Kinetic of dissolution of hydroxyapatite crystals in the presence of extracts of some medical plants

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Abstract: The mechanism of dissolution of hydroxyapatite (HAP) crystals was studied using constant composition techniques. HAP seeds were prepared and analyzed using XRD, FTIR, SEM, SSA and chemical composition which confirmed the HAP structure and ca/p =1.66. The dissolution of HAP crystals seeds was studied at σ =0.6, I=0.15 mol dm^{-3} (using NaCl electrolyte), pH=7.4, t=37^0C and 51mg of crystal seeds. It was found that the dissolution process of HAP crystals was followed surface-controlled mechanism ($n \simeq 2$). The low value of Ea=2.5kJ/mole and independence of dissolution rates on the fluid dynamics supported the suggestion of surface- mechanism. The effect of aqueous extracts of Hibiscus (Hb), Barely (Br), *Ammi visnaga* (kh) and *Niggilla sativa* (N.S) on the dissolution process of HAP crystals at the experimental conditions was studied. It was found that concentrations as low as 10^{-7} mol dm^{-3} decreased the dissolution rates of HAP crystals. The order of inhibition was Hb>Br>Kh>N.S. The additive adsorbed on the active sites on HAP crystal surface blocking them, reducing their numbers and decreased the dissolution rates of HAP crystal seeds. The validity of application of Langmuir-isotherm supported the surface mechanism and formation of mono molecular layer on the HAP crystal surface. The values of Kl in case of the presence of Hb, Br, Kh, and N.S were: 3.27, 3.10, 2.50 and 2.35×10^7 in case of the presence of Hb, Br, Kh and N.S respectively. The effect of change of pH, I, σ and t on dissolution rates of HAP crystals in the presence of 10^{-7} mol dm^{-3} of N.S was studied. Increasing the values of pH or the values of ionic strength of the medium reduced the inhibitory effect of additive. The order in the presence of N.S was $n \simeq 2$, Ea=3.06KJ/mol and Concentration of 7×10^{-7} mol dm^{-3} of it, complete adsorption of N.S ions on the HAP crystals surface was found, which supported the surface- mechanism.

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1. Introduction.

Calcium phosphates are mineral component of bone and teeth. As much there is great interest in understanding the physical mechanisms that under- lie their growth, dissolution and phase stability.

Apatite is dominant phosphate phase found in mineralized tissue. Most of injectable calcium phosphate cements used evolves to an apatitic calcium phosphate during the setting reaction [(1).]

Oxalate, phosphate, uric acid, and urate are found in urinary calcui as either apatite ($Ca_5(PO_4)_3OH$) or brushite ($CaHPO_4.2H_2O$). calcium hydroxyl apatite, $Ca_{10}(PO_4)_6.(OH)_2$. HAP, plays important role in soil science and is the main constituent of inorganic part of bone and teeth material[(2)]

HAP, is the most stable calcium phosphate salt under physiological conditions. This model compound has been used to study hard tissue calcification such as bone and teeth and in many undesirable cases of pathological mineralization of particular cartilage, cardiac values and kidney stones[(3)].

One of the most relevant properties of HAP is its high resistance to dissolve in water, which along with cellular structure of natural bone produces an excellent biomaterial, as well as appropriate components for design and the synthesis of alternative materials such as composites prepared by the growth of minerals on different substrates like silica[(4)].

Recently, the bad effects of medicine still have strong discussion and studying between medical and medicine scientists in all over the world. This makes some scientists to call for returning to natural and medical plants for treatment the diseases.

Medicinal plants, which are old famous popular medicine and approximately using in wide range by Egyptians on their diet.

The natural products from medicinal plant or an animal origin in general have high surface activity. This investigation was aimed to study the effect of aqueous extracts of Hibiscus, Barely, *Ammi visnaga* and *Niggella sativa* on the rates of dissolution of HAP crystals.

2- Experimental procedure

2-1 Analytical grade chemicals

Grade A glassware and doubly distilled deionized water were used. Solutions of carbonate free

sodium hydroxide (J.T. Baker chemical company) and hydrochloric acid (El-Naser pharmaceutical chemical company) were prepared. Solutions of calcium chloride. (Fisher Scientic Company) and dipotassium mono hydrogen phosphate (Baker chemical company) were prepared by weighing suitable amounts of the salts and dissolving in a definite volumes of deionized distilled water. These solutions were then filtered through Millipore filter pads (0.22m, Millipore filters), quantitatively transferred to grade A volumetric flasks and diluted to the required concentrations using deionized distilled water concentration of $CaCl_2$ was determined using atomic absorption and using cation-exchange resin (Dowix-50). K_2HPO_4 concentration was determined using flame photometry.

The extracts of plants were made by using soxtic system HT, 1043. Extraction unit made in Sweden.

The fresh plant was dried in shad, and then crushed the dried plant, and the samples were inserted into extraction unit. The extract was evaporated at 60°C, dried and stored. A suitable volume of saturated solution was taken to prepare solution of different concentrations by dilution.

2-2 preparation of HAP crystals

The seed of HAP crystals were prepared as described previously[3].

The seed crystals subjected to XRD, SEM, FTIR and chemical analysis.

2-3 Dissolution experiments

Dissolution experiments of HAP crystals were carried out in water thermostated double- walled Pyrex glass vessel using potentiostatic measurements using Metrohm combititrator (model 718 stat titrino. Emf measurements were made by calcium ion –selective electrode (CH-9101 Herisau) in conjugation with a calomel electrode (model 90.02 Orion). The electrodes were checked before and after each dissolution experiments.

The cells were maintained at required temperature by circulating thermostated water through outer jackets. and nitrogen gas were bubbled through the cell to exclude CO_2.

In dissolution experiments, a measured volume of NaCl was transferred to the cell followed by definite volume $CaCl_2$ solution, then slow addition of known volume of K_2HPO_4 solution and the total volume of the cell completed using the suitable volume of deionized water.

The dissolution experiment starts by adding a suitable weight of prepared seed crystals. Samples were periodically withdrawn and filtered using 0.22m filter for chemical analysis.

3- Results and Discussion

Hydroxyapatite and calcium phosphate coatings have been used primarily to alter implant substances, with the assumption that improved osteointegration of the implants can be achieved. In particular, the crystallinity of hydroxyapatite and other calcium phosphate ceramics are of great interest because of their dissolution properties. It is has been reported that amorphous or smaller imperfect crystals are subject to a higher dissolution rate than crystalline compounds. The dissolution characteristics of calcium phosphate materials used for providing bioactive coatings on medical and dental implants are being activity addressed[5]. Calcium phosphate biomaterials differ in their solubility or extent of dissolution. ACP>α-TCP>β-TCP>CdA>HAP.

Dissolution of HAP has already been performed to characterized the dissolution behavior of it. HAP is the most stable phase of calcium phosphate having definite composition and definite crystallographic structure.

Since the transformations of calcium phosphate phases are dissolution-precipitation, so understanding of the mechanism of dissolution of them are therefore of considerable importance. Dissolution reactions are very important in technical and physiological systems. For many sparingly soluble salts (MaAb), the rate of dissolution normalized for seed surface area, can be expressed by the equation:

$$R = d[MaAb]/dt = kS\sigma^n \quad (1)$$

Where:

k: is the dissolution rate constant.

S: is proportional to the number of dissolution sites available on the seed crystals.

n: is the effective order of reaction.

σ: Is the relative degree of undersaturation,

The relative degree of under saturation, σ may be expressed by the equation:

$$\sigma = 1-(IP/Ksp)^{1/9} \quad (2)$$

Where:

IP is the HAP ionic product which equal to

$(a_{Ca^{2+}})^5(a_{PO_4^{3-}})^3\, a_{OH^-}$, where (a)denotes to activity.

Ksp: is the solubility product of the HAP $=2.35\times10^{59-}$

The ionic strength, I, is defined by means of the following equation:

$$I = 1/2 \sum Z_i^2 C_i \quad (3)$$

Where:

Z_i: is the ionic charge and

C_i: is the molar concentration of each one of the ions in solutions.

It should be observed that the ion activity coefficient by Debye-Huckel model is determined by the charge and total ionic force in solution and not in principle by its own solution concentration.

If we fix the ionic force experimentally for a solution to a constant value, we can obtain the activity coefficients of the species using equation (3) and

extened Debye-Huckel equation, as proposed by Davies:

$$-\log Y_Z = AZ^2[\ (I^{1/2}/\ 1+I^{1/2})-0.3I] \quad (4)$$

Where:

Yz: is activity coefficient of ion (Z),

A: is Dybe-Huckel constant that includes factors such as solvent dielectric constant, T(absolute temperature), the Boltzman constant, the ionic radius and the factor of conversion of the common natural logarismthes, for water. At $t=25^0C$, A is similar to 0.5. So, obtain the solubility product.[4]

In equation (3) when z = 0, so uncharged ion don't contribute in increasing ionic strength and $y_z=1$.

In the present study, dissolution of HAP crystals was investigated at σ =0.6, $t=37^o$ C, I=0.15 mol dm^{3-}, using NaCl as a background electrolyte, and at pH=7.4 using constant- composition method, in which the temperature, the ionic strength, the degree of undersaturation and solution speciation remain constant during the entire experiment, so the rate of dissolution of HAP crystals can be attributed to the change in HAP undersaturation during the reaction. The prepared HAP seeds were analyzed using XRD, FTIR, SEM, TGA and chemical analysis from which confirm the HAP structure and also the molar ratio ca/p was 1.69.

The rates of dissolution of HAP crystals are determined from the slops of lines from the plots of volume of titrant added to the time (table 1). Plotting log R against log σ for dissolution process (σ= 0.316-0.891), (fig(1) the order of reaction was $n\tilde{-}2$ which suggested surface-controlled mechanism. The rates of dissolution of HAP crystals were proportional to the mass of seed HAP crystals used to intiate the reaction (table(1). The in sensitivity of dissolution rates of HAP crystals to the changes in fluid dynamics pointed to the surface-controlled mechanism. Changing the stirring rates between 100 and 500 pm has no effect on dissolution rates suggesting that the rate-deterring step of the process is not bulk diffusion from bulk solution to the crystal surface[6]. The stirring rate may have little influence on the fluids shear forces at crystal surfaces. The particles will tend to move with fluid flow. The rates of dissolution of HAP crystals were also proportional to the weight of inoculating seed used to initiate the dissolution process which confirm the surface mechanism. The low value of activation energy, Ea, of dissolution of HAP crystals at experimental conditions, Ea=2.5 k cal/mol also supported the surface mechanism. Dissolution process, such as found in healthy bone remodeling, or as found in diseases such as caries and esteoporsis, reflect and underlying chemistry that has been biologically manipulated to favor resorption. In heterogeneous reactions of solid-liquid systems, the soluble reactants diffuse across the interface and/or through the porous solid layer. Subsequently, chemical reactions occur, followed by desorption of soluble products, which diffuse away from the reaction surface. These steps take place consecutively, and one or more of them may be rate-determining. Adsorption from solutions containing several ions is a complicated process associated with the competition of at least two components. The solvent and the solute species, the surface of the mineral attracts the counter ions in the solution, and a layer is formed, which is firmly attached around the particle, called the sterm layer. Additional counter ions are also attracted by the surface, but they are repelled immediately by the sterm layer as well as by other counter ions trying to approach the particle. This dynamic equilibrium induces the formation of a diffuse layer of counter ions whose concentration is high near to the surface; however it is gradually decreased with the distance and reaches equilibrium with the counter ions in the solution. In addition, there is a lack of co-ions in the neighborhood of the particle, since these ions are repelled by the surface.[8] Crystallization and dissolution processes of calcium phosphate were affected both the solution speciation as well as the surface of the mineral. The parameter as supersaturation, pH, ionic strength and the ratio of cations to anions. supersaturation are the most crystallization parameters, the supersaturation ratio, S, is given by:

$$S = \frac{IP}{k_{sp}}\ ,\ S_{HAP} = \frac{[Ca^{2+}]^5[PO_4^{3-}]^3[OH^-]}{k_{sp}} \quad (5)$$

For S=1, the mineral and solution are in equilibrium, for S<1, the solution is under saturation and the mineral will dissolve, and for S > 1, the solution is supersaturation and the mineral will grow. The second parameter, pH affects both the solution as well as the mineral surface. In the solution, a shift to lower PH will lower the saturation state by shifting the balance of phosphate species from PO_4^{3-} to HPO_4^{2-} to $H_2PO_4^-$. At the mineral surface, the pH can shift the surface charge by changing the distribution of proton and hydroxyl groups hydrating the interface. For hydroxyapatite the point of zero charge (in solutions without calcium) occurs at pH=7.3, For more alkaline solutions the surface is negatively charged where as for more acidic, it is positively charged. However, ions other than H^+ and OH^- can adjust surface charge and in calcium containing solutions, the Ca^{2+} ions bind to the surface for pH > 7, leaving the surface neutral rather than negative (provided calcium remains in solution).

The third parameter is the ionic strength which plays a role in screening both ion -ion electrostatic interactions in solution (which is accounted for by the

activity coefficient) and electrostatic interactions between ions in solution and the surface. In most biological systems, the ionic strength is near 0.15M corresponding to Debye length of approximately 1 nm.

Temperature is generally an important crystal growth parameter, many solubility products and association constants are temperature dependent thus so is the solution speciation. Temperature also affects the kinetics of adsorption, desorption and diffusion. However, in biological systems, temperature is regulated and remains nearly constant (37 C^o for humans) thus while temperature can be used as a tool *in vitro* to measure the activation barriers (Ea), it is not a control used in vivo. Parameters such as ratio of Ca/p acknowledges that growth rates may not be dictated simply by the supersaturation and the surface energies but rather that kinetics may play a role. In principle ion ratios can affect growth rates, growth shape and transformation of meta-stable phases. The growth of multi-species crystal relies on the relative rates of adsorption and desorption of the various ions or growth units that make up the unit cell. Most body fluids are richer in phosphate than calcium with serum being the exception. In serum Ca/P ratio is near that found in bone (1:66)` and likely plays a role in creating crystals with this stiometry during the body cycle of bone reformation. HAP is supersaturated in all fluids over almost their entire range with the result that under most conditions our bones and teeth are stable[8]. The charge of apatite surfaces will arise as a result of various dissolution and hydrolytic reactions which occur when the solid is suspended in aqueous solution. For hydroxyapatite these reactions can be represented by:

$Ca_{10}(PO_4)_6(OH)_2$	$\rightleftharpoons$	$10Ca^{2+}+6PO_4^{3-}+2\ OH^-$	$\rightarrow$	(1)
$Ca^{2+} + OH^-$	$\rightleftharpoons$	$CaOH^+$	$\rightarrow$	(2)
$CaOH^+ + OH^-$	$\rightleftharpoons$	$Ca(OH)_{2aq}$	$\rightarrow$	(3)
$PO_4^{3-} + H^+$	$\rightleftharpoons$	HPO_4^{2-}	$\rightarrow$	(4)
$HPO_4^{2-} + H^+$	$\rightleftharpoons$	$H_2PO_4^-$	$\rightarrow$	(5)
$H_2PO_4^- + H^+$	$\rightleftharpoons$	H_3PO_4	$\rightarrow$	(6)
$Ca^{2+} + HPO_4^{2-}$	$\rightleftharpoons$	$CaHPO_4^0$	$\rightarrow$	(7)
$Ca^{2+} + H_2PO_4^-$	$\rightleftharpoons$	$CaH_2PO_4^+$	$\rightarrow$	(8)

At equilibrium

$\mu_i` = \mu_i`` = \mu_i```$ where,

$\mu_i`$ = electropotential of bulk solid,

$\mu_i``$ = for bulk solution.

$\mu_i```$ = for solid surface.

I = subscript refers to any one of ionic species.

Due to slow diffusion of lattice ions in solid at room temperature, it is probable that the thermodynamic equation will only be established between the surface and solution phases. The change in surface potential can be measured by the change in activity of H^+ in the bulk solution. At PZC there is little tendency of H^+ to diffuse into or out of solid on acid side of PZC adsorption of protons by the surface occurs and slow diffusion of H^+ into the solid could result while, on alkaline side of PZC, adsorption of OH^- would result in a slow diffusion of H^+ from inside the solid to the surface. At pH values below PZC, the solid surface will adsorb H^+ ions and the PH of the solution will rise whereas the reverse will occur at pH values above the PZC. The changes in H^+ activity in solution are also influenced to some degree by hydrolytic reactions of dissolved ions in solution. If solid /solution ratio (or more correctly solid surface area/solution ratio) is sufficiently high, however, the solution hydrolytic reactions will be insignificant in relation to surface adsorption reactions and a reliable estimate of the PZC can then be obtained. Calcium and phosphate are also potential determining and the effect of equilibrating hydroxyapatite in the presence of various levels of these ions [9]. Dissolution of HAP crystals illustrated by equation[10]:

$Ca_5(PO_4)_3OH \rightleftharpoons 5Ca^{2+}_{aq} + 3PO_4^{2-}_{aq} + OH^-_{aq}$ (1)

According to Rootare et al [11], the dissolution of HAP crystals are:

$Ca_{10}(PO_4)_6(OH)_2+6H_2O$	$\rightleftharpoons$	$4Ca_2(HPO_4)(OH)+2Ca^{2+}+2HPO_4^-$	$\rightarrow$	(2)
$Ca_2(HPO_4)(OH)_2$	$\rightleftharpoons$	$2Ca^{2+}+HPO_4^{2-}+2OH^-$	$\rightarrow$	(3)

Assuming the solution next to the surface of HAP crystal to be saturated and Ca/P=1.67 and considering the transport of OH^-,$(PO_4)^{3-}$ ions from the surface and trans port of H^+ ions to the surface as a coupled diffusion phenomena, the H^+ ions are consumed in the boundary layer and H_2O, HPO_4^{2-} ions and $H_2PO_4^{2-}$ ions are formed[3].

The rate of dissolution of HAP crystals may be depends on the rate of which H^+ ions can be delivered to the dissolving surface. H^+ ions as shown by Gray[12], be transported from the bulk. solution to the crystal surface, either in the form of free ions or in the form of weak acid which dissociates at the crystal surface. The rate-determining process for a rotating HAP may be:

1- Transported of H^+ ions from bulk to crystal surface,

2- Surface process,

3- Transport of dissolved substance from the volume or a combination of these processes.

For pH in physiological pH controlled by surface mechanism[13]. The rate-controlling, step is the formation of dissolution nuclei, which depends on H^+ ions, this dependence on H^+ ions concentration is explained by H^+ ion catalysis of exchange of phosphate between crystal surface and the solution[14].

In the present study the rates of dissolution of HAP crystals were studied at $\sigma = 0.6$, I=0.15 mol dm^{3-}, t=37 C^o and at different PH-values. From the study it was found that the increase of dissolution rates of HAP crystals with increasing the pH of the medium.

H^+ ions diffuse to the HAP crystal surface and protonated the phosphate sites forming HPO_4^{2-} or HPO_4^{2-} on the crystal surface[8].

When HAP crystals dissolved in water, both Ca^{2+}, PO_4^{3-} and OH^- ions are formed. The Ca^{2+} ion do not exist as such but form an aqua-calcium complex ion, $[Ca(H_2O_6]^{2+}$ in the present of water. The pK value of aqua- calcium ion is 12.6 and it is quite likely that the reaction of this ion in solution is the loss of a proton to give hydroxo-aqua-calcium complex ion, $[Ca(OH)(H_2O)_5]^+$, therefore, it is believed that must have been a significant effect of acidity of the coordinated water in the aqua-calcium complex ion on the phosphate and OH^- ions.

The ($a_{H^+_{surface}}$ / $a_{H^+_{bulk}}$) ratio decreased with increase of pH of the medium which indicate the increasing effect of solute transport on the dissolution rates of HAP crystals.

Ionic strength of the medium is important parameter affecting dissolution and crystallization processes. Neutral electrolytes such as NaCl and KCl have pronounced effect on the growth and dissolution of HAP crystals. Decreasing the ionic strength of medium, increased SSA of HAP crystals. the neutral electrolyte effectively reduced the degree of supersaturation of solution in crystallization of HAP crystals[15].

Ionic strength influences the step speed by changing the activity coefficients of ion in solution. This depends on anions / cations from background electrolytes. These changes were treated using Davis equation (4).

The effect of change of ionic strength values of the medium on the rates of dissolution of HAP was investigated Fig. (2). Dissolution of HAP crystals at $\sigma = 0.6$, pH =7.4, t=37 C^o and at ionic strength range of I = (0.05- 0.5 mol dm^{3-}) was studied.

It was found that, the rates of dissolution of HAP crystals were increased by increasing, the values of ionic strength of the medium. The changing of the ionic strength of the medium indicated that the dissolution process of HAP crystals is electrostatic in its nature.

AFM studies demonstrated that, background electrolytes enhance the calcite dissolution rate and the magnitude of this enhancement is determined by the nature and concentration of the electrolyte through modifying water structure dynamics as well as solute and surface hydration. At relatively high ionic strength, Cl^- ions determine the solvent structure around Ca^{2+} and calcium removal from the surface structure, rate limiting step for dissolution. Moreover, a high concentration of Cl^- ions increases the etch pit density [16].

Additives play an important role in the theory of dissolution and crystallization. Additives or foreign ions may be accelerate the rate of growth owing to the formation of less soluble phases, such as fluoroapatite in the presence of fluoride ion. On the other hand foreign ions may retard the growth rate as a consequence of:

a) Increasing the ionic strength, hence the solubility of the calcium phosphate phases,

b) Complexing with the calcium or phosphate lattice ions, thereby preventing them from participating in the precipitation reactions,

c) Adsorption at growth sites on crystal surface.

In practice, most crystallization and dissolution inhibitors are effective at very low concentrations. Some additives may retard or accelerate the rate of precipitation or dissolution, while others may only change the morphology of growing crystals. The material which when added to the medium in small amounts, reduce or stop the rate of reaction is called inhibitor.

The structures of phosphates compounds allow various substitutions of cationic and anionic groups and thus give rise to new families of phosphatic materials of great importance in chemical and natural science [17]. The molar Ca/P ratios reflect the

substitution of foreign ions in crystal lattice, an event that has been shown to be common for calcium phosphates. The high degree of substitution of apatites may be due to the diffusion of ions in or out of OH^- channels within the structure, facilitating the exchange of OH^- ions for water or for other ions. These channels at the crystal surfaces form parallel grooves containing adsorbed water, phosphate, OH^- and foreign ions. Ion vacancies in Ca^{2+} or OH^- positions compensate for excess or depleted charges due to substitution. In particular, the high stability and flexibility of the apatite structure accounts for the great variety of possible cationic and anionic substitutions.

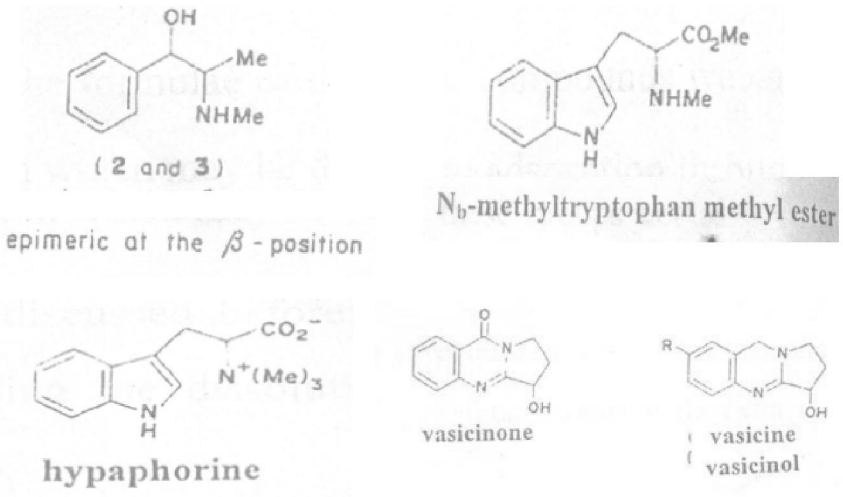
The effect of *Hibiscus* (Hb), *Ammi visnaga* (Kh), *Barely* (Br) and *Nigella sativa* (N.S) on the rates of dissolution of HAP crystals at σ =0.6, t=37C°, I=0.15 mol dm^{3-} and pH=7.4 using 51mg seed crystals was studied (fig (4). From the fig, we can find that. these additives inhibited the dissolution rates and the order of inhibition was: Hb>Br>Kh>N.S. Also, Concentration of additives as low as 10^{-7} mol dm^{-3} decreased the rates of dissolution of HAP crystals. The low values of concentrations of additives which inhibited the rates of dissolution of HAP crystals, supported the surface –controlled mechanism.

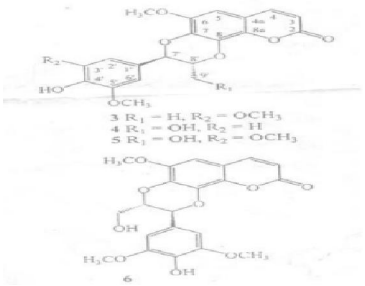
Medicinal plants and its constituents used as therapeutics drugs in health management since ancient time and large population of the world relies on traditional medicine for primary health treatment.

Methanol extract of *Hibiscus* contains some compounds:

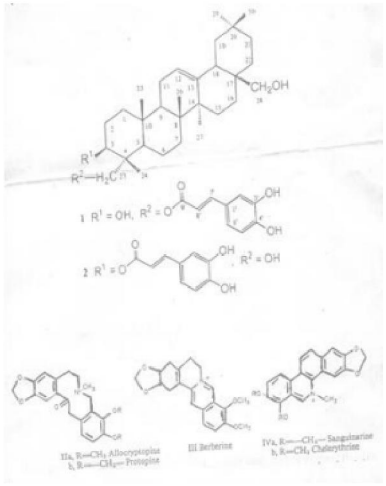
Table 1. Phytochemicals of *H. sabdariffa*

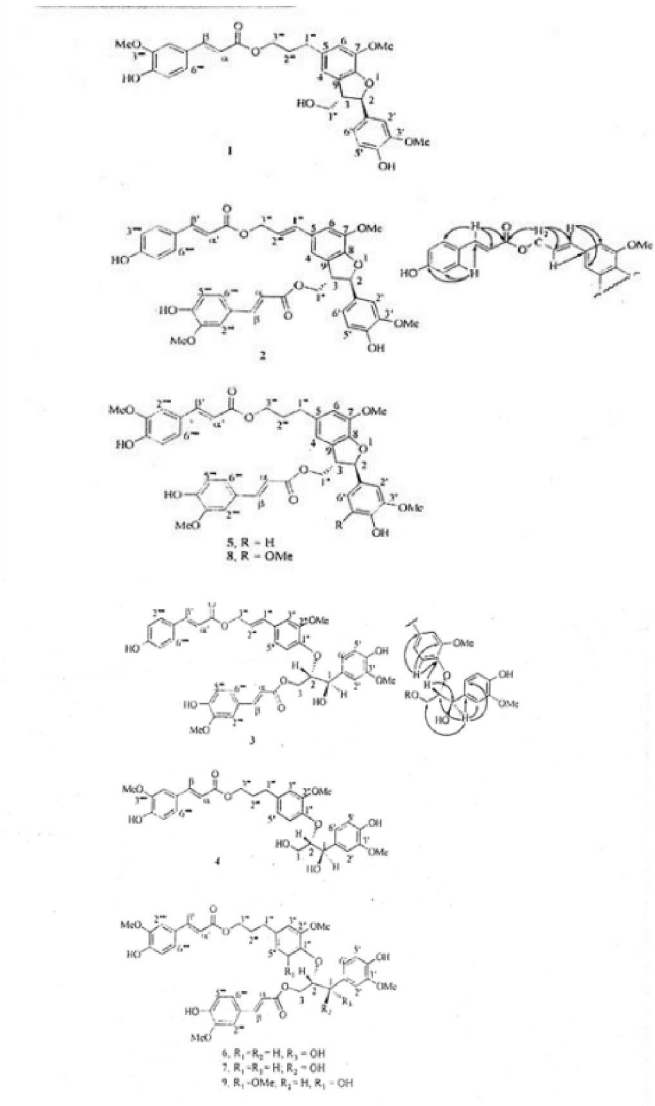
Part of the plant	Chemical constituents
Flower	Carbohydrates, arabinans, mannose, sucrose, thiamin xylose mucilage e, niacin peclin, proteins, fat, arabinogalactans, rhamnogalacturans, riboflavin, β-carotene, phytosterols, citric acid, ascorbic acid, fruit acids, maleic acid, malic acid, hibiscic acid, oxalic acid, tartaric acid, (+) -allooxycitronic acid-lactone, allohydroxycilric-acid, glycolic acid, utalonic acid, protocatechuic acid, cyanidin-3-glucoside, cyaniding-3-sambubioside, cyanidin-3-xyloglucoside, delphinidin, delphinidin-3-glucoside, delphinidin-3-sambubioside, delphinidin-3-xyloglucoside, delphinin, gossypetin, gossypetin-3-glucoside, hibiscetin, hibiscin, hibiscitrin, sabdaretin, sabdaritrin, fibre (crude), resin, fibre (dietery), minerals and ash.
Seed	Starch, cholesterol, cellulose, carbohydrates, campesterol, β-silosterol, ergosterol, propionic acid, pentosans, pelargonic acid, palmitoleic acid, palmitic acid, oleic acid, myristic acid, methanol, malvalic acid, linoleic acid, sterculic acid, caprylic acid, formic acid, stearic acid, cis-12,13-epoxy-cis-9- octadecenoic acid, isopropyl alcohol, isoamyl alcohol, ethanol, 3-methyl-l- butanol, fibre and minerals.
Leaf	α- Terpinyl acetate, anisaldehyde, β-carotene, β-sitosterol, β-D-galactoside, β-sitosteryl benzoate, niacin, fatl, isoamyl alcohol, iso-propyl alcohol, methanol, 3-melhyl-l-butanol, benzyl alcohol, ethanol, malic acid, fibre and ash
Fruit	α-Terpinyl acetate, pectin, anisaldehyde, ascorbic acid, calcium oxalate, caprylic acid, citric acid, acelic acid, ethanol, formic acid, pelargonic acid, propionic acid, isopropyl alcohol, methanol, benzyl alcohol, 3-methyl-l- butanol, benzaldehyde and minerals.
Root	Tartaric acid and saponin





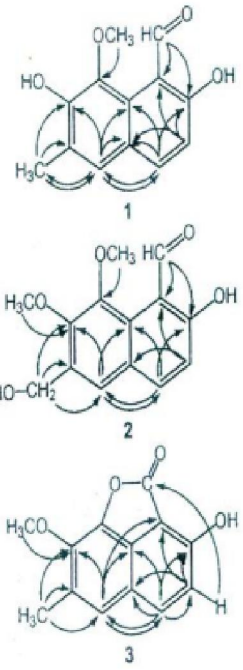
According to the formulae of these compounds, we can expect the ease of inhibition which rnay be due to adsorption through COO'. OH, - H groups which was discussed before: these groups act as a good medium for inhibiting the dissolution process of HAP crystals. Also the methnolic extract the following compounds were found to exist in the extract:



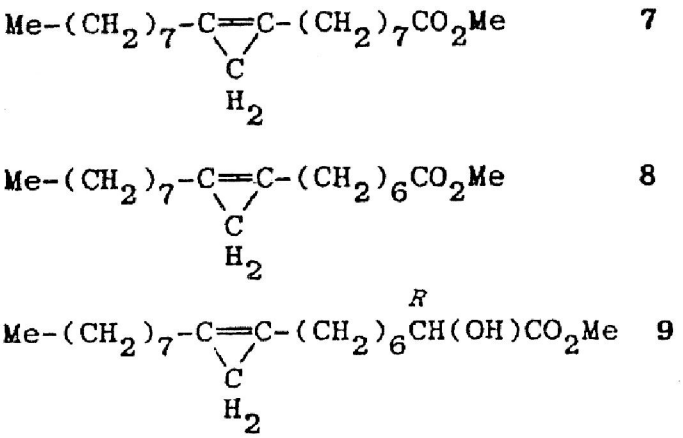


Lignans 1 – 9 from Hibiscum connabinus and major connectivities.

The compounds found in Methanolic extract of Hibescuse are shown in the following scheme:



Structures of syriacusins A (1), B (2) and C (3). Arrows indicate correlations between ^{1}H and ^{13}C



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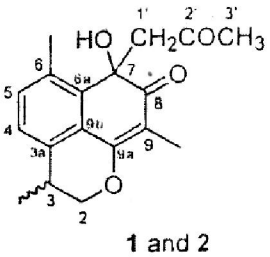
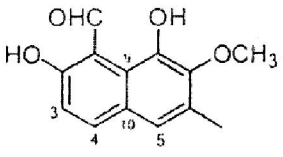


1 and 2



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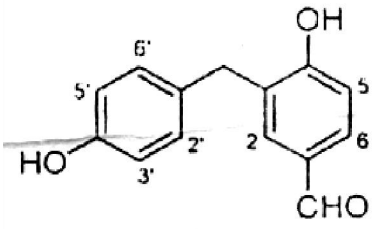

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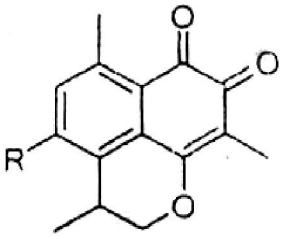
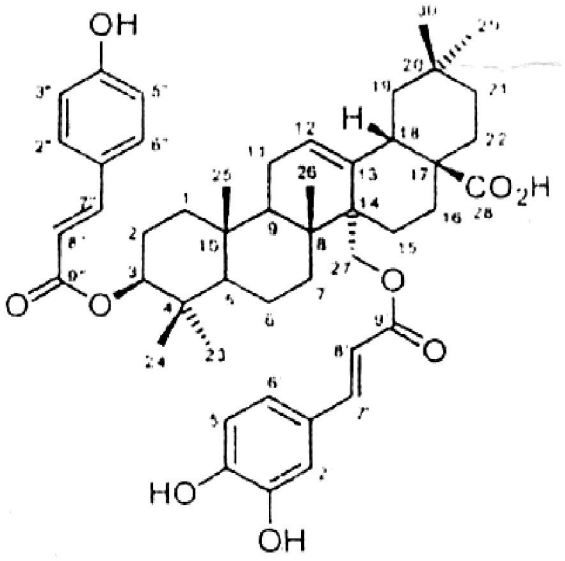


18: R = H
19: R = OH



5 (3-O-coumaroyl-27-O-caffeoyl)
29 (3,27-di-O-caffeoyl)
30 (3-OH-27-O-*trans*-feruloyl)

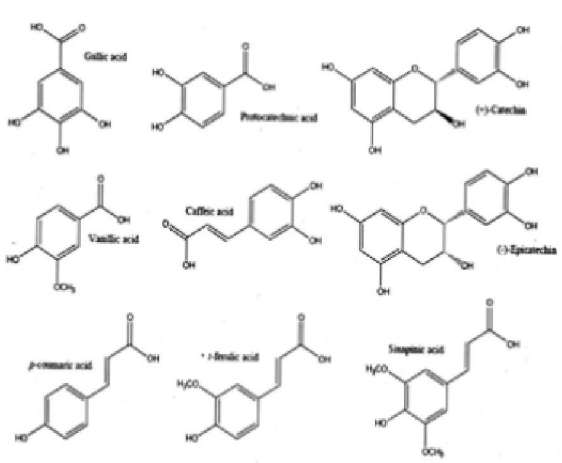
Barley is an abundant source of phenolic compounds and can be consider as an excellent dietary matrix of natural antioxidants for disease prevention and health promotion. Additionally, barley phenolics are very important due to their influence in several stages of the brewing process and the overall beer stability (e.g., formation of haze, color taste, filtration, foam stability and redox state) it is known that over 60% of the total polyphenolic content, found in beer comes from barley.

Barley contains different classes of phenolic compounds, such as benzoic and cinnamic acid derivatives, proanthocyanidins, quinines, flavonols, chalcones,flavanones, and amino phenolic compounds. They can be found in a free, esterified or in an insoluble bound form and they are quantitatively distributed between different tissues of the grains. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) and p-coumaric acid (4-hydroxycinnamic acid) are the major low – molecular weight phenolic acids in barley grains, mainly found in the outer layers (husk, pericarp, testa and aleurone), but also detected in endosperm. Other bound phenolic acids found in barley are vanillic, sinapinic, and p-hydroxy benzoic acids. Barley grains also contain arrange of flavan-3-ols from monomers ((+)-catechin and (-)-epicatechin), dimmers (prodelphinidin B3 and procyanidin B3, and trimers (procyanidin C2), up to higher-molecular weight flavonoid-derived tannins.

The methanolic extract of barley contained low molecular weight carbohydrates. Carbohydrates are inhibitor for crystallization and dissolution of HAP crystals, the presence of -OH group make them good inhibitors, it also contains cellulose, ferulic and chlorogenic acids which containing-OH groups. The over growth of calcite on cellulose was done selectively, On macromoles, possibly through active sites formation at ionizable functional group (OH)-Cellulose found in the extract is trimethyle cellulose and so the presence of -OCR) groups in the methyl cellulose groups also increase the inhibitory effect of barley cellulose.

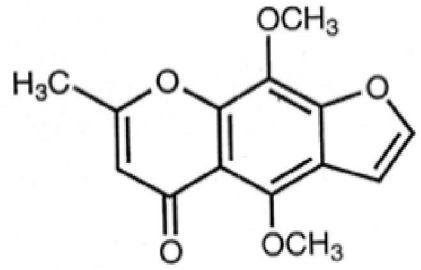
From barley three proteins of molecular weight 50.000, 40.000 and 25000 were separated in which the major group subunit has the major terminal amino acid sequence. The amino acids in barley composition are. Asp, Thr, Ser, GIu, pro, Gly, Ala, Val, Sys, Met, leu, Tyr, PIe, Ly, His, Arg. Also it consists of amides of asparagines and glutamine, higher glycine phenolic acid (Ferulic and chlorogenic acid) and cutin.. Barley grain contains starch, dietary fibers, protein and low molecular weight carbohydrate (glucose, mannose, galactose arabinose and xylose) uronic acid, β- glycan and fat klason lignin. Analysis showed that barley contains 4-11% arabinoxylose, 3-7% mixed linkage (1-, 3), (1-4), β- glycan and smaller amounts of cellulose and ligning. β-glycan in barley is composed of cellotriosyl and cellotetrosyl units joint by single (1-3) linkage.

Structure of the ohenolic compounds present in barley extract:

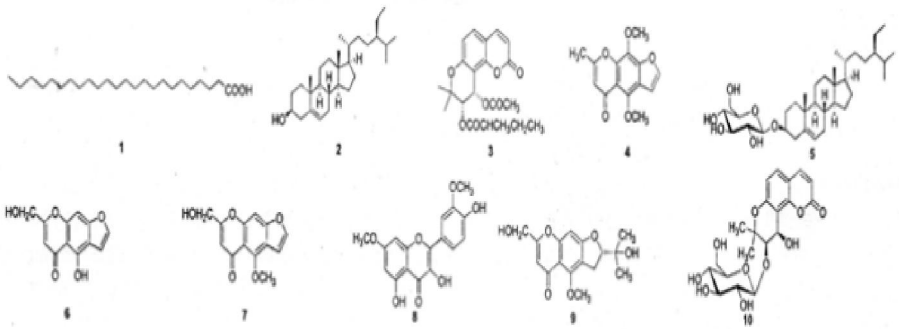


Ammi sativa:

A visinga is perennial herb widely distributed in Mediterranean area. Among Egyptians people, it is called Khilla, chellah or kella, while in Europe, the plant has often been referred to as toothpick herb or Bishop's weed. Turkish people referred this plant as "dis tu,","Kilir" and hiiam. The compounds isolated from methanol extract of the plant were: Khellin (4,9-dimclhoxy-7-mclhyl-5H-furo[3.2-g] [1] benzopyran-5-one) is obtained the fruits and seeds of the plant *Ammivisnaga*. The fruits contain about I percent of this compound. Pure khellin occurs in colorless, odorless needle-shaped crystals, having a bitter taste. It has also been called visammin by some authors. *Ammivisnaga* is, commercial medicinal plant: mainly grown In Mediterranean, areas in open fields. The extract of fruits has been widely employed as herbal medicine in the treatment of coronarv diseases and bronchial asthrma. Khellin is used as a spasmolytic agent in the therapy' of asthma and angina petoris and recently its use has been proposed for the treatment of vitiligo and psoriasis. Studies on the photogenic and mutagenic activity of khellin have also been reported.



Chemical structure of khellin.

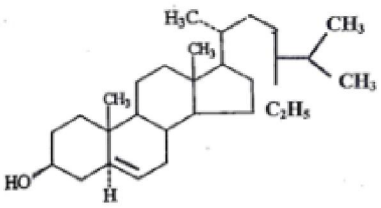


Chemical structures of isolated compounds.

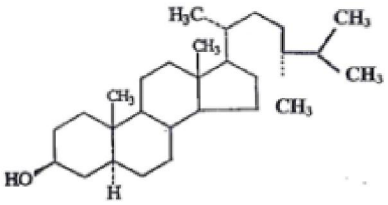
Nigella sativa (black seeds), (N.S), is a natural annua, herb has long been used as natural medicine for treatment of many acute, as well as chronic conditions. Also it is used in foods in Egypt and Arabian region.

Nigella sativa can heal every disease except death.

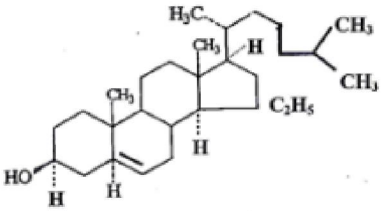
The Methanol extract of (N.S) contains the following compounds:



II. Stigma sterol

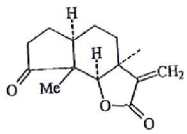


III. Stigma sterol

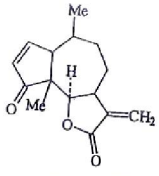


I. Cholesterol

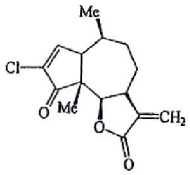
2,3-Dehydro-11-hydroxy methylene Ambromaritolide (XIV)



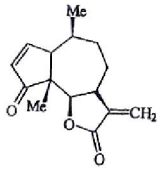
Damsin (I)



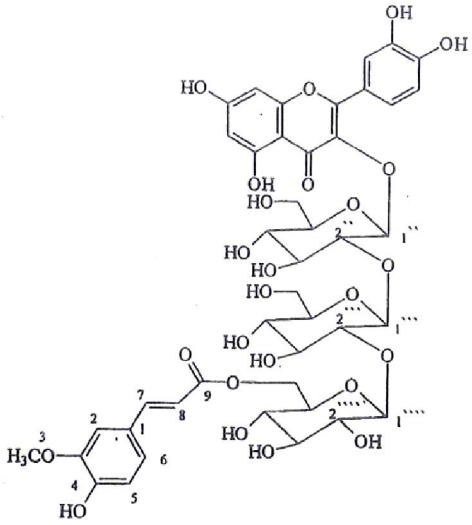
Ambrosin (II)



Chloroambrosin (III)

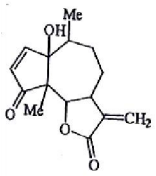


Neoambrosin (IV)

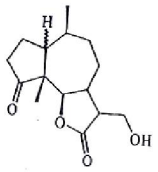


Quercetin-3-O-(6-feruloyl)-β-glucopyranosyl (1→2)-O-β-galactopyranosyl(1→2)-β-glucopyranoside

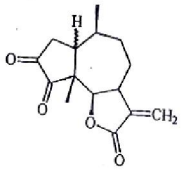
(XI)



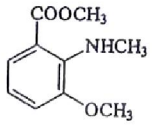
Parthenin (V)



13-Hydroxy damsin (VI)

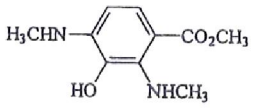


3-Keto dams



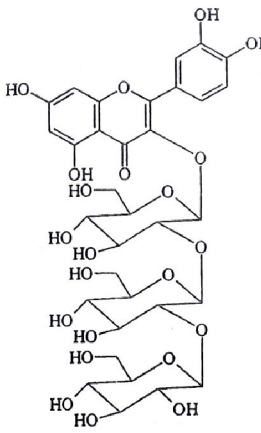
Damascenine

(XII)

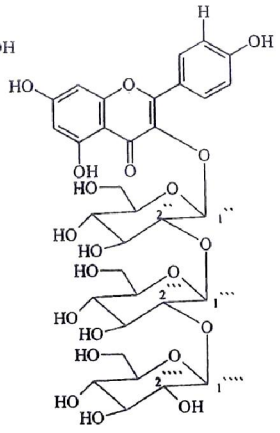


Damascinine

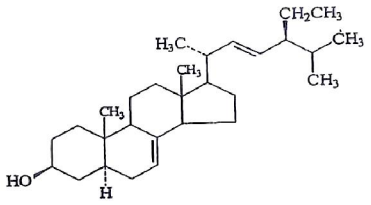
(XIII)



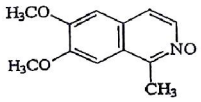
Quercetin-3-O-β-glucopyranosyl
(1→2)-O-β-galactopyranosyl
(1→2)-β-glucopyranoside
(IX)



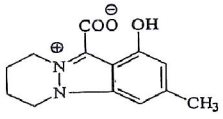
Kaempferol-3-O-β-glucopyranosyl
(1→2)-O-β-galactopyranosyl
(1→2)-β-glucopyranoside
(X)



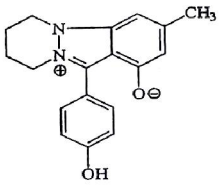
α-Spinasterol
(V)



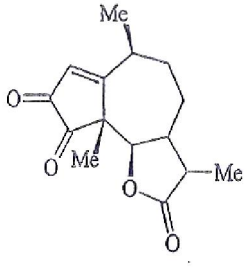
Nigellinine N-oxide
(VI)



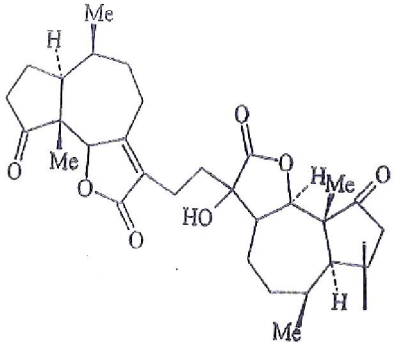
Nigellicine
(VII)



Nigellidine (VIII)



4-Hydroxy-12,6-pseudoguaianolide
(XV)



Anhydro coronopolin (XVI)

From methanol extract of N.S we can expect that the influence of = 0-, OCH_3, N-N, -OH, -COO-, -H3CO-, -NO groups could be of the cause its inhibitory effect on the rates of dissolution of HAP crystals. The presence or these groups may act as a good inhibitor for Ca^2 active sites which decrease the dissolution rates of HAP crystals. Also was found that in surface controlled and generally in all physical adsorption on the surface, when the additive is less soluble in water, it will be good inhibitor and make good blocking to the active sites on the surface of crystals, due to competition with water.

Table 2. Chemical composition, including active principles, of N. sativa seed

Group	Sub-group	Components
Fixed oil (32-40 %) (Gad et al., 1963; Babayan et al., 1978; Salama 1973; Staphylakis and Gegiou 1986) Volatile oil (0.4-0.45 %) (Enomoto et	Unsaturatedfattyacids	Arachidonic, eicosadienoic, linoleic, linolenic, oleicand almitoleic acid. Palmitic, stearic and myristic acid. Beta-sitosterol, cycloeucalenol, cycioartenol, sterol esters and sterol glucosides

al., 2001; **El Dakhahakhany 1963; Ghosheh** et al., 1999)	Saturated fatty acids	Nigellone, thymoquinone, thymohy, droquinone, dithymoquinone, thymol, carvacrol, **α& β-pinene, d-Limonene,** d-citronellol, p-cymeneand-(2-methoxypropyl)-5-methyl-1,4-benzenediol6,16-18
Proteins (Babayan et al., 1978) (16-19.9 %)		Arginine, glutamic acid, leucine, lysine, methionine, tyrosine,
Alkaloids (Atta-ur- Rehman et a!., 1985; Atta-ur-Rehman et al., 1995)	Amino acids	**proline and threonine, etc.13**
Coumarins (Atta-ur-Rehman et al., 1985; Atta-ur-Rehman et al., 1995; El-Zawahry, 1964; Drozed etal., 1973)		Nigellicine, nigellidine, nigellimine-N-oxide 6-methoxy-coumarin, 7-hydroxy-coumarin, 7-oxy-coumarin
Saponins (Kumara and Haul 2001; Ansari et al., 1988)		Alpha-Hedrin, Steryl-glucosides, acetyl-steryl-glucoside
Minerals (1.79-3.74 %) (El-Zawahry, 1997; Babayan et al., 1978)		Calcium, phosphorous, potassium, sodium and iron
Carbohydrates (33.9%) Fiber (5.5 %), Water (6 %) (Haq, et al., 1999; El-Zawahry 1997)	Triterpenes, Steroida	

Table 3. Physicochemical properties of the Moroccan Nigella seeds oil.

Cold press-extracted	**Solvent- extracted**	
Free fatty acids (as oleic %)	0.9 ± 0,2	2.3 ± 0.5
Iodine value (g of I_2/100g)	128 ±2	126 ±'4
K_{232}	1.585 ±0.008	2.21 ± 0.01
K_{270}	2.370 ± 0.003	2.771 ± 0.009
PV(Meq0_2/kg)	3.4 ± 0.5	11.4 ± 2.5
Refractive index at 20 °C	1.468 ± 0.003	1.473 ± 0.002

Table 4. Moroccan Nigella seed oil fatty acid composition(%).

Fatty acid	Cold press-extracted	Solvent- extracted
Myristic acid(C14:0)	1±0.1	0.2±0.1
Palmitic acid(C16:0)	13.1±0.2	11.9±0.2
Palmitoleic acid(C16:1)	0.2±0.1	0.2±0.1
Stearic acid(C18:0)	2.3±0.1	3.2±0.1
Oleic acid(C18:1)	23.8±0.1	24.9±0.5
Linoleic acid(C18:2)	58.5±0.1	56.5±0.7
Linoleic acid(C18:3)	0.4±0.1	0.2±0.1
Arachidic acid(C20:0)	0.5±0.1	0.2±0.1
ΣSaturated fatty acids	16.8±0.5	15.5±0.5
ΣUnsaturated fatty acids	82.9±0.5	82.1±0.5

The additive' s molecules, ions are adsorbed on active sites on HAP crystals, blocking them and reducing their numbers and decreased the dissolution rates of HAP crystals. The positive parts or groups of the additive, adsorbed on the negative ions on the HAP crystal surface and vice versa.

The sorption process can be described by four-consecutive steps:

1-Transport in the bulk of solution,

2- Diffusion through the solution to the external surface of crystal surface (also called diffusion of additive molecules),

3- Particle diffusion in the liquid contained in the pores and in the sorbate along the pore walls,

4- Sorption and desorption within the particle and on external surface.

Generally, steps (1) and (4) occur rapidly so that the rate –controlling steps becomes step 2, step 3, or the combination of them. Since, the mechanism was surface –controlled one, so we suggest that step (3) may be the rate –determining step.

In the classic description of Pinng mechanisms is based on impurity adsorption at surfaces, step or kinks In this case, impurities act as blockers at the sites where they adsorb, preventing the crystal step from propagating locally and thus causing a straight step to become scalloped. As step curve, their velocity is reduced until they eventually stopped when their radius of curvature reaches the critical radius. In this model, the degree of inhibition depends on the undersaturation or supersaturation and the blocker concentration on the surface. Higher concentration of a dsorbate. cause a greater reduction in velocity and

this effects is more pronounced. at lower supersaturations or undersaturations[8]. A good model crystal for studying the several aspects of interactions between additives and crystals, Skirtic *et al* had summarized following:

1) Importance of molecular size of the additive i-e small or macromolecules, number of functional groups in the molecules and the overall charge in the growth or dissolution of crystals.

2) Importance of a structural fit between the organic molecules and the ionic structure of a particular crystal face, this decides the order of inhibition or reaction at a particular crystalline face. It may affect various crystalline face expected to the solution. As a result, it may change the morphology of the growing crystal.

3) Influence of the hydration layer exposed on the surface of the crystals. Such structural hit exists between distances of carboxylic groups in the polaspartic β-sheet and distance of neighboring calcium ions from two adjacent layers constitute one Ca-HPO_4 bi-layer lying beneath the hydrated layer parallel to be (0.10) plane [19]. In adsorption of phosphonates on gypsum, the suggested mechanism of the process were:

1) Chemical bonding (complexiation) of Ca^{2+} ions at the crystal surface with the phosphonate group.

2) Formation of hydrogen bridges between the hydroxyl groups and either the sulfate ions or the crystal water molecules at the gypsum crystal surface [20],

The inhibition of growth rates of HAP by serine explained by attraction of positively charged prontonated amion group adsorbed on the negatively sites on HAP crystal surface [21],.

In dissolution of HAP crystal in the presence of Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} and Cd^{2+} it was found that the rates of dissolution decreased due to adsorption of metal ions on active sites on the surface of the crystals blocking them so decreasing their numbers on the crystal surface[3]. The molecular weight, the atomic size of the caions in the inhibitors affected their inhibitory effect of them.

Adsorption of the inhibitor on the surface of crystals may occur by competition between the active sites on the surface of crystal similar to that of the inhibitor charge In dissolution of HAP crystas in the presence of some cations, it was interpreted as competition between the cation and Ca2+ ions active sites on surface of HAP crystals which depended on the size of cations replace the Ca^{2+} ions at the surface[3]. Sulfonic anions replaced the sulfate lattice of gypsum crystal surface enhancing the inhibiting capacity of the additive [22].

In homogeneous nucleation of gypsum in the presence of polyacrylate, it was suggested that $–COO^-$ of the additive adsorbed on $Ca2^+$ active sites on the gypsum crystals surface [23]. The negatively charged –C-N functional group in polymer adsorbed on Ca^{2+} active sites of HAP crystal surface [24].

From SSA measurements at p/p0=0.9525 it was found that SSA of HAP crystals seed was 35.72 m2/g, and in case of the presence of black seeds (N.S) was 58.79 m2/g, the total pore volume was reduced in the presence of N.S from 8.548e-02 → 1.274e-01 and the average pore radius were reduced from 4.785e+01 A → 4.32205e+01 A. from which the N.S additive adsorbs on the pore of HAP crystal surface reducing their volume and their radius.

The validity of applying Langmuir isotherm supported the surface-mechanism. The familiar expression describing the Langmuir-adsorption of solute is given by:

$$\theta = K_l [c] / (1+K_l) [c] \quad (6)$$

Where: θ: is the fraction of the surface covered by the adsorbing solute,

C: is the solute concentration of adsorbate

K: is the affinity constant for solute-surface interaction.

In the presence of additive (adsorbate), the rate, Ri, is proportional to the fraction of the surface free from adsorbed additive (1-θ) and given by:

$$R_I = Ro\ (1-\theta) \quad (7)$$

Where Ro: is the rate of reaction in the absence of additive. On substituting in eq.(6) and upon further rearrangement yields:

$$Ro\ (Ro-Ri) = 1/(K_l[c]) + 1 \quad (8)$$

From eq.(8), this model predicts a linear relationship between Ro(Ro-Ri) and 1/[c], with an intercept of unity indicates. That the Langmuir-isotherm satisfactory described the inhibitory effect of the additive ions. fig(5) indicated the relation between Ro(Ro-Ri) and 1/[c] in the case of the presence of Hb, Br, Kh and N.S. at the experimental conditions. The best fit linear relation and an intercept of unity strongly suggested that the mechanism of inhibition involves that proposed for the Langmuir adsorption namely the formation of a monomlecular blocking layer of additive ions at the dissolution sites on the crystal surface. Affinity constants in case of the dissolution of HAP crystals in the presence of Hb, Br, Kh and N.S were: 3.27, 3.10, 2.59 and 2.35×10^7 M which supported the order of inhibition of dissolution of HAP:

△ Hb > Br > Kh > N.S. △

The values of of ΔG of the dissolution of HAP crystals in the presence of Hb, Br, Kh and N.S were:

-54.30,-52.75,-52.48 and -51.93 KJ/mol in case of the presence of Hb, Br.Kh and N.S respectively.

The values of K_{ads} in dissolution process of HAP crystals in the presence of Hb, Br.Kh and N.S were: 2.56, 1.39, 1.25 and 1.01 respectively.

The values of Kl, Δ G and K_{ads} reflected the good adsorption of these additives on dissolution sites on HAP crystal surface blocking them and reducing the dissolution rates.

The effect of change of experimental conditions on the inhibitory effects of additives was studied. The order of dissolution of HAP crystals in the presence of N.S was n $\simeq$ 2 which supported the surface – mechanism.

The effect of changed values of pH in the presence of N.S was studied. The rate of dissolution of HAP crystals in the presence of N.S (Ri) increased by increasing pH values of the medium i.e the inhibitor has good effect at low pH values.

The effect of change of values of ionic strength of medium on dissolution rates of HAP crystals at experimental conditions was studied, the rate increase by increasing the values of ionic strength of the medium which indicated that the dissolution reaction was electronic in its action.

Concentration of 7×10^{-7} mol dm^{-3} of N.S complete inhibited the dissolution of HAP crystals which indicate the low numbers of active sites on HAP crystal surface and supported the physical adsorption mechanism.

Table (1): Effect of change of degree of under saturation on the rate of dissolution of calcium phosphate at 37◦C, pH =7.4, I= 0.15 mol dm^{-3} using emf method

Exp No	σ	-log σ	R /10^{-6} mol $dm^{-1}m^{-2}$	- log R
1	0.8	0.0969	1.961×10^{-6}	5.708
2	0.7	0.1549	2.4×10^{-6}	5.618
3	0.6	0.2218	3.67×10^{-6}	5.435
4	0.5	0.3010	5.4×10^{-6}	5.265
5	0.4	0.398	9.02×10^{-6}	5.045
6	0.3	0.5229	1.58×10^{-5}	4.801
7	0.25	0.6020	2.9×10^{-5}	4.678
8	0.2	0.6989	3.48×10^{-5}	4.459

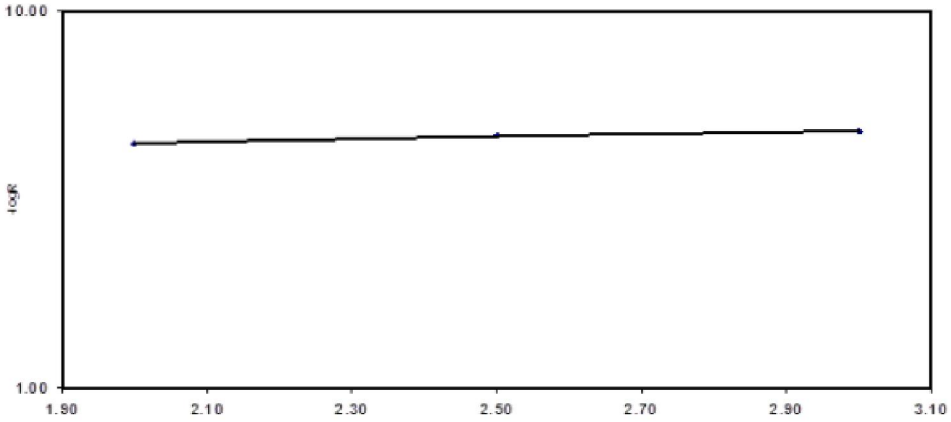


Fig (1): plots of –logR against –logσ of dissolution of calcium phosphate crystals at t=37C^{o} and pH=7.4

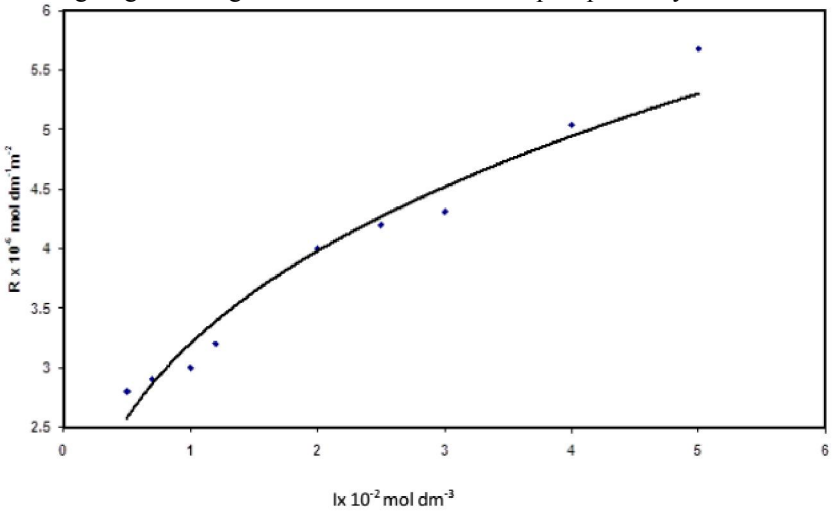


Fig (2): Effect of ionic strength(I) on the rate of dissolution of calcium phosphate at t=37 C,^{o}=0.6 and pH7.4

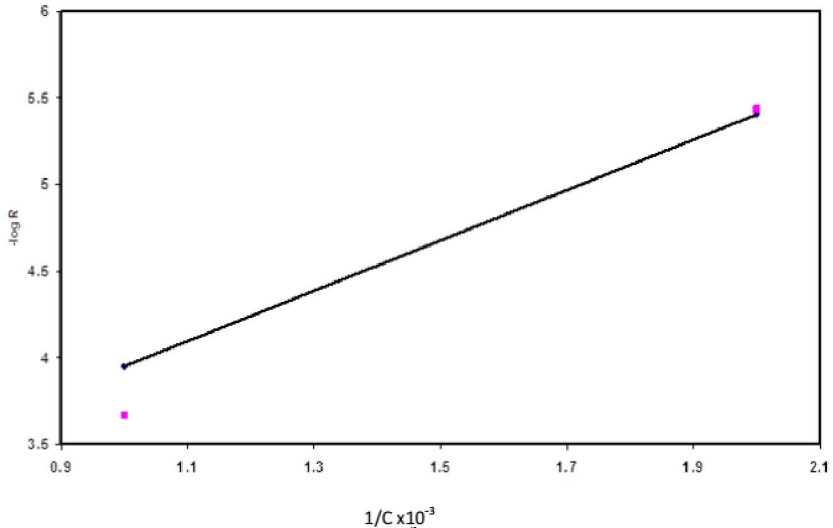


Fig (3): plot of-log R against –log1/T×10 $^{-3}$ at t=37 C^{o},51 mg seed and I=0.15 mol dm^{-3}

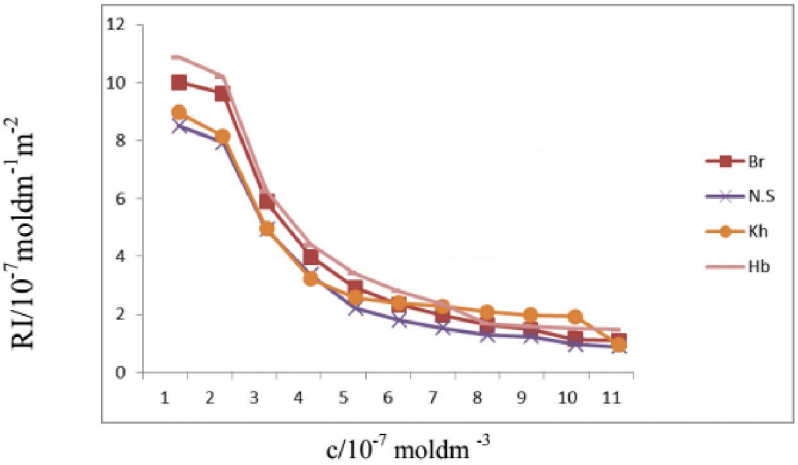


Fig (4): plots of rates of dissolution of calcium phosphate crystals against [additive]$^{-1}$ in the presence of Hb, Br, Kh and N.S at t = 37^{0}C, 51mg seed
I =0.15 moldm^{-3} and σ =0.6 and pH = 7.4 using emf method

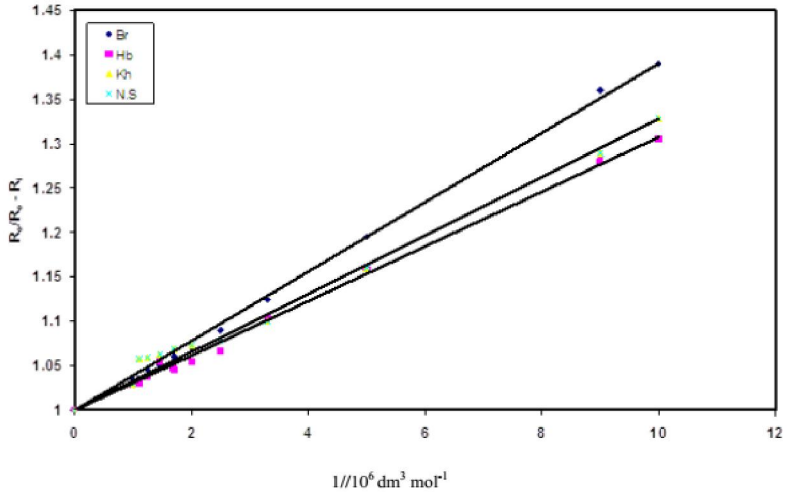


Fig (5): plots of Ro/(Ro-Ri) against [additive]$^{-1}$ for dissolution of calcium phosphate at t=37 C^{o}, I=0.15 mol dm^{-3} and σ=0.6 in the presence of Hb, Br, Kh and N.S

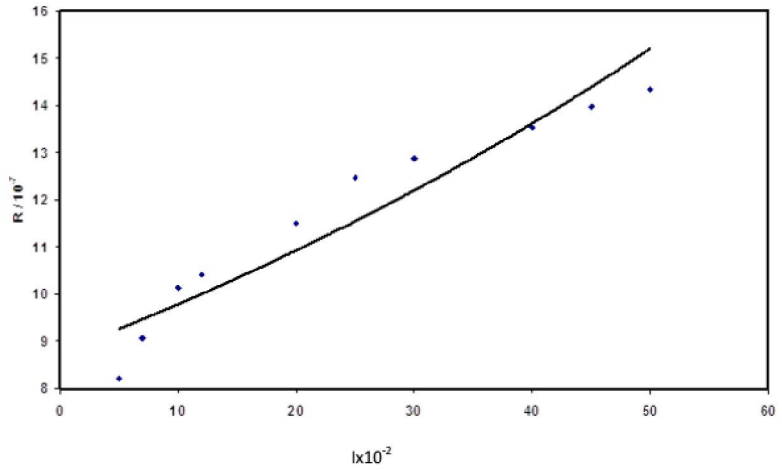


Fig (6): plot of rates of dissolution of calcium phosphate against ionic strength in the presence of N.S at t=37 C°, 51mg seed and pH=7.4

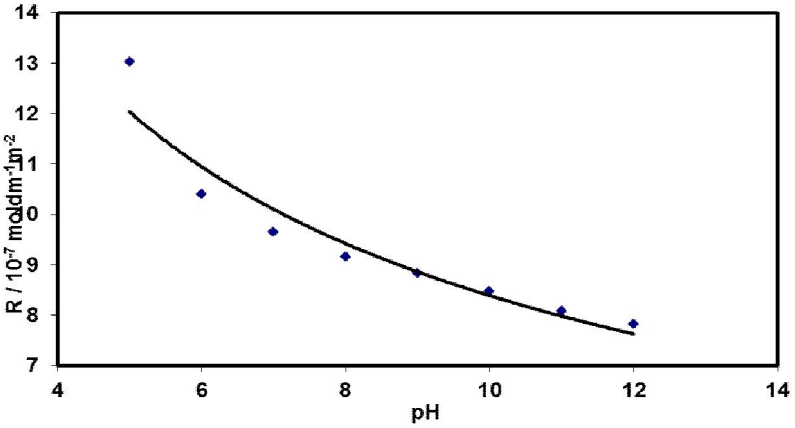


Fig (7): plot of rates of dissolution of calcium phosphate against pH in the presence of N.S at t=37^{-o}, I=0.15 mol dm^{-3} and σ=0.6

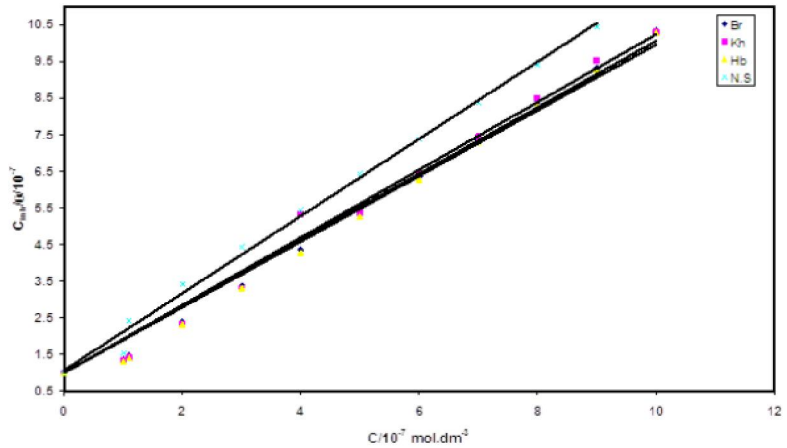


Fig (8) plots of surface coverage (θ) of calcium phosphate against in the presence of different concentrations of Hb, Br, Kh and N.S

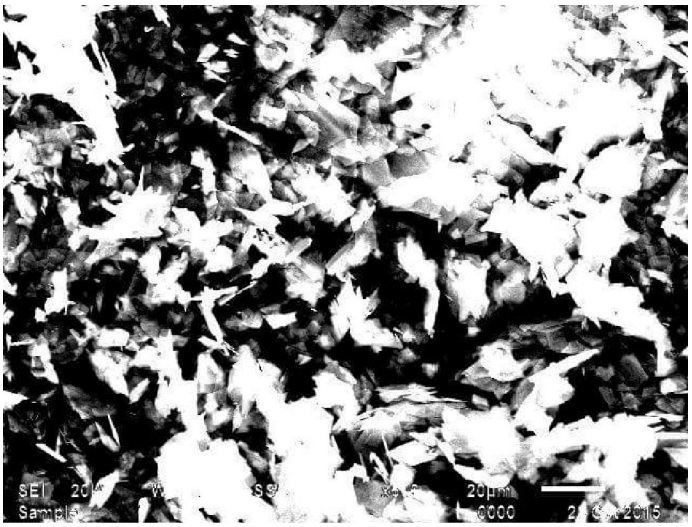


Fig: (9) SEM of calcium phosphate crystal

Fig (10) SEM of calcium phosphate crystal in the presence of N.S

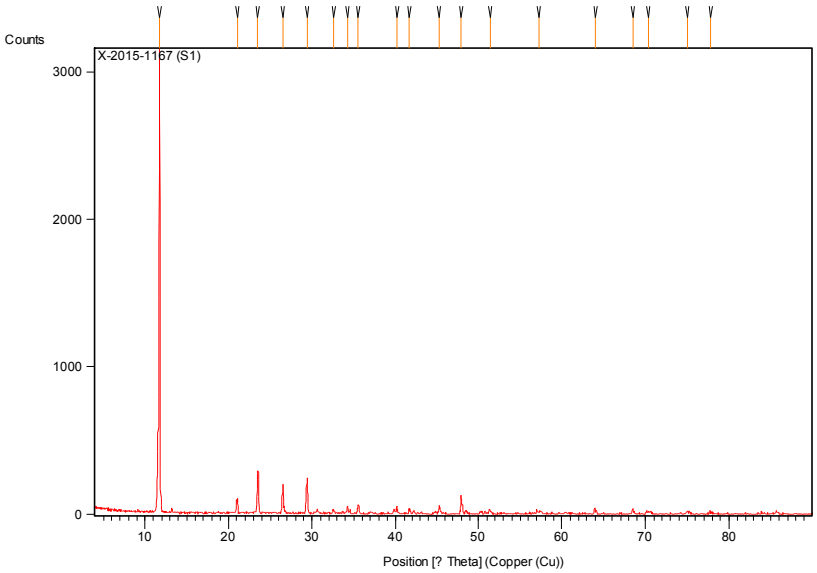


Fig (11): X-ray powder diffraction for calcium phosphate crystal

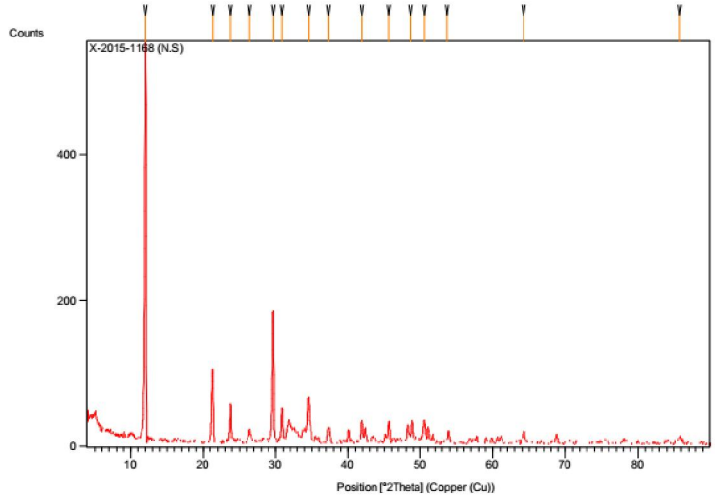


Fig (12): X-ray powder diffraction for calcium phosphate crystal in the presence of N.S

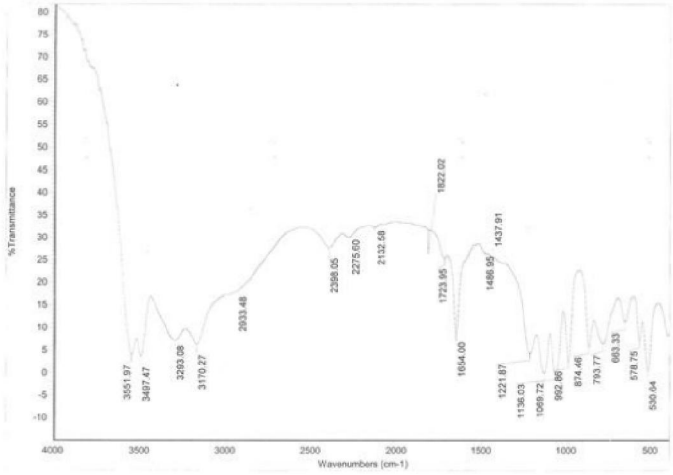


Fig (13): FT-IR analysis of calcium phosphate crystal

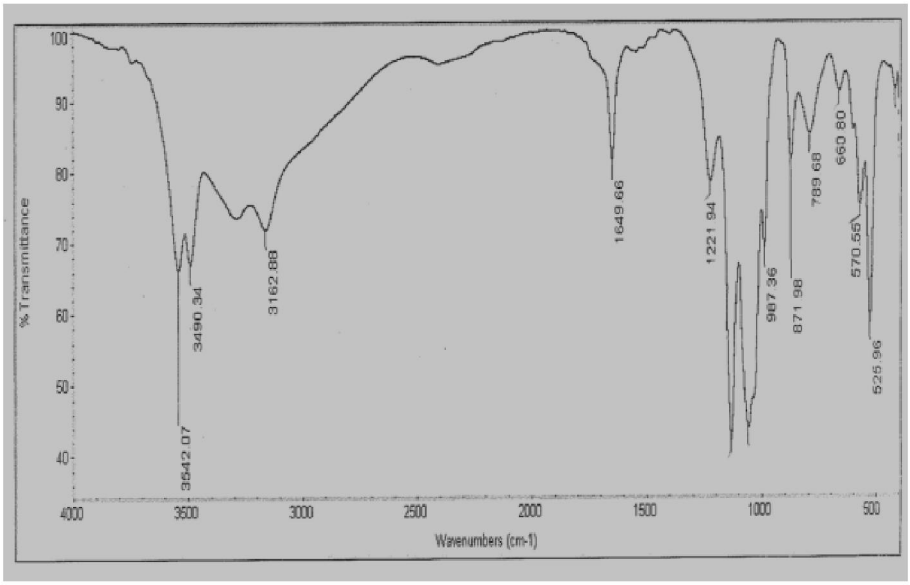


Fig (14): FT-IR analysis of calcium phosphate crystal in the presence of N.S

Conclusion:

The dissolution rates of HAP crystals under experimental conditions followed surface –controlled mechanism with n=2. The stirring dynamics did not affected the dissolution rates of HAP crystals. Ea of process was 2.5KJ/mol. increasing the values of pH and ionic strength of the medium, increased the dissolution rates of HAP crystals. The methanolic extracts of medicinal plants of Hb, Br, Kh and N.S inhibited the dissolution rates of HAP crystals by the order: Hb>Br>Kh>N.S.

The affinity constants. G and K_{ads} in the presence of inhibitors supported the mechanism. The order of dissolution rates of HAP in the presence of 10^{-7} mol dm^{-7} mol dm^{-3} of N.S was $n \tilde{-} 2$ and Ea =3.06 KJ/mol, the inhibition decreased by increasing the values of pH or ionic strength of the medium. The radius of pores of ions on HAP crystal surface reduced in the presence of N.S additive which supported the surface mechanism.

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