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Original Article

The effect of some Herbal extracts on the dissolution of calcium oxalate monohydrate

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Abstract

The mechanism of kinetic dissolution of calcium oxalate monohydrate (COM) crystals was investigated in the absence and presence of aqueous extract of *peganum harmala* (Ph) *Ambrosia martiama* (seed) (Am) and *petroselinum Sativum* (Ps) seed, at 37^{o}C and I = 0.15 mol dm^{-3} NaCl using constant composition technique. All experiments was carried out at different relative undersaturation (σ = 0.04 – 0.16). The suggestion of predominately surface Controlled mechanism is also supported by the observed low value of the activation energy Ea = 4.0 kcal/mol. The effective order of dissolution reaction is determined in absence and presence of aqueous extract of additive where parabolic rate low with n – 2. The dissolution was measured and compared with and without the aqueous extracts and the order of inhibition of dissolution was found to be ph > Am>Ps respectively.

Key word: dissolution, inhibition, calcium oxalate monohydrate and herbal extracts.

Introduction

The mechanism of calcium oxalate renal calculi formation has attracted the medical scientists because its widespread clinical occurrence and difficulty of treatment [1] .The exact mechanism of the formation of calcium oxalate stones is not completely understood and a number of questions about promoting or inhibiting factors still remain unanswered [2]. The main difficulty encountered in the study of all dissolution processes is the understanding of the rate controlling step. Two phenomena occur simultaneously in the dissolution of a crystal in aqueous solution. First the crystal surface in contact with the solution is disturbed into substances which are liberated at the interface and dissolved; this is called the solid surface process. Secondly, aggressive substances are transported from the solution to the surface through interfacial layer while dissolved substances are transported to the bulk of solution this is generally called bulk transport or solution diffusion process. A number

of studies have been carried out to understand the effect of various additives such as amino acid [3], carboxylic acids [4], plant extracts [5] and metallic ions [6].

In the present work, we have obtained the effect of aqueous extracts of Ph, Am and Ps on the inhibitory activity dissolution of calcium oxalate monohydrate. Dissolution rate were measured over range of undersaturation which reflect the percentage of dissolution inhibition of COM.

Material and Methods

Solutions were prepared from analytical grade chemicals (El Nasr – Pharmaceutical Chemical Company) and distilled deionized water. Calcium chloride and sodium oxalate solutions were analyzed by passing aliquots through ion exchange resin (Dewix-50) in the hydrogen form and titrating the eluted acids with standardized sodium hydroxide solution of suitable concentration using phenolphthalein as indicator. All solutions prepared and stored in Pyrex vessels. Calcium oxalate seed was prepared in details by adding 1L of (0.01 M) CaCl2 solution to 1L of sodium oxalate (0.01M) at 25 ^{0}C at a rate of 500 ml/hour. The sodium oxalate solution was constantly stirred throughout the addition. The seed suspension was allowed to age with stirring for 24 hours then filtered and the seed crystals were washed with deionized distilled water to remove surface contamination due to chloride and oxalate ions. The seed crystals were aged for 30 days then refiltered and carefully washed with deionized distilled water and this process was repeated several times. The seed was then filtered and dried. The seed crystal was characterized as $CaC_2O_4 . H_2O$, by x-Ray powder diffraction (copper K x radiation, Phillips XRG 3000 Diffractometer) fig (1), and Thermo gravimetric analysis fig (2). Particle sizes, measured by single point BET nitrogen adsorption at 77 k was 3.37 m^2g^-1.

Fig. (1), X-ray difractogram of calcium oxalate monohydrate crystals.

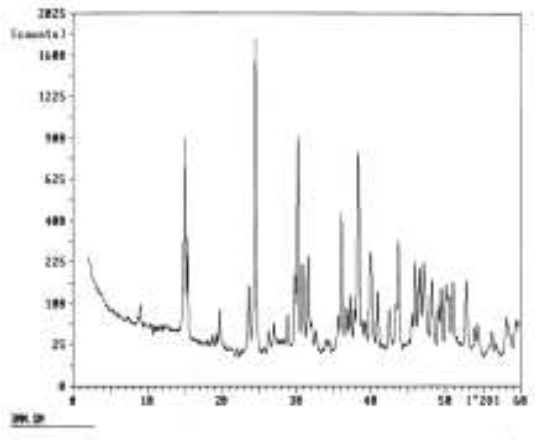
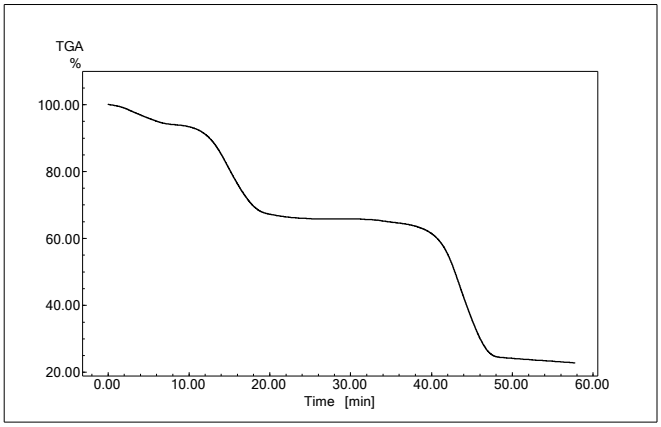


Fig. (2). TGA thermogram of calcium oxalate crystals.



Techniques

Dissolution experiments were carried out in double walled Pyrex glass vessel thermostated at 37 ± 0.1 C. The vessel contents were stirred in presence of nitrogen gas bubbling to exclude atmospheric CO_2.

A measured volume of deionized distilled water was transferred to the cell and a known volume of NaCl was added, then a definite volume of calcium chloride solution was added, then a 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 (6.5 + 0.05) using standrized sodium hydroxide solution and/or hydrochloric acid solution. Satisfactory stability of undersaturation solution was verified by constant emf reading for at least 30 minutes. Samples were periodically with draw and filtered through Millipore filters (0.22 M) for solution analysis.

Result and Discussion

Dissolution reactions are very important in technical and physiological systems. An understanding of the mechanism of dissolution of COM is important in elucidating the factors leading to renal stone formation.

In the present work, the rates of dissolution of COM (K_{so}= 9.899x10^{-8}) have been investigated at 37˚C in absence and presence of trace amounts of natural products from medicinal plants in undersaturated solution.

The relative undersaturation, σ, is defined by

$$\sigma = (\pi_0^{1/2} - \pi^{1/2}) / \pi_0^{1/2} \qquad (1)$$

In which π is the molar concentration product of calcium oxalate, $[Ca^{2+}]$ $[O_x^{2-}]$, in the solution and π_0 the solubility value at the same ionic strength (0.15 mol L^{-1} NaCl in the present work .In order to study the kinetics of dissolution reaction of calcium oxalate monohydrate, it is necessary to take into account the association of calcium and oxalate ions in equation (2).

$$Ca^{2+}{}_{(aq)} + C_2O_4{}^{2-}(aq) \longrightarrow CaC_2O_{4(aq)} \qquad (2)$$

The thermodynamic association constant given by:

$$K = \frac{[CaC_2O_4]}{[Ca^{2+}]\,[C2O^{2-};_4]} \cdot \frac{1}{f^{2};_Z} \qquad (3)$$

Where f_z is the activity coefficient of a divalent cation and anion. The relative undersaturation σ, for a solution containing equal calcium and oxalate ions (present work) can be defined:

$$\sigma = \left(\left[Ca^{2+}\right]_t - \left[Ca^{2+}\right]_{eq}\right) / \left[Ca^{2+}\right]_{eq} \qquad (4)$$

The subscripts "t" and "eq" are values at time "t" and at equilibrium respectively. The thermodynamic solubility product :

$$K_{sp} = [Ca^{2+}]\,[C_2O^{2-};_4] \,.\, f^{2};_z \qquad (5)$$

Where :

f_z : is the activity coefficient of divalent ion

The activity coefficients of divalent cation and anion were assumed to be equal and were obtained using the extended Debye Huckel equation proposed by Davis [7].

$$-\log f_z = AZ^2\left[\sqrt{\frac{I}{1+I}} - 0.31\right] \qquad (6)$$

Where: Z: is the charge on the ion (valence), I: is the molar ionic strength and A: is constant.

Dissolution experimental conditions are summarized in Table (1) in which T_{Ca} and T_{ox} are the total molar concentration of calcium and oxalate respectively. The dissolved amount of calcium oxalate

monohydrate was calculated from the titrant addition where the slope of these lines reflects the rates of dissolution are summarized in Table (1).

Table (1) : Dissolution of calcium oxalate crystals T_{ca}+2 : T_{ox}-2= 1 : 1 at t = 37°C and ionic strength 0.15 mal dm^{-3} (NaCl) using EMF.

Exp No.	Tca^{+2} / 10^{-4}	10^2 σ	Seed /mg	Rate /10^{-9} mol min^{-1} m^2
10	1.914	4	10	2.250
11	1.894	5	10	3.310
12	1.854	7	10	5.903
13	1.814	9	10	8.740
14	1.794	10	10	10.682
15	1.774	11	10	12.315
16	1.735	13	10	16.513
17	1.695	15	10	20.208
18	1.814	9	50	8.750
19	1.814	9	90	8.735
20	1.814	9	300	8.721
21	1.814	9	500	8.715
22	1.814	9	700	8.711
23	1.814[a]	9	10	8.740
24	1.814[b]	9	10	8.751

a)Stirring rate (200) r.pm. b) Stirring rate (500) r.pm

For many sparingly soluble salts, M_a and A_b the rate of dissolution, normalized for surface area, can be expressed by equation 7. [8].

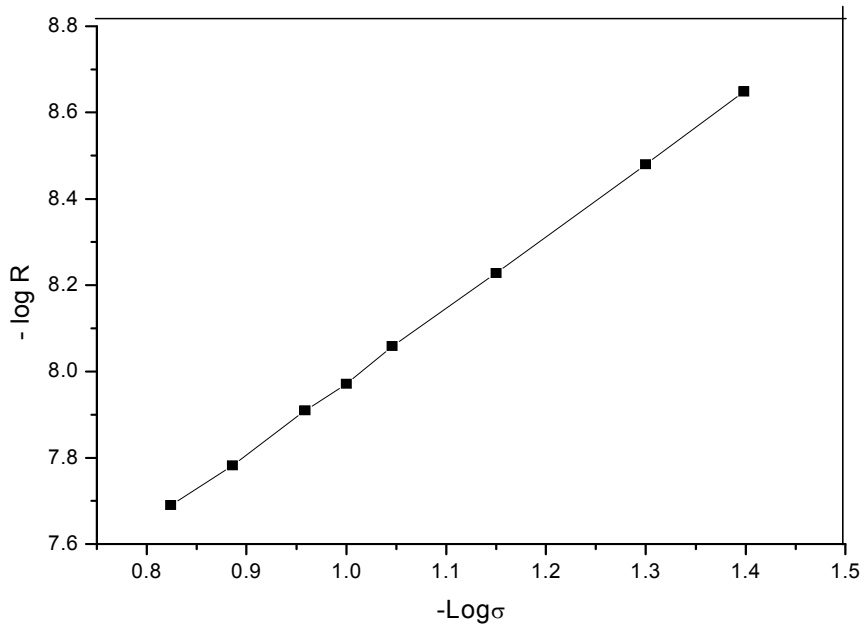
$$R = d [Ma Ab] / dt = Ks\sigma^n \quad (7)$$

In which K is the dissolution rate constant, s is proportional to the number of dissolution sites available on the seed crystals, and n is the effective order of reaction, determined from the slope of typical plots of – log R against a – log σ as shown in Table (2), Fig (3).

Table (2) : Effect of undersaturation, σ , on the rate of dissolution of calcium oxalate crystals at t = 37 ˚C.

Exp No.	$10^2\,\sigma$	-log σ	Rate /10^{-9} mol min^{-1} m^2	-log R
25	4	1.3980	2.250	8.648
26	5	1.3000	3.311	8.480
27	7	1.1500	5.904	8.228
28	9	1.0460	8.740	8.059
29	10	1.0000	10.682	7.971
30	11	0.9586	12.315	7.910
31	13	0.8861	16.513	7.782
32	15	0.8240	20.209	7.690

Fig(3): Plots of –Log R against –Logσ for dissolution of calcium oxalate monohydrate at 37°C



It was found that the order of dissolution of COM crystal was n ~ 2 suggesting surface controlled mechanism over a range of relative undersaturation 0.04 - 0.16. The suggestion of predominantly surface. Controlled process may also be supported by the observed independence of the rates of dissolution of COM crystals on the changes in the rate of stirring (fluid dynamics) as shown in Table (1) expts [(23,24)]. However, this evidence may be inconclusive for such small particles for which changes the stirring rate may have little influence on the fluid shear forces at crystal surfaces shown in Table (1). The particles will tend to move with the fluid flow. The rates of dissolution of COM crystals were found to be affected by the weight of inoculating seed used to initiate the dissolution process which may confirm the surface controlled mechanism.

It has been observed [9], that there was a change with mechanism of the dissolution of calcium oxalate monohydrate from diffusion to surface controlled reactions as the degree of saturation was decreased. In general, the rates of crystallization and dissolution of COM [10-12], and divalent metal ion salts [13–14] are markedly inhibited by added substances. The possible inhibitory effects of some water extracts from medicinal plants on the dissolution rate of COM were investigated. Additives may play an important part in the theory of supersaturated and undersaturated solutions, the effects of *peganum harmala* (Ph), *Ambrosia maritime* (Am) and *petro selenium sativa* (Ps) extracts on the rates of dissolution of COM were studied. The additives may have a number of effects on the process of precipitation and dissolution of COM crystals, this effect are: (i) interact chemically with the crystal surface to form complexes. (ii) Change in the characteristics of the adsorption layer of solid solution interface. (iii) Being adsorbed on the crystal surface and physically block the active dissolution sites. (iv) Alter the surface charge or surface energy of the crystals. In general a specific inhibition of the rate of dissolution is expected to take place at much lower concentration of the additives molecular than for simple complexation.

The effect of Ph, Am and Ps on the rates of dissolution of COM crystals at 37 C, σ, 0.09, I = 0.15 mol dm^{-3} and stirring rates of 200 r.p.m, were studied. The results are listed in Table (3).

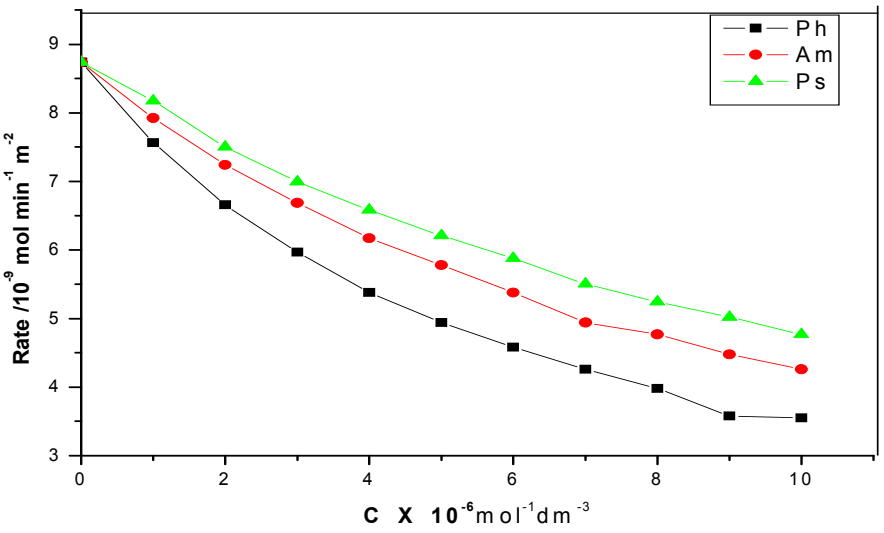
Table (3) :The effect of degree of undersaturation on the rate of dissolution of COM crystals in presence Ph, Am, Ps., at t=37 ˚C , Ionic strength I = 0.15 mol dm^{-3} σ =0.09.

Exp No.	T_{Ca} / 10^{-4} mol dm^{-3}	[Gg]/10^{-6} mol dm^{-3}	$[Gg]^{-1}$ / 10^{-5}	Rate /10^{-9} mol min^{-1} m^{-2}	R_0 / R_0 - R_I
100		-	-	8.740	-
101	1.814	1.00 Ph	10.000	7.567	7.450
102	1.814	2.00 Ph	5.000	6.659	4.200
103	1.814	3.00 Ph	3.333	5.966	3.150
104	1.814	4.00 Ph	2.500	5.379	2.600
105	1.8154	5.00 Ph	2.000	4.940	2.300
106	1.814	6.00 Ph	1.6700	4.578	2.100
107	1.814	7.00 Ph	1.4300	4.258	1.950
108	1.814	8.00 Ph	1.2500	3.981	1.836
109	1.814	9.00 Ph	1.1000	3.575	1.692
110	1.814	10.00 Ph	1.000	3.551	1.684
111	1.814	1.00 Am	10.000	7.924	10.700
112	1.814	2.00 Am	5.000	7.241	5.830
113	1.814	3.00 Am	3.333	6.689	4.260
114	1.814	4.00 Am	2.500	6.170	3.400
115	1.8154	5.00 Am	2.000	5.778	2.950
116	1.814	6.00 Am	1.670	5.379	2.6
117	1.814	7.00 Am	1.430	4.940	2.299
118	1.814	8.00 Am	1.250	4.768	2.200
119	1.814	9.00 Am	1.100	4.477	2.049
120	1.81 4	10.00 Am	1.000	4.258	1.949
121	1.814	1.00 Ps	10.00	8.o73	13.100
122	1.814	2.00 Ps	5.000	7.501	7.050
123	1.814	3.00 Ps	3.333	6.992	5.000
124	1.814	4.00 Ps	2.500	6.582	4.050
125	1.814	5.00 Ps	2.000	6.207	3.450
126	1.814	6.00 Ps	1.670	5.875	3.050
127	1.814	7.00 Ps	1.430	5.503	2.700
128	1.814	8.00 Ps	1.250	5.244	2.500
129	1.814	9.00 Ps	1.100	5.021	2.350
130	1.814	10.00 Ps	1.000	4.768	2.200

The rates of dissolution of COM crystals in the presence of aqueous extract of Ph and Am and Ps plotted against [additive] are shown in Fig (4), such plotting indicate the influence of different concentration of additives on the dissolution rates from the fig (4), we can seen that when concentration of additives was increased the rate of dissolution rates decreased due to blocking of the active sites on the crystal surfaces by the additive molecules of extracts. Table (4) indicates that when concentration as low as 10^{-6} the percentage of inhibition of dissolution of COM crystals are 51 .

36, 51 . 28 and 47.62 for Ph, Am and Ps respectively. The results suggesting the order of inhibition follow the sequence: Ph > Am > Ps

Fig. (4). Plots of the rate of dissolution of COM against additive for Ph, Am, Ps at σ = 0.09



A specific inhibition of the rate of dissolution is expected to take place at much lower concentrations of the additives molecular than for simple complexation.

Anions of additives molecule inhibit the rate of dissolution by adsorption on the cationic sites on the surface. The adsorption of anions on the surface reducing the number of empty sites to receive dissolving lattice oxalate anions.

By similarly:

Ca^{2+} (sol) ⟶ Ca^{2+} (ads) (8)

$O^{2-}{}_{;x}$ (sol) ⟶ $O^{2-}{}_{;x}$ (ads) (9)

Ca^{2+} (ads) + $O^{2-}{}_{;x}$ (ads) ⟶ CaC_2O_4 (ads) (10)

CaC_2O_4 (ads) ⟶ CaC_2O_4 (lattice) (11)

The inhibitor might be specifically adsorbed at lattice sites and thus preventing the transfer of COM units between the adsorbed state on the crystal lattice as in step:

$$Ca\ C_2O_4\ (ads) \longrightarrow CaC_2O_4\ (lattice) \qquad (12)$$

In general, inhibitor molecules exert their influence through adsorption at active dissolution sites on the crystal surfaces. Chelating anions may be adsorbed at cationic sites and inhibit the dissolution when present at very low levels.

The major constituents of *peganum harmala* include three alkaloids harmalin, harmin and harmalal and B-carbolin alkaloide harmalini [15, 16]. Also, B-carbolin lactams harmalanine and harmalacidine [17], flavonids [18], oxamid [19] and anthraquinone glucoside [20, 21], isolated from seeds of *peganum harmala*. Damsin, ambrosin,parthenin,neoambrosin,minor lactones[22], Ambrosin , Damsin and sesquiterpene lactone isolates of *Ambrosia maritima L* [23], coumarin [24]. Aqueous extract of *Petrosalnium Sativa L* has glycosidea flavonoids such as apiine , apiol, apigenin [25], and the flavonoides have antioxidant effects [26] .

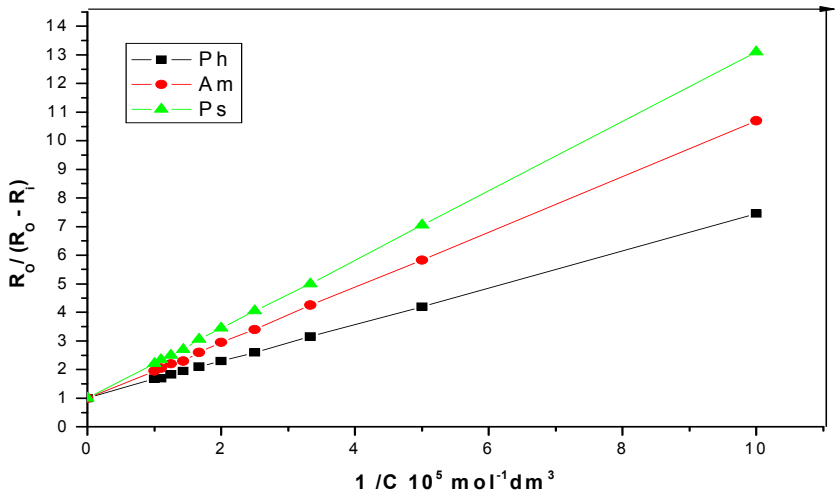
These bimacromolecule seem to play an important role in the inhibition of COM crystals. It can be seen that the aqueous extract of peganum harmala Ambrosia Martina and petroselinum sativa produce significant amount of inhibition on the dissolution of COM crystal as shown in Fig (4). It may be assumed that the interaction between the COM and the additives depends on their hydrophobicity and electrostatic attraction between anionic and cationic sites on the surface of COM crystal groups. The polyelectrolyte and macromolecules presence in biological fluids may adsorb on the active dissolution sites on crystal surfaces blocking them and thus directly influencing the rates of dissolution [27].

Adsorption of additive in the process of dissolution strongly depends on the nature of substrate. The amount of bound dissolution factor increased almost linearly with its concentration in the solution. The adsorption can be interpreted in terms of a Langmuir-type isotherm [28], leading to an equation of the form.

$$R_0 / (R_0 - R_i) = (K_L C)^{-1} \qquad (13)$$

In which R_i and R_0 are the rates of dissolution in the presence of inhibitor respectively, K_L is the adsorption affinity, and C is the concentration of additive. Typical adsorption plots according to eq (13) in fig. (5), and table (3) conform the applicability of this simple adsorption isotherm at all undersaturation studied.

Fig. (5) Plot of $R_0/(R_0 - R_i)$ against [1/C]of dissolution of COM in presence of Ph, Am, Ps at σ = 0.09.



The values of the adsorption affinity constant K_L are1.64 x 10^6 , 1.03x10^6 and 8.33 x 10^5 $mol^{-}1$ for peganum harmala, Ambrosia maritime and petro selenium sativa at relative undersaturation σ = 0.09, the values reflect the high adsorption affinity at low undersaturation in the presence of Ph and Am inhibitors. The observed increased of inhibition of surface controlled dissolution with decreasing undersaturation is especially interesting if inhibitor molecules are adsorbed at active sites on the crystal surface between the advancing dissolution steps, the reaction can proceed provided that the adjacent adsorbed molecules are separated by a distance greater than that of the critical each pit.

The effect of inhibitor may be described as the prevention or strong retardation of the nucleation of each pit in areas around the adsorbed inhibitor molecules. Due to interaction with inhibitor, lattice ions in these areas will be strongly attached to the crystal surface. It sufficient inhibitor molecules are adsorbed on to the surface, the whole crystal may be inactivated and no dissolution will occur. Prevention of retardation of dissolution may occur by preferential adsorption of the inhibitor molecules at the edges of the subcritical each pits forming on the surface thus preventing their development by beyond the critical size. The linear relationship and intercept of unity, strongly suggest that the mechanism of COM formation or dissolution may be the formation of a monolayer blocking layer of additive ions at the dissolution sites on the crystal surface of COM. So, the additives act as inhibitor through adsorption on to the active sites on the crystal surface of COM.

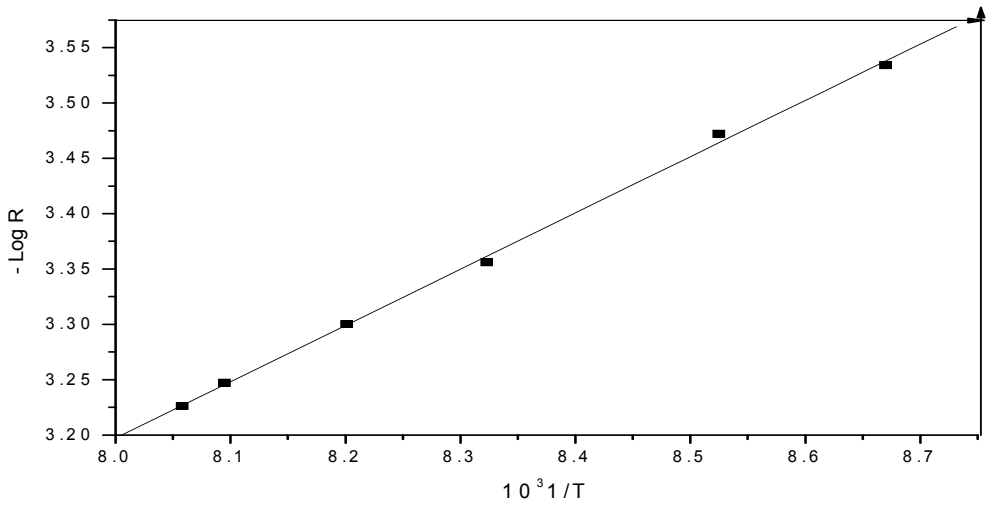
The effect of temperature on the rate of dissolution of COM in the presence of aqueous extracts of *peganum harmala* L., at σ = 0.09, PH=6.5 ±0.05 are illustrated in table (4), plotting 1/T versus log R as indicating in fig. (6), linear relationship obtained. The activation energy, the slope of line, for

dissolution process is found equal 4.0 K. cal/mol [29]. The small value of activation energy and the independence of the rate of dissolution on the mode and stirring rate rule out bulk diffusion of electrolyte to the crystal surface as rate determining step and support the suggestion of surface controlled mechanism.

Table (4) : Effect of temperature on the rate of dissolution of calcium oxalate crystals at σ = 0.09, I = 0.15 mol dm^{-5}.

Exp No.	T/K	Rate /10^{-9} mol min^{-1} m^2	-log R	10^3 K/T
33	310	8.740	8.058	3.226
34	308	8.041	8.0947	3.247
35	303	6.293	8.201	3.300
36	298	4.757	8.323	3.356
37	288	2.989	8.525	3.472
38	283	2.164	8.665	3.534

Fig. (6). Plots of –log R against 1/T for dissolution of calcium oxalate monohydrate crystals σ = 0.09 in the presence of (ph)



It obvious from the results obtained in the present work the occurrence of calcium oxalate calculi in the body is more complex phenomenon occurring under dynamic condition in which urine

continuously flows. This in vitro study provides basic information to identify the potent inhibitors. Both of these aqueous extracts contain many complex macro-bi molecules; these molecules give high inhibition effect in the dissolution of COM.

Conclusion

Dissolution of COM crystals was studied using constant composition method. It was found that the dissolution of COM follows surface controlled mechanism. The inhibition of dissolution of COM crystal by the effect of aqueous extract of Egyptian herbal medicinal plants peganum harmala, Ambrosia maritime and petroselinum sativa was studied, the aqueous extract of peganum harmala produced maximum inhibition of COM crystal dissolution followed by the aqueous extract of Ambrosia maritime, in vitro conditions

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