

## Preparation and *in vitro* characterization of tretinoin-containing microspheres suited for dermatological preparations

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### Abstract

Preparation of tretinoin-containing microspheres with the aim of reducing undesirable skin reactions towards trans retinoic acid (TRA) and thus, improving patient compliance is of particular interest. These microspheres have to be smaller than 30  $\mu\text{m}$  to avoid causing gritty feel when applying on the skin, and slow the release of drug to minimize its skin side effects. Following preliminary evaluations on process conditions for preparation of microspheres by an emulsification-solvent extraction technique, a 2<sup>3</sup> full factorial design was employed to investigate the influence of various formulation variables including cellulose acetate (CA) concentration, polyvinyl alcohol (PVA) concentration and TRA to polymer weight ratio on physical properties and release behavior of microspheres. The particle size of microspheres increased with increasing CA concentration and TRA to polymer weight ratio, but was not much influenced by PVA concentration. The drug encapsulation efficiency increased in microspheres made at higher PVA concentration or with lower CA and TRA concentrations. Examining release data by Korsmeyer-Peppas equation indicated that the Fickian mechanism was basically involved in the drug release from all formulations. In general, a slower drug release rate (i.e. greater mean dissolution time) and smaller dissolution efficiency values were obtained where lower PVA concentration, higher CA concentration or lower drug to polymer weight ratio were used to prepare microspheres. Our results indicated that TRA-containing microspheres of suitable drug release profile and optimal particle size can be prepared by a simple emulsification-solvent extraction technique, provided that the process conditions are appropriately adopted and that polymer, emulsifier and drug concentrations are properly selected.

**Keywords:** Tretinoin; Microspheres; Emulsification-solvent extraction; Cellulose acetate

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### INTRODUCTION

Tretinoin or all-trans retinoic acid (TRA) is a metabolite of vitamin A which is indicated primarily in the topical treatment of comedonal and papulopustular acne vulgaris. It also has been used to treat mottled hyperpigmentation, roughness, and fine wrinkling of photodamaged skin and to induce remission in acute promyelocytic leukemia. In addition, topical TRA has been tried in a wide range of skin conditions such as rosacea, keratinisation disorders, pigmentation disorders, and some neoplastic disorders. Despite having these potentials TRA suffers several disadvantages which can strongly limit its utility. Its very low

water solubility may put restriction on the formulations which may be used for its efficient delivery, while its photodegradability can cause skin sensitization. Furthermore, topical TRA often causes transitory stinging, erythema, peeling, edema, dryness, or itching, which can result in poor patients compliance (1,2). To overcome problems associated with topical delivery of TRA various formulation approaches have been employed which include liposomes (3-6), niosomes (7), inclusion complexes (8,9), and solid lipid nanoparticles (10). Micro-encapsulation techniques have also been employed for incorporation of various topical drugs (11). The particulate delivery systems may be considered for topical

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delivery of medications because of their potentials of providing a controlled release rate and enhanced deposition of drug into the hair follicles, where drug is most needed. In view of this, microspheres seem to be a suitable candidate for topical controlled delivery of TRA, through which the severity of drug side effects may be reduced. The possibility of employment of various polymers and techniques for preparation of microspheres also makes them a potential candidate for encapsulation of drugs of various physico-chemical characteristics. The main aim of the present study was to develop a simple emulsification-solvent diffusion method for preparing TRA-containing microspheres and to evaluate the effects of formulation variables on microspheres physical characteristics and drug release behavior. This was preceded by a series of preliminary investigations in which the optimal experimental conditions were assessed.

## MATERIALS AND METHODS

### *Materials*

TRA and Polyvinyl alcohol (PVA) were purchased from Merck (Darmstadt, Germany). Cellulose Acetate (CA) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetone, ethyl acetate, isopropyl alcohol, potassium dihydrogen phosphate and disodium hydrogen phosphate were all of analytical grade and obtained from Merck.

### *Preparation of microspheres*

Microspheres containing TRA were prepared using an emulsification-extraction technique. Preliminary studies were first conducted to evaluate the influence of various experimental conditions such as temperature, stirring rate, state of saturation of external phase and organic to aqueous phase volume ratio on the microsphere particle size and production yield. Microspheres were then prepared at preset conditions as follows (Fig. 1): briefly, certain amounts of CA were dissolved in 50 ml mixture of acetone and ethyl acetate (2:3) to produce 2% or 4% w/v polymer solutions, as indicate in Table 1. TRA was dissolved in the polymer solutions so as to

obtain various drug/polymer ratios of 0.15 or 0.3 (w/w). A solution of PVA in water (60 ml, 1% or 2% w/v) was prepared, saturated with ethyl acetate, and placed in an ice-bath. Drug/polymer solution was dripped into the PVA solution, while being stirred using a paddle mixture at 1000 rpm for 60 min. The emulsion obtained was removed from the ice-bath and subjected to gradual addition of 340 ml 1% PVA solution while stirring the mixture at the same speed at room temperature for 1 h. The microspheres so formed were separated from the mixture by centrifugation (Segorita BHG 110, England) at 10,000 rpm for 5 min. To further remove remaining organic solvent from the microspheres and harden their walls, 200 ml 0.1% PVA solution was added to the microspheres and stirring the mixture was carried out under the conditions previously described for another 2 h. The microspheres were separated by centrifugation, washed with water, dried at room temperature, and then kept in a dark vessel in a desiccator (12-15). The supernatants of all centrifugation steps were pooled and kept for TRA analysis.

### *Experimental design*

A 2<sup>3</sup> full factorial design was used to study the influence of independent variables, polymer (CA) concentration, emulsifier (PVA) concentration, and drug/polymer weight ratio, on the dependent variables such as drug release, entrapment efficiency and particle size. The microspheres formulations together with their measured responses are reported in Table 1 and 2.

### *Particle size analysis*

Microspheres were sieved using a series of standard test sieves consisting Tyler sieve no. 32, 60, 100, and 270, shaken on a mechanical sieve shaker (Retsch, Model Etac BD, Germany). The weight of fractions retained on each sieve was measured. In another experiment a small amount of microspheres was suspended in distilled water on a microscope slide. The particle size was obtained by measuring the diameter of individual particles using an optical microscope (Leitz, model HM-LUX3, Germany), equipped with a digital camera (Nikon, Model HFX-DX)

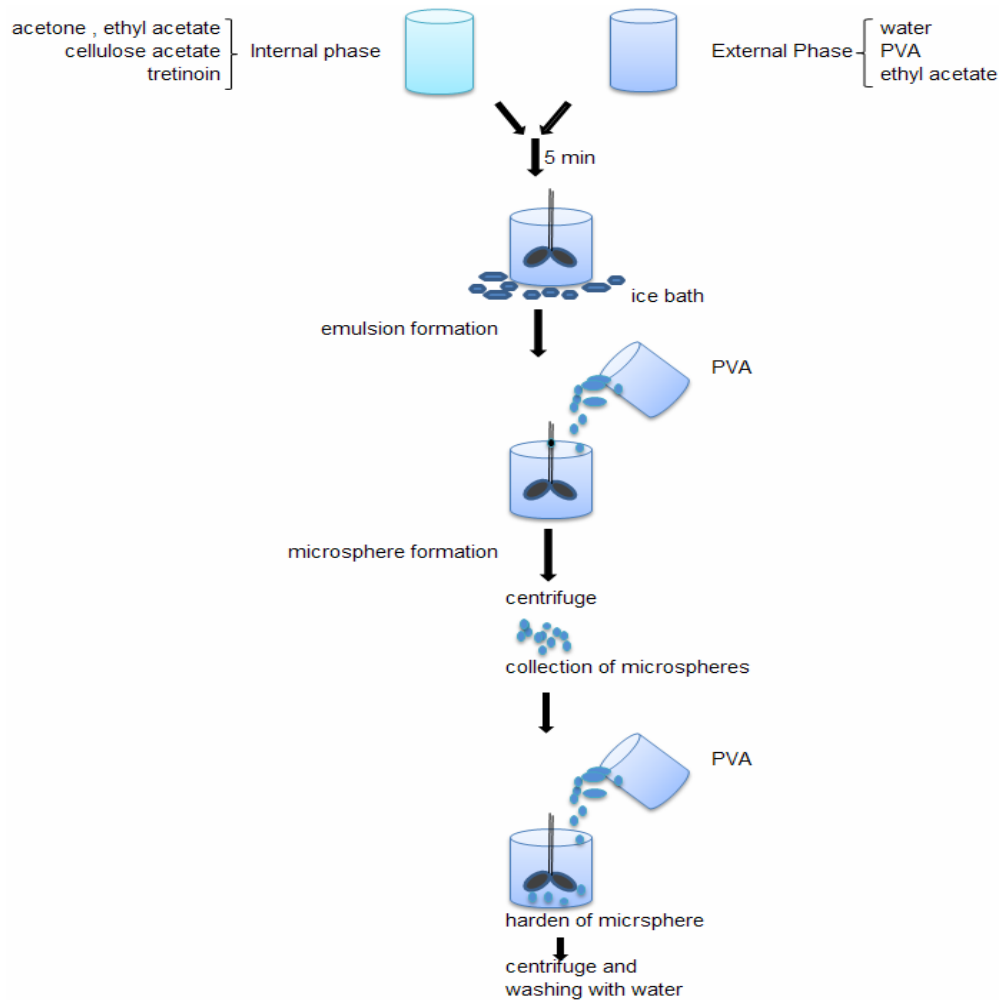


Fig. 1. Schematic presentation of microspheres preparation steps

Table 1. Experimental microsphere formulations in the  $2^3$  full factorial design

Formulation Codes	CA <sup>1</sup> (% w/v)	PVA <sup>2</sup> (% w/v)	TRA <sup>3</sup> /CA (wt ratio)
C <sub>2</sub> P <sub>1</sub> T <sub>0.15</sub>	2	1	0.15
C <sub>2</sub> P <sub>1</sub> T <sub>0.30</sub>	2	1	0.30
C <sub>2</sub> P <sub>2</sub> T <sub>0.15</sub>	2	2	0.15
C <sub>2</sub> P <sub>2</sub> T <sub>0.30</sub>	2	2	0.30
C <sub>4</sub> P <sub>1</sub> T <sub>0.15</sub>	4	1	0.15
C <sub>4</sub> P <sub>1</sub> T <sub>0.30</sub>	4	1	0.30
C <sub>4</sub> P <sub>2</sub> T <sub>0.15</sub>	4	2	0.15
C <sub>4</sub> P <sub>2</sub> T <sub>0.30</sub>	4	2	0.30

<sup>1</sup>cellulose acetate, <sup>2</sup>polyvinyl alcohol, <sup>3</sup>tretinoin

(6). A micrometer slide was employed to calibrate the size of particles in the photos taken by the camera. The diameter of 625 particles was measured and averaged.

Table 2. Formulations variables and their levels in a  $2^3$  factorial design

Coded Levels	Process variables and their		
	CA <sup>1</sup> (%)	PVA <sup>2</sup> (%)	TRA <sup>3</sup> /CA (Wt ratio)
Lower (-1)	2	1	0.15
Higher (+1)	4	2	0.30

<sup>1</sup>cellulose acetate, <sup>2</sup>polyvinyl alcohol, <sup>3</sup>tretinoin

#### Determination of drug content

To 50 mg TRA-containing microspheres 25 ml acetone:ethyl acetate (2:3) solution was added. The mixture was stirred for 6 h, while being completely protected from light. The solution obtained was assayed for TRA content by UV-spectrophotometry (Shimadzu, model UV Mini-1240CE) at 340 nm after appropriate dilution with the solvent system. The drug content and encapsulation efficiency

(EE) were calculated as follows:

$$\% \text{Drug content} = (\text{amount of TRA loaded in microspheres} / \text{weight of microspheres}) \times 100$$

Eq. 1

$$\% \text{EE} = (\text{amount of TRA loaded in microspheres} / \text{initial amount of TRA}) \times 100$$

Eq. 2

### Drug release from microspheres

The study of drug release from microspheres was carried out using the USP dissolution test apparatus No. II. An amount of 50 mg microspheres was placed in 900 ml dissolution medium comprising phosphate buffer (pH 7.4): isopropyl alcohol (8:2) which was maintained at  $37 \pm 0.1$  °C and stirred at 70 rpm. Samples (3 ml) were withdrawn at predetermined time intervals and assayed spectrophotometrically at 340 nm. The same volume (3 ml) of the dissolution medium was replaced after each sampling.

The influence of CA aging on drug release also was demonstrated by comparing TRA release from selected formulations freshly prepared and those prepared and kept in a desiccator at a dark place and room temperature for 6 months.

### Analysis of release data

The mechanism of drug release was evaluated from  $M_t/M_\infty = Kt^n$ , the semi-empirical equation proposed by Korsmeyer-Peppas (12).

In this equation,  $M_t/M_\infty$  is the fraction of drug released up to time  $t$ ,  $K$  is the kinetic

constant and  $n$  is the diffusional exponent, an indicative of the release mechanism. In order to compare release profiles of TRA from different formulations, dissolution efficiency and mean dissolution time, as model-independent approaches were also employed. All data were analyzed by ANOVA, followed by Fisher-LSD multiple comparisons test.

## RESULTS

### Preparation of microspheres

The investigation of some process and formulation variables in preliminary experiments indicated that the microspheres of optimal particle size range and uniformity were obtained (data not shown) where a lower volume of PVA aqueous solution was used, the external (aqueous) phase was saturated with ethyl acetate and emulsification was performed under ice-bath condition.

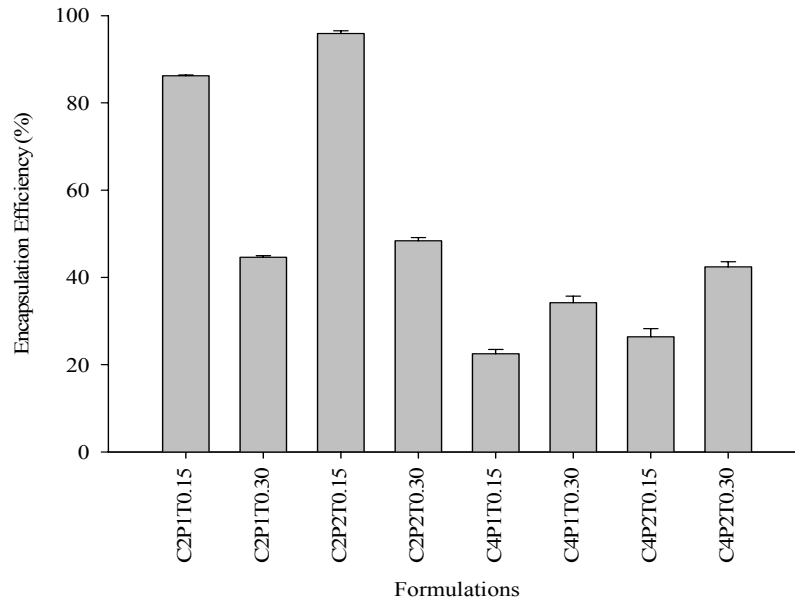
The microsphere production yield, calculated from the ratio of the weight of microspheres obtained to the initial weight sum of polymer and drug used, was about 37-52% yield for formulations made with 4% CA, and close to 100% for other formulations. This indicated that only low amounts of feeding materials were wasted during this procedure.

The influence of formulation variables on different characteristics of microspheres, investigated using a  $2^3$  factorial design, is summarized in Table 3. As is seen, all responses were significantly affected by the polymer concentration (CA) and the drug/polymer weight ratio.

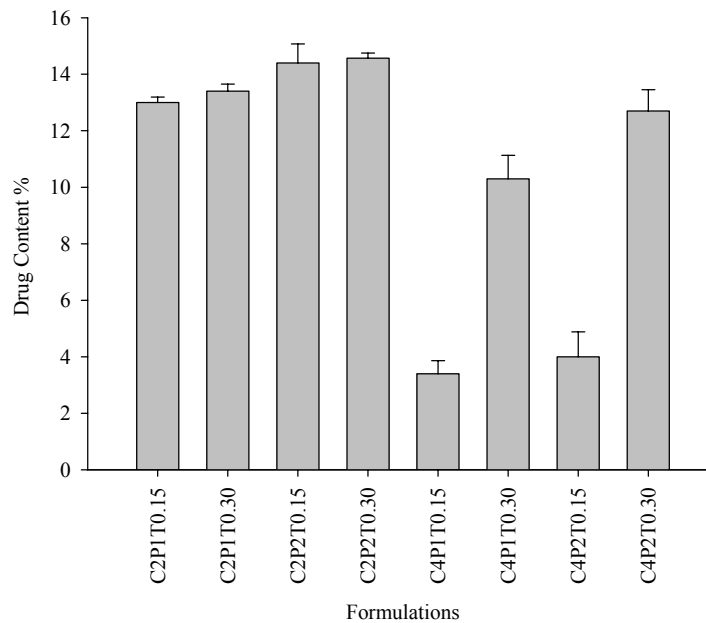
**Table 3.** The summary of statistically significant formulation variables<sup>1</sup> and their interactions

Responses	Factors					
	CA <sup>2</sup> [A]	PVA <sup>3</sup> [B]	TRA <sup>4</sup> /CA [C]	AB Interaction	AC Interaction	BC Interaction
DE <sub>8</sub> % <sup>5</sup>	S (-)	-	S (+)	-	S (+)	-
MDT <sup>6</sup> (min)	S (+)	S (-)	S (-)	-	S (-)	S (-)
EE <sup>7</sup> (%)	S (-)	S (+)	S (-)	-	S (+)	-
Drug Content (%)	S (-)	S (+)	S (+)	-	S (+)	-
Size (µm)	S (+)	-	S (+)	-	-	-

<sup>1</sup>Statistically significant formulation variables (with  $P < 0.05$ ) were marked with "S". The plus sign in the bracket indicates the positive effect on the value of the response as a function of the increase in the level of the factor. The minus sign indicates the opposite, <sup>2</sup>cellulose acetate concentration, <sup>3</sup>polyvinyl alcohol concentration, <sup>4</sup>tretinoin to cellulose acetate weight ratio, <sup>5</sup>dissolution efficiency after 8 h, <sup>6</sup>mean dissolution time, <sup>7</sup>drug encapsulation efficiency



**Fig. 2.** Encapsulation efficiency of TRA in various microsphere formulations. Results are expressed as mean  $\pm$  SD (n=3).



**Fig. 3.** TRA content of various microsphere formulations. Results are expressed as mean of drug amount in 100 g microspheres  $\pm$  SD (n=3).

The Fig. 2 indicates that formulations made with 2% CA resulted in significantly higher encapsulation efficiencies than with 4% of the same polymer ( $P < 0.01$ ). Using the lower percent of TRA/CA (i.e. 0.15 as compared to 0.3) in formulations made with 2% CA also produced higher encapsulation efficiencies

( $P < 0.001$ ). However, this difference was not seen in formulations made with 4% CA. The TRA encapsulation efficiency was higher in all the formulations made with 2% PVA as compared to 1% PVA ( $P < 0.01$ ).

The percent of drug content of microspheres is depicted in Fig. 3. In a similar manner with the

results of encapsulation efficiency, higher drug contents were obtained in formulations made with lower CA content. (i.e. 2%). However, no significant difference in the drug content of the aforementioned formulations was observed at various TRA/CA ratios in each pair group (e.g. C2P1T0.15 vs C2P1T0.3). In contrast, such differences were significant in microspheres made with 4% CA. In almost all formulations, compared pairwise, microspheres of higher drug content were obtained where a higher

PVA concentration (i.e. 2% cf 1%) was employed.

The analysis of particle size results indicated statistically insignificant effect of PVA concentration. The influence of CA concentration and TRA/CA ratio on the particle size is depicted in Fig. 4 and 5. As can be seen microspheres of smaller sizes were produced where both CA concentration and TRA/CA ratio were set at their lower values.

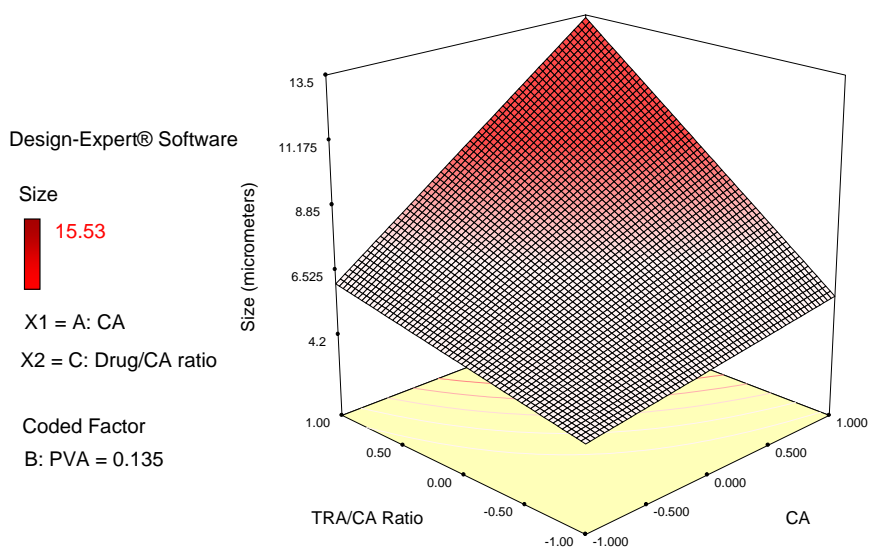


Fig. 4. The microsphere size as functions of polymer (CA) and drug/polymer (TRA/CA) ratio in a 3-D surface view.

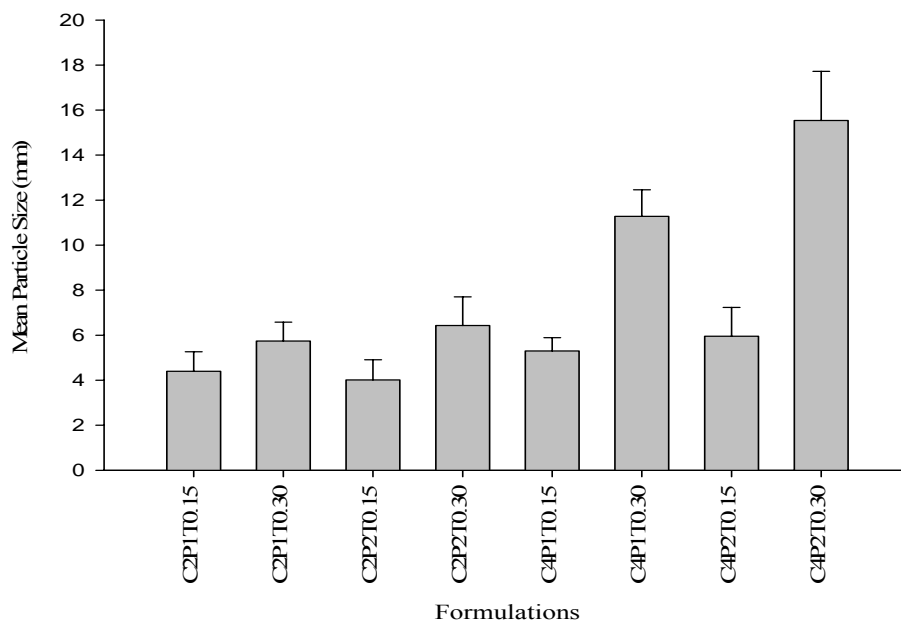
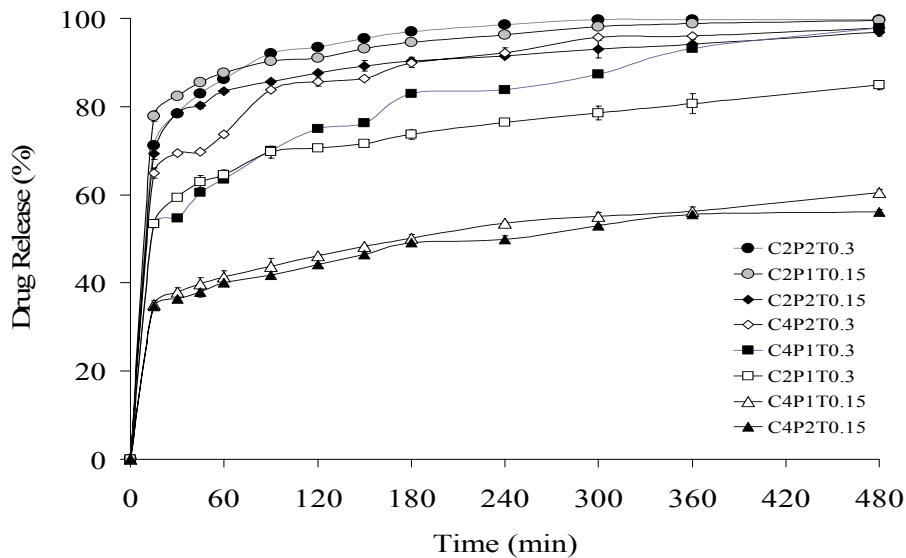


Fig. 5. Particle size of various microsphere formulations, presented as mean ± SD (n=3).

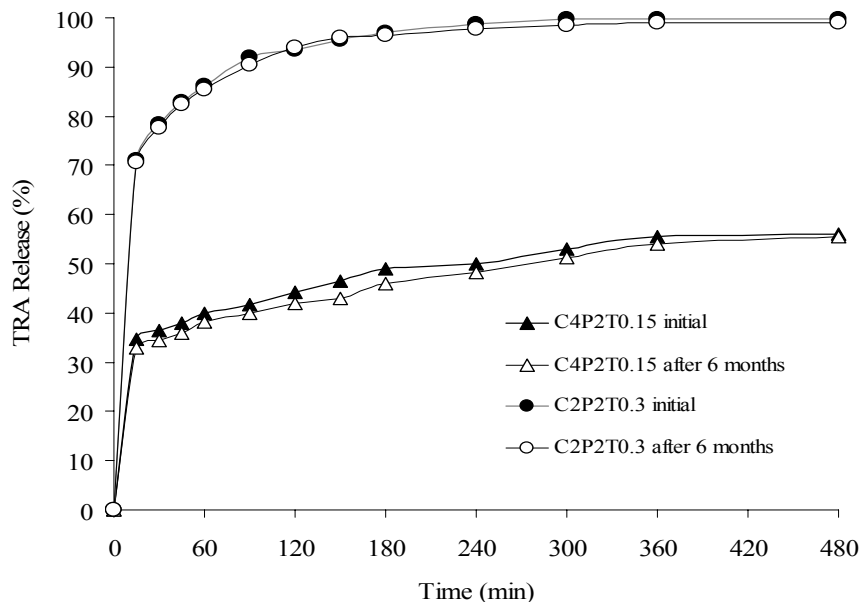
**TRA release from microspheres**

The release of TRA from microspheres is depicted in Fig. 6. The release pattern of TRA from all formulations was consisted of a burst effect at the early stage, followed by a slower release at the later stage. As can be seen, microspheres loaded with lower TRA ratio (i.e. 0.15) released smaller amount of drug during the course of study than microspheres of

higher TRA ratio of 0.3. In contrast, the extent of drug release was often greater from formulations comprising 2% CA than 4% CA. However, the concentration of PVA did not seem to cause much effect on the drug release. The comparison of TRA release from freshly-prepared microspheres and those of six-months of age are shown in Fig. 7.



**Fig. 6.** Release of TRA from various microsphere formulations in dissolution medium comprising phosphate buffer (pH 7.4): isopropyl alcohol (8:2) at 37 °C. Each point represents mean  $\pm$  SD (n=3).



**Fig. 7.** Release of TRA from representative microsphere formulations, immediately or six months after preparation, in dissolution medium comprising phosphate buffer (pH 7.4): isopropyl alcohol (8:2) at 37 °C. Each point represents mean  $\pm$  SD (n=3).

**Table 4.** Release parameters of tretinoin from different microsphere formulations (n=3)

Formulation Codes	MDT <sup>1</sup> ± SD	DE <sub>8</sub> % <sup>2</sup> ± SD
C <sub>2</sub> P <sub>1</sub> T <sub>0.15</sub>	115.6 ± 3.5	91.9 ± 0.3
C <sub>2</sub> P <sub>1</sub> T <sub>0.30</sub>	157.1 ± 2.4	72.9 ± 1.0
C <sub>2</sub> P <sub>2</sub> T <sub>0.15</sub>	122.5 ± 11.5	87.7 ± 0.8
C <sub>2</sub> P <sub>2</sub> T <sub>0.30</sub>	80.1 ± 1.4	92.8 ± 0.1
C <sub>4</sub> P <sub>1</sub> T <sub>0.15</sub>	178.8 ± 9.5	50.0 ± 0.6
C <sub>4</sub> P <sub>1</sub> T <sub>0.30</sub>	173.3 ± 3.9	80.1 ± 0.3
C <sub>4</sub> P <sub>2</sub> T <sub>0.15</sub>	160.7 ± 3.8	47.9 ± 0.1
C <sub>4</sub> P <sub>2</sub> T <sub>0.30</sub>	123.7 ± 6.0	87.1 ± 0.2

<sup>1</sup>MDT: mean dissolution time (min), <sup>2</sup>DE<sub>8</sub>%:Dissolution efficiency up to 8 h of release study

TRA dissolution efficiency up to 8 h of release study (DE<sub>8</sub>%) and mean dissolution time (MDT) of all formulations are given in Table 4. The ANOVA test showed that microspheres made with higher CA% generally had greater MDT and smaller DE<sub>8</sub>% of TRA ( $P < 0.05$ ). The opposite effect, however, was seen with increasing TRA/polymer ratio in the formulations.

## DISCUSSION

### Preparation of microspheres

The method used to prepare microspheres produced a 37-52% yield in formulation made with 4% CA, but a high yield of close to 100% in other formulations. This indicated that only low amounts of feeding materials were wasted in this procedure.

A very important criterion for dermatological preparations is to be devoid of particles causing a gritty feel when applying on skin. This is generally achieved when particles suspended in a topical vehicle have sizes smaller than 30 μm (15). Thus, our preliminary studies were aimed to assess the process conditions in which microspheres of desired size range could be obtained. Reducing the temperature of external phase was shown to play an important role for this purpose. Acetone existing in the internal phase can readily diffuse into and then evaporate from the aqueous external phase. Particles of smaller sizes were obtained in preliminary studies (data not shown) where emulsification

was performed in an ice-bath as compared to the room temperature. Since the temperature controls the speed of solvents evaporation, and thus particle formation (16), the emulsification was carried out under ice-bath condition for all experiments. Another process condition which was investigated in the preliminary experiments was the influence of saturation of aqueous phase with ethyl acetate on the particle size. Microspheres produced tended to have larger sizes where the external (aqueous) phase was devoid of ethyl acetate. Under this condition, organic solvents of emulsified droplets can diffuse out more quickly into the aqueous phase and form larger particles. In contrast, where ethyl acetate-saturated aqueous solution was used as the external phase, a longer time was needed for the organic solvents within the dispersed droplets to enter the aqueous phase resulting in formation of particles of smaller sizes during emulsification stage (15). Therefore, in all experiments the aqueous phase used was saturated with ethyl acetate.

Statistical examination of the particle size data indicated that the combinatory effect of polymer concentration (CA%) and TRA/polymer weight ratio had a substantial influence on the particle size. The significantly larger sizes of particles of formulations C<sub>4</sub>P<sub>1</sub>T<sub>0.30</sub> and C<sub>4</sub>P<sub>2</sub>T<sub>0.30</sub> than of other formulations may be partially attributed to the higher viscosity of organic phase and thus formation of larger organic phase droplets during emulsification step. This is in agreement with other workers (16,17) who obtained microspheres of larger sizes where they used higher concentrations of polymers. The increase in TRA/CA weight ratio was another factor which affected the microsphere particle size. This was also observed by Young-Il Jeong and co-workers (18), and could be possibly resulted from the change in hydrophobicity, and consequently the surface tension magnitude of the dispersed organic droplets containing a larger amount of TRA. Significantly greater encapsulation efficiency of TRA in microspheres of C<sub>2</sub>P<sub>1</sub>T<sub>0.15</sub> and C<sub>2</sub>P<sub>2</sub>T<sub>0.15</sub> than that in C<sub>2</sub>P<sub>1</sub>T<sub>0.3</sub> and C<sub>2</sub>P<sub>2</sub>T<sub>0.3</sub> ( $P < 0.001$ ) respectively, (Fig. 2) can



be explained by the fact that lower amounts of TRA have a greater chance of entrapment in the same amounts of polymer (CA) used for preparation of microspheres. The encapsulation efficiency of TRA in formulations mentioned above also showed an increase where a 2% PVA concentration was used, as compared to 1%. This can be attributed to easier and more efficient emulsification step in the presence of the optimal 2% PVA solution (19). The lower encapsulation efficiency of TRA in formulations made with 4% CA, compared with 2% polymer (Fig. 2), seemed to be due to the high viscosity produced by the former, impeding efficient drug entrapment during emulsification step. Lower microsphere production yields of about 37-52% in formulations made with 4% CA, compared to about 90-100% obtained for other formulations also indicates the difficulty posed by higher concentrations of CA in preparing microspheres.

#### ***Drug release from microspheres***

An *n* value of less than 0.43 obtained by examining TRA release data using Korsmeyer-Peppas equation indicated that the Fickian mechanism was basically involved in the drug release from various formulations. This is in agreement with Soppimath and co-workers (20) who also reported the same mechanism for drug release from microspheres made with CA.

The burst effect seen in TRA release from microspheres (Fig. 6) may be attributed to the deposition of drug at or close to the outmost parts of the microspheres walls (21). In the case microspheres are incorporated into a topical vehicle, the drug released as burst can provide the initial therapeutic concentration needed, whereas the remaining drug released at later stage can maintain drug concentration needed for a longer period.

Pairwise comparisons of formulations with similar PVA concentration and drug/polymer weight ratio but only different at CA concentration indicated that microspheres prepared from higher CA concentrations generally produced significantly greater MDT and smaller DE values ( $P < 0.05$ ), as expected. This can be attributed to the thicker wall in

such microspheres, which causes the slower drug release rate (i.e. greater MDT) and lower extent of drug release during the same length of time (i.e. smaller DE) (22,23). Smaller MDT and greater DE values were often obtained for microspheres prepared from similar CA and PVA concentrations, but with higher drug concentrations. This seems to be due to the lower availability of polymer at higher drug loading, leading to a faster drug release. The pairwise comparisons also indicated that microspheres made at higher PVA concentration generally released the drug more completely and more quickly, as indicated by their greater DE and smaller MDT values ( $P < 0.05$ ). It can be conceived that at higher PVA concentration microspheres are formed more easily, and accordingly the drug is released from such microspheres more readily, due to the surfactant effect of PVA (21,24). Similar profiles of TRA release from microspheres, immediately or six months after preparation (Fig. 7), indicated that microspheres maintained their integrity and stability during the course of study.

#### **CONCLUSION**

Following preliminary studies for finding desirable experimental conditions, a full factorial design study was carried out which indicated that TRA-microspheres of smaller sizes were obtained with lower CA and TRA concentrations. A slower drug release rate (i.e. greater MDT) and smaller DE value was generally obtained where TRA-containing microspheres were prepared at lower PVA concentration or from higher CA or lower drug concentrations. These results indicate that TRA-containing microspheres of suitable drug release profiles and optimal particle size for dermatological preparations can be produced by proper selection of polymer, emulsifier and drug concentrations as well as appropriate adoption of process conditions, using an emulsification-solvent extraction technique.

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