-translational modification of the protein sequence. An appreciable number of eukaryotic proteins are acylated by the covalent addition of myristate (a C14-saturated fatty acid) to their N-terminal residue via an amide linkage (Towler *et al.,* 1988).

The highest point of hydrophilicity (Hopp and Woods, 1981) was detected in the predicted protein encoded by UVISmC2 insert from amino acid 33 to amino acid 38. Hydrophilicity is associated with antigenic determinants because hydrophilic regions are on the outside of a protein. Also, the most Polar Regions may provide the greatest interactions for bonding between the antigen and antibody. This finding may be in agreement with the assumption of Francis and Bickle (1992) of the presence of immuno-dominant epitope(s) at the 5' end of the gene encoding 21.7kDa antigen, based on the observation that all of the separately derived clones encoding 21.7 kDa produced a similarly sized product corresponding to the whole gene.

In this study, the encoded by UVISmC2 clone (21.7 kDa) was isolated from cercariae cDNA expression library. Northern blot analysis demonstrated that mRNA for Sm21.7 is present in the sporocyst, 3 hours schistosomula and adult stages (Francis and Bickle, 1992). In addition, the SMS01 was isolated from schistosomula 25 days (Ahmed *et al.,* 2001). Hence, the 21.7 kDa antigen is expressed in all post snail stages of the S. *mansoni* life cycle.

Immunocytochemical localization on cryosections of adult worms using specific antibodies produced by the immunization of rabbits with purified SMS01 protein (21.7 kDa) showed that this antigen is located in the tegumental region and dispersed among the parenchymal cells of the adult schistosome parasite (Ahmed *et al.,* 2001).

Since the tegument is the outer covering of the parasite and serves as an interface between the host immune system and the parasite, the antigens associated with the tegument would be the major focus for development of a vaccine and/or immunodiagnostic reagents for schistosomiasis (Bergquist, 1990). So, the localization of the 21.7 kDa protein in the tegumental and subtegumental layers and its expression in all post snail stages of the parasite draw the attention to the importance of this antigen.

In the vaccinated rabbit serum (VRS) model, this antigen has been isolated several times using different strategies which means that this antigen may be one of the target antigens for protection in this model. In addition, this antigen was designated "vaccine dominant" as the antigen was recognized preferentially by mouse vaccine sera compared with mouse infection sera (Francis and Bickle, 1992).

On the empirical vaccination level, the 21.7 kDa antigen encoded by UVISmC2 succeeded to induce a good degree of protection in experimental animals. Immunization experiments in mice have revealed 40-70% protection against challenge infection with S. *mansoni* cercariae. Also, the antigen may be considered an antipathology vaccine since it plays an important role in the reduction of granuloma formation (Ahmed *et al.,* 2001). In experimental schistosomiasis, because sterilizing immunity has not yet been attained and because the disease is largely caused by the host's response to eggs, vaccination to reduce

granulomatous inflammation and fibrosis is suggested as an alternative approach (Botros *et al.,* 1996). All of these findings support the importance of this antigen, 21.7 kDa, encoded by UVISmC2 in the irradiated cercariae as a vaccine candidate.

UV light was reported to induce mutations (Griffiths *et al.,* 1996). DNA was identified as the principle biological target for UV irradiation (Imlay and Linn, 1988; Wales and Kusel, 1992). Therefore, the changes detected in the sequence of the 5' and 3' noncoding regions raise many questions about their reason and significance. As there is little data dealing with the molecular basis of the UV irradiated vaccination model, it is not easy to ascertain that these changes are a direct response to the effect of UV irradiation. If these changes were related to the effect of UV irradiation, why were they restricted to the noncoding region sequences and what are their significance.

Also, the results of the present study have shown no direct effect of UV irradiation on the codon sequence of the ORF of encoded by UVISmC2 insert. Wales and Kusel (1992) postulated that irradiation distorts the structure of schistosmular proteins, converting normally weak immunogens into highly immunogenic conformations. Such abnormal, non-native antigens may be created by the absorption of high-energy radiation and by reaction with oxygen radicals or the defective proteins may be produced following synthesis on RNA and DNA templates damaged by the irradiation. Also, Wales *et al.,* (1993) reported that UV irradiation causes an immediate and striking alteration in the carbohydrate antigens expressed by schistosome larvae and postulated that the structure of glycocalyx antigens released by irradiated larvae is modified in a way that alters the pattern of processing by the proteolytic enzymes of the antigen presenting cells resulting in presentation of new antigenic determinants to T helper, stimulating their potent protective immunity.

Hence the results of the present study, in the light of these postulations, expose to view those defects or abnormalities in the post translational processing of the proteins should be a point of consideration in the UV vaccination model. Further studies, however, are required to substantiate this suggestion. On the other hand, as the reported changes in encoded by UVISmC2 sequence were restricted to the noncoding sequence, characterization of the other identified positive clones may shed some light in this respect, keeping in the mind that radiation has haphazard and unoriented effects.

6. Conclusion

In conclusion, the proven value of irradiation as attenuating agent for schistosomiasis deserves to be examined by various means. The importance of antigen 21.7 kDa encoded by UVISmC2 as a candidate vaccine is currently under investigation. Although it is well known that irradiated cercariae is an effective vaccine against schistosomes in the experimental model, the actual effect of irradiation on DNA, RNA and protein to produce this protection is unknown. In this work for the first time we have try to isolate post-irradiation gene transcripts encoding antigens with immunogenic potentiality. So far in the schistosomiasis research a post-irradiation expression library has been constructed that induce the UV affected transcriptions. This library was immunoscreened with protective sera from UV irradiated rabbits to isolate genes encoding immunogenic antigens which may acquire its immunogenicity after UV irradiation due to unknown mechanism.

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Efficacy of combined SMS01 DNA and protein as a cocktail vaccine against *Schistosoma mansoni* infection

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Abstract: Schistosomiasis control currently relies primarily on chemotherapy which is both expensive and temporary; therefore there is an urgent need for an effective vaccine. One of the main strategies for vaccine development is the identification of specific antigen(s) that elicit highly protective immune response in immunized hosts. Several defined vaccine candidate antigens of *Schistosoma mansoni* have shown promise in animal vaccination experiments. In a previous study, single-gene vaccination with SMS01 recombinant protein was shown to elicit partial protection against *Schistosoma mansoni* challenge infection. Here we show that the vaccination of mice with a SMS01 (Sm21.7) vaccine cocktail significantly enhance protective responses against *S. mansoni* infection. To evaluate the usefulness of combined SMS01 DNA and protein as a cocktail vaccine against infection, the vaccination models were applied using DNA vaccination and SMS01 fusion protein or both. Consequently, the gene coding for SMS01 immunogenic protein inserted into mammalian expression vector pcDNA1, used as DNA vaccine, in combination with recombinant protein produced in pET-3a system. Then the ability of these combinations to induce a protection against *S. mansoni* infection was analyzed according to worm reduction rate and egg reduction rate after vaccination of mice. In addition the level of IgG antibody response was determined by ELISA. Results showed a significant reduction in worm burden in animals immunized with protein, DNA or combined vaccine, has been observed as 63%, 55% and 46% respectively compared to the control group. ELISA results showed that all the vaccinated groups have produced a high IgG titre and the highest IgG titre was produced by fusion protein group when compared to the control. Antifecundity effects of the three treatments have been observed, and the oogram pattern indicated that the dead ova were very high and the highest level was obtained using fusion protein. In spite of this result, the advantages of DNA vaccination model cannot be denied as being easier and economic. Thus, vaccination against *S. mansoni* remains a long-term prospect because wide ranges of time and tremendous amount of work are needed for continuous development and optimization of vaccine cocktails. [The Journal Of American Science. 2007;3(4):113-126]. (ISSN: 1545-1003).

Key Words: Schistosoma *mansoni*; Liver worms; SM21.7; Vaccine; SMS01; vaccine cocktails

1.Introduction

Schistosomiasis is still a major helminthes infection at the beginning of the 21st century and an important public health problem in many countries. As the second major parasitic disease in the world after malaria, schistosomiasis affects 200 million people, 800 million being exposed to the risk of infection (WHO 2002). It is also estimated that 20 million individuals suffer from severe consequences of this chronic and debilitating disease responsible for at least 500.000 deaths per year (Capron *et al.* 2002a). In Egypt, there is extensive documentation that the government's efforts have been successful in reducing both the prevalence and morbidity of this disease (Engles et *al.* 2002). However, schistosomiasis is still endemic in rural areas of Egypt and in spite of the low endemicity level, transmission still occurs. Four species are of direct medical importance to man; *S. mansoni*, *S. haematobium*, *S. japonicum* and *S. mekongia.* Despite the development of active and relatively safe drugs, the development of human schistosomiasis vaccine is recognized as priority to

complement existing control measures (Bickle et *al.* 2001; Bergquist 1995). Although praziquantel is an effective drug for the treatment of schistosomiasis, reinfection and the drug resistance of the parasite have become a problem. Therefore, the development of an effective vaccine against schistosomiasis is important to control this disease (Bergquist *et al*.2002).

In the past few years, many vaccine strategies have focused on defense against invasion of cercariae, to reduce worm burden by inducing humoral immunity with schistosome vaccine candidates, but the high-level antigen induced-specific antibodies could not adequately protect the host from infection (Bergquist 1998; Chen *et al.* 2003). Vaccination can be targeted towards either the prevention of infection or the reduction of parasite fecundity. A reduction in worm numbers is the "gold standard" for anti-schistosome vaccine development. However, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeted on parasite fecundity and egg viability seems to be entirely relevant (Capron *et al*. 2002b). Several promising candidate vaccine antigens have been characterized and their primary sequences derived for *S. mansoni*. These antigens include the glycolytic enzyme triose-phosphate isomerase (*Sm* TPI) (dos Reis MG *et al*. 1993 and Reynolds *et al*. 1994), a 28 kDa glutathione-S-transferase (*Sm*28) (Balloul *et al*.1987 and Boulanger *et al.* 1991), Sm20.8 (Mohamed *et al.*1998), the myofibrilar protein paramyosin Sm97 (Pearce *et al*.,1988), an integral membrane protein *Sm*23 (Da'dara *et a*l. 2002) *S. mansoni* calpain (Karcz *et al*. 1991) and *S.mansoni*(Ahmed *et al.* 2001).

Nucleic acid vaccination against schistosomiasis has lately been investigated using a panel of plasmids encoding schistosome antigenic proteins such as Sm21.7 (Ahmed *et al*. 2006), Sjc26GST (Zhou *et al.* 2005), Sj62 kDa (Zhang *et al.* 2007), *Schistosoma japonicum* paramyosin (Fonseca *et al*. 2005) and *Schistosoma mansoni* 23(Da'dara *et al.*2001), 28 GST (Dupre *et al.* 1997). In such a previous work, it was estimated that SMS01 (Sm21.7) has induced high level of protection against *S. mansoni* challenge infection (Ahmed *et al.* 2001). The goal of this research was to study the response of using recombinant DNA and recombinant protein as a vaccine in the protection of experimental animals challenged wit *S.mansoni* infection. To achieve that, gene coding for immunogenic protein inserted in mammalian expression vector, used as DNA vaccine, in combination with recombinant protein of this gene produced in pET-3a system.

2.Materials and Methods

2.1.Mice, parasites, and infection

An Egyptian strain of *S. mansoni* was maintained in golden hamsters, *Biomphalaria alexandrina* snails and animals were purchased from the Schistosome Biologic Supply Center, Theodor Bilharz Research Institute (Giza, Egypt). All animal presented here had been approved by the local government based on national regulations. We have used female Swiss albino mice (N = 70, age: ~4 weeks, weight: ~18 g) and New Zeeland female rabbits (3.5-4.5Kg). Animals were kept in groups under environmentally controlled conditions (temperature: ~25°C; humidity: ~70%; 12 hour light/dark cycle) and had free access to water and food.

2.2. Preparation and purification of recombinant SMS01 DNA

The DNA was prepared according to Ahmed *et al.* (2001). Briefly, a pair of primers was synthesized according to the DNA sequence of the SMS01, *Bam*H1 adaptors linked to forward and reverse primers and the Kozark sequence was added to the position of initiator. The forward primer was 5′CATCTGGATCCATGGATAGTCC and the reverse 5′TAACGGATCCCTAGTTACTTGG. The amplified sequence was ligated into the eukaryotic expression pcDNA1/Amp expression vector (Invitrogen, Corp, SanDiago, CA), which was previously digested with *Bam*H1 and treated with alkaline phosphates. The structure was verified by

restriction digestion and sequencing. Large-scale preparation of the plasmid was carried out by using the alkali lysis method, followed by double banding on CsCl-EtBr gradient (Sambrook *et al.,* 1989). Then DNA was resuspended in phosphate buffer saline (PBS) for vaccination. The SMS01-pcDNA1 plasmid encoding the full length SMS01 was used throughout these experiments.

2.3. Expression of recombinant protein in pET-3a system

The recombinant protein was expressed in pET-3a system using IPTG method according to (Studier *et al.* 1986; Rosenberg 1987). In brief, the bacterial colony, which contained the recombinant plasmid with SMS01 DNA sequence cloned into pET-3a, was cultured overnight in LB-Amp medium. The culture was then diluted 1:10 (V/V) in fresh LB medium and grown for *2-3* hrs at 37°C (till OD 600 was equal to 0.6). Then the IPTG was added to the final concentration 2mM and the incubation was continued for further 3hrs. The cells were centrifuged at 6,000 rpm (Sorvall, GSA rotor) at 4°C for 10 minutes and the pellet was resuspended in 1:50 (V/V) of the total volume of the bacterial culture in PBS, and then sonicated for 2-3 minutes on ice. The crude lysate was centrifuged at 12,000 rpm in Sorvall SS34 rotor at 4°C for 10 minutes and clear supernatant was further clarified by filtration through 0.45µm filter according to Smith and Johnson (1988).

2.4. Purification of recombinant protein using ion exchange chromatography

Recombinant protein was purified from the bacterial lysate by passing over a cation exchange column, SP-Sephadex C-50 (Pharmacia). The protein was eluted with a pH gradient (pH from 7.2 to 10.8) made with 0.1 M Tris-glycine buffer. The SMS01 recombinant protein was eluted at pH 9.4 (corresponding with the predicted PI of the protein). The eluted fractions were evaluated by SDS-PAGE to identify the fractions of the purified protein.

2.5. Detection of the purified fused protein using SDS-PAGE

Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the fused and purified SMSO1 protein (Laemmli 1970; Russel and Blair 1977). In brief, separated gels were composed of 10% acrylamide and stacking gel was formulated as *5%* acrylamide. Electrophoresis was carried out at a constant 150 volt in an electrode buffer pH 8.3. The gel was used for Western blot analysis or stained with coomassie brilliant blue for 30 minutes.

2.6. Western blot analysis

To detect the antigenic proteins immobilized on nitrocellulose membranes, the sensitive technique of Brunette (1981) was carried out. Recombinant proteins were separated on SD S-PAGE and transferred onto on PVDF membrane in a transfer unit (Mini Trans Blot Bio-Rad) using lx transfer buffer. The transfer was carried out at 80 volt for 2 hrs at 4°C. The membranes were blocked with 5% non-fatty dry milk in TBST for 2hrs then, incubated for 60 minutes with the primary antibody (rabbit serum) at room temperature with gentle agitation. Then the blots were washed with 3 changes of TBST and incubated at room temperature for 1 hr with alkaline phosphatase conjugated secondary antibody (goat anti rabbit IgG fraction). Furthermore the membranes were washed 3 times with TBST for color development solution (BCIP/NBT) until signals become clearly visible. Finally the membranes were rinsed with TBST then immersed in stopping solution for 1 minute air dried and stored protected from light.

2.7. Preparation of antibodies against SMS01 fusion protein

The purified fused protein SMS01 was used as an antigen to immunize New Zealand female rabbits (Green and Manson 1992). For primary immunization, the antigen in PBS was emulsified with complete Freund's adjuvant (100µg/animal) and used to inject the animals subcutaneously in multiple locations. Four weeks later, a booster dose of the antigen (50 µg/animal) emulsified with incomplete Freund's adjuvant (without the bacterial extract) was injected. After three weeks, an activating dose of the antigen emulsified with

incomplete Freund's adjuvant was injected into the animals. Blood samples were collected by ear vein puncture 2 weeks after each immunization and sera were used for western blot analysis.

2.8. Vaccination experiments

In order to assess the importance of SMS01 as a vaccine candidate, the groups of female Swiss albino mice were injected intramuscularly. The first group was injected with (50 µg /mouse) with purified SMS01-pcDNA1 DNA. The second group was injected with the SMS01 recombinant protein emulsified with incomplete Freund's adjuvant. The mixed group was injected with a cocktail vaccine (DNA and protein) as discussed before, but a space of time was left between the two injections to reduce the mortality rate. It has been recorded that death may be occurred at once if both injections were given at the same time. For that we can say that a chemical shock may take place in this case. Then the fourth group (control) of mice was not vaccinated. In each group, the zero time was detected briefly after which the first injection was performed. Three weeks later, after the first injection each mouse in each group was boosted with either 50 µg DNA or 50 µg proteins or with both as in case of the cocktail group. The animals were also boosted for a second time as to predict the best immunological response. Blood samples were collected and the sera were tested by ELISA for the production of antibodies against recombinant antigen

2.9. Challenge infection and evaluation of the worm burden

Vaccinated and control groups of mice, three weeks after the last immunization, were exposed to 100 *S. mansoni* cercariae for challenge by the tail immersion method (Oliver and Stirewalt 1952). Six weeks later, the animals of each group were necropsied and worms were recovered from the hepatic and portomesenteric vessels using the perfusion technique and the percent of protection was calculated using the formula (% Protection = (C-T/C) X 100). Where(C: the mean worm burden of the control animals, T: the mean worm burden of the tested animals) and dead animals were excluded.

2.10. Enzyme-Linked Immunosorbant Assay (ELISA)

The ELISA was done as described by **Hillyer *et al* (1979).** Briefly, microtitre plates were coated with 3-5µg/ml of recombinant SMS01 protein in 0.05M carbonate buffer pH9.6(l00 µl /well), and incubated at room temperature overnight. Plates were washed twice with PBS/Tween (2-3 minutes each)then dried on tissue paper. The plates were blocked for non specific binding with PBS containing 1% BSA (100 µl/well). Then plates were incubited at 37°C for one hour followed by 3 washes with PBS/Tween. Addition of 100µl of diluted serum/well was added followed by incubation for 1.15 hr at 37°C. Washing 3 times with PBS/Tween (l00 µl /well) was repeated then 100µl/well of diluted secondary antibody (1µl/ml in PBS-Tween) was added and plates were incubated at 37°C for 45 minutes. Subsequently, the plates were washed with PBS/Tween for 3 times with shaking. Substrate solution (NBT and BCIP) was added and the plates were left in dark for 30 minutes. The reaction is finally stopped using 0.4N NaOH(l00µl/well) and the absorhance was measured at 405 nm using Bio Tek ELISA reader (Roitt *et al*. 1998).

2.11. Oogram pattern and tissue egg load

Three fragments of the small intestine (from the middle part of the small intestine) were cut longitudinally, washed with saline, compressed between two microscope slides, and examined under a low-power microscope. A total of 100 eggs per animal were observed, and the stage of each egg and the mean number of the different stages were recorded. The number of eggs/gm liver or intestine was calculated according to (Pellegrino et al.,1962). Viable eggs were counted and classified according to their degree of development into the following stages: Stage I: Embryo, one third the diameter of the egg shell. Stage II: Embryo, one half the diameter of the egg shell. Stage III: Embryo, two thirds the length of the egg shell. Stage IV: Embryo, occupying the entire egg shell.

The mature egg contains a fully, developed miracidium. Dead egg, appearing as semi transparent, granular or black eggs, were also counted.

The tissue egg load was determined as described by (Kloetzel et al.,1967). Where, 0.3 g of liver and small intestine was taken from each mouse and digested overnight in 5 mL KOH (5%). Then, after complete digestion, the samples were vortexed, and three aliquots of 100 µL each were examined microscopically. Subsequently, all *S. mansoni* eggs were counted. The hepatic and intestinal tissue egg loads were determined by multiplying the number of eggs in each 100µL sample by the total volume of KOH and dividing this value by the weight of the sample in gram.

2.9.3 Statistical analysis

Statistical significance was determined by student's t-test and significance was determined using a P value<0.05 as being significant.

3.Results

3.1 Identification of SMS01-pcDNA1

The SMS01 gene was amplified by PCR as (approximately 500 bP)which is the right size of the gene and was confirmed by sequence and restriction enzymes digestion as shown in Figure(1).

3.2 Expression and purification of protein

The SMS01 expressed protein by pET-3a system was purified on a cation exchange column using pH gradient. SMS01 protein was eluted at pH9.4 is the PI of the protein. The eluted fractions were analyzed by SDS-PAGE and confirmed by western blot analysis as a single band using sera from rabbit immunized with SMS01 recombinant protein (Figure 2).

3.3 Worm burden and the capacity of protection

The number of worms burden in sacrified animals in different groups was shown in Fig (3). Results showed that the vaccinated mice with SMS01 fusion protein have produced the best level of protection (63%) against infection with *S. mansoni* and vaccinated mice with SMS01 DNA have brought such a considerable protection level (about 55.36%). On the other hand, a protection of about 46.81% can be deduced in mice vaccinated with both of SMS01-pcDNA and the SMS01 fusion protein. The statistical analysis of the data obtained from the worm counts in all animals in the study is shown in Table (1). There were significant differences in worm burden between all vaccinated groups and the control($P < 0.001$). In addition a significant difference was also found between the group of vaccinted with fusion and the mixed group ($P < 0.01$).

3.4 Ova count and antifecundity effects

Ova count was determined as it is an indication for the antifecundity effects. Ova count in different groups was briefly illustrated in Figure **(4).** The immunized mice using SMS01 fusion protein as a vaccine against *S. mansoni* infection, has proved to be such a successful antifecundity vaccine as it has produced a remarkable reduction of ova count in liver (mean = 2630 ± 1135.59) and in intestine (6000 ± 1536.91). The reduction of ova count in immunized mice with DNA was (mean = 3684.21 ± 712.79) in liver and (mean=7684.21 ± 1500.09) in intestine respectively. The mixed group was produced the lower reduction of ova count (mean = 5094.73 ± 2084.19 in case of liver and mean=9368.42 ± 2962.92 in intestine) when compared to control group.

Statistical analysis of the data obtained from the ova counts in case of liver and intestine from all animals in different groups is summarized in Tables (2) and Table(3). Ova count in both of liver and intestine was proved to be highly significant with respect to the control in all vaccinated groups ($P < 0.001$). Concerning ova count in liver, noticeable difference could be seen between the DNA and protein groups ($P < 0.05$), as well as between the mixed group and each of the later ones ($P < 0.01$, $P < 0.001$ respectively). While in case of

the intestine, there was a considerable difference between the DNA and protein groups ($P < 0.01$). A significant difference could also be deduced between the mixed group and each of the DNA and the protein groups. As in case of the liver, same difference could be deduced between the mixed group and each of the other two groups.

3.5 Oogram pattern

The oogram pattern was considered to be such a very useful test, as to make such an accurate differentiation between groups concerning different stages of ova found after vaccination . In this work , it was found that the total number of dead ova could assign at which level the vaccine used was successful (Figure %). Results showed that all vaccinated groups have produced a highly significant increase in the number of dead ova with respect to the control ($P < 0.001$). Where the number of dead ova (Figure 5) was remarkably high (mean = 69.9 ± 5.40) in mice vaccinated with SMS01 fusion protein when compared with both DNA group (mean = 55.76 ± 4.24) and the mixed group (mean = 41.2 ± 4.25). The protein group has also produced such a considerable reduction in the immature stage (mean = 21.03 ± 6.76). In addition a noticeable significant difference could be found between the mixed group and each of the other two groups in case of dead ova ($P < 0.001$)(Tables 3 and 4).

Results showed that all the vaccinated groups have produced a significant reduction in the mature stage with respect to the control group. The mean number of the mature stage was, (9.39 ± 3.64), (10298 ± 5.05) and (13.03 ± 5.64) in mice vaccinated with protein, DNA and mixed group respectively when compared to the control group (Fig 5). The mature stage was highly reduced in case of all groups ($P < 0.001$) with respect to the control).

The protein group has produced the best significant difference in case of the immature stage ($P < 0.01$). Also, a slightly significant difference could be denoted concerning the mixed group ($P < 0.05$). Upon comparing between groups: A very high significant difference could be seen between the DNA group and the protein one ($P < 0.002$ in case of immature stage, $P < 0.001$ in case of dead ova). In addition, results showed no significant difference between the mixed group and the protein one ($P < 0.1$) concerning the mature stage. There is a significant difference between the mix group and the DNA group ($P < 0.01$) as well as the protein one ($P < 0.001$) was observed in immature stage (Tables 3 and 4).

3.6 Detection of Anti SMS01 IgG in immunized mice

ELISA was considered to be such a successful test for the detection of antibody titers in vaccinated group of animals (Table 5). Results showed that the highest IgG titre (mean = 1.77 ± 0.144) was obtained in vaccinated animals with fusion protein. While the DNA group has produced a considerable antibody titer (mean = 1.57 ± 0.28), the mixed group, has produced the lower IgG titre (mean = 1.38 ± 0.39) as showed in Figure (5). Statistical data as showed in Table (5) revealed that, all groups have produced a significant difference with respect to the control ($P < 0.001$). The IgG antibody titre in the protein group exceeds both that found in both of the DNA group and the mixed group by such a remarkable significant difference ($P < 0.01$, $P < 0.001$ respectively). A slightly low significant difference could be observed between the DNA group and the mixed group one ($P < 0.1$)

4. Discusion

Human schistosomiasis, a chronic and debilitating parasitic disease of the tropics, is ranked second after Malaria in terms of public health importance. At present, there is no vaccine available and chemotherapy is the cornerstone of schistosomiasis control. Praziquantel is the drug of choice (Utzinger *et al.* 2001). Inspite of safe and efficacious drugs, schistosomiasis still ranks high on the list of endemic diseases of public health importance in the world, in part due to rapid reinfection rates which demand frequent

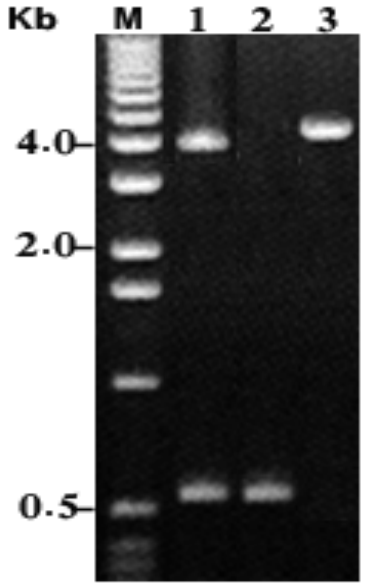

Fig (1): Agarose gel electrophoresis for the digestion product of recombinant pcDNA1-SMS01 clones DNA to determine the insert presence. M: 1Kb DNA marker. Lane (1) represents DNA of recombinant pcDNA1 clones digested with *BamHI*. Lane (3) PCR products of SMS01. Lane (3): SMS01 digested with *EcoRI*.

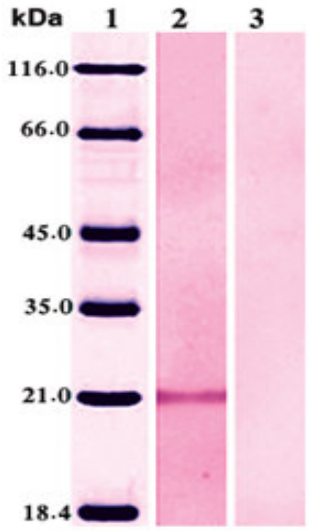

Fig (2): Western blot analysis showing the reactivity of vaccinated rabbit serum with SMS01 purified protein compared to the non recombinant pET-3a vector. Lane 1: High molecular weight protein, Lane (2): Specific band at molecular size of 21.7 kDa which is the molecular weight of the fused protein. Lane (3) Non recombinant bacterial lysate with no response.

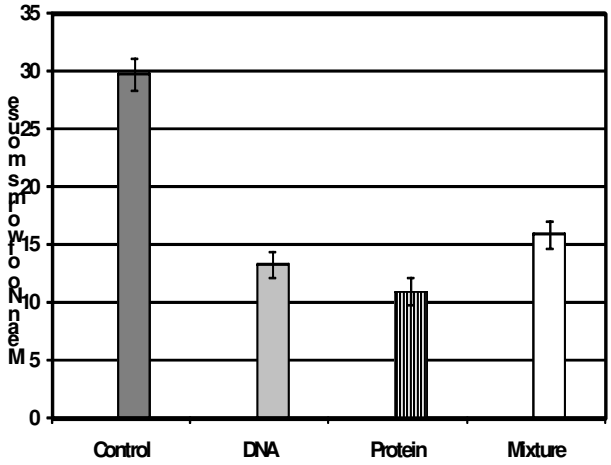

Figure (3): Changes of worm burden in immunized and control mice sacrificed six weeks after challenge infection. The percentage of protection was calculated by perfusions of adult worms at six week post-challenge infection. The vaccinated mice with SMS01 protein have reached the maximum level of protection (63%). While the DNA groups exhibit a considerable level of protection (55.36%), the lowest level (46.81%) was obtained by the mixed group compared to the control group.

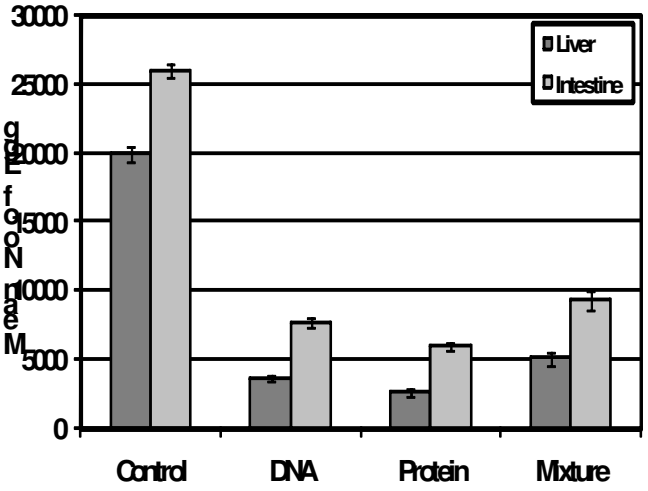

Figure (4): Changes in ova count in immunized and control mice (livers and intestines) sacrificed six weeks after challenge infection. Vaccinated mice with SMS01 has proved to be a successful antifecundity and produced a remarkable reduction in ova count in livers (mean=2630±113.90) and intestines (mean=600±1536.91). SMS01 DNA revealed a level of reduction about (mean =3684.21±721.79) in livers and (mean =7684.21±1500.09) in intestines, and finally the mixed group denoted a level of reduction in livers (5094.73±2084.19) and (9368.42±2962.92) in intestines when compared to the control group.

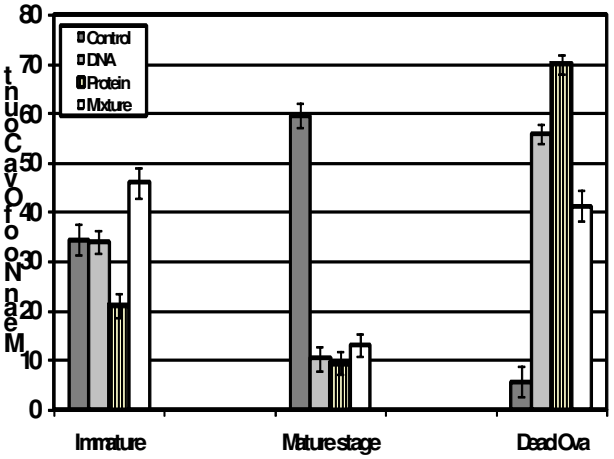

Figure (5): The oogram pattern in immunized and control of mice vaccinated with either the DNA or the protein or both and sacrificed six weeks after challenge infection. The number of dead ova was remarkably high in the protein group (mean=69.9± 5.40) when compared with DNA group (mean =55.67 ±4.24) or Mixed group (mean= 41.2±4.25). The protein group has also produced such a considerable reduction in the immature stage (mean = 21.03 ± 6.76). The mean number of the mature stage was, (mean=9.39 ± 3.64), (mean=10298 ± 5.05) and (mean=13.03 ± 5.64) in mice vaccinated with protein, DNA and mixed group respectively when compared to the control group.

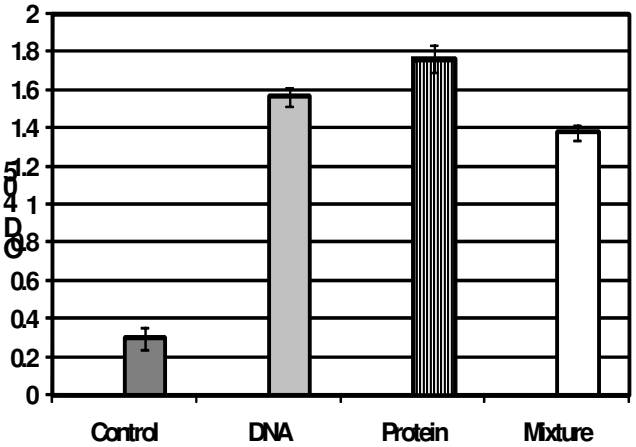

Figure (6): IgG antibody responses against crude adult worm antigens (SWAP) in mice immunized animals compared to the control groups at six weeks post-challenge and control. The protein group have produced the highest IgG titre (mean=1.77 ±0.14), considerable titre was produced by DNA group (mean=1.57 ±0.28). While mixed group was reached a lower tire (mean =1.38 ±0.39).

Table (1): Statistical analysis of Changes of worm burden in immunized and control mice sacrificed six weeks after challenge infection.

Group A	Group B	Mean difference	Standard error (±)	Significance
Control	DNA	16.45	2.23	< 0.001
	Protein	18.80	2.23	< 0.001
	Mix	13.90	2.23	< 0.001
DNA	Control	-16.45	2.23	< 0.001
	Protein	2.35	1.82	< 0.1
	Mix	-2.55	1.82	< 0.1
Protein	Control	-18.80	2.23	< 0.001
	DNA	-2.35	1.82	< 0.1
	Mix	-4.90	1.82	< 0.01
Mixed	Control	-13.90	2.23	< 0.001
	DNA	2.55	1.82	< 0.1
	Protein	4.90	1.82	< 0.01

Table (2): Statistical analysis in ova count in immunized and control livers of mice sacrificed six weeks after challenge infection

Group (A)	**Group (B)**	**Mean difference**	**Standard error (±)**	**Significance**
Control	**DNA**	**16315.78**	**580.4**	**< 0.001**
	Protein	**17370.00**	**575.4**	**< 0.001**
	Mix	**14905.26**	**580.4**	**< 0.001**
DNA	**Control**	**-16315.78**	**580.4**	**< 0.001**
	Protein	**1054.21**	**475.97**	**< 0.05**
	Mix	**-1410.52**	**482.04**	**< 0.01**
Protein	**Control**	**-17370.00**	**575.42**	**< 0.001**
	DNA	**-1054.21**	**475.97**	**< 0.05**
	Mix	**-2464.73**	**475.97**	**< 0.001**
Mixed	**Control**	**-14905.26**	**580.45**	**< 0.001**
	DNA	**1410.52**	**482.04**	**< 0.01**
	Protein	**2464.73**	**475.977**	**< 0.001**

retreatment. In addition the potential development of drug resistance emphasizes the need for a long-term approach such as the development of a protective vaccine (Da'dara *et al.* 2001).

One of the main strategies for vaccine development is based upon the identification of larval stage antigens that elicit highly protective immune response in vaccinated hosts (Hota-Mitchell *et al*. 1999). One of these important genes,

Table (3): Statistical analysis of Changes in ova count in immunized and control intestines of mice sacrificed six weeks after challenge infection

Group (A)	Group (B)	Mean difference	Standard error (±)	Significance
Control	DNA	18315.78	792.58	< 0.001
	Protein	2000.00	785.72	< 0.001
	Mix	16631.57	792.58	< 0.001
DNA	Control	-18315.78	792.58	< 0.001
	Protein	1684.21	649.92	< 0.01
	Mix	-1684.21	658.20	< 0.01
Protein	Control	-2000.00	785.72	< 0.001
	DNA	-1684.21	649.92	< 0.01
	Mix	-3368.42	649.92	< 0.001
Mixed	Control	-16631.57	792.58	< 0.001
	DNA	1684.21	658.20	< 0.01
	Protein	3368.42	649.92	< 0.001

Table (5): Statistical data of the ELISA readings in immunized and control mice sacrificed six weeks after challenge infection.

Group (A)	Group (B)	(t) test	Significance
Control	DNA	-19.94	< 0.001
	Protein	-40.14	< 0.001
	Mix	-11.62	< 0.001
DNA	Control	19.94	< 0.001
	Protein	-2.75	< 0.01
	Mix	1.70	< 0.1
Protein	Control	40.14	< 0.001
	DNA	2.756	< 0.01
	Mix	3.88	< 0.001
Mixed	Control	0.36	< 0.001
	DNA	-1.70	< 0.1
	Protein	-3.88	< 0.001

SMS01 (Sm2 1 .7) was identified as located in the tegumental region and dispersed among the parenchyma tissue of liver worms schistosome parasite (Ahmed *et al.* 2001). Since the tegument is the outer covering of the parasite and serves as an interface between the host immune system and the parasite the antigen associated with the tegument would be the major focus for deve1opnent of vaccine and/or immunodiagnostic reagents for schistosomiasis (Bergquist 1992). Therefore localization of the 21.7 kDa protein in tegument and subtegumental layers would likely confirm the importance of this protein as a target of all host's protective response to S. *mansoni* infection (Ahmed *et al. 2001)*

In this study to evaluate the efficacy of combined SMS01 DNA and protein as a cocktail vaccine against *Schistosoma mansoni* challenge infection, the vaccination models were applied using DNA vaccination and fusion protein or both. Thus SMS01 gene was cloned into the eukaryotic expression vector pcDNA1/Amp as to estimate its protective capacity. This vector, with its CMV promoter, has high transcription and expression levels in different mammalian cells (Wang *et al.* 1998). The expression of SMS01 recombinant protein was carried out in the pET-3a vector, and recombinant SMS01 protein was purified according to Ahmed et al. (1997).

In this work DNA vaccine was injected intramuscularly as it appeared to generate the best immune response (Fynan *et al.* 1993) while protein was injected intraperitoneally. All groups in the immunization experiments were challenged with 100 cercariae 4 weeks after the last boost and six weeks later worm burdens were analyzed, to detect their ability for protection against *S. mansoni* infection. Results showed that in all vaccinated groups induced statistically significant levels of protection to challenge infection. The groups of mice vaccinated with SMS01 DNA have significantly reduced the worm burden by (55.36%, $P < 0.001$) and by (46.81%, $P < 0.001$) in mixed group (Figure 3). The highest significant reduction levels were obtained by mice vaccinated with recombinant protein (63.08%, $P < 0.001$). No significant difference between the vaccinated groups could be detected, except in case of the recombinant protein and the mixed group ($P < 0.01$). A remarkable production of specific anti-SMS01 antibodies in female Swiss albino mice was found in all vaccinated groups, which was confirmed by ELISA ($P < 0.001$) in all groups, with respect to the control group (Table 1).

A significant difference in IgG titre could be detected between groups of mice vaccinated with DNA and recombinant protein ($P < 0.01$). Such a

noticeable difference could be detected between the mixed vaccinated groups with both of the DNA and the recombinant protein vaccinated group ($P < 0.1$ and $P < 0.001$ respectively). These data are in agreement with Anderson's hypothesis, which supported the idea of antibody involvement in the augmented protection of multiply vaccinated C57Bl/6 mice. Multiple vaccinations with irradiated cercariae led to an increase in the level of protection from 59 to 82%. Since antibody titre was elevated, it was concluded that the additional protection was the result of antibody mediated mechanisms (Anderson *et al.* 1999).

Our results showed that all vaccination groups of mice have induced an antifecundity effect. The total ova count in livers and intestines

Table (4): Statistical data of oogram pattern in immunized and control in mice sacrificed six weeks after challenge infection

Group (A)	Group (B)	Stage	Calculation of (t) test	Significance
Control	DNA Protein Mix	Immature Stage	0.10 2.91 -2.45	< 0.1 < 0.01 < 0.05
Control	DNA Protein Mix	Mature Stage	11.94 12.63 11.07	< 0.001 < 0.001 < 0.001
Control	DNA Protein Mixed	Dead Ova	-32.59 -34.41 -23.06	< 0.001 < 0.001 < 0.001
DNA	Control Protein Mixed	Immature Stage	-0.10 3.82 -3.46	< 0.1 < 0.002 < 0.01
DNA	Control Protein Mixed	Mature Stage	-11.94 0.45 -1.13	< 0.001 < 0.1 < 0.1
DNA	Control Protein Mixed	Dead Ova	32.59 -6.53 7.64	< 0.001 < 0.001 < 0.001
Protein	Control DNA Mixed	Immature Stage	-2.91 -3.82 -7.97	< 0.01 < 0.002 < 0.001
Protein	Control DNA Mixed	Mature Stage	-12.63 -0.45 -1.70	< 0.001 < 0.1 < 0. 1
Protein	Control DNA Mixed	Dead Ova	34.42 6.53 13.20	< 0.001 < 0.001 < 0.001
Mixed	Control DNA Protein	Immature Stage	2.45 3.46 7.97	< 0.05 < 0.01 < 0.001
Mixed	Control DNA Protein	Mature Stage	-11.07 1.13 1.70	< 0.001 < 0.1 < 0.1
Mixed	Control DNA Protein	Dead Ova	23.06 -14.53 -13.20	< 0.001 < 0.001 < 0.001

were significantly reduced in all groups compared to the control ($P < 0.001$). The mice groups vaccinated with recombinant protein showed the best antifecundity effect when compared to either the DNA or the mixed vaccinated group in liver. A noticeable difference in case of liver could be seen between the DNA and protein group ($p<0.05$), as well as between the mixed group, DNA and protein groups ($p<0.01$ and $p<0.001$) respectively. There was a considerable difference in case of intestine could be seen between the DNA vaccinated groups and protein group ($p<0.01$). Also, a significance difference could be deduced between the mixed group and each of DNA group and protein group. As in case of liver, same difference could be deduced between the mixed group and DNA or protein group. Furthermore concerning the viability of ova and oogram, it was concluded that the recombinant protein vaccinated groups showed the best protection model with respect to the control; it was the only group that showed a significant decrease in the immature stage ($P < 0.01$).

Our results showed that a successful decrease in the mature stage as well as a significant increase in the number of dead ova could be observed in all groups ($P < 0.001$) compared to the control. The mature stage was reduced in all groups ($p<0.001$) compared to the control group. The protein group has produced the best significant difference in the immature stage ($p<0.01$). Also, a slightly significance difference could be denoted concerning the mixed group ($p<0.05$). A very high significant difference could be seen between the DNA group and protein group ($p<0.001$ in case of dead ova. Noticeable significant difference could be found between the mixed group and the DNA group ($p<0.01$) as well as the protein group ($p<0.001$). There is no high difference could be seen between the mixed group and the protein group ($p<0.1$).

In genetic vaccination, the DNA vector carrying the genetic code for a pathogenic antigen is taken up into cells and transcribed in the nucleus. Messenger RNA is translated into protein in the cytoplasm. The gene product (protein antigen) is ultimately degraded by proteosomes into intracellular peptides. Being produced in the host cell, the antigen is processed through the MHC class I system and thus stimulates a cell mediated/cytotoxic T-cell response when presented on the cell surface (Gilsdorf 1994 ; McDonnell *et al.* 1996). In spite of the fact that this work has proved that vaccination with recombinant SMS01 created the best protection (vaccination) model against *S.mansoni* we can't deny the advantages of DNA vaccination criteria vaccination with DNA is probably more simple and cost-effective than with conventional protein preparations, thus raising hopes for use against infectious diseases in developing countries, where DNA vaccination may therefore, become the poor man's gene therapy(Chlichlia *el al.*2002).

5. Conclusion

The cocktail vaccination model was considered very successful against *Leishmania major* either by using plasmid DNA encoding TSA/LmSTI1 leishmanial fusion proteins (Ameen 2007) or by using DNA encoding cysteine proteinases (Rafati *et al.* 2001). In this work, the cocktail vaccination model has produced a successful protection results with respect to the control. On the other hand, it could be denoted that each of the DNA or the protein vaccination models was still more protective against *S. mansoni* than the cocktail one. Still, there is a long way from an ideal vaccine as we not deny that experimental animals vaccination models can not adequately represent the human situation.

Actually studies on experimental models have been highly productive and are still much needed but may not adequately represent the human situation. SMS01 may be such a crucial antigen, and several strategies remained to be tried such as; changing vaccination protocols (amount of vectors. route of infection, number of boosters and intervals), vectors (choosing stronger promoter) and adjuvant molecules (JL-12 for example), check protection after chemotherapy. We may still have a long way from an ideal vaccine that gives complete protection against schistosome infection but hopefully progressing in the right direction.

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