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تأثير المستخلصات المختلفة لنبات البقدونس علي معدل ذوبان وتكوين بللورات اكسالات الكالسيوم

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الملخص العربي

في هذا البحث تم دراسة تأثير مستخلصات نبات البقدونس علي معدل ذوبان وتكوين بلورات اكسالات الكالسيوم حيث تم عمل مستخلصات للبقدونس في اوساط مختلفة من الكلوروفورم والايثانول والماء، وقد تم تحضير تركيزات مختلفة من هذه المستخلصات وكانت النتائج كالتالي :-

١- المستخلصات المختلفة لنبات البقدونس تمتلك خواص بيولوجية تعمل علي تثبيط معدل الذوبان لبلورات اكسالات الكالسيوم .

٢- إن تأثير المستخلص المائي للبقدونس **Pa(a)** يعمل علي تثبيط معدل الذوبان لبلورات اكسالات الكالسيوم اعلي من مستخلص الايثانول **Pa(e)** ثم يلية مستخلص الكلوروفورم **Pa(c)** .

٣- إن تأثير المستخلص المائي للبقدونس علي معدل ترسيب بلورات اكسالات الكالسيوم كان اعلي من المستخلص الايثانولي ثم يليه مستخلص الكلوروفوم.

EFFECT OF DIFFERENT EXTRACTS OF PARSELY ON THE RATE OF DISSOLUTION AND CRYSTALLIZATION OF CALCIUM OXALATE CRYSTALS

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ABSTRACT: ***This research aims to prepare some natural extracts from parsley and study its ability to inhibit on the rate of dissolution and crystallization of kidney stones of calcium oxalate. Three extracts of parsley were prepared, aqueous extract Pa (a), ethanolic extract Pa (e) and cloroformic extract Pa (c). The potentiometric measurements, dissolution experiments and crystal growth measurements were studied on the these extracts. The results of this study showed that these extracts have ability to inhibit the rate of solubility of calcium oxalate stones, and the highest extract has the ability to inhibit the rate of formation of calcium oxalate in Pa (a) followed by Pa (e) followed by Pa (c) .***

Keywords: ***Parsely, Crystallization, Dissolution, Crystal growth.***

INTRODUCTION

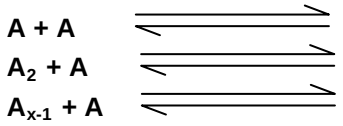
Calcium oxalate is one of the main constituents of deposits in urinary tract [(13)]. Crystallization of calcium oxalate is of particular interest not only from the theoretical point of view but also because of its biological importance [(14)].

The mechanisms of dissolution and precipitation are important in oceanography, crystallography, geology, metallurgy, industrial purification of chemicals, photography, ceramic, polymer sciences and destination technology [(4,28,7,21)]. The formation of submicroscopic particles (nuclei) of the new phase from a supersaturated solution is particularly interesting, but relatively difficult to observe experimentally.

The formation of submicroscopic particles (nuclei) of the new phase from a supersaturated solution is particularly interesting, but relatively difficult to observe experimentally.

It begins only when certain supersaturation has been reached and proceeds very rapidly[(2)] . If the concentration of the solute ions or molecules is high enough, some clusters become sufficiently large and become

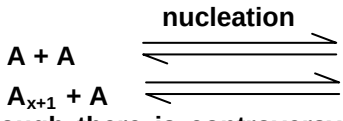
consolidated into small crystallites, where upon the supposedly irreversible crystal growth ensues. The largest cluster which may exist before spontaneous crystallization is usually refereed to as the critical cluster, or nucleus, so in terms of molecular aggregation the process may be represented as follows:



$A + A \rightleftharpoons A_2$

$A_2 + A \rightleftharpoons A_3$

$A_{x-1} + A \rightleftharpoons A_x$ **(Critical cluster or nucleus)**



nucleation

$A + A \rightleftharpoons A_{x+1}$

$A_{x+1} + A \rightleftharpoons$ **Crystal growth**

Although there is controversy over whether growth and dissolution are reciprocal processes, [3] it is possible that the latter involves the following processes [33].

1. Detachment from a dissolution site.
2. Diffusion along surface away from the dissolution sites.
3. Description from the surface.
4. Bulk diffusion.

When studying inhibition of crystal growth and dissolution in natural systems, some fundamental problems have to be considered:

1. How should the effect of inhibition of mineralization and demineralization processes be detected and described ?
2. How can the effect of inhibitors be explained ?

In discussing the effect of additives upon the rate of crystallization and dissolution, the following factors must be considered:

1. The additive may form stable complexes with one or more, of the crystal lattice ions of the precipitated phase. The effective concentrations of the free ions would therefore be reduced and the solubility increased. For this factor to be important, the concentration of additives have to be probably of the same order of magnitude as that of the crystal lattice ions.
2. The additive may be adsorbed at growth or dissolution sites and thus prevents further precipitation or dissolution. If adsorption takes place only at certain sites, the morphology of the growing and dissolving crystals may be changed. Thus, in extensive studies of the crystallization of potassium dihydrogen phosphate, Mullin and co-worker [20] showed that the habit of the crystals formed is influenced not only by pH and supersaturation, but also by the presence of low concentrations of foreign cations such as Cr^{3+}, Fe^{+3} and Al^{3+}.
3. The additive, if ionic, will influence the ionic strength of the solution and hence the effective solubility of the precipitated phase. In practice,

however, most crystallization and dissolution inhibitors are effective at very low concentrations [25-12], while others may only change the morphology of the growing crystals [16].

Molecules or ions can be adsorbed on crystal surfaces, they usually influence the rates of crystallization and dissolution. Some of these additives may accelerate or retard the rates of precipitation and dissolution, while others may only changes the morphology of growing crystals [10].

Modern researches proved that natural plants have the capacity of curing diseases more than chemical drugs and it does not have any bad side effects. This illustrates that it is important to use natural plants and extracts of natural plants in preparing medicine so we find that advanced countries are comitating how to import natural plants (seeds, oils, extracts) to decrease the dependence on the chemical drugs. In a previous work the dissolution of COM crystals was studied in presence of some alcoholic extracts of parsely.

Materials and Methods

Preparation of seed:

Calcium oxalate seeds were prepared by adding one liter of 0.1 M calcium chloride solutions to one liter of sodium oxalate solution (0.1 M) at 298 K at a rate of 250 ml per half an hour. The sodium oxalate solution was constantly stirred throughout the addition. The seed suspension was allowed to age with stirring for one day and was then filtered and the seed crystals were washed with deionized distilled water to remove surface contamination essentially chloride and oxalate ions. The seed crystals were aged for one month., then were refiltered and washed further with deionized distilled water and (his process was repeated several times. The seeds were then filtered and dried. The seed material was then subject to x-ray powder diffraction studies, scanning electron microscope and the determination of specific surface area (SSA).

Measurements of surface area:

In the present work, the specific surface area (SSA) was determined by the BET method applying equation (1):

$$\mathrm{SSA} = 1/\mathrm{W}\left(1 - P/P_o\right)\left(\mathrm{S_g}/S_{gc}\right)\mathrm{V_c}\,\frac{\mathrm{N^o}A_{cs}\,\mathrm{P_o}}{\mathrm{RT}} \quad \text{..... (1)}$$

where:

W : the weight of solid.85
S_g : the desorption single area.
S_{gc}: the single area of calibration.
V_c : the volume of calibration.
N° : Avogadro's number. :
A_{cs}: the cross-sectional area of adsorbate molecule.

P_o : the ambient pressure.

It should be noted that the specific surface area measured by the BET gas adsorption method can be different from those measured by Oilier methods[(6)].

X-ray diffraction:

The solid phase of calcium oxalate was characterized by x-ray powder diffraction using Cu-K radiation. The solid sample was well ground and mixed with internal standard, potassium bromide the ratio of about 4:1 by weight. The sample and standard were filled in a rectangular cavity (1.5 cm × 1.0 cm × 0.05 cm) of a 3.8 cm × 3.8 cm × 0.2 cm aluminum solid holder and were slowly scanned at a speed of 10 / 4 20 = 10° to 90° as shown in Fig (1).

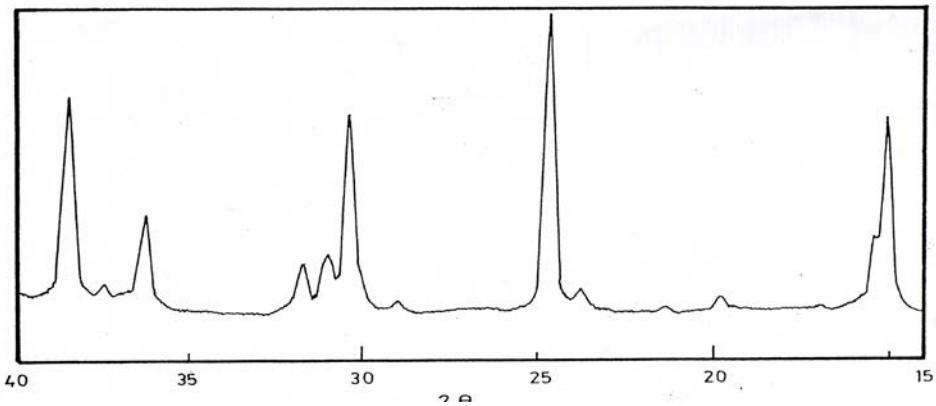


Fig. (1): X-ray powder diffraction studies for calcium oxalate crystals.

IR Spectra were recorded by the IR Spectrum of $CaC_2O_4 . H_2O$ fig. (2).

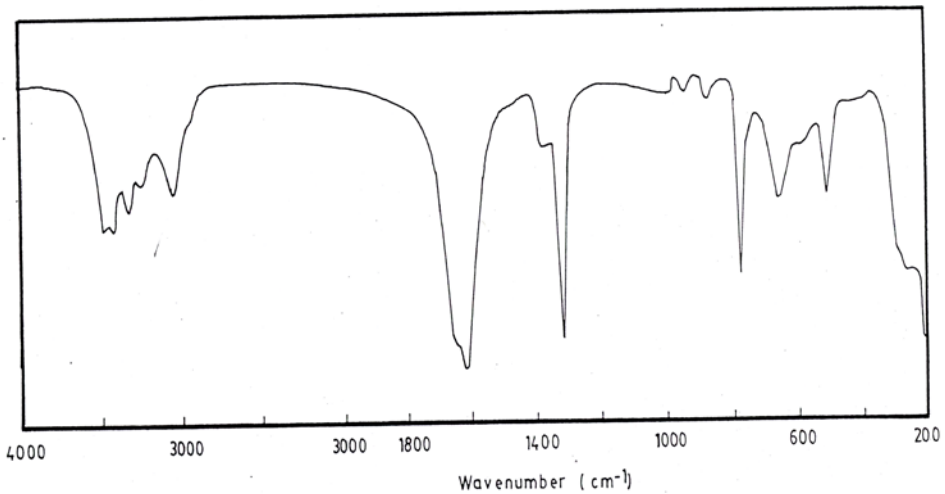


Fig. (2): IR spectrum of calcium oxalate.

Extraction and isolation of different extracts from parsely:

Extraction is used to isolate soluble matter such as crude fat, additives, pesticides and critical minor constituents from complex material. The hexan extract was mold first, secondary chloroform extract, then ethanolic extract and at last aquas extract of parsely, they were made by using (soxtic system HT, 1043. Extraction Unit) made in Evleden. The extraction unit is supplied with eruice unite 1044 with hot oil to achieve solvent evaporation. The tube connecting the extraction and service units is also equipped with an air pump to evaporate the last traces of solvent from extraction cups. The manner of extraction can be summarized as follow:

The fresh plants were dried in shad then crushed, by using the (Micro-Feiniile-Culaui) MFC. Then the samples were inserted in a thimble and then into the extraction unit. The extract was evaporated using rotavap at 60 °C, dried and stored.

Preparation of extract solution:

Chlorofomic and alcoholic extracts were prepared by dissolving in suitable volume of ethanol then completed by deionized distilled water. A suitable volume of saturated solution were taken to prepare different concentrations by dilution. Aqueaus extract was also prepared by dilution of saturated solutions to get different concentrations.

Dissolution experiments:

The crystal dissolution experiments were carried out in water thermostated double-walled pyrex glass vessels. The cells were maintained at the required temperature (37 °C) by circulating thermostated water through the outer jackets. The cell contents were stirred with a magnetic stirrer and presaturated with nitrogen gas bubbled through the solutions during the experiments to exclude carbon dioxide.

In dissolution experiments, a measured volume of deionized, distilled water was transferred to the cell and a known volume of sodium chloride was added, then definite volume of calcium chloride solution was added followed by slow addition of known volume of sodium oxalate solution over a period of five minutes. The total volume was usually 300 ml and the pH was adjusted to the required value (6 $\pm$ 0.05) using standard sodium hydroxide solution or standard hydrochloric acid solution. Satisfactory stability of the undersaturated solution was verified by constant pH reading for at least 30 minutes in experiments using pH-state, and by stability of EMF reading also at 30 minutes in potentiostate experiments. Following the addition of dry seed crystals, dissolution began immediately and combined pH glass

electrode was used to control the addition of titrant solution consisting of 0.15 M sodium chloride in experiments using pH-state, while calcium ion selective electrode in conjunction with calomel reference electrode were used in experiments using potentiostate.

Crystal growth measurements:

Crystal growth experiments were carried out in water thermostated double-walled pyrex glass vessel at (37°C). A measured volume of dc ionized distilled water was transferred to the cell followed by definite volumes of sodium chloride and calcium chloride solutions, then a known volume of sodium oxalate solution was added slowly with constant stirring. The total volume was usually 300 ml and the pH was adjusted to pH = 6.5 $\pm$ 0.05 by using standard solution of sodium hydroxide or hydrochloric acid. The stability of supersaturated solution was verified by constant EMF reading for at least 30 minutes. Then the dry seed was added and crystal growth began immediately. The calcium ion selective electrode in conjunction with Δg / Δg Cl electrode (Model 9050 Metrohm) were used to control the addition of tiirant solution consisting of (2.15×10^{-4} M) calcium chloride and (2.15×10^{-4} M) sodium oxalate solutions with definite volume of 1 M sodium chloride solution.

RESULTS AND DISCUSSION

The rates of dissolution of COM crystals was studied in Fig. (3) indicates the typical plot of extent of Ca^{2+} dissolved in the presence of aqueous extract [Pa(a)], ethanolic extract [Pa(e)] and cloroformic extract of parsley [Pa(c)], as a function of time. The rates of dissolution of COM crystals in the presence of Pa(a), Pa(e) and Pa(c) plotted against [additive] Fig. (4) such plotting shows the influence of Pa(a), Pa(e) and Pa(c) on the dissolution rates. It can be seen that concentration of Pa(a) as low as 10^{-5} ml dm^{-3}, markedly reduce the dissolution rates by at least 64.5 % times compared to that in absence of additive at the same relative under saturation (σ = 0.09) from the same fig. it is seen that the concentration of Pa(e) as low as 10^{-5} mol dm^{-3}, markedly reduced the dissolution rates by at least 60.98 %, and the concentration of Pa(c) as low as 10^{-5} mol dm^{-3}, markedly reduced the dissolution rates by at least 53.98 % times compared with that absence of additive at the some under saturation (σ = 0.09) when the concentrations of the additive was increased, the rates of dissolution decreased due to blocking of active sites on the crystal surfaces by the additive molecules. Adsorption of the molecules at active dissolution sites on the crystal surfaces (Thresnold effect) may be induced through adsorption by much lower concentrations of additive molecules.

The dwretic effect of parsley in flok medicine determines the mechanism of action of the herb. Rats offered on aqueous parsley extract to drink,

eliminated a significantly larger volume of urine per 24 h as compared to when they were drinking water the findings were supported by the results of other experiments in situ kidney prefusion technique which demonstrated also a significant increase in urine flow. Parsley extract, was shown on the other hand to reduce the activation of Na^+, K^+ such an inhibition would decrease apical cellular Na^+ reabsorption hence lowering K^+ secretion decrease K^+ concentration according to Rossl Beier *et al.* [8] referred to the aqueous extract of parsley contains.

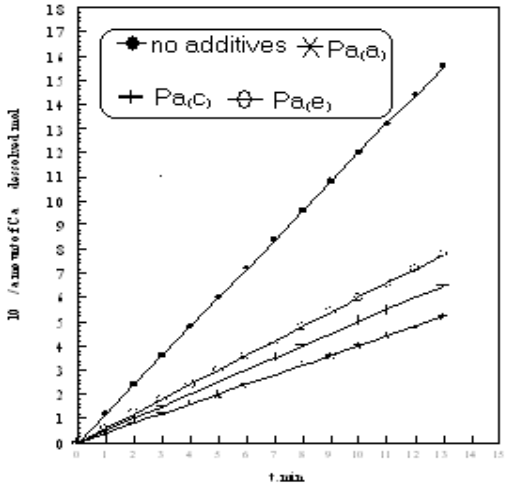


Fig. (3) : Plots of amount of calcium dissoluted against time in the presence of Pa(a), Pa(e), Pa(c).

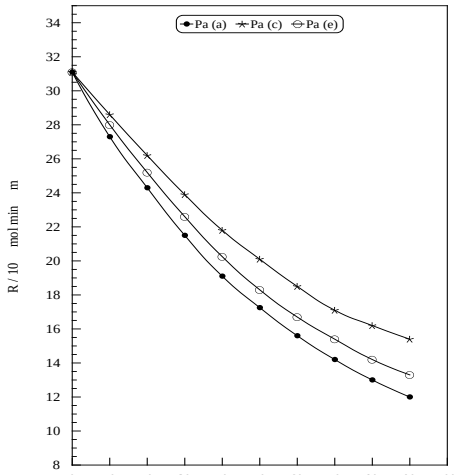
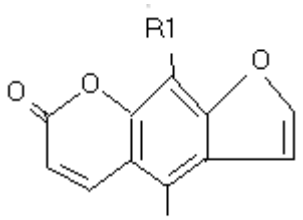
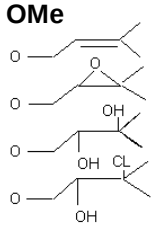
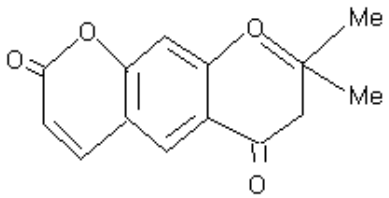


Fig. (4) : Plots of rate of dissolution of COM against [additive] for different extracts of Pa.



Compound	R^1	R^2
1. Psoralen.	H	H
2. Bergapten.	H	OMe
3. Xanthotoxin.	OMe	H
4. Isopimpinellin.	OMe	OMe
5. Isoimperatorin.	H	
6. Oxypeucedanin.	H	
7. Oxypeucedenin hydrate.	H	
8. Saxalin.	H	
9. Graveolone.	H	O



From the previous studies it can be found that the increase of the basicity of amino acids decreased the rate of dissolution of COM previously. It was found that the rates of dissolution of COM crystals at 37 °C in presence of negative charged groups or ions as NH^{2-}, Cl^- or OH^- were effectively reduced[26].

Effect of aqueous extracts of Pa:

The aqueous extract of parsley contain more than hydroxyl group, the presence of –OH group makes good inhibitors, it is also containing $O\text{-}CH_3$ and the presence of $O\text{-}CH_3$ groups increases the inhibitory effect [27]. From

the chemical composition of Pa(a) we expect that it is a good inhibitor for both dissolution and crystallization process of COM.

The presence of ascorbic acid, which may adsorb on Ca^{2+} active sides on the crystal surface leads to decrease the dissolution rate. Both eosin and fluorescein are negatively charged xanthene based heterocycles and have a deprotonated carboxyl group on the phenyl ring at neutral pH (the pH of COM supersaturated growth in solution). It is likely that both dianionic and monoanionic species of these fluorophores are present in the crystal growth solutions. The fluorescence energy maxima are insensitive as exciled state deprotonation of the monoanion compounds as is the case with fluorescence. The negative charge on the fluorophores could be attracted to the positively charged calcium ions of the lattice [23].

Kinetics of calcium oxalate may be interpreted in terms of at least two factors:

1. **Specific adsorption on the crystal surface.**
2. **Sequestration of calcium ion in the solution.**

The applicability of Langmuir model is illustrated in Fig. (5) satisfactory linear relationship is obtained which confirm that the additive is adsorbed at the active sites on the crystal surface. Applying Langmuir equation supports the assumption of surface controlled mechanisms. Chelating anions may be adsorbed at cationic sites and inhibit the dissolution when present at very low levels. The value of adsorption affinity constant, KL is determined to be (0.09×10^5 dm^3 mol^{-1}). The value of KL reflects high adsorption affinity at the same relative under saturation.

Effect of ethanolic extract of parsley:

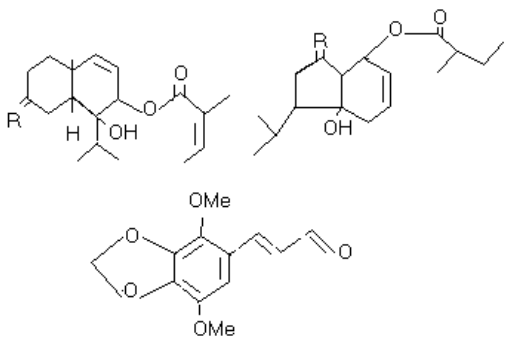
According to Dittrich *et al.* [17] the ethanolic extract of parsley contained Nicotinic acid-N-glycoside and 12-OXO phytodienoic acid and 6-o-melonylapin, celleluse, ethyl format.

The inhibition effect of Pa (e) could be attributed the influence of COO^- group, -N- and $CONH_2$ groups, as is previous studies it was found that COO^-, $CONH_2$, -N- groups acted as good inhibitors [5,10].

The presence of nicotinic acid, cellelluse, ethyl format and malonate of apin, which may get adsorbed on the calcium ion sites on the crystal surface, would lead to decrease of dissolution rate.

Effect of chloroformic extract of Pa:

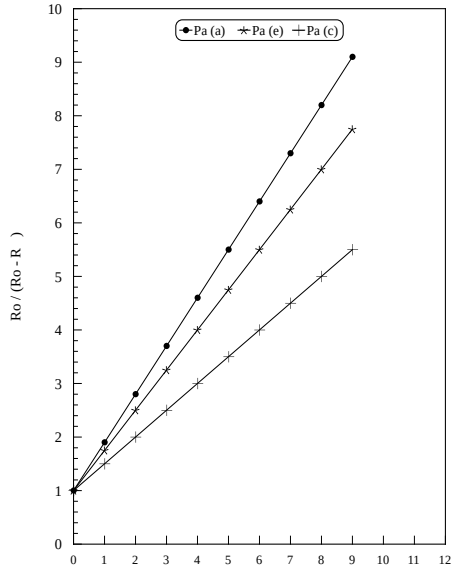
Pa(c) contained the following compounds according to Giovani *et al* [24,15].



R = O

R = H

From the chemical compounds in Pa(c) we can find that the OH and OME groubs in these components may adsorb at calcium ions on crystal surface [26], and blocking these active sites, so inhibit the dissolution of COM crystals.



$(I / C)\ 10^{-6}\ mol^{-1}\ dm^{-3}$

Fig. (5): Plots of Ro / Ro –Ri against (additive) of dissolution of COM in presence of Pa(a),Pa(e),Pa(c) using emf.

Study of the effect of crystallization of COM in the presence of parsely extracts :

Additives of both an organic or inorganic nature play an important role in crystallization processes. It is important to know how the additives influences the crystallization process. The type and number of polar functional groups contained in additives molecule. Hydrophobic and hydrophilic regions, the molecular weight and concentration of additives and a close match between the spacing of acid groups and the spacing of cations of the crystal surface are considered among the factors that influence crystallization. It is proposed that the additives have two functions:

a- They could inhibit crystal growth by binding to the growth sites of the crystals.

b- They could act as a heterogeneous nucleator.

Proteins are known to play a key role in regulating biomineralization by controlling the shape size and often the phase of inorganic crystals [22,1,18,23,29]. Mechanisms by which organic molecules regulate biomineralization processes at both nucleation and growth stages include matrix assisted orientation of the crystal, face selective surface adsorption, and control of the crystal phase. Acidic proteins have been shown to get adsorbed and be overgrown by crystals of calcite, providing evidence that biopolymers do indeed co-crystallize with the mineral constituents of biogenic crystals. The mechanism by which macromolecules incorporate into the lattice of single biomineral crystals remains unknown, and little information is available regarding the structure of incorporated proteins or their orientation with respect to the unit cell of the inorganic crystal. Shedding light on the interactions between proteins with inorganic host lattices will aid in nanoscale materials design including the growth of composite crystals [30].

In general, changes in the rate of crystallization produced by the addition of foreign substances may result either from ocmplexation of the inhibitor, usually a chelating or sequestering agent, with the lattice cation and by adsorption of the molecules at active sites at the crystal surfaces. The latter of "threshold effect" may be induced through adsorption at much lower concentrations of the additive molecules. The influence of the inhibitors on crystal growth must be studied under highly reproducible conditions by the constant. Composition method described by Nancollas *et al.* [9,26], which is employed in the present study. According to the work of Hamza *et al.* [11], the rates of crystallization and dissolution of alkaline earth metal salts are markedly inhibited by the addition of certain organic molecules. Recently attention has been paid to the influence of certain amino acids in the study of COM crystallization [17,31], which act as nucleator modifiers and growth retardants. This takes place through the binding of the amino acid with

calcium or oxalate ions or by its adsorption on the specific crystal faces, a phenomenon, which alters the nucleation rates at the crystal faces[11].

In the present work the rate of crystallization of COM is studied in the presence of different extracts of some natural plants e.g. ethanolic, aqueous and chloroformic extracts of parsely fig. (6) that the rate of crystallization of COM in the presence of ehtanolic, aqueous and chloroformic extracts of parsely decreases with the successive addition of the extracts; the effectiveness of inhibition is in the order:

Pa(a) > Pa(e) > Pa(c)

It is also observed that when the volume of additive increased, the rate of crystallization decreased as shown in fig. (7), it is also indicates the same arrangement of parsely inhibition on COM crystallization as:

Pa(a) > Pa(e) > Pa(c)

Simply, by applying the Langmuir adsorption fig. (8), the influence of parsely extracts can be interpreted in terms of their selective adsorption at the growth sites on crystal surface. The value of KL for Pa(a) was found to be 5.67×10^4 dm^3 mol^{-1} and in Pa(c) to equal 4.35×10^4 dm^3 mol^{-1} and KL for Pa(e) to equal 5.01×10^4 dm^3 mol^{-1}.

The values indicate high adsorption affinity in this order:

Pa(a) > Pa(e) > Pa(c)

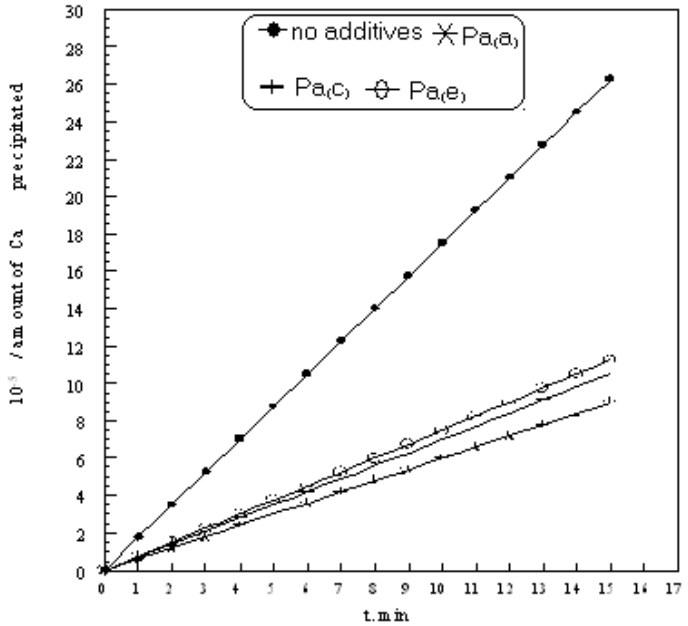


Fig.(6): plots of amount of calcium oxalate preceptitated against time in presence of some different Pa extracts.

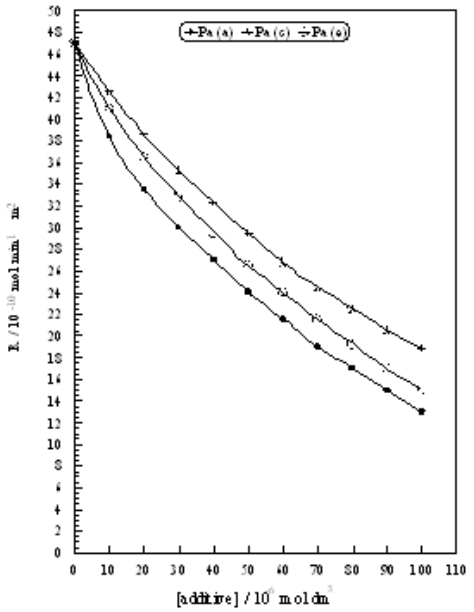


Fig.(7): Plots of rate of crystal growth of calcium oxlate against [additive] for Pa(a), Pa(e), Pa(c).

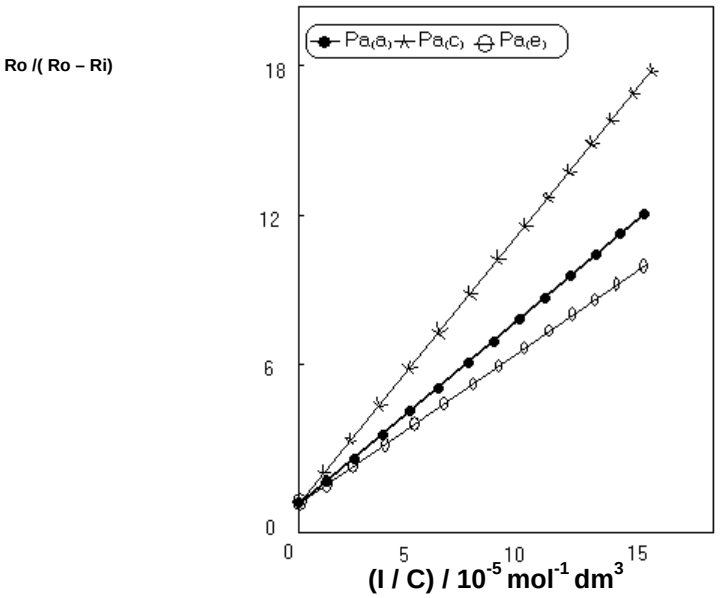


Fig.(8): Values of Ro/Ro-Ri against [additive] of crystal growth of COM in presence of Pa(a),Pa(e),Pa(c) at 37 ^{o}C and σ = 0.4 using emf.

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تأثير المستخلصات المختلفة لنبات البقدونس علي معدل ذوبان وتكوين بللورات اكسالات الكالسيوم

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الملخص العربي

في هذا البحث تم دراسة تأثير مستخلصات نبات البقدونس علي معدل ذوبان وتكوين بلورات اكسالات الكالسيوم حيث تم عمل مستخلصات للبقدونس في اوساط مختلفة من الكلوروفورم والايثانول والماء، وقد تم تحضير تركيزات مختلفة من هذه المستخلصات وكانت النتائج كالتالي :-

١- المستخلصات المختلفة لنبات البقدونس تمتلك خواص بيولوجية تعمل علي تثبيط معدل الذوبان لبلورات اكسالات الكالسيوم .

٢- إن تأثير المستخلص المائي للبقدونس **Pa(a)** يعمل علي تثبيط معدل الذوبان لبلورات اكسالات الكالسيوم اعلي من مستخلص الايثانول **Pa(e)** ثم يلية مستخلص الكلوروفورم **Pa(c)** .

٣- إن تأثير المستخلص المائي للبقدونس علي معدل ترسيب بلورات اكسالات الكالسيوم كان اعلي من المستخلص الايثانولي ثم يليه مستخلص الكلوروفوم.