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Polymeric nanoparticles composed of fatty acids and polyvinylalcohol for topical application of sunscreens

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Abstract

Benzophenone-3 (BZP) or oxybenzone is widely used in many cosmetic formulations, such as sunscreen lotions or emulsions, shampoos and hair sprays. The nature of the vehicle used can enhance or block the percutaneous absorption of UV filter. In this work, we hydrophobically modified polyvinylalcohol 10 000 (PVA) with fatty acids (FAs) to obtain PVA–FA derivatives for the preparation of lipophilic polymeric nanoparticles able to prevent BZP movement towards the skin. Synthesized PVA–FA derivatives were confirmed by H^1 NMR. Nanoparticles loaded with BZP were prepared using a solvent extraction method. The particle size was monitored by means of dynamic light scattering measurements. In-vitro skin permeation studies were performed.

Introduction

Nanoparticles are colloidal particles ranging in size from 10 to 1000 nm, and they are extensively employed as drug delivery systems (Kreuter 1991; Lee et al 2003). They have several advantages over conventional drug carriers: small particle size, ease of administration, drug targeting to the specific body site, solubilization of hydrophobic drug. Lipophilic polymeric nanoparticles could be employed as carriers for active cosmetic ingredients and, in particular, they could limit their transcutaneous absorption (Sayre et al 1990; Dunford et al 1997; Ricci et al 1998). In this study, we synthesized lipophilic polymers composed of polyvinyl alcohol (PVA) and various fatty acids (FAs) and investigated, in-vitro, the influence of the different nanoparticles prepared on percutaneous absorption (Barry 1983; Walters et al 1998) of the model sunscreen benzophenone-3 (Treffel & Gabard 1996; Gupta et al 1999). PVA was selected as a starting material for the preparation of such polymers due to its biocompatibility and the possibility for substitution through chemical linkage to its oxy-residues able to modify its physico-chemical properties (Orienti et al 2001). We substituted PVA, at two different substitution degrees (40% and 80%), with saturated FAs – myristic, palmitic, stearic and behenic acid – to give to the polymer sufficient lipophilicity to allow preparation of nanomatrices for sunscreen delivery. We examined the effects of various PVA–FA derivatives on the formation of polymeric nanoparticles, physico-chemical properties and skin permeation characteristics.

Materials and Methods

Materials

Polyvinyl alcohol (MW = 10 000 Da, 80% hydrolyzed) was from Sigma, myristoyl chloride, stearoyl chloride, palmitoyl chloride, behenoyl chloride, Span 80, Tween 80 and benzophenone-3 (BZP; 2-hydroxy-4-methoxybenzophenone) were from Fluka and all the solvents employed were from Carlo Erba (Milan, Italy).

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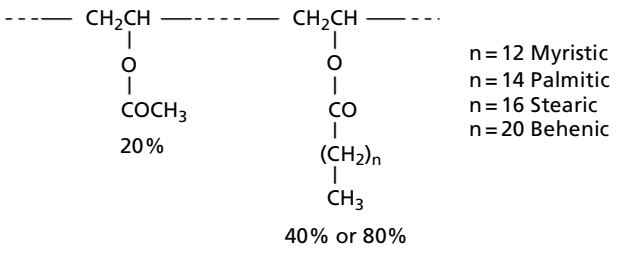

Figure 1 Chemical structure of PVA–FA derivatives.

Synthesis of PVA–FA derivatives

The PVA–FA derivatives (Figure 1) were prepared by dissolving PVA (80 mmol of monomer) in 100 mL of *N*-methylpyrrolidone (Giménez et al 1999). The solution was supplemented with pyridine (80 mmol), dimethylaminopyridine (8 mmol) and acyl chloride (32 mmol) to obtain a 40% molar ratio acyl chloride–hydroxyvinyl monomer (PVA–MIR40, PVA–PALM40, PVA–STEA40 and PVA–BEH40) or acyl chloride (64 mmol) to obtain an 80% molar ratio acyl chloride–hydroxyvinyl monomer (PVA–MIR80, PVA–PALM80, PVA–STEA80 and PVA–BEH80). Immediately, a precipitate of the pyridinium salt was observed. The solution was stirred for 48 h at room temperature. The precipitate of pyridinium salt was removed by filtration and the substituted polymer was separated by precipitation into water. The precipitate obtained was purified by re-precipitating twice from ethanol into water, and then dried under vacuum to constant weight.

Polymer characterization

The degree of substitution of the polymer was determined by H^1 NMR using a Gemini 300 instrument and recording the spectrum in $(CD_3)_2SO$.

Preparation of the polymeric nanoparticle suspensions

Polymeric nanoparticles containing BZP were prepared by a solvent extraction method: 0.50 g of the substituted polymer and 0.50 g of BZP were dissolved in 2 g ethanol and injected in 47 g distilled water under ultrasonication (5 min at 40% maximal amplitude at 37 °C). Two millilitres of each nanoparticle suspension were used for the in-vitro skin permeation studies. For better comparison, a standard formulation was prepared with 0.18 g Span 80 and 0.32 g Tween 80 as suspending agents and 0.50 g BZP with the same preparative method.

Photon correlation spectroscopy (PCS)

Particle size distributions were measured by PCS using a Brookhaven 90-PLUS instrument with an He–Ne laser beam at a wavelength of 532 nm (scattering angle of 90°) (Chen & Huang 1985). Polymeric nanoparticle suspensions and BZP suspension were used for particle size measurement without filtering.

Determination of the amount of BZP placed on the skin in the permeation studies

The amount of BZP available for skin absorption in skin permeation studies, starting from 2 g of all the suspensions analysed, was determined supplementing these systems with 25 mL of *N*-methylpyrrolidone to solubilize the polymer and provide drug release from the nanoparticles. The solutions were subsequently filtered (Albet, Valencia, 0.45 μm) and the amount of drug was detected by HPLC.

In-vitro skin permeation studies

The diffusion of BZP across excised pig-ear skin (obtained from the local slaughterhouse) was studied using a static diffusion cell based on the Franz design. The diffusion cell consisted of donor and receptor chambers between which the skin was positioned. Only the segments of thickness 1.50 ± 0.05 mm were selected for the study. The area available for diffusion was 10.7 cm^2 and the receptor chamber volume was 100 mL. The receptor chamber, containing a saline solution, was maintained at 37.0 ± 0.5 °C throughout the experiment. The contents were continuously agitated by a small bar magnet. At time zero, 2 g of each suspension were placed on the skin in the donor compartment. At predetermined time intervals the receiver phase was withdrawn and replaced with blank buffer to maintain sink conditions (Fernandez et al 2000a, b) and the amount of BZP was analysed by HPLC. Moreover, at the end of each application time (6 h) the skin was removed, rinsed with water, gently dried with a cotton swab and weighed. Following the addition of 5 mL methanol, the skin sample was subjected to ultraturrax (Leica T25, Milan, Italy) treatment for 5 min. Subsequently, the suspension was centrifuged at 15 000 rev min^{-1} (ALC 4239R, Milan, Italy). Then 2.5 mL from the supernatant was desiccated by vacuum rotation and the remainder was redissolved in 1 mL methanol. Sunscreen content was finally determined by HPLC.

Chromatographic conditions

Chromatographic separations were performed using a Shimadzu (model SPD-10A) liquid chromatograph connected to a UV–Vis detector (SPD-10AV) and to a computerised integration system, ChromatoPlus (Shimadzu, Kyoto, Japan). Manual injections were made using a Rheodyne 7125 injector with a 20-μL sample loop. Separations were obtained on a C18 Phenomenex Luna (4 μm, 150 × 4.60 mm i.d.) (Chemtek Analitica, Bologna, Italy) column at room temperature using methanol–water (80:20 v/v) at a flow rate of 0.8 mL min^{-1}. UV absorption was read at 257 nm.

Statistical analysis

All the data are the means of results from five experiments ± s.d. Statistical data analysis was performed using a

one-way analysis of variance. In all cases, post-hoc comparisons of the means of indivdual groups were performed using Tukey's HSD test. A significance level of $P < 0.05$ denoted significance in all cases.

Results

Polymer characterization

Proton assignments for substituted PVA in $(CD_3)_2SO$ (relative to dimethyl sulfoxide δ 2.50) were: δ 5.00 ppm = CH (vinylic proton bounded to the acetylic group of PVA), δ 0.96 ppm = CH_3 (myristoyl), δ 0.92 ppm = CH_3 (palmitoyl), δ 0.82 ppm = CH_3 (stearoyl), δ 0.91 ppm = CH_3 (behenoyl). The level of substitution, calculated from the H^1 NMR spectrum, was determined as 39.3% for PVA–MIR40, 38.8% for PVA–PALM40, 37.0% for PVA–STEA40, 35.2% for PVA–BEH40, 79.7% for PVA–MIR80, 78.9% for PVA–PALM80, 76.6% PVA–STEA80, 74.9% for PVA–BEH80. These data were obtained by comparing the signal of myristoyl (δ 0.96 ppm), palmitoyl (δ 0.92 ppm), stearoyl (δ 0.82 ppm) and behenoyl (δ 0.93 ppm) protons to that of vinylic proton bounded to the acetylic group (δ 5.00 ppm) present at 20% in the PVA.

Photon correlation spectroscopy (PCS)

The mean diameter of the nanoparticles increased with increasing acylic chain length and increasing substituition degree of the polymers (Table 1). The mean diameter of BZP suspension was lower than those of polymeric nanoparticles, indicating the ability of the surfactant to suspend the sunscreen in the aqueous phase.

Determination of the amount of BZP placed on the skin in the permeation studies

The amount of BZP present in the 2 g of suspension placed on the skin was similar for all the formulations analysed (Table 2).

In-vitro skin permeation studies

The transcutaneous permeation of BZP from the different nanoparticles suspensions and from BZP suspension is shown in Figure 2. BZP suspension provided higer fractional amounts with respect to all the nanoparticles suspensions, indicating the nanoparticles' ability to limit sunscreen absorption. Moreover, nanoparticles with a low degree of substitution provided the highest amounts of BZP in the receiver compartment. Among these, nanoparticles with short chain length provided higher amounts of BZP than nanoparticles with high chain length. There was a correlation between the size of the nanoparticles and the fractional amount of BZP recovered in the skin 6 h after topical application (Figure 3): the amount of BZP decreased with increasing substitution degree and, for each degree of substitution, increasing nanoparticles size. This indicated the ability of low-substituted formulations to enhance the location of sunscreen in the epidermis, achieving high protection. The permeation parameters of the different nanoparticles suspensions (Table 3) were obtained by plotting BZP mass per cross-sectional area diffused as a function of time and performing linear regression analysis on the data. Absence of lag times and highest fluxes were observed for BZP suspension and PVA–MIR40, indicating the ability of these systems to provide rapid saturation of the skin and increased diffusibility of BZP. For nanoparticles with a high degree of

Table 1 Nanoparticles and BZP suspension mean diameter (nm).

BZP	PVA–BEH		PVA–STEA		PVA–PALM		PVA–MIR	
	40%	80%	40%	80%	40%	80%	40%	80%
223 ± 15	405 ± 15	440 ± 25	380 ± 8	399 ± 12	357 ± 5	373 ± 5	312 ± 10	339 ± 7

Data are presented as means ± s.d., n = 5.

Table 2 Amount of BZP (mg) placed on the skin in the permeation studies.

BZP	PVA–BEH		PVA–STEA		PVA–PALM		PVA–MIR	
	40%	80%	40%	80%	40%	80%	40%	80%
19.6 ± 0.3	19.6 ± 0.2	19.3 ± 0.6	19.5 ± 0.8	19.4 ± 0.2	19.3 ± 0.5	19.7 ± 0.5	19.9 ± 0.3	19.2 ± 0.7

Data are presented as means ± s.d., n = 5.

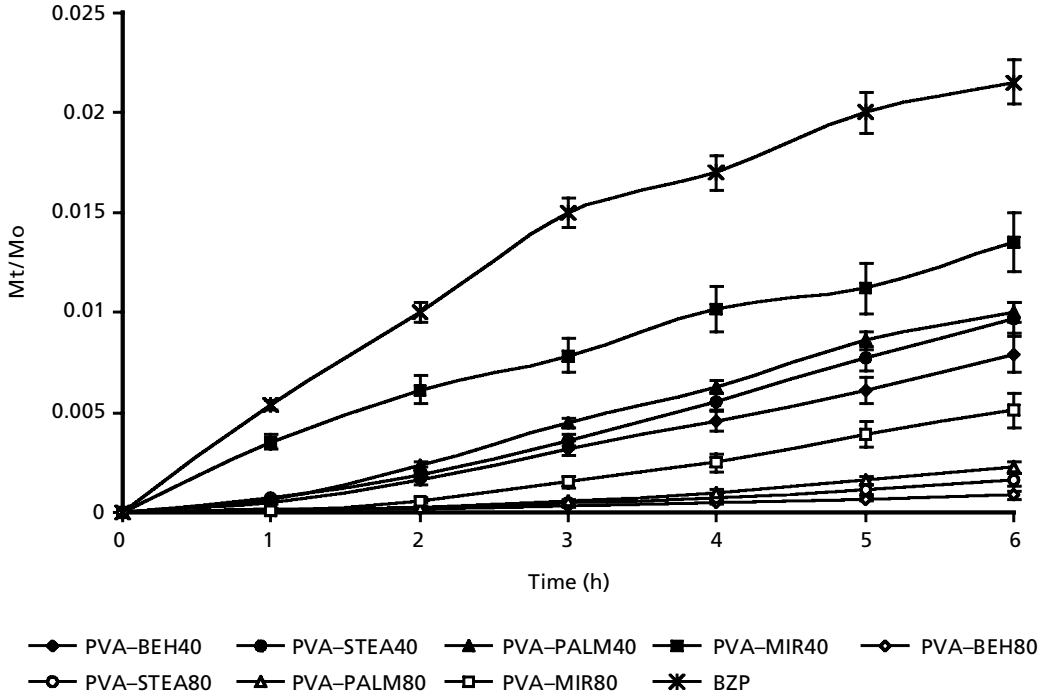

Figure 2 Transcutaneous permeation of BZP (Mt/Mo, fractional amount) from the different nanoparticles suspensions, ±s.d., across pig-ear skin. Mt, amount of BZP recovered at each time interval; Mo, amount of BZP present in the suspension placed on the skin surface.

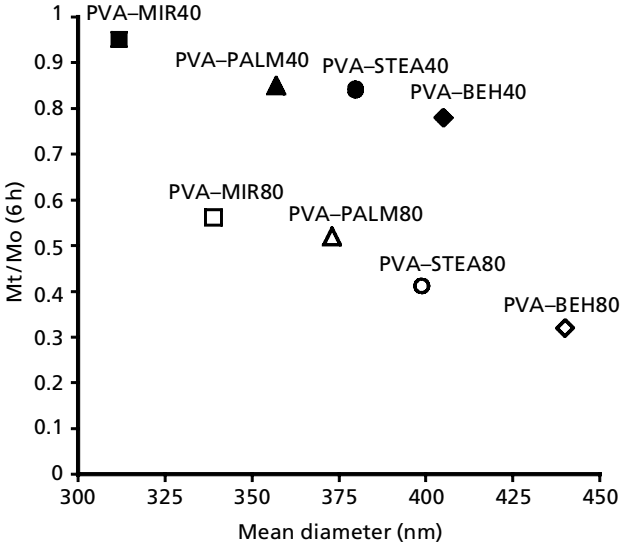

Figure 3 Correlation between the size of nanoparticles and BZP fractional amount recovered in pig-ear skin 6 h after topical application.

substitution, longer lag times and lower fluxes than nanoparticles with a low degree of substitution revealed their tendency to limit BZP movement towards and across the skin.

Discussion

Nanoparticles are colloidal polymeric particles with a therapeutic agent or a functional molecule distributed in their matrix, encapsulated or simply bound to their surface (Aukunuru & Kompella 2002). Several biodegradable or biocompatible polymers have been investigated for the formulation of nanoparticles. In this work, PVA 10 000, substituted with different acyl chains at different degrees of substitution, was chosen for the formulation of nanoparticles carrying a UV-filter using a solvent extraction method. The purpose of this study was to develop a new formulation that can limit BZP penetration into the skin and into the systemic circulation. In fact, sunscreens are intended to act on the surface of the skin and the degree of penetration depends strongly on the physico-chemical properties of the active compound and of the nature of the vehicle in which the sunscreen is applied. Different PVA derivatives can provide polymeric nanoparticles with different physico-chemical and functional properties.

The nature of the substituents and the degree of substitution, varying the properties of the polymer network, influence particles' size during their preparation and particularly modify their ability to interact with the skin surface. The variation in nanoparticle size is more affected by the nature of the substituents than the degree of substitution. In fact, myristoyl and palmitoyl substituents provided smaller particles than stearoyl and behenoyl substituents. This behaviour can be attributed to an

Table 3 Permeation parameters of BZP from the different nanoparticle suspensions across pig-ear skin.

		Flux ($mg\,h^{-1}\,cm^{-2}$) $\times 10^{-4}$	Lag time (h)
BZP		3.10 ± 0.02	—
PVA–BEH	40%	1.01 ± 0.09	0.74 ± 0.08
	80%	0.21 ± 0.03	1.68 ± 0.09
PVA–STEA	40%	2.06 ± 0.04	0.65 ± 0.04
	80%	0.33 ± 0.04	1.75 ± 0.06
PVA–PALM	40%	2.15 ± 0.05	0.50 ± 0.02
	80%	0.52 ± 0.10	1.70 ± 0.04
PVA–MIR	40%	2.70 ± 0.05	—
	80%	1.03 ± 0.04	1.85 ± 0.10

Data were obtained by plotting the mass of the sunscreen per cross sectional area diffused as a function of time and applying linear regression analysis to the data ($R^2 > 0.990$ for all the equations calculated) and are presented as means ± s.d., n = 5.

enhanced viscosity of the inner ethanolic phase of the preparative dispersions in the presence of longer acylic-chain polymers. As concerns permeation studies, all nanoparticles produced decreased flux through the skin and also increased lag times with respect to the BZP suspension. This behaviour agrees with the topic of sunscreen vehicles, which should be able to block percutaneous absorption of UV filter. Moreover, BZP lag times and BZP flux through the skin from the different systems were more affected by the degree of substitution than the nature of the substituents. In particular, systems with a low degree of substitution provided faster saturation of the skin and higher permeation profiles than those with a high degree of substitution, indicating the ability of these nanoparticles to permit BZP movement towards and across the skin. The ability of these polymers to interact with the skin (Orienti et al 2000) is due to their amphiphilic properties conferred by the concomitant presence of hydrophilic (hydroxyvinyl chains) and lipophilic (fatty acid chains). Probably, the low degree of substitution confers a suitable hydrophile–lipophile balance for the interaction of the polymer with the skin.

Conclusions

Substituted PVA, at two different degrees of substitution (40% and 80%), with saturated fatty acids (myristic, palmitic, stearic and behenic), can be employed for the preparation of nanomatrices for sunscreen delivery. These systems can prevent the movement of benzophenone-3 towards the skin, thereby limiting its percutaneous absorption. In particular, nanoparticles with a low degree of substitution seem to be the best candidate for enhancing sunscreen location in the epidermis, while nanoparticles with a high degree of substitution seem to prevent BZP percutaneous absorption.

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