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IN VITRO RELEASE PROPERTIES OF CAFFEINE.I.EFFECT OF CONCENTRATION AND TYPE OF OINTMENT BASE*

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ABSTRACT

Caffeine has recently been found to cure atopic dermatitis, presumably by increasing skin levels of cAMP.In the light of these findings, its release from different ointment bases at varying concentrations was investigated in vitro. The ointment bases used were a petrolatum (named as petrolatum A), a PEG ointment (USP XVIII), a hydrophilic ointment (USP XVIII), and a w/o type emulsifying ointment. It was incorporated into ointment bases at 1,5,10,20 and 30 % (w/w) concentrations, by simple trituration technique.

Release experiments were carried out at 37°C,with diffusion cells which were placed in distilled water filled beakers.

For all caffeine concentrations used, the release was highest from the PEG ointment. It decreased with the hydrophilic ointment, the emulsifying ointment, and

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petrolatum A, in that order. From both petrolatum base and the PEG ointment, release of caffeine increased significantly with increasing concentrations. As for the hydrophilic and emulsifying ointments, release patterns were found to be independent of concentration for some percentages of caffeine.

INTRODUCTION

It has been found recently that caffeine can be used to cure atopic dermatitis (1,2). Its supression of immediate hypersensitivity reactions was explained with its β-adrenergic effect (3). By local application, it probably inhibits cyclic nucleotide phosphodiesterase which degrades cyclic adenosine -3',5'-monophosphate (cAMP), thus increases levels of cAMP (4,5). This increment therefore eases the skin problems of atopic subjects (3,6).

This effect was firstly reported by Kaplan et.al. (1,2,3) where caffeine was used at 10 to 30% concentrations in hydrophilic ointment. They have not given the particulars of neither the method of preparation nor the choice of concentration and the ointment base.

Knowing the dependence of efficacy of an ointment on the type of ointment base(7-16) and the concentration of the drug (7,13,17-19), it was felt that the topical application of caffeine from different ointment bases at varying concentrations deserved further investigation. First of all, its release from different ointment bases was planned to be looked into in vitro.

This paper describes the method of in vitro release of caffeine from an oleaginous (petrolatum),two emulsion type (o/w and w/o) and a water-soluble (PEG

ointment) base at 1,5,10,20 and 30 % (w/w) concentrations. It also compares release patterns and released amounts of caffeine for obtain an optimum condition for in vivo applications.

EXPERIMENTAL

Materials

An anhydrous caffeine[1] was used throughout the experiments. IR spectrum of caffeine exhibited inflections only at the same frequencies as the reference standards. Its UV spectrum showed maxima and minima similar to those of reference standards given in the literature and the absorptivity of the sample at the maximum near 272 nm is within the same height of the absorptivity generally reported. Its melting point was 236°C. The rest of the purity controls according to Pharmacopeia standards were found to be satisfactory.

The particle size distribution of caffeine, determined by sieving was found to be within the 45-180 µm range. However the majority of particleswere around 180 µm.

Deionized glass distilled water was used for the experiments. Its pH was 6.0 and conductivity of 1.1 µMHOS.

A petrolatum of unknown source, named as petrolatum A was used as an oleaginous base. It was in accordance with USP XVIII requirements. Propylene glycol[1], polyethylene glycol (PEG) 400[2], PEG 4000[1], liquid paraffin[1], 1-octanol[1], sorbitan mono oleat[3], sodium lauryl sulphate and stearyl alcohol used without further purification. The dialysis membrane[4] used was from the same roll for all the experiments.

Solubilitiy Determinations

The solubility of caffeine was determined in water at 25°C. About 400 mg of caffeine was placed in 15 ml of water, in 50 ml screw capped tubes. The tubes were placed horizontally on a shaker in a water-bath, and shaked at 95 rpm until the equilibrium was reached. This stage was determined by repetitive sampling. The concentration determination was made by UV absorption measurements.

Solubility of caffeine in PEG 400 and liquid paraffin were determined because they were the major components of different type of ointment bases and the solubility of caffeine in these vehicles could give an indication to its solubility in other similar semisolid or solid components of the ointments used.

These solubility determinations were made by incorporating known amounts of material in increasing amounts in a known quantity of solvent. The solubility values of caffeine in the PEG 400 and liquid paraffin were accepted as the highest concentrations that could be completely solubilised in the referred solvents.

Partition Coefficient

10 ml of 1-octanol was added to 10 ml of 0.0824 M caffeine solution, in 50 ml screw-capped tubes. The tubes were shaken on a horizontal shaker at 37°C until no difference was observed between the repetitive samplings. The water and octanol phases were separated and assayed for caffeine concentration.

The results of these experiments are shown in Table 1.

Preparation of Ointments

The ointments were prepared by simple trituration. Petrolatum A was used as supplied. Hydrophilic and

TABLE 1

Some Physicochemical Properties of Caffeine

Solubility	
In water (25°C)	0.103 M
In PEG 400 (37°C)	1 %
In liquid paraffin (37°C)	0.001 %
Partition coefficient (1-octanol/water)	0.464

polyethylene glycol (PEG) ointments were prepared according to USP XVIII requirements. In hydrophilic ointment preservatives were not used to prevent a possible interference. The water in oil (w/o) type emulsion base that contains 64 parts white petrolatum, 6 parts sorbitan monooleat (Span 80) and 30 parts water was prepared according to the method given by Whitwoth (19).

Incorporation of caffeine powder to the ointment bases was carried out using a mortar and pestle. In order to obtain 1,5,10,20 and 30 % (w/w) concentrations, caffeine was accurately weighed and powdered in a mortar. Freshly prepared ointment base was incorporated on to it by geometric dilution until a homogeneous mixture was obtained. The mixture then was left at ambient temperature for sixteen hours before it was placed in the diffusion cells. All the ointments prepared were of suspension type.

During the diffusion measurements, to control a possible interference of the ointment bases with that of caffeine, ointments having no caffeine were prepared to be used as blanks.

Diffusion Cell :

Diffusion cell which was used in this study was similar to that of Ayres and Laskar(13). It was made of

inert glass with 1.8 cm of internal diameter and 1.5 cm of depth. A 6.0 cm glass rod was attached to the back of the device from the center in order to suspend it into diffusion medium.

The diffusion cell was placed at the center of a beaker with a 4.5 cm diameter and 8.0 cm depth. The diffusion surface was placed at 3.0 cm height from the bottom of the beaker. The beaker was filled with 100 ml of distilled water and its top was covered with a watch glass to prevent possible evaporation. A small hole was bored at the center of the watch glass through which the glass rod extruded. The exposed part of the glass rod was held tight by means of a piece of sponge hence the movement of the device was prevented throughout the experiment. Whole system was placed in a 37^{o}C water bath (Figure 1).

Procedure

The diffusion cell was filled with each ointment to the top by means of a spatula to ensure that there are minimum amount of air bubles left in the system. The excess was removed using a spatula to obtain an even surface. Then a cellophane membrane which was left in distilled water for sixteen hours, was placed on the surface of the ointment. It was carefully pressed to ensure a complete contact of the membrane with the ointment and tightly secured.

The device was then immediately placed in the beaker and the whole system was placed in a water bath. No attempt was made to stir the "receiving sink" during the experiments except at the times of sampling.

Caffeine concentration in distilled water at definite time intervals was the measured spectro - photometrically at 272 nm. For this, 1.0 ml samples

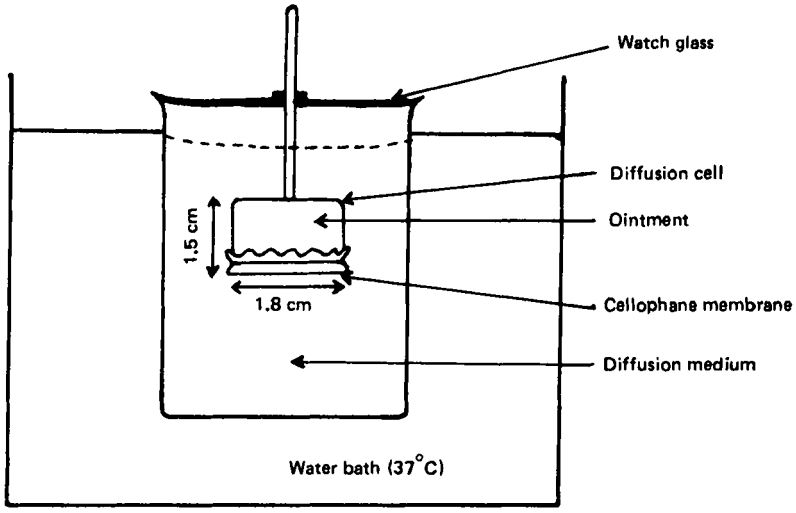


FIGURE 1
Schematic Diagram of Diffusion Apparatus Used For Release Experiments.

were withdrawned by raising the top at first 15 and 30 minutes and later at hourly intervals up to six hours. The system was stirred quickly at each sampling and was replaced, after the addition of 1.0 ml of distilled water to its original position. The dilutions were made for direct spectrophotometric readings as found necessary.

Black runs demonstrated that there were no interferences of the bases at spectrophotometric readings of 272 nm. Stability controls showed that there were no measurable degradation of caffeine during the experimental period.

Repeatibility of the Experiments

Repeatibility of the experiments was controlled using petrolatum base with 10 % caffeine (Table 2).Each experiment was repeated six times. The variation coefficients of the first 15 and 30 minutes sampling were 18 and 9. Beginning from second hour the variations decreased to 5 and stabilised thereafter.

TABLE 2

Repeatibility of the Experiments

Time	Mean of the Amount of Released Caffeine ± S.E(n = 6)	Variation Coefficient
15 Min.	5.33×10^{-6} M ± 0.39×10^{-6}	18.2
30 Min.	6.73×10^{-6} M ± 0.25×10^{-6}	9.3
1 Hr.	8.06×10^{-6} M ± 0.28×10^{-6}	8.6
2 Hr.	1.23×10^{-5} M ± 0.03×10^{-5}	6.4
3 Hr.	1.50×10^{-5} M ± 0.03×10^{-5}	5.6
4 Hr.	1.80×10^{-5} M ± 0.03×10^{-5}	4.3
5 Hr.	1.91×10^{-5} M ± 0.03×10^{-5}	4.5
6 Hr.	2.12×10^{-5} M ± 0.04×10^{-5}	5.1

n: Number of experiments.

To reduce possible errors which might be introduced by the measuring technique, each experiment was repeated 5 to 15 times whenever necessary. The means for each set of measurements were calculated and these were used to plot the respective curves.

Statistical Analysis

Release results obtained with all bases at varying caffeine concentrations were compared statistically using Student's T test to asses the differences between the experimental points corresponding to identical values on the time axis.A significant difference between the preparations was accepted to exist if all the experimental points were different except the first three points at the 5 % level.

In addition, all the results obtained with different ointment bases at varying concentrations were investigated to find the best curve fitting by computer. Therefore all the release data were fitted to normal,

log-log and q versus $\sqrt{t}$ curves to obtain a satisfactory fitting for all the available data.

RESULTS AND DISCUSSION

Release rates of the insoluble drugs in their ointment bases generally follow the well-known equation of Higuchi (20,21) which is,

$$Q = \sqrt{Dt\ (2A-C_s)C_s} \qquad \text{Eq.1}$$

In this equation Q defines the amount of drug released per unit area at time t, A is the concentration of drug expressed in units/cm^3, C_s is its solubility as units/cm^3 in the external phase of the ointment and D is the diffusion coefficient of drug molecule in the external phase. Because in the ointments of the suspension types, the concentration of the drug is generally greater than its solubility ($A > C_s$), the equation given above can be more simplified to give Equation 2,

$$Q = \sqrt{2\ ADC_s t} \qquad \text{Eq.2}$$

which theoretically proves that the amount of drug released is proportional to the square root of the amount of drug in per unit volume, diffusion coefficient, solubility of the drug and the time.

Therefore when Q versus t are drawn, generally straight lines are obtained, but they are not always linear (9,19). When logarithm of the amount of drug released is plotted against the logarithm of diffusion time (22) or the amount released versus the square root of the time, better fittings are seen in the literature (7,13,15).

Therefore the present results were fitted to the equations given above by means of a computer and

straight lines with highest correlation coefficients were obtained when the amount released is plotted against the square root of the time.

The release from PEG ointment was an exception to that for which, the log-log fitting could be satisfactorily used. Therefore, it can be suggested that the release from all the investigated ointment systems are vehicle controlled with possible exception of PEG ointment.

Effect of Concentration on the Release Properties of Caffeine

The release of caffeine from petrolatum A is given in Figure 2. The dependence of release rate and released amounts on caffeine concentration is clearly seen in this figure. According to the statistical evaluation, the released amounts for respective concentrations of caffeine are significantly different from one another.

Figure 3 shows the release from a w/o type emulsion ointment. In these release curves, the amount of released caffeine increased up to 10 % concentration. In these series, there is no significant difference between the released amounts of 10 and 20 %, 10 and 30 % and 20 and 30 % concentrations.

The release from the hydrophilic ointment is given at Figure 4. Although a concentration dependence of release is followed up to 10 % caffein concentration, statistical evaluation of this data showed no difference between 5 and 10 %, 5 and 20 % and 10 and 20 % concentrations. Release from the hydrophilic ointment which contained 30 % caffeine showed a significant decrement.

On the other hand, PEG ointment follows the same general trend (Fig.5). In this ointment base, an

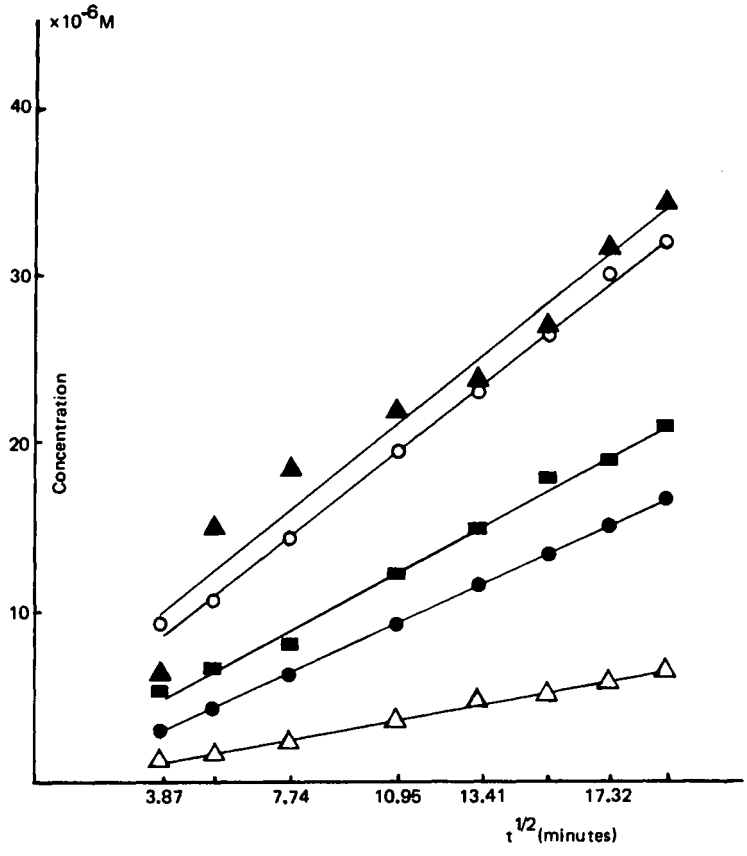


FIGURE 2

Release of Caffeine at Varying Concentrations From Petrolatum A.
Key: Δ , 1%(w/w); ● ,5 %; ■, 10 %; ○ , 20% ; ▲,30 % Caffeine.

increment in the released caffeine is observed up to 20 % concentration. No significant difference of release was found between 20 and 30 % concentrations.

For PEG ointments the release pattern demonstrated a better fitting to the log-log arrangement than the usual Q versus $\sqrt{t}$ arrangement as it is mentioned previously (Fig.6). This can be explained rearranging Equation 2 as follows;

$$q = Q.A' = A' \sqrt{2 AC_s Dt} \qquad \text{Eq.3}$$

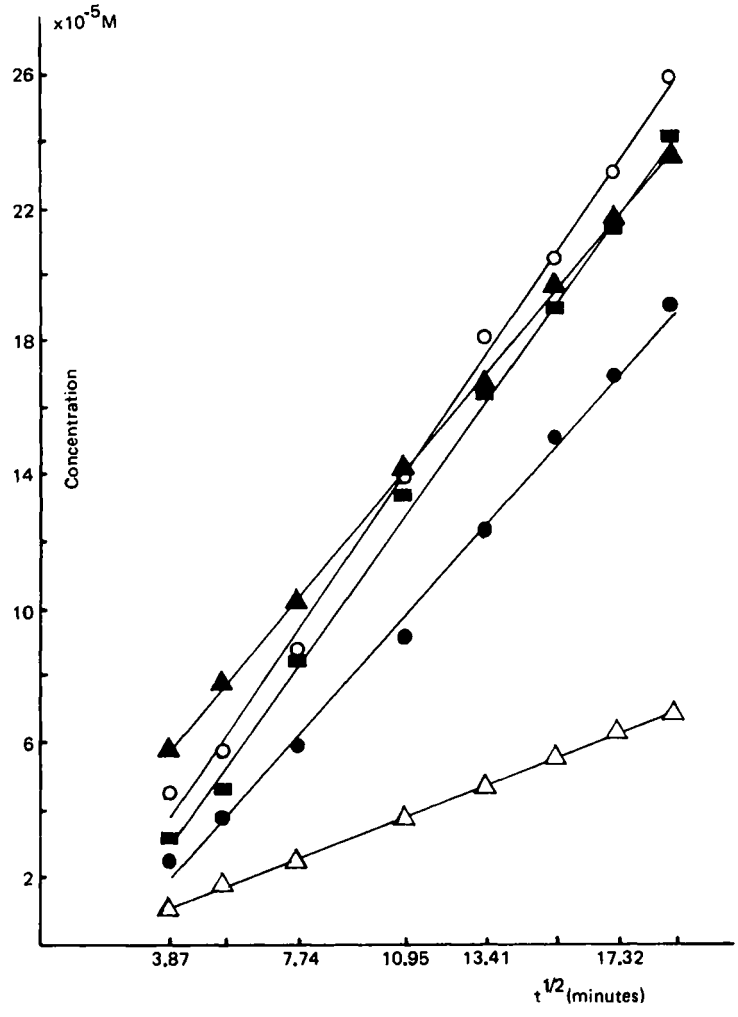


FIGURE 3

Effect of Caffeine Concentration on Release From a w/o Type Emulsion Base.
Key: Δ,1%(w/w); ●,5% ; ■,10% ; ○,20%; ▲,30 % Caffeine.

where, q is the amount of drug released from the whole area at time t, Q is the amount of drug released per unit area at time t and A' is the diffusion area.

According to Eq.3, solubility of the drug (C_s), diffusion coefficient (D) and the diffusion area (A') must be constant throughout the diffusion period.

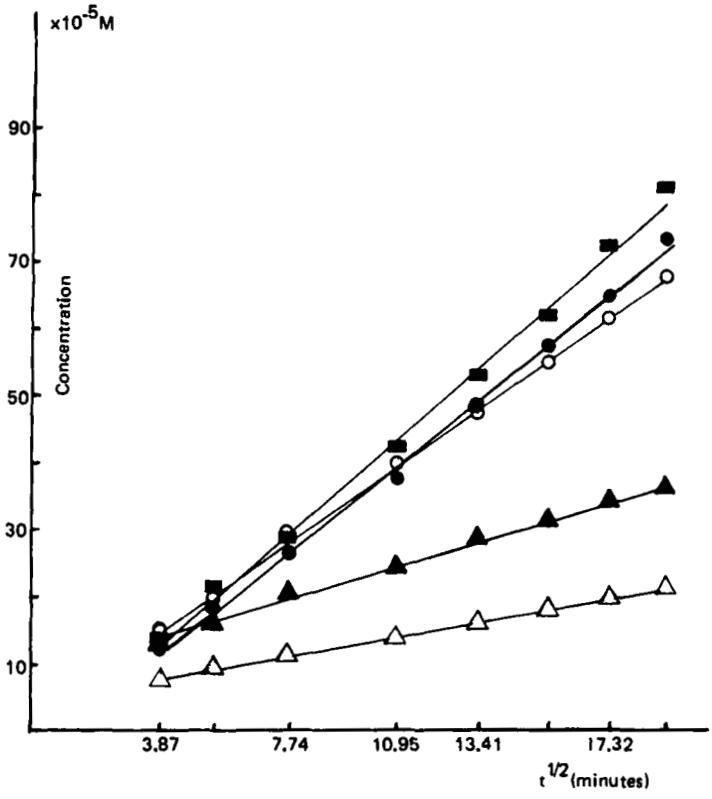


FIGURE 4
Release of Caffeine at Different Concentrations From Hydrophilic Ointment (USP XVIII).
Key: △,1%(w/w); ●,5%; ■,10 %; ○,20% ; ▲,30 % Caffeine.

However, hydrophilic character of PEG ointment would cause the absorption of water to such extent that eventually the whole ointment would become a solution. In fact, during the experiment it was observed that the PEG ointment absorbed water too much that the surface of the membrane become visibly concave and towards the ends of the diffusion experiments the whole ointment turned to be a solution. Therefore a deviation from Eq.3 can be expected due to the variations of the values A', C_s and D during the experimental period.

Figs. 2 to 6 suggest that, the occurance of concentration dependency is observed consistently only

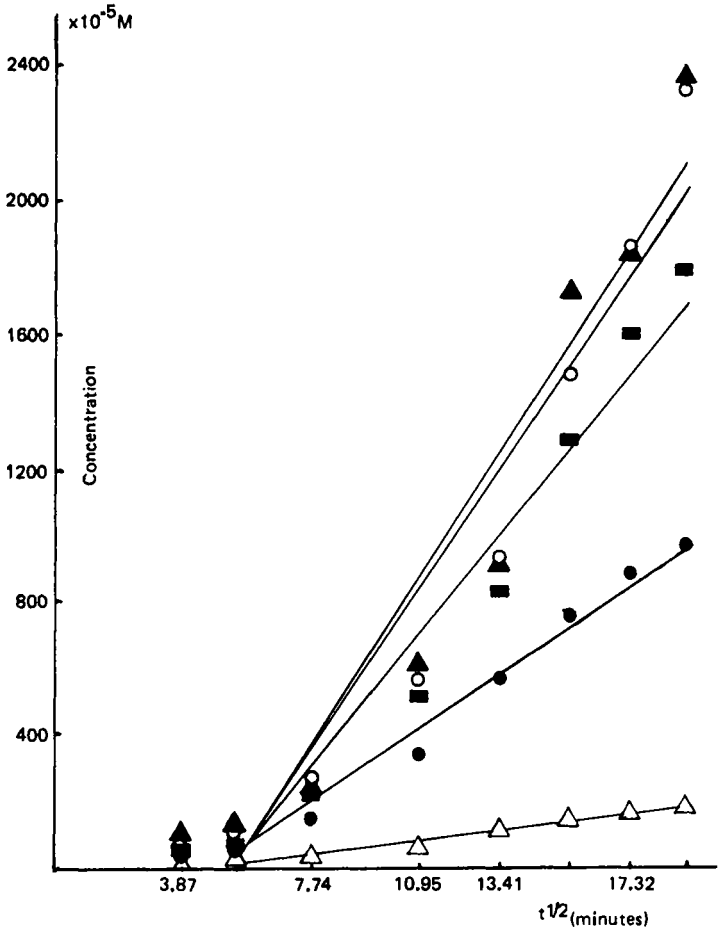


FIGURE 5
Effect of Caffeine Concentration on Release From PEG Ointment (USP XVIII).
Key: Δ,1%(w/w); ●,5%; ■,10 %; ○,20%;▲,30% Caffeine.

with petrolatum base. For the rest of the ointments used, such dependence only observed up to certain concentrations, from then onwards irregular variations occured.

The irregularities are most obvious in the emulsion type of ointments. Microscopical examination of those exhibited a great increase in the size of caffeine crystals similar to the crystal sizes of hydrated caffeine earlier observed. As both ointments

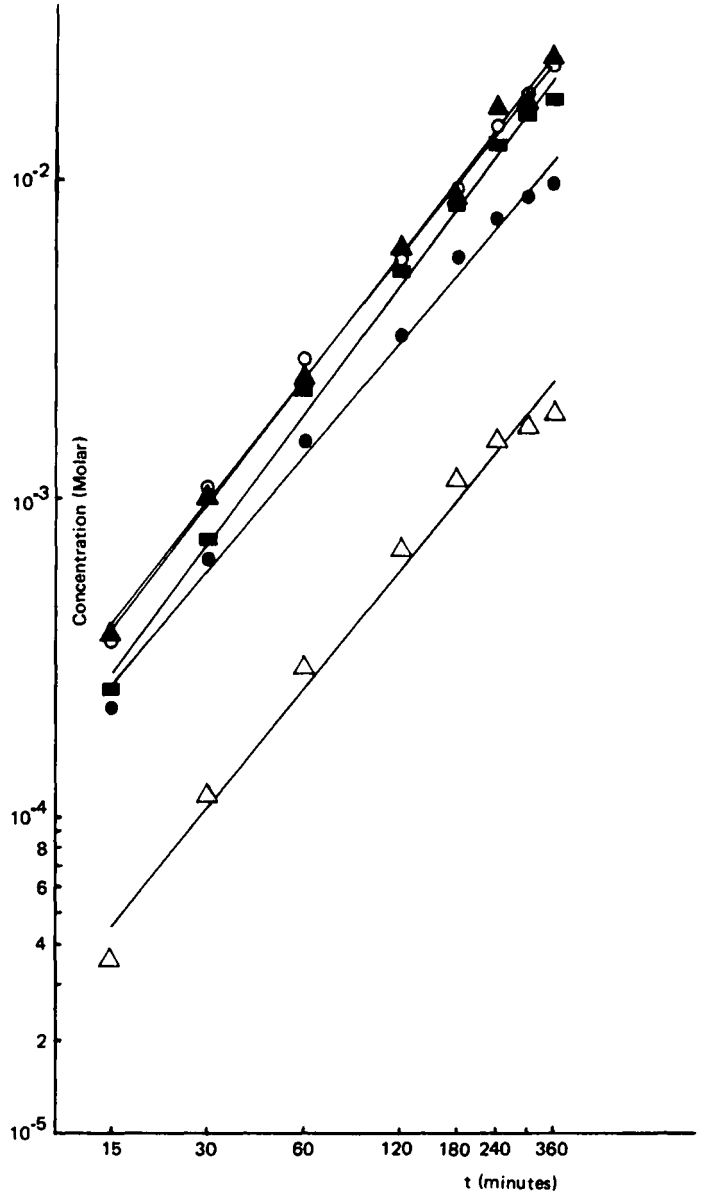


FIGURE 6
Logarithmic Representation of Release From PEG Ointment.
Key: Δ,1%(w/w); ●,5%; ■,10%; ○,20 %; ▲ ,30 % Caffeine.

contained water in their structure hydration of caffeine crystals can be expected.

Figs. 7 and 8 show the microscopical apparences of caffeine crystals in anhydr and emulsion type of ointments. As seen in these figures caffeine crystals show a great increase in size in hydrophilic ointment and w/o emulsion type ointment while they are in original shape and size in anhydrous bases.

As already mentioned, the hydration was found to cause a rapid and enormous increase in the particle size in emulsion type ointments.

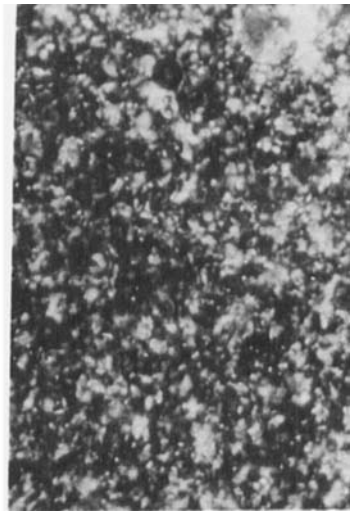
The particle size distribution under these conditions can very likely change. In the hydrophilic and emulsifying ointments therefore, at higher caffeine concentrations, this increment in size and change in size distribution can obscure the effect of caffeine concentration on release.

Since in our experiments and in the literature (23), no thermodynamic differences between anhydrous and hydrated caffeine was found the insignificant differences or the decrement in the released amounts in the more concentrated ointments might be due to the utilization of some of the incorporated caffeine to enlarge its crystals. Thus, the rest of caffeine might act as a lesser concentration of drug added to in the system.

Effect of Ointment Bases

When the solubility of caffeine in PEG and liquid paraffin is considered, a maximum release can be expected from petrolatum if the rule of "the release from the bases in which the drug is insoluble is higher than the bases in which it is soluble" is applicable. However, our results seem to be contrary to this generalization.

Petrolatum A base

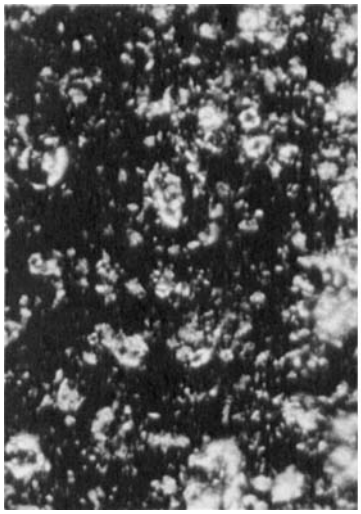


Petrolatum A base with 1 % caffeine

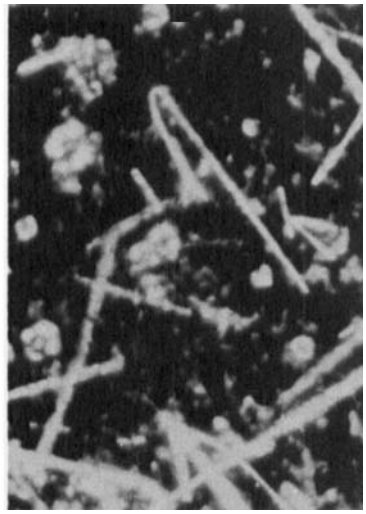
PEG ointment base

PEG Ointment base with 1 % caffeine.

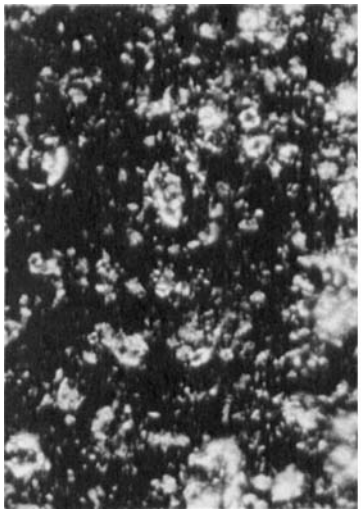
FIGURE 7
Microscopical Apparences of Caffeine Crystals in Anhydrous Ointment Bases (Magnification: X 143).



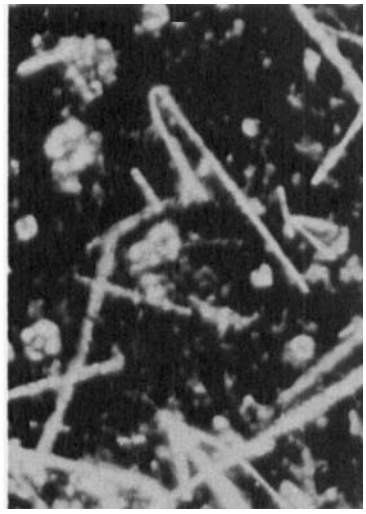
w/o Type emulsion base



w/o Type emulsion base with 1 % caffeine.



Hydrophilic Ointment base



Hydrophilic Ointment base with 1 % caffeine.

FIGURE 8
Microscopical Apparences of Caffeine Crystals in Emulsion Type of Ointment Bases (Magnification: x 143).

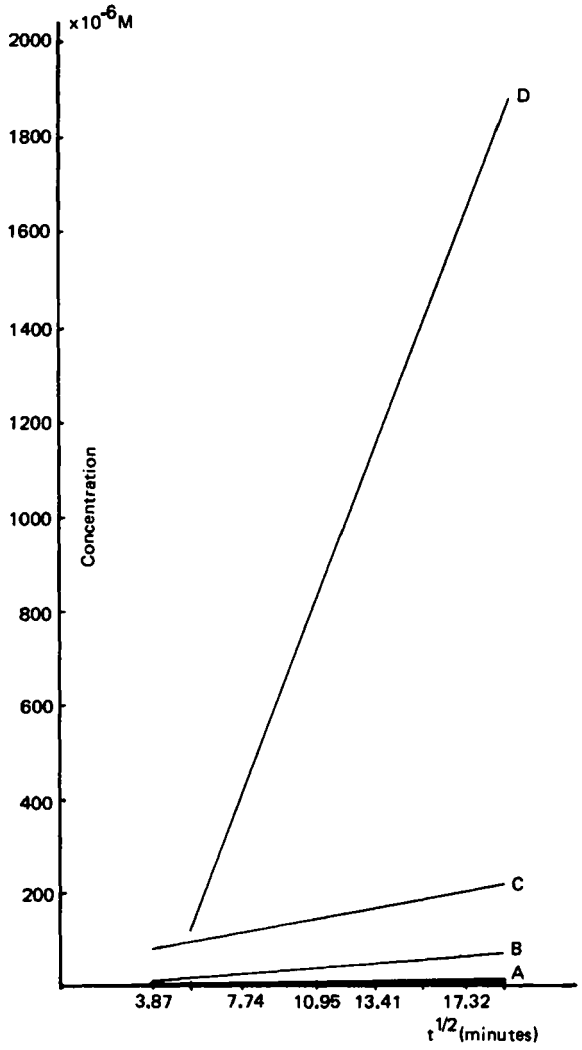


FIGURE 9
Effect of Ointment Base on Release of 1 % (w/w) Caffeine. Key: A, Petrolatum A; B, w/o Type Emulsion Base; C, Hydrophilic Ointment; D, PEG Ointment.

In Figs. 9 and 10, the release of 1 and 30 % caffeine from different ointment bases are given. For all caffeine concentrations investigated, the release was highest from PEG ointment. It was decreasing with hydrophilic ointment, water in oil type emulsion base and petrolatum A in that order. In fact, looking

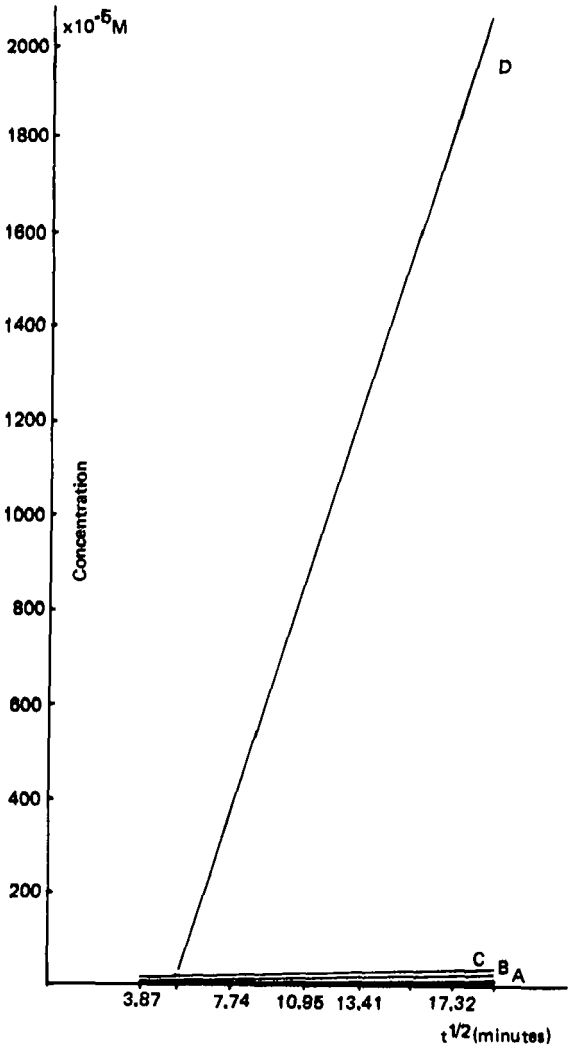


FIGURE 10
Effect of Ointment Base on Release of 30 % (w/w) Caffeine Key: A, Petrolatum A; B, w/o Type Emulsion Base; C, Hydrophilic Ointment; D, PEG Ointment.

through the Figs. 2 to 10, it can be predicted that from the bases in which caffeine is partially soluble, release is faster and the released amount of drug is more.

The reason for the maximum release from PEG ointment is likely to be the water-soluble character and

high osmotic pressure of this base. This phenomenon can also explain the lag-time seen in the release patterns from the PEG ointments (Figs. 5,6,9,10).

Similar findings were reported by Ayres and Laskar (13), Plakogiannis and Yaakob (15), Spang-Brunner and Speiser (16) using different drugs. Therefore a prediction about which base might release the drug best, does not seem to be possible for the ointment types under consideration. But in general, solubility of caffeine in polyethylene glycol and its water miscibility as well as hydrophilic ointment and water in oil type emulsion base may be contributing factors in achieving improved release rates compared with petrolatum Å.

Kaplan et.al.(1,2,3) whose results were cited at the introduction, used caffeine in a hydrophilic ointment at 30 % concentration. According to the results obtained "in vitro" in the present study, the release will be highest from PEG ointments and 20 % concentration would seem to be the optimal concentration as there is no significant difference between 20 % and higher concentrations.

In in vivo experiments, absorption properties of the drug is as important as the release properties. Therefore, the same results may not be applicable in vivo. However, it seems reasonable to expect a certain degree of correlation between our results and the in vivo absorption studies which we intend to investigate further.

FOOTNOTES

[1]) Merck, Germany

[2]) B.D.H. England.

[3]) Atlas Co., U.S.A.

[4]) E.I. du Pont Nemours and Co.,

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