

THE EFFECT OF CYCLODEXTRINS ON THE IN VITRO CHARACTERISTICS OF SEMISOLID FORMULATIONS OF *all-trans* RETINOIC ACID

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Abstract

The aim of this study was to prepare a formulation would be more efficient and better patient compliance and alternative to topical preparations currently used in the market. For these purpose inclusion complexes were prepared with two different cyclodextrins. Aqueous solubility, X-ray diffraction analysis and DSC analysis were carried out on the inclusion complexes. Besides the two different semisolid vehicles were formed by using inclusion complexes that were selected. In vitro drug release from formulations prepared was investigated using Franz diffusion cells in pH 6.0 buffer solution. Formulations prepared with inclusion complex exhibited significantly higher drug release when compared to the other formulations contained free drug and commercial preparations. The diffusion coefficient, permeability coefficient and vehicle/membrane partition coefficient were calculated using the data obtained from statistical analysis. All result taking accounts, hydrogel type formulations prepared with beta-cyclodextrin inclusion complex were seen as more appropriate vehicle for clinical trial to acne vulgaris patients.

Key words: *all-trans* Retinoic acid, Inclusion complexes, Beta-cyclodextrin, 2-Hydroxypropyl beta-cyclodextrin (Encapcin®), In vitro drug release.

all-trans Retinoik Asidin Yarıkatı Preparatlarının *İn vitro* Özellikleri Üzerine Siklodekstrinlerin Etkisi

Bu çalışmanın amacı, topikal tedavide kullanılan piyasa preparatına alternatif, çok daha etkin ve hasta uyuncu daha iyi olan bir formülasyonun hazırlanmasıdır. Bu amaçla 2 farklı siklodekstrin ile inklüzyon kompleksleri hazırlanmıştır. Bu kompleksler üzerinde çözünürlük, X-ışını kırınımı ve DSC analizleri gerçekleştirilmiştir. Bunun yanısıra, seçilen kompleksler kullanılarak 2 farklı özellikte yarıkatı formülasyonlar hazırlanmıştır. Bu formülasyonlardan *in vitro* etkin madde salımı Franz difüzyon hücresi kullanılarak pH 6.0 tampon çözeltisinde gerçekleştirilmiştir. İnkluzyon kompleksleri ile hazırlanan formülasyonlar serbest etkin madde içeren formülasyonlar ve piyasa preparatlarından çok daha fazla etkin madde salımı göstermiştir. Elde edilen veriler kullanılarak difüzyon, permeabilite ve sıvağ/membran partisyon katsayıları hesaplanmıştır. Bütün sonuçlar dikkate alındığında, beta-siklodekstrin inklüzyon kompleksi ile hazırlanan hidrojel formülasyonlarının akne vulgarisli hastalar üzerinde yapılacak klinik denemeler için daha uygun olduğu görülmektedir.

Anahtar kelimeler: *all-trans* Retinoik asit, İnkluzyon kompleksleri, Beta-siklodekstrin, 2-Hidroksipropil-beta-siklodekstrin, *In vitro* etkin madde salımı.

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INTRODUCTION

all-trans Retinoic acid (tretinoin - RA) is used successfully in the topical treatment of acne vulgaris and in some disorders of keratinization. Its mechanism of action includes enhancing epithelial turnover, promoting the drainage of existing comedones and inhibiting formation of new comedones (1). The side effects of RA such as dryness and irritation reduce the effectiveness of therapy by limiting patient compliance.

RA has also numerous drawbacks, such as poor stability in the presence of air and light, and insolubility in water. In the literature, a great number study was existed for the preparation of cyclodextrin complexes with all-trans retinoic acid to overcome its stability problems and to improve solubility (2-4). But a formulation contained the inclusion complex was not yet took place in the current acne treatment.

Cyclodextrins are cyclic oligosaccharides which have a hydrophilic outer surface and a hydrophobic central cavity. The cavity of the cyclodextrins is occupied by included water molecules both in crystalline state as well as in aqueous solution. In aqueous solutions cyclodextrins are capable of forming inclusion complexes with many drugs, which are less polar than water, by taking up into the central cavity either entire of molecule or one part of it (5-6). The phenomenon is widely named as molecular encapsulation (7). The cyclodextrins and their inclusion complexes have been widely used in pharmaceutical field such as (i) to increase the water solubility of hydrophobic molecules, (ii) to increase the physical and chemical stability of guest molecules, (iii) to reduce or prevent skin irritation, (iv) to increase the absorption of various compounds into skin.

Montassier et al. (8) prepared complexes of RA with BCD, HPBCD and DIMEB using the co-precipitation method. They reported that an increase in aqueous solubility was noticeable. But there has not been any study regarding the in vitro release of the preparation including RA-cyclodextrin complex.

Loftsson et al. (9) showed that it is possible to obtain effective transdermal delivery of lipophilic drugs from aqueous cyclodextrin vehicles. Due to cyclodextrin complexation, the concentration of free drug in the vehicles is low. However, the drug molecules within the complex are in a fast equilibrium with the free drug molecules out in the solution, resulting in effective delivery of the drug to the skin surface.

In some cases the complexation efficiency is not very high. Redenti et al. (10) reported that it is possible to enhance the efficiency through the formation of multicomponent complex systems. It is known that water-soluble polymers enhance the complexation efficiency of a wide variety of guest molecules through stabilization of the drug/cyclodextrin complex, and that they increase the aqueous solubility of the natural cyclodextrins (11).

The objective of the present work was to develop the novel formulation using drug-water soluble polymer-cyclodextrin ternary system to maintain clinical efficacy for the topical treatment of acne vulgaris. This formulation prepared is suggested to be feasible than commercially available topical RA products because of reduced local irritation and increased patient compliance.

MATERIALS AND METHODS

Materials

RA was kindly supplied by Roche (Turkey). β -cyclodextrin (BCD) and Encapcin® (2-hydroxypropyl- β -cyclodextrin, DS=3.0) were kindly provided respectively from Chinoin (Hungary) and Jansen (Belgium). Polyvinyl prolidone (Kollidone 30, MW= 30000-50000, PVP), Cremophor EL, Carbopol 940 and Carbopol 1342 were purchased from Basf, Fluka and Biesterfeld, respectively. Methanol and acetonitrile (HPLC grade) were purchased from Merck Chemical Company (Germany). All ingredients used were pharmaceutical grade.

Phase-solubility studies

The phase-solubility studies were carried out with respect to Higuchi and Connors Method (12). For this purpose, excess amount of RA was added to the aqueous CD solutions at various concentrations (0-30 mM for Encapcin® and 0-15 mM for BCD) and stirred with a magnetic stirrer at 25±1°C. After equilibrium was reached the samples were filtered through 0.45 µm membrane filter and diluted. The molar concentration of drug was plotted versus the molar concentration of CD and the phase-solubility diagrams of RA were obtained.

Calculation of complex stability constant

The complex stability constants were calculated by using slope values of the straight line of the phase-solubility diagrams and aqueous solubility value of RA with respect to the following equation (Eq 1) as described in literature (12):

$$K_{1:1} = \frac{\text{slope}}{S_0(1-\text{slope})} \quad \text{Eq.1 (where } S_0 \text{ is the solubility of RA in absence of CD).}$$

Preparation of CD complexes and physical mixtures

RA is a labile drug against to air and light. In order to prevent the degradation of free RA with light and air, some steps in this study used were carried out into a special chamber through nitrogen gases and under yellow light conditions. The other steps used for inclusion compound was studied only yellow light because of the prevention of any drug loss.

Inclusion complexes were prepared using equimolar amounts of RA and cyclodextrin (CD). A 1:1 molar ratio was used successfully for complexation in our previous study (13). RA amount was 265 mg and 229 mg for complexation with BCD and Encapcin® respectively.

Kneading and modified methods were selected for preparing inclusion complexes. According to the kneading method, CD was wetted with a small amount of water to form a slurry mixture. Then RA was added in portions to this mixture and stirred until a homogeneous mixture maintained for 15 minutes. The later method was modified from the method previously reported by Loftsson et al. (14). Briefly, RA was added to aqueous solutions containing 10% (w/v) CD and 0.1 % (w/v) PVP, as a water-soluble polymer to alter complexation efficacy. The suspension obtained in sealed containers was saturated by sonication in an ultrasonic bath for one hour and then autoclaved at 121°C for 20 minutes in order to speeding the reaction. After the suspension was cooled until the room temperature, it was stirred for five days to maintain the equilibration. Afterwards the water was removed by evaporation under the vacuum for 24 hours. The solid product obtained was gently ground with a pestle and mortar to produce equal particles content.

Physical mixtures were also prepared in the 1:1 molar ratio by simple mixing of both components with pestle and mortar in order to serve as reference.

Confirmation of the complex formation

Raw materials, physical mixtures and complexes were subjected to a series of physicochemical analyses. IR spectrophotometry (JASCO FT/IR 420, Japan) was carried out on potassium bromide disks, from 4000 to 400 cm⁻¹. Differential scanning calorimetry (Rheometric Scientific DSC Gold Plus, USA) was carried out at scanning rate of 20°C/min. (25-250°C) with nitrogen (20 ml/min.) as purging gas. X-ray analyses (Rekagu D-max 2200, Japan) were carried out using an x-ray generator equipped with copper anticathode of 80 mA power and 40 kV.

Determination of RA content in inclusion complexes

The determination of RA contained in the complex was carried out by spectrophotometrically. Because of the in solubility of BCD in alcohol, dimethyl sulfoxide (DMSO) was chosen as a solvent for BCD and its complexes whereas absolute alcohol chosen as a solvent for Encapcin® and its complexes. The validation of assay method was performed in both solvents. The samples of BCD and Encapcin® diluted with DMSO and absolute alcohol respectively. The samples contained BCD were analyzed at 361 nm because of DMSO, and the others were analyzed at 337.5 nm (15).

Determination of solubilized drug

The aqueous solubility experiments were performed in order to increasing solubility effect of CDs on the RA. For this purpose the method described by Higuchi and Connors (12) were carried out to determine the solubility of RA, physical mixtures and complexes in water. The excess amount of RA or the corresponding amount of the inclusion complex and physical mixtures was added to distilled water and the suspension obtained was mixed by magnetic stirrer at 200 rpm for 24 h. The medium was maintained $20 \pm 1^\circ\text{C}$ by using water bath with temperature regulator. The samples were filtered through membrane filter (0.45 μm), diluted and analyzed spectrophotometrically above mentioned.

Preparation of the formulations

In this study, two different type of vehicles such as hydrogel and o/w emulsion were prepared. All formulations contained 0.025% RA or corresponding amount of inclusion complex. Hydrogel and o/w emulsion codes and compositions are listed in Table 1.

Table 1. Composition of hydrogel and o/w emulsion formulations (%w/w).

Ingredients	Hydrogel			Oil in Water Emulsion	
	F1	F2	F3	F4	F5
RA	0.025	0.025	0.025	0.025	0.025
Carbopol 940	-	0.5	0.5	-	-
Carbopol 1342	0.5	-	-	-	-
Triethanolamine	0.5	0.5	0.5	-	-
Liquid paraffin	-	-	-	20	20
Cetyl alcohol	-	-	-	1.5	1.5
Stearyl alcohol	-	-	-	1.5	1.5
Span 60	-	-	-	6	6
Tween 60	-	-	-	4	4
Methylparaben	-	-	-	0.15	0.15
Propylparaben	-	-	-	0.15	0.15
Cremophor EL	2	2	2	2	2
Glycerin	1	1	-	-	-
Propylene glycol	-	-	2	7	9
Ethanol 96%	10	10	10	10	10
Butylated hydroxytoluene	0.05	0.05	0.05	0.1	0.1
EDTA- disodium	0.05	0.05	0.05	0.05	0.05
Purified water (qsp)	100	100	100	100	100

B and E coded hydrogels and o/w emulsions were prepared with two following inclusion compounds:

B-RA : BCD complex that containing 0,025 g RA,

E-RA: Encapcin® complex that containing 0,025 g RA

Preparation of gel formulations

Briefly, Carbopol 940 or Carbopol 1342 (0.5% w/w) were dispersed in distilled water by using a propeller-type Stir-Pak mechanical stirrer (Cole-Parmer Ins. Co., Chicago, IL). Later on alcoholic solution of BHT (Butylated hydroxytoluene) was added to glycerin/propylene glycol. Other hand the free RA or CD complexes were dissolved in Cremophor EL. Glycerin/propylene glycol containing alcoholic BHT solution and Cremophor EL solution with RA or its complexes were added to Carbopol dispersion while continuous stirring. It was stirred until obtained uniform appearance. Carbopol gels were neutralized by addition of triethanolamine to achieve gel formation.

Preparation of o/w emulsion formulations

Preparations of o/w emulsion were made using conventional hot emulsion technique. Shortly, oleaginous phase consist of liquid paraffin, cetyl alcohol, stearyl alcohol and Span 60 was heated to 75°C on the water bath. Aqueous phase including distilled water, Tween 60, glycerin/propylene glycol and antimicrobial protective agent was also heated in same manner as oleaginous phase. During the emulsion process, separately RA or its CD complexes were dissolved in Cremophor EL. After the emulsion prepared was cooled to the room temperature, the mixture containing RA or its complexes were added to emulsion by dispersing with magnetic stirrer. Afterwards alcoholic BHT solution was added onto the emulsions and mixed until uniform mixture achieved was.

In vitro release studies

In vitro diffusion of RA from two different bases formulations and two commercial preparations was investigated using Franz diffusion cells.

Two different commercial preparations were selected for comparison with in vitro drug release. The commercial preparations are coded as follows commercial preparation with gel bases (containing 0.025% RA), CM1; commercial preparation with cream bases (containing 0.1% RA), CM2.

Accurate weight of formulation containing free RA or its complexes was placed into the donor compartment and evenly distributed over the membrane surface. The receptor compartment was filled with acetate buffered saline pH 6.0 containing 1% Cremophor EL (polyoxyl 35 castor oil). Cremophor is a nonionic surfactant which used to ensure that solubility in the receptor solution would not limit penetration through skin. Bronaugh and Stewart (16) reported that diffusion characteristics of membrane are unaffected by Cremophor exposure. The receptor phase was agitated 300 rpm by means of a magnetic stirrer and the temperature of the cells was maintained at $32 \pm 1^\circ\text{C}$. The volume of the receptor compartment is 28 ml and an affective diffusion area is 2.54 cm^2 . As a diffusion membrane, a synthetic cellulose acetate membrane with a pore size of $0.45 \mu\text{m}$ was used after being treated with receptor solution for 1 hour. Throughout the experiment, the donor chamber surface was occluded; thus, any evaporation from the formulation was avoided. Samples ($500 \mu\text{l}$) were withdrawn from the receptor phase at predetermined times over a 4 h period and replaced with fresh receptor phase. At the end of the experiments, the samples were mixed with 1 ml absolute ethanol and were analyzed by HPLC for determining RA content.

Studies were performed in triplicate and the mean values were used for analysis of the data.

Permeation profiles were constructed by plotting the total amount of RA transported across the semi synthetic membrane ($\mu\text{g}/\text{cm}^2$) against time ($\text{h}^{1/2}$). The release rates of RA (k) were calculated from the slope of the graph and expressed as $\mu\text{g}/\text{cm}^2/\text{h}$.

Assay of RA by HPLC

RA was detected by HPLC (Hewlett Packard – HP1100) equipped with a $20 \mu\text{l}$ sample loop injector. Samples were chromatographed using a $250 \times 4 \text{ mm}$ i.d. stainless-steel column packed with octadecylsilane C18 (LiChrosorb RP-18, $5 \mu\text{m}$, Merck, Darmstadt, Germany) and with a

flow rate of 2.0 mL/min. The mobile phase, filtered through a 0.45 µm filter prior to use, was consisted of methanol:acetonitrile:water:glacial acetic acid in the ratio of 80:10:10:0.5 (v/v) (17).

The column effluent was monitored using a UV detector set at 338 nm. Under these conditions the retention time of RA was 3.8 min.

Calibration curves were constructed from the absolute alcoholic RA stock solutions. The curves were linear ($r^2=0.998$). The LOD and LOQ values, one of the parameter of analytical method validation, were 6.75×10^{-3} ng and 2.07×10^{-2} ng per ml respectively.

The mathematical evaluation of in vitro drug release data

The analysis of the in vitro data on the release of RA from hydrogel and o/w emulsion bases was carried out using the Higuchi equation, which is valid when the release of drug from the base is less than 30 % (18). The diffusion coefficient of RA from different bases was calculated using Eq. 2:

$$Q = 2 C_0 \cdot A \cdot (Dt/\pi)^{1/2} \quad \text{Eq. 2}$$

where Q is the amount of drug released (µg); A is the area of the diffusion membrane (cm²); C₀ is the initial concentration of drug in the base (mg/cm³); D is the diffusion coefficient (cm²/h); and t is the time (h).

The permeability coefficient values of RA for each base were calculated according to Fick's law of diffusion (18)(Eq. 3):

$$Q = P \cdot A \cdot C_0 \cdot t \quad \text{Eq. 3}$$

where P is the permeability coefficient (cm/h) and the other parameters are the same as for Eq. 2.

The vehicle/membrane partition coefficients were calculated by Eq. 4:

$$K_{V/M} = P \cdot h / D \quad \text{Eq. 4}$$

where $K_{V/M}$ is the permeability coefficient (vehicle/membrane) (cm/h); h is the thickness of the membrane (cm) and D is diffusion coefficient (cm²/h).

Statistical analysis

The in vitro data was subjected to statistical analysis using SPSS 11.5 software for ANOVA following Student –Newman Keuls multiple comparison test. P value of less than 0.05 was considered as evidence of a significant difference.

RESULTS AND DISCUSSION

Solubility

The typical phase solubility diagram of RA obtained into aqueous BCD and Encapcin® solutions is shown in Figure1. The solubility of RA increases linearly as a function of the CD concentration. Thus, the phase solubility diagram is of Higuchi's A_L type, and formation of a RA:BCD and RA:Encapcin® 1:1 complex can be assumed. At room temperature the aqueous solubility of RA and its complex was determined and results are shown in Figure 1. The apparent solubility constants of RA with BCD and Encapcin® were estimated to be 281.94 M⁻¹ and 219.08 M⁻¹ respectively, as determined by phase solubility method.

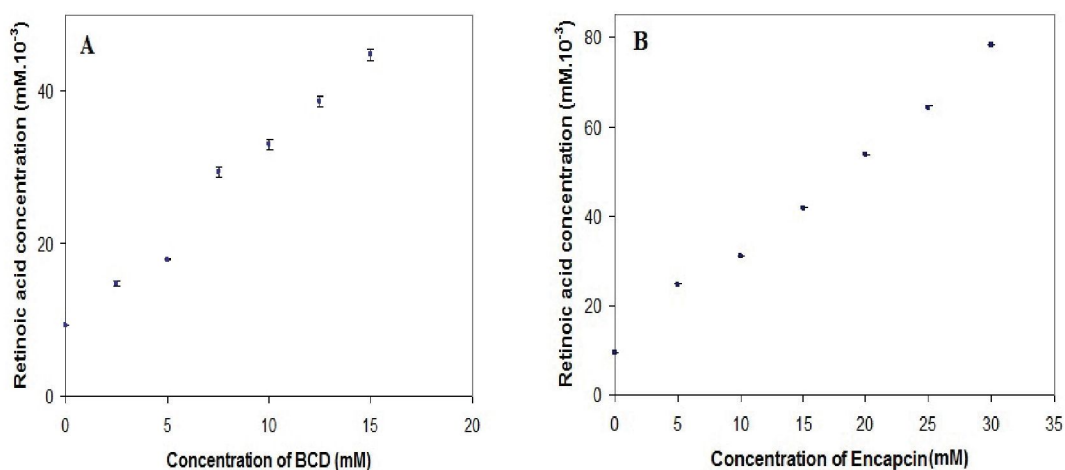


Figure 1. The phase solubility diagrams of RA with BCD (A) and Encapcin® (B).

The water solubility of RA and their physical mixtures and inclusion complexes prepared with BCD and Encapcin® at molar ratio of 1:1 was determined by the solubility studies. The results are shown in Table 2. As it is seen in the table 2, preparing the physical mixtures and inclusion complexes with both CD at a 1:1 molar ratio increased the solubility of RA. Aqueous solubility was more enhanced with Encapcin® (17.37-fold) than BCD (11.07-fold).

Table 2. Results of solubility experiments (n = 6).

	Physical Mixture		Kneading		Modified Method	
	Water solubility (µg/mL)	Enhancement	Water solubility (µg/mL)	Enhancement	Water solubility (µg/mL)	Enhancement
RA	2.77 ± 0.37	-	-	-	-	-
BCD	20.20 ± 1.02	7.29	29.81 ± 4.15	10.76	30.67 ± 9.00	11.07
Encapcin®	9.84 ± 0.31	4.40	16.97 ± 2.76	6.13	48.12 ± 5.33	17.37

Physicochemical characterization

The IR spectrums and X-ray powder diffraction patterns of RA indicate the transformation of RA from the crystalline to the amorphous state by formation of an inclusion complex with BCD and Encapcin® (19).

More evidence of the complex formation was obtained from the DSC thermograms (Figure 2). The endothermic peak at 185°C is equivalent to the melting point of RA completely or partially disappeared in the inclusion complex samples with BCD and Encapcin®, respectively.

All these results suggest a probable inclusion of RA in the cavity of CDs.

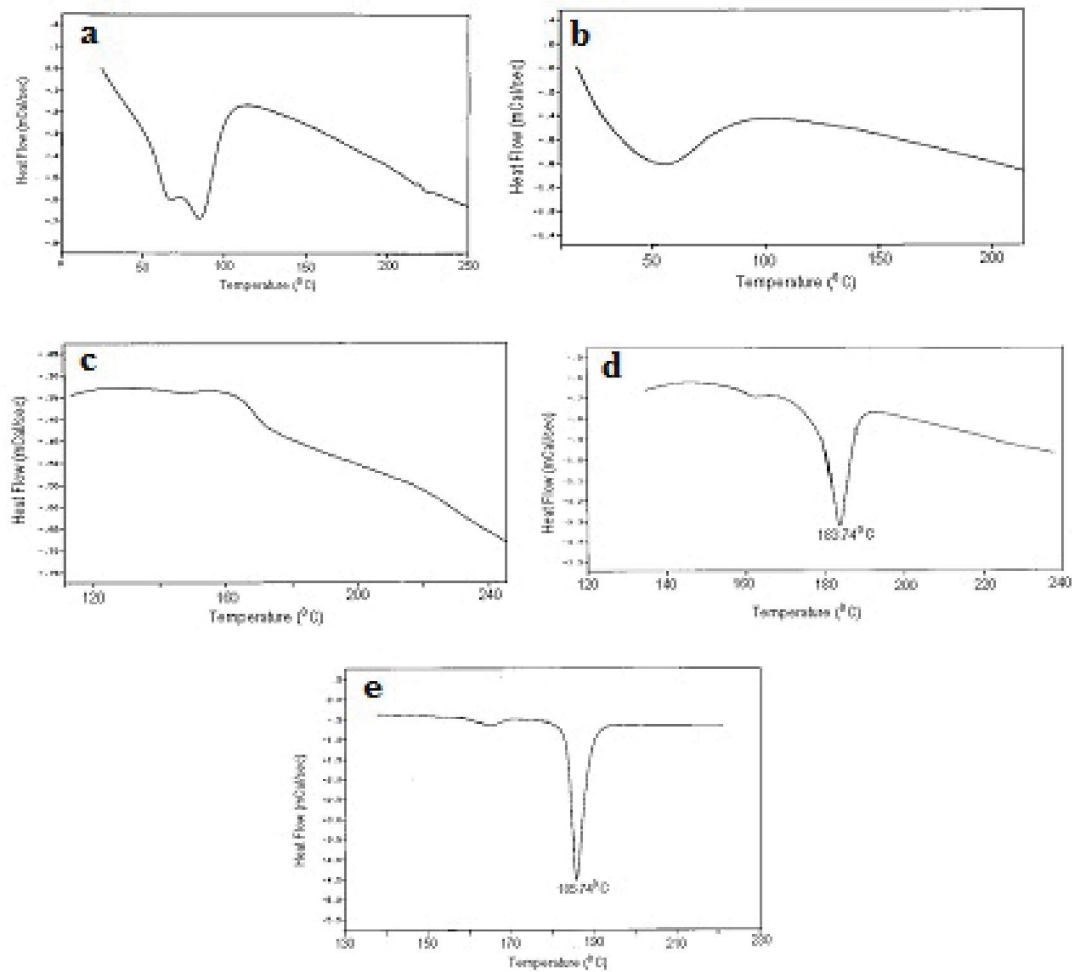


Figure 2. DSC thermograms of RA with B-CD and Encapcin®, (a) B-CD, (b) Encapcin®, (c) B-CD: RA 1:1 complex, (d) Encapcin®: RA 1:1 complex, (e) RA alone.

In vitro drug release

The release behavior of the BCD and Encapcin® complexes from hydrogel and o/w emulsion bases was compared with a free RA and two commercial preparations. Figures 3 and 4 show the amount of drug released from formulations containing free RA or its complexes as a function of the square root of time. The linearity of the plots may indicate that release of RA is diffusion-controlled. It is evident that the release rate of RA was significantly increased by complexation, particularly with BCD from hydrogel formulations rather than o/w emulsion formulations.

The rank order for in vitro release of RA was found to be: hydrogel bases > CM1 > o/w emulsion bases > CM2.

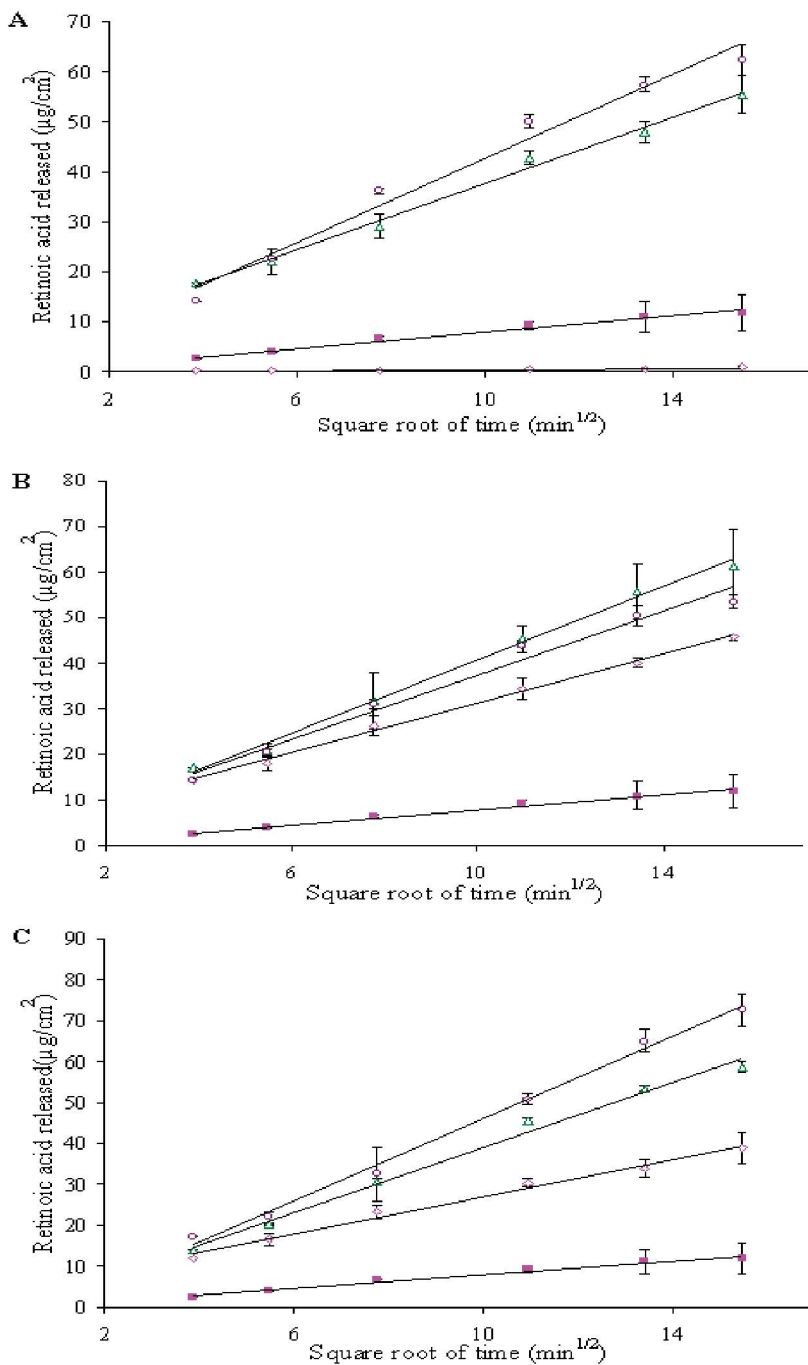


Figure 3. In vitro release of RA from hydrogel bases and CM1 as a function of the square root of time. F1 coded formulations (A); F2 coded formulations (B); F3 coded formulations (C), (◇) free RA, (○) B-CD: RA 1:1 complex, (△) Encapcin®: RA 1:1 complex, (■) CM1 in all plots.

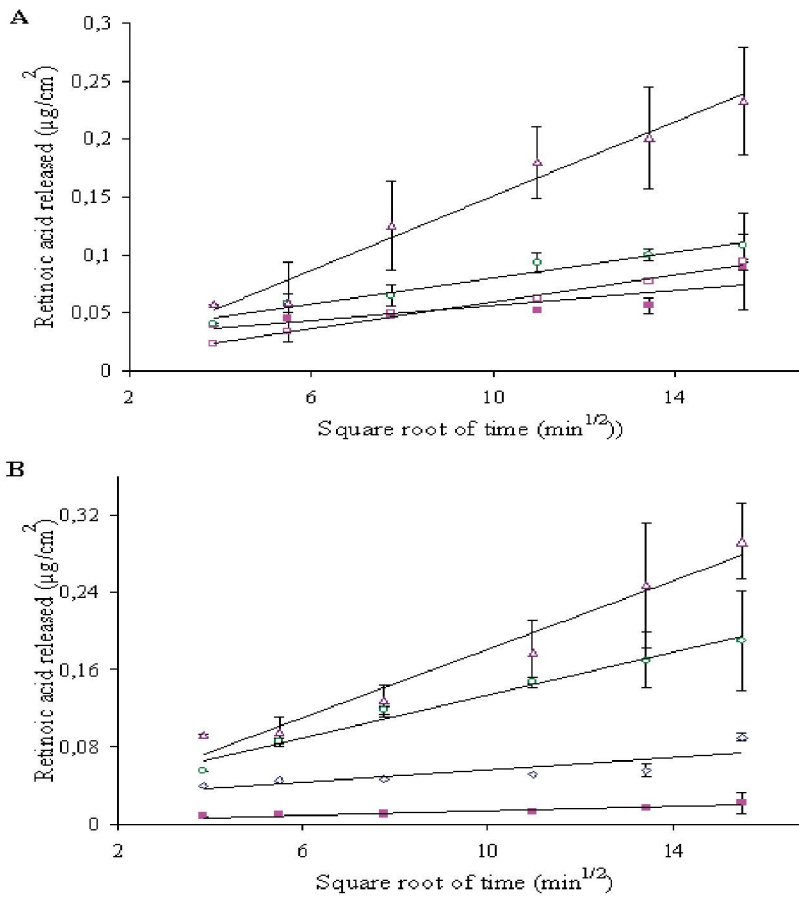


Figure 4. In vitro release of RA from o/w emulsion bases and CM2 as a function of the square root of time. F4 coded formulations (A); F5 coded formulations (B), (◇) free RA, (○) B-CD: RA 1:1 complex, (△) Encapcin®: RA 1:1 complex, (■) CM2 in all plots.

According to the in vitro release study, F2 and F3 coded formulations containing free RA showed greater drug release than CM1, as opposed to F1 coded formulations containing free RA, which showed less drug release than CM1. In vitro RA release was increased in all hydrogel formulations containing complex with BCD and Encapcin® (Table 3a). It was shown that the F3B coded formulation had the greatest release. Increased releases from F3-coded formulations was attributed to the presence of propylene glycol in the composition as well as inclusion complex was presented in formulation. Schlichting et al. (20) reported that propylene glycol with lauryl alcohol and ethoxylated lauryl alcohol, components of w/o emulsions, contributed most of the solubility characteristics of the RA cream.

The o/w emulsion type formulations, although these formulations showed greater drug release than CM2, they did not show an increase to the same extent as with hydrogel type formulations. However, F5-coded formulations were seen as favorable both cosmetically and regarding in vitro drug release. The difference between these formulations (F2- and F3-coded) and F1-coded formulations is that the former contain a high ratio of propylene glycol. In these formulations the amount of propylene glycol was increased from 7% to 9%, targeted to enhance in vitro drug release. Thus, the effect of penetration enhancer was obtained as with hydrogel type bases, as reported by Schlichting et al. In addition, the authors reported in the same paper that RA, which is relatively insoluble in o/w, was preferably high at concentrations of 0.10% or more in o/w. But this concentration could alter the side effects such as drying and irritating. However dose can reduce by using formulation contained cyclodextrin complex.

Table 3a. The permeation parameters for hydrogel formulations and CM1.

Formulations	Release rate constant, k ($\mu\text{g}/\text{cm}^2 \cdot \text{h}^{-0.5}$)	Released amount (%)	Diffusion coefficient, D ($\text{cm}/\text{h} \cdot 10^{-3}$)	Partition coefficient, $K_{V/M}$	Permeability coefficient, P (10^{-2})
CM1	6.25	0.03	0.49	0.29	0.96
F1	0.292	0.07	0.0012	6.31	0.045
F1E	24.7	7.63	7.69	0.75	3.86
F1B	31.68	9.04	12.65	0.58	4.85
F2	20.5	6.99	5.30	0.90	3.19
F2E	26.41	8.54	8.79	0.69	4.05
F2B	28.18	8.60	10.01	0.67	4.46
F3	25.03	4.96	7.89	0.74	3.90
F3E	29.99	8.28	11.33	0.61	4.64
F3B	37.46	10.2	17.68	0.50	5.90

In order to obtain meaningful information for the drug release, the release kinetic model of the formulations was evaluated kinetically. Drug release from formulations were fitted the Higuchi kinetics model (Q/\sqrt{t}). The diffusion coefficients were calculated by Higuchi equations (21- 23). The calculated diffusion coefficient, permeability coefficient and partition coefficient for the formulations are shown in Table 3a and 3b.

These results indicate a direct dependence of the release rate at the diffusion coefficient, which in turn the dependence on other factors such as the solubility of the drug in the base. Therefore, a greater release of drug is expected when there is less affinity of the drug to the base as in the case of the F3B hydrogel formulation, which gave the highest diffusion coefficient value of $17.86 \times 10^{-3} \text{ cm}^2/\text{h}$ (Table 3a).

The permeability coefficient values of RA for each base were calculated according to Fick's law of diffusion. The highest value of $5.9 \times 10^{-2} \text{ cm/s}$ was obtained for the F3B formulation (Table 3a).

The partition coefficient factor is considered one of the important parameters for the estimation of the interaction of drug with vehicle and receiving medium. The partition coefficient values for all formulations are given in Tables 3a and 3b. It was observed that RA had a lower partition coefficient in hydrogel bases or less affinity to the base than o/w emulsion bases. Therefore, the drug had a faster release from this particular base.

Table 3b. The permeation parameters for o/w emulsion formulations and CM2.

Formulations	Release rate constant, k ($\mu\text{g}/\text{cm}^2 \cdot \text{h}^{-0.5}$)	Released amount ($\% \cdot 10^{-2}$)	Diffusion coefficient, D ($\text{cm}^2/\text{h} \cdot 10^{-7}$)	Partition coefficient, $K_{V/M}$ (10^2)	Permeability coefficient, P (10^{-5})
CM2	0.051	0.055	0.02	1.62	2.20
F4	0.045	1.22	0.26	0.42	7.20
F4E	0.124	3.27	1.94	0.15	19.20
F4B	0.05	1.41	0.32	0.36	7.60
F5	0.017	0.37	0.04	1.46	2.40
F5E	0.138	4.31	2.40	0.14	22.40
F5B	0.089	2.82	0.10	0.20	13.60

As shown in Figures 3-4, the preparation of inclusion complex of RA with cyclodextrin had a significant effect on the enhancement of the in vitro drug release. On the other hand, while the release profile was increased to a greater extent with BCD complexation than with Encapcin® from hydrogel formulations, it increased to a lesser extent from o/w emulsion formulations. Because the aqueous solubility of the Encapcin® complex is greater than that of the BCD complex, enhanced in vitro release for o/w emulsion formulations may be obtained. In our study, diffusion coefficients were determined using Equation 3, because in vitro drug release ranged between 0.0013-10.55%. According to the Higuchi theory, Equation 3 is valid if the percentage released is less than 30% of the total drug in the vehicle. Depending on drug release, greater diffusion coefficients were calculated by Higuchi equation with respect to hydrogel formulations versus o/w emulsion formulations.

According to calculated permeability coefficients, there was no significant difference among the hydrogel formulations which contained complexes ($p > 0.05$). But the permeability coefficients of the free drug were significantly different that of the complexes ($p < 0.01$). The rapid dissolution of the BCD and Encapcin® complexes resulted in an increase in the net amount of RA permeating into the receptor phase. The results indicated that the enhanced release by BCD and Encapcin® is mainly due to the lower affinity and faster dissolution of the complex in all prepared formulations. The penetration of RA was decreased with complexation and this effect was well correlated with decrease in the apparent partition coefficient of RA (Tables 3a-b).

For the o/w emulsion formulations, lower permeability coefficients were observed depending on occlusive and emollient substance.

According these results, it was demonstrated that hydrogel formulations had the highest diffusion coefficient and the lowest partition coefficient. Hydrogel type formulations were seen as an appropriate vehicle for retinoids and similar drugs.

CONCLUSION

The results of this work indicate that the enhancing effect of the complexation with cyclodextrins on the skin permeation of RA can be mainly ascribed to an increase in the solubility of the drug and to increase in the diffusion and permeability coefficient. With regard to results, we may conclude that the RA:BCD complex can be used to prepare alternative formulation would be more effective and well-tolerated for the treatment of acne vulgaris.

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