

Gelatin microspheres for topical delivery of Vitamin A palmitate

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ABSTRACT Vitamin A palmitate is widely used in skin care products, but the major problem associated with these formulations is stability against photooxidation. Hence an attempt was made to increase the stability of vitamin A palmitate by encapsulating it in gelatin microspheres. Gelatin microspheres were prepared using coacervation method and process was optimized using 2³ factorial design. Drug loaded microspheres were incorporated in carbopol gel for controlled delivery over a period of 24 hrs. Microspheres were evaluated for percent drug entrapped, particle size, surface morphology and in vitro drug diffusion studies for drug loaded microspheres and gel formulations using dialysis membrane and rat skin. Effect of stirring time, speed of stirring, concentration of gelatin on particle size was also investigated. Microspheres were found to be spherical having smooth surface and particle size in range of 100-350- μ m. Highest drug entrapment of 67% was achieved with gelatin: drug (1:2). Increase in speed of agitation decreased the particle size of microspheres. Drug release from microspheres followed Higuchi kinetics while gel formulation showed zero order release profile. The developed microspheres showed drug release for a period of 24 hr at a controlled rate and the stability of Vitamin A palmitate was also increased by encapsulation method.

Keywords: Vitamin A palmitate, Microencapsulation; Gelatin Microspheres; Coacervation; Zero order release; Topical gels.

Introduction

Vitamin A derivatives, due to their unique characteristics are expected to play a major role of the 21st century cosmetics. Because of the interest in anti aging in general and healthy skin in particular. Vitamin A and its derivatives have long been used as essential vitamins for healthy skin. Vitamin A acts on DNA to promote healthy keratinocytes with better horny layer, reduction of excessive pigmentation and increased collagen formation and elastin formation^{1,2}.

Retinyl palmitate is the most important form of vitamin A for the skin because more than 80% of the vitamin A normally found in skin as retinyl palmitate. Even when one applies retinol or retinoic acid to the skin, it is normally converted to retinyl palmitate

and stored in the skin. Retinyl palmitate is the least irritant version of vitamin A, for this reason it is used most regularly in various antiaging products³. The major problem associated with vitamin A and its derivatives is their stability against photooxidation.

Hence the aim of research project was to enhance the stability of vitamin A palmitate by encapsulating it in microspheres. Encapsulation increases the stability of the drug and also delivers the drug in controlled form.

Experimental

Materials

Vitamin A palmitate was obtained as gift sample from US Vitamins, food grade gelatin, formaldehyde 40%, isopropyl alcohol, liquid paraffin and glycerin were purchased from S. D. Fine Chemicals.

Carbopol 974 p was obtained as a gift sample from Noveon.

Microencapsulation method

Vitamin A Palmitate and gelatin were weighed as given in the formulation table 1. About 10 ml of aqueous gelatin solution was prepared by heating the polymer in water at 55° C, which is the water phase. Vitamin A palmitate was dispersed in the polymer with controlled constant stirring for about 15 minutes, such that the drug was properly dispersed in the gelatin solution. The oil phase consists of a mineral oil such as liquid paraffin containing sorbitan monooleate (4%) and magnesium stearate (8%) dispersed in it. The water phase consisting of drug dispersed in the polymer solution was added drop wise in the oil phase with constant stirring. The vessel was then immediately stepped in ice bath and stirring continuously for 20 min. Microspheres obtained were dehydrated by the addition of isopropyl alcohol with gentle stirring⁴⁻⁷. Then the microspheres were decanted and redispersed in isopropyl alcohol. Formaldehyde solution in isopropyl alcohol (1% v/v) was added with stirring and the microspheres were then recovered by decantation, washed thrice with isopropyl alcohol followed by twice with acetone, dried at room temperature and stored in air tight container or refrigerator.

Optimization of Vitamin A Palmitate microspheres

Vitamin A Palmitate loaded gelatin microspheres were optimized using 2³ factorial design at two levels. Various parameters like effect of concentration of gelatin: drug ratio, speed of agitation, concentration of cross linking agent on formation of microspheres were studied as given in table 1 and experimental design for the same is shown in table 2. Gelatin was used in concentration ranging from 5-20 % w/v. Cross linking agent was used in 1-4 % w/v concentration and its effect on in-vitro drug release and surface morphology of drug was studied⁸⁻¹⁰.

Microspheres characterization

Particle size analysis

Size and shape of drug-loaded microspheres were analyzed by image analysis and optical microscopy.

Scanning Electron Microscopy

Surface morphology of microspheres was studied using scanning electron microscopy, JEOL, Japan. The microspheres were mounted on sample stub and coated with gold film (~ 200 nm) under reduced pressure (0.133 Pa)¹¹.

Entrapment Efficiency

To determine the entrapment efficiency, drug loaded microspheres were first washed with cyclohexane. The solution was then filtered and analyzed for unentrapped Vitamin A Palmitate. The intact microspheres were then triturated and immersed in 0.1 N HCL overnight and warmed for 30 mins at 50°C. The resultant dispersion was then separated using cyclohexane, filtered and analyzed at 322 nm using UV-Visible spectrophotometer. The total drug content in microspheres was calculated using given formula,

Total drug content = Unentrapped drug + Entrapped drug.

Table 1: Level and parameters selected for optimization process

Process parameters	Associated variable	Higher level (Code +1)	Lower level (Code -1)
Concentration of drug:gelatin	X1	1:4	1:2
Stirring rate (rpm)	X2	1200	800
Concentration of continuous phase	X3	200 ml	50 ml

In vitro release profile

Drug release from microspheres was determined in phosphate buffer: Tween 80 (80:20 v/v) (pH 7.4). 5 g of microspheres were placed in 900 ml release medium, stirring at 75 rpm. 5 ml samples were removed at different time intervals and analyzed spectrophotometrically at 322 nm.

Preparation of gel formulation

A series of gel formulations containing Vitamin A palmitate microspheres were formulated using carbopol 974 p in concentration range of 0.5-1.5 %. The carbopol was neutralized with triethanolamine. Triethanolamine is one of the commonly used neutralizing agents in cosmetic gels as it is non irritant. Gels were stabilized by adding preservatives like methyl and propyl paraben.

Conventional gels were prepared by same procedure as described above, instead of microspheres pure Vitamin A palmitate was dispersed in carbopol.

In vitro diffusion studies form gel formulations

Drug release from drug loaded microsphere gel formulation and a conventional gel formulation containing Vitamin A palmitate using Keshary-Chein cell containing 20 ml of release medium Phosphate buffer:Tween 80 (80:20). About 1 g of topical gel formulation was spread on the dialysis membrane and placed on Keshary-Chein cell ¹²⁻¹⁴. The release medium was maintained at 37±0.2°C. About 1 ml of sample was removed at different time intervals and replaced with the same amount of fresh medium each time. In vitro diffusion studies were also carried out on rat skin. The data obtained was fitted with various mathematical models.

Stability studies

Stability studies were performed on developed formulation as per ICH guidelines for period of three months. The formulations were evaluated for appearance, drug content and in-vitro drug diffusion studies.

Results and discussion

Particle size analysis and surface morphology

Gelatin microspheres of vitamin A palmitate prepared in this study were nearly spherical and had smooth surface, which was confirmed from SEM (Figure.1). The mean particle size of gelatin microspheres was 10-100 µm. About 90 % of microspheres were in narrow range of 25-40 µm, which is suitable enough for topical formulations. It was observed that as the concentration of gelatin: drug ratio increases aggregates of microspheres were obtained further increase in concentration gave lump formation. Stirring rate was inversely proportional to particle size. As the stirring speed is increased above 1200, only fibres were obtained. Since less time is there for microsphere formation. Increasing concentration of cross linking agent retards the drug release. Crosslinking agent hardens the microspheres because of which drug release is decreased. The optimum concentration of crosslinking agent was found to be 1%w/v.

Table 2: Experimental Factorial Design

Experiments	X1	X2	X3	Characterization of drug loaded microspheres		
				Particle size ^a (µm)	EE ^b (%)	Yield (%)
M1	+1	-1	-1	200 ± 4.5	20 ± 2.6	50
M2	+1	+1	-1	235 ± 3.7	33± 2	62
M3	+1	-1	+1	330 ± 6.7	41.2± 3.7	40
M4	-1	-1	+1	80 ± 3.9	43 ± 3	50
M5	-1	+1	+1	78 ± 5.7	52.6 ± 1.9	51
M6	-1	+1	-1	50 ± 4.5	27± 2.5	56
M7	-1	-1	-1	42 ± 2.4	67 ± 2.7	89
M8	+1	+1	+1	45 ± 6.8	51 ± 3.6	47

X1: Concentration of gelatin: drug X2: Stirring rate, X3: Concentration of continuous phase
EE: Encapsulation efficiency; ^aMean ± SD, n = 10; ^bMean ± SD, n = 6

Table 3: Fitting of drug release data according to various mathematical models

Formulations	Mathematical model					
	Zero order		First order		Higuchi	
	R ²	r	R ²	r	R ²	r
Vitamin A palmitate loaded microspheres	0.8765	0.8812	0.8991	0.9145	0.9878	0.9923
Gel loaded with vitamin A Palmitate microspheres	0.9876	0.9898	0.8967	0.9134	0.8613	0.8779
Plain vitamin A palmitate	0.8985	0.8989	0.9767	0.9798	0.9656	0.9787

Entrapment efficiency

Batch M7 gave highest drug entrapment of 67% as compared to other batches. Hence this batch was selected as the optimized batch for gel formulations.

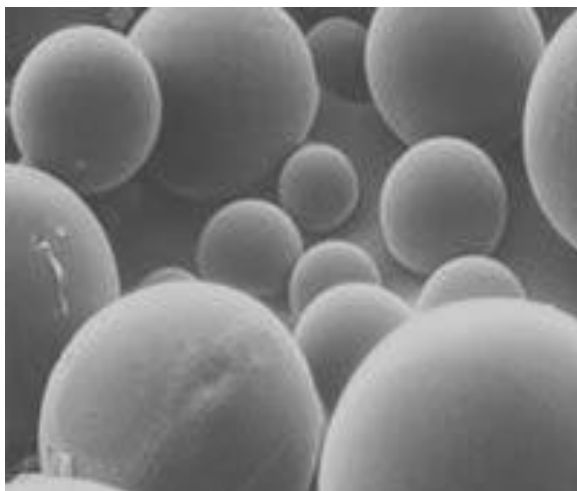


Figure 1: Scanning Electron Microscopy of Vitamin A palmitate loaded microspheres at 400 X magnification

Drug content and in-vitro release profile

Drug content of microspheres was approximately 90% (w/w). Figure 2, shows the profile of drug release from gelatin microspheres and conventional gel using dialysis membrane. As can be seen in this figure, nearly 70% of the drug content of microspheres was released in 12 h. In this figure drug release from the gel containing microsphere and the plain gel is compared. It can be seen that drug release from the gel containing microsphere and conventional gel formulations are not the same. Almost same results were obtained using rat skin.

Drug release from the microsphere gel follows zero order kinetics with r-value of 0.9876 (Table 3), while drug release from conventional gel followed both Higuchi model of release kinetics and first order release. After 18 hrs more than 80% of Vitamin A palmitate was released from microsphere gel formulation. Zero order release from a topical dosage form is very much favored, as drug is released slowly and linearly.

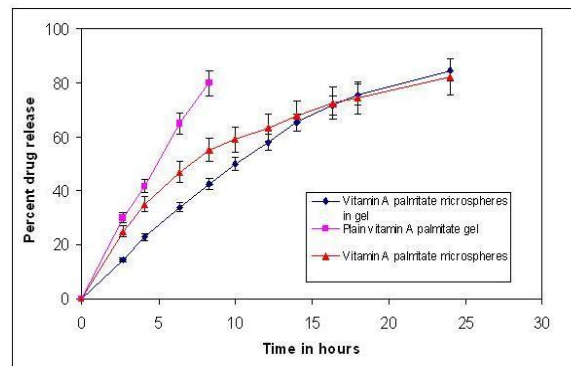


Figure 2: *In vitro* diffusion studies using dialysis membrane, Mean and SD, n = 6

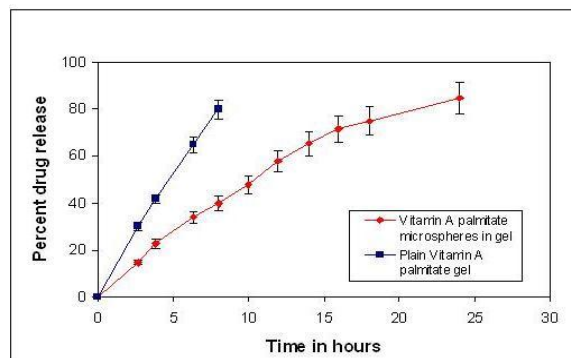


Figure 3: *In vitro* diffusion studies using rat skin, Mean and SD, n = 6

Stability studies

The developed formulations were found to be stable for period of three months and there was no change in drug content and appearance of the formulation. Hence it was concluded that the stability of the drug was increased by microencapsulation.

Conclusion

Stability of vitamin A palmitate was found to increase by microencapsulating it in gelatin microspheres. The developed gel formulation released drug at controlled rate for prolonged period of time with zero order rate kinetics.

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