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Topical application of albumin microspheres containing vitamin A
Drug release and availability

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Summary

Egg albumin microspheres containing vitamin A (15.7 ± 0.8% w/w; size 222 ± 25 μm) were produced by an emulsion method. These particles were used to prepare O/W creams of vitamin A. The in vitro and in vivo drug release of a microencapsulated vitamin A cream was studied and compared with a non-microencapsulated vitamin A cream. The in vivo study in six volunteers shows that these microspheres were able to remain on the skin for a long period of time, and as a consequence they were able to prolong the release of vitamin A. The relative availability of the microencapsulated vitamin A cream, compared with the non-microencapsulated vitamin A cream was 78.2 ± 7.3%.

Introduction

In recent years, a great number of different colloidal particles, such as microcapsules, microspheres and liposomes, have been proposed and used in topical formulations as drug carrier vehicles. It has been claimed that these new drug vehicles can improve and control the drug release from conventional topical formulations. Furthermore, the massage effect of these particles in the skin can have a cleaning and stimulant effect (Horino et al., 1985; Yanagida and Murotani, 1985).

Although the application of these colloidal particles in dermatology is of great interest, there are few papers about the characteristics of these new vehicles for topical formulations and most of the background is based on different patents.

One of the problems in the study of the possible efficacy of a topical formulation in dermatology is the limited number of in vitro/in vivo correlation investigation. This is due to the fact that most of the in vitro dissolution systems used are oversimplified simulations of the complex processes involved in drug application and absorption from the skin (Abdou, 1989). For this

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reason, the use of in vivo experiments is always required in order to judge the efficacy of a topical formulation.

Theory

In vivo drug release

There are several methods available to study in vivo drug release (Barry, 1988). We have chosen one based on the assay of the remaining formulation present on the skin at different times.

The amount of drug released at time t (X_t) can be expressed as the difference between the initial drug (X_0) and the residual drug on the skin (X_r) at that time.

$$X_t = X_0 - X_r \quad (1)$$

X_r can be calculated by removing and weighing the formulation remaining on the skin (M_r) and subsequent assay of the formulation (C_r):

$$X_r = M_r \cdot C_r + X_e \quad (2)$$

Therefore, Eqn 1 can be expressed as:

$$X_t = X_0 - M_r \cdot C_r - X_e \quad (3)$$

If we consider X_t as a percentage of the initial dose, then

$$X_t\,(\%) = \frac{X_0 - M_r \cdot C_r - X_e}{X_0} \cdot 100 \quad (4)$$

In order to study the in vivo drug release of topical formulations, small samples of such preparations can be placed on the skin. At different times the residual formulation on the skin can be removed, weighed and tested. Eqns 1–4 can then be used to determine the in vivo drug release.

Materials and Methods

Materials

The following materials were obtained from the indicated sources. Reactive grade: retinol palmitate (Roche); ovalbumin (Ovosec); Cremophor EL (Basf); isooctane (Sharlau-Ferosa); Lanol CTO and 14M (Seppic); and isopropyl myristate and Span 85 (Sigma). Analytical grade: methanol HPLC (Scarlau-Ferosa); hydroxynaphthol blue (Sigma); hydrochloric acid, sodium acetate and glacial acetic acid (Merck).

Methods

Assay of vitamin A An HPLC method was used (DeLeenheer et al., 1979). The HPLC apparatus was a Hewlett-Packard HP 1084 with a fixed UV detector at 254 nm.

Microencapsulation 1 g of vitamin A was solubilized with 4 g of Cremophor EL and dissolved in 20 ml of water. 10 ml of this vitamin A solution were added to 10 ml of a 20% w/v ovalbumin solution. Then, 100 ml of isooctane with Span 85 (1% v/v) were added and the system was stirred for 15 min in order to produce an emulsion. The emulsion was then heated to 60 °C for 15 min and on coagulation, albumin microspheres were isolated by decantation. Subsequently, they were dried at 45 °C for 12 h. The microspheres were sieved through a 250 μm sieve and then stored at 5 °C in a closed container in the dark.

Appearance The appearance of the microspheres was evaluated by optical microscopy (Optiphot, Nikon).

Available drug loading in the microspheres An accurately weighed amount of microspheres were crushed in a morter and then suspended in 50 ml of pH 5 buffer. The amount of vitamin A released at 12 h in the dark was evaluated as the available drug loading in the microspheres.

Formulation of the o/w creams 0.4 g of Cremophor EL were added to 5 g of Lanol CTO and 4 g of Lanol 14M. The mixture was heated to 50 °C and homogenized. Subsequently, water also heated to 50 °C was added to 100 g. Two different creams of vitamin A were formulated: one contained microencapsulated vitamin A, the other comprised vitamin A solubilized with Cremophor EL. Both creams had a vitamin A concentration of 10 mg/g. In order to determine the error of the recovery process, another cream containing a non-absorbable chemical ($CaCO_3$) was prepared.

In vitro drug release Samples were placed in a

container of 19.6 cm^2 and immersed in 100 ml of pH 5 buffer (BP, 1988) (sodium acetate/glacial acetic acid) at 35 °C under continuous mechanical stirring at a rate of 50 rpm. Samples were taken at different intervals, filtered and then assayed.

In vivo drug release The o/w creams of microencapsulated and non-microencapsulated vitamin A were tested by six informed healthy young people (five males and one female; age, 24–28 years). The study was conducted in a randomized and crossover manner; six areas of 2×2 cm^2 were located on the underside of the forearm of each volunteer to limit the skin surface area to 4 cm^2. An accurately weighed amount (approx. 100 mg) of each formulation was placed on each of the limited skin surfaces. The non-absorbed residual content of formulation on the skin was removed at 30, 60, 90, 120, 150 and 180 min. The residual contents of the formulations were weighed and then assayed. The amount of drug release, expressed as a percentage of the initial dose, was calculated according to Eqn 4.

Determination of the error (X_e) in the recovery

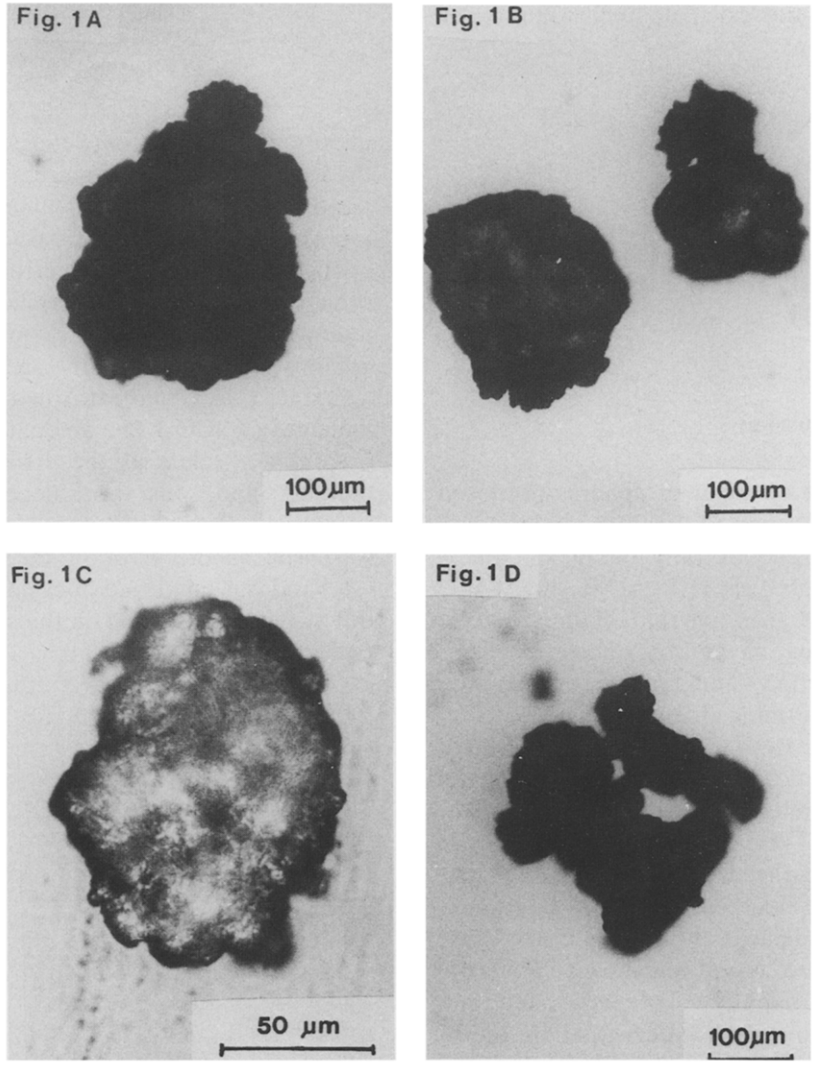

Fig. 1. (A–D) Albumin microspheres containing vitamin A.

process from the skin A non-absorbable chemical ($CaCO_3$) was formulated into a cream with characteristics similar to those of creams containing vitamin A. The cream containing $CaCO_3$ was administered to each of the six volunteers as described above. The error of the method (X_e) was calculated as the difference between the amount of $CaCO_3$ administered to each volunteer (X_0) and the recovered portion (X_r): $X_e = X_0 - X_r$.

Relative bioavailability The relative bioavailability (F) of the microencapsulated vitamin A cream compared with the non-microencapsulated vitamin A cream was calculated according to the following equation:

$$f\,(\%) = \frac{X_{t,\mathrm{MAVC,3h}}}{X_{t,\mathrm{NMVAC,3h}}} \cdot 100$$

where $X_{t,\mathrm{MAVC,3h}}$ and $X_{t,\mathrm{NMVAC,3h}}$ denote the total amount of vitamin A released at 3 h from the microencapsulated and non-microencapsulated vitamin A cream, respectively.

Results and Discussion

The mean size of the microspheres produced by the previously reported emulsion method was 222.1 ± 25.2 μm and the amount of vitamin A available for release was $15.7 \pm 0.8\%$. Fig. 1A–C shows the appearance of the albumin microspheres containing vitamin A. These particles have a rough surface and tend to agglomerate with each other forming clumps (Fig. 1D).

Fig. 2 demonstrates the in vitro drug release of different formulations. Under our experimental conditions no membrane was used: the vehicle of the formulation is the factor which controls the rate of release of the drug (Barry, 1988). Fig. 2 indicates that the microencapsulation of vitamin A causes a slight delay in the release of the drug, however, this effect is not significant ($P < 0.01$) for the incorporation of microspheres into the cream formulation. In the latter situation, the in vitro release rate depends on the release from the cream and the effect of microencapsulation is less important than and outweighed by the effect of the cream. These results are consistent with the rapid drug release from albumin microspheres reported by Brophy and Deasy (1984).

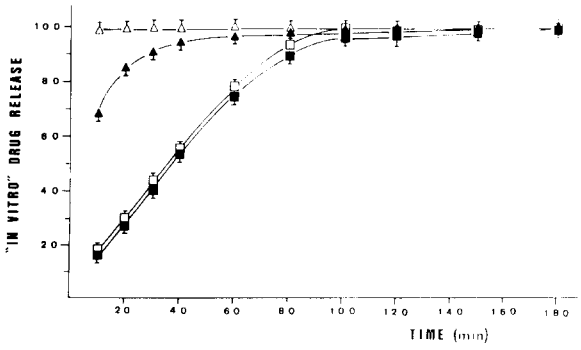

Fig. 2. In vitro drug release of different formulations of vitamin A in pH 5 buffer at different times. (△) Solubilized vitamin A, (▲) albumin microspheres containing vitamin A, (□) o/w cream of vitamin A, and (■) o/w cream of microencapsulated vitamin A.

In order to assess the error of the recovery process in the in vivo experiments, a cream containing a non-absorbable chemical ($CaCO_3$) was administered to each of the six volunteers.

Table 1 lists the data on the non-recovered amount of $CaCO_3$. The amount of non-recovered $CaCO_3$ was taken as the error of the recovery method and was not dependent on time (ANOVA, $P < 0.001$). A mean value of 16.1% was used as the error of the recovery method (X_e) and was employed in calculating the amount of vitamin A remaining on the skin (X_r) at different times (Eqns 3 and 4).

The cumulative in vivo drug release (Fig. 3) from the two different creams, with and without

TABLE 1

Mean values and standard deviations of non-recovered $CaCO_3$ at different times

Time (min)	Non-recovered $CaCO_3$
30	14.6 (7.1)
60	17.1 (7.2)
90	16.4 (7.5)
120	17.6 (10.2)
150	16.7 (7.2)
180	14.3 (10.5)

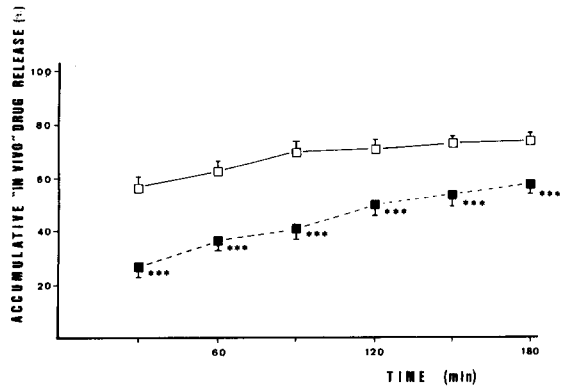

Fig. 3. Mean values and standard deviation of the cumulative in vivo drug release of the two formulations at different times (□ ——— □) o/w cream of vitamin A, (■ - - - - - - ■) o/w cream of microencapsulated vitamin A. *** Significant (ANOVA, $p < 0.001$).

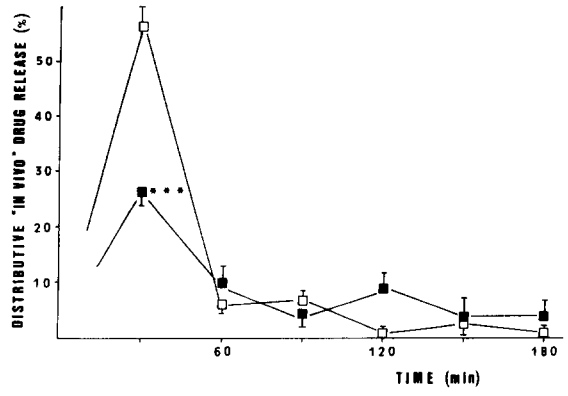

Fig. 4. Mean values and standard deviation of the distributive in vivo drug release of the two formulations at different times. (□) o/w cream of vitamin A, (■) o/w cream of microcapsulated vitamin A. *** Significant (ANOVA, $p < 0.001$).

microencapsulated vitamin A, shows significant differences ($P < 0.001$). These results can also be expressed in the form of a distribution (see Fig. 4). Fig. 4 demonstrates that the most important difference between the two formulations is in the vitamin A release during the first 30 min. Most of the retinol palmitate ($72.4 \pm 4.2\%$) is released from the non-microencapsulated vitamin A cream during the initial 30 min whereas only $40.5 \pm 2.4\%$ is released from the microencapsulated cream. The slower rate of release from the microencapsulated vitamin A cream can have various therapeutic effects. This may be useful in attempting to minimize possible inhibition of the process of keratinization due to excess vitamin A (Marcus and Coulston, 1990). It may also be of use for the preparation of sustained-release formulations in order to prolong the duration of drug release. However, in the case where the rate of release is too slow, such formulations can have poor bioavailability.

Figs 3 and 4 clearly demonstrate that the rate of drug release from the microencapsulated vitamin A cream is slower as compared with the non-microencapsulated formulation. The relative bioavailability during the first 3 h was evaluated as $78.2 \pm 7.3\%$. Fig. 4 indicates that, during the final stages of the in vivo study, the microencapsulated vitamin A cream releases drug at a higher rate than that of the non-microencapsulated formulation.

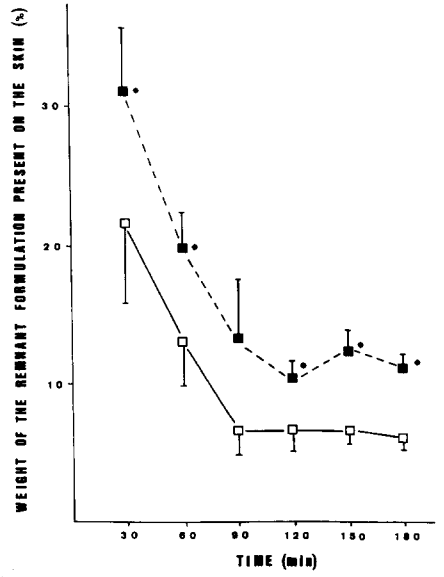

Fig. 5. Mean values and standard deviation of the weight of the remnant formulations present on the skin (expressed as a percentage of the initial weight) at different times (□ ——— □) o/w cream of vitamin A, (□ - - - - - - □) o/w cream of microencapsulated vitamin A. * Significant (Student's t-test $p < 0.05$).

Fig. 5 shows plots of the weight of the remnant formulation present on the skin at different times. The absorption of the micoencapsulated vitamin A cream formulation occurs to a lower extent than that of the non-microencapsulated cream. The difference between both formulations varied between 5 and 7%, and could be due to the presence of microspheres. The proportion of microspheres in the microencapsulated vitamin A formulation was 7.1%. Furthermore, the presence of these particles in the residual formulation on the skin was examined by optical microscopy. Ovalbumin microspheres do not penetrate the skin and tend to remain on the surface, where they release vitamin A.

It can be concluded from the present work that egg albumin microspheres of size 222 ± 25 μm, containing vitamin A ($15.7 \pm 0.8\%$), can be useful for topical formulations of vitamin A. The in vitro study showed that, during the first 3 h, the microspheres could remain on the surface of the skin, and as a consequence, were able to prolong the release of vitamin A. The relative bioavailability of the microencapsulated vitamin A cream, as compared with the non-microencapsulated formulation was $78.2 \pm 7.3\%$.

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