Drug Delivery, 9:147–152, 2002

1071-7544/02 $12.00 + .00
DOI: 10.1080/1042650029009564 7

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healthsciences

Poly(Vinylalcohol-Co-Vinyloleate) for the Preparation of Micelles Enhancing Retinyl Palmitate Transcutaneous Permeation

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The amphiphilic properties of poly(vinylalcohol) substituted with oleic acid was evaluated to assess the possibility to prepare polymeric micelles in an aqueous phase containing a hydrophobic core able to host lipophilic drugs such as retinyl palmitate and thereby enhance its transcutaneous absorption in the stratum corneum. The effect of the increased drug absorption suggests the possibility of interaction between the substituted polymer and the components present in the intercorneocyte spaces. Correlations between the drug concentration in the preparative mixture, micelle size, and drug permeation were evaluated to establish the best functional properties of the micellar systems enhancing retinyl palmitate absorption. Transcutaneous absorption increased with decreasing micelle size, and micelle size decreased on decreasing the drug concentration in the preparative mixture.

Keywords Amphiphilic Properties, Polymeric Micelles, Poly(Vinylalcohol) Derivatives, Retinyl Palmitate, Transcutaneous Permeation

Amphiphilic copolymers are now of growing scientific interest because of their ability to entrap lipophilic drugs in their inner core (Jones and Leroux 1999). These systems may be used for parenteral drug targeting (Orienti et al. 1998; Yokoyama 1998), prolonging the permanence of short half-life drugs in circulation (Yu et al. 1998), or increasing the bioavailability of poorly soluble drugs both for oral (La, Okano, and Kataoka 1996) or transdermal routes (Lian and Lin 2000). The transdermal route, in particular, is improved in the presence of carriers entrapped in the intercorneocyte spaces on the skin surface, thus providing release of the loaded drug as long as the corneum has been renewed.

Our work describes the use of poly(vinylalcohol) substituted with oleic acid as a micellar carrier for retinyl palmitate in the transdermal route. Retinyl palmitate serves as a test drug because of its high lipophilicity that makes it an excellent candidate for polymeric micelles, and studies on this drug have revealed its preventive effect in skin tumors (Abdel-Galil, Wrba, and El-Mofty 1984). The physicochemical properties of the substituted polymers were evaluated in solution and correlated to the functional properties of the micellar carrier-drug system to establish the best conditions favoring the transdermal absorption of lipophilic drugs.

MATERIALS AND METHODS

Materials

Polyvinyl alcohol (PVA) (Mw = 10000, 80% hydrolized), retinyl palmitate, 4-dimethylaminopyridine (DMAP), pyridine, N-methylpyrrolidone (NMP), ethylene glycol dimethyl ether (glyme), 4′,5′-dibromofluorescein, and oleyl chloride were purchased from Fluka (Milan, Italy); tetrahydrofuran (THF) and acetone were from Carlo Erba (Milan, Italy). Acetonitrile (HPLC grade) was from Romil Pure Chemistry (Cambridge, Britain). Phenomenex Luna C18(2) column was obtained from Chemtek Analitica (Bologna, Italy). Other organic and inorganic chemicals were commercially available and used without further purification.

Synthesis of Substituted PVA

PVA (40 mmol of monomer) was dissolved in 50 ml of NMP (Giménez et al. 1999). The solution was supplemented with pyridine (40 mmol), DMAP (4 mmol), and oleyl chloride (2 mmol), to obtain a 5% molar ratio (acyl chloride:hydroxyvinyl monomer). A precipitate of the pyridinium salt was observed immediately. The solution was stirred for 48 hr 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 reprecipitating twice from ethanol into water, and then dried under vacuum to constant weight.

Received 29 October 2001; accepted 20 December 2001.
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Polymer Characterization

The degree of substitution of the polymer was determined by elemental analyses performed using a Perkin Elmer model 240 B elemental analyzer and by H^1-NMR using a Gemini 200 instrument and recording the spectrum in a $(CD_3)_2SO$: D_2O 10:1 (v:v) mixture.

Preparation of Polymeric Micelles

The micelles were prepared by dissolving 100 mg of the substituted polymer in 1 ml glyme and injecting this solution in 9, 19, 29, 39, 49, 59, 69, 79, 89, and 99 ml PBS under ultrasonication (5 min at 40% maximal amplitude at 37°C). Polymeric micelles containing retinyl palmitate were prepared by adding 20 mg (PVA_{OL}-RetP20), 40 mg (PVA_{OL}-RetP40), 60 mg (PVA_{OL}-RetP60), 80 mg (PVA_{OL}-RetP80), or 100 mg (PVA_{OL}-RetP100) of the drug to the polymer solution (100 mg/ml) in glyme. Then this solution was injected in 39 ml of PBS under ultrasonication under the same conditions, obtaining different concentrations of the drug in the external aqueous phase (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml). Finally, the suspensions of the polymeric micelles were filtered by a 0.22-micron filter (Millex-HV, Millipore). For comparison, a standard formulation (RetP20) was prepared with only 20 mg retinyl palmitate through the same preparative method, obtaining a concentration of 0.5 mg/ml of the drug in the external aqueous phase.

Dynamic Light Scattering Measurements

The size of all the micelles prepared (before and after filtration) was monitored at 37°C for 6 hr (time generally required for topical applications) using a Brookhaven 90-PLUS instrument equipped with a 50 mW He-Ne laser (532 nm). Measurements were carried out by fixing the scattering angle at 90°C. Results were the combination of three 5-min runs for a total correlation function (ACF) accumulation time of 15 min. The diffusion coefficient was evaluated from the time autocorrelation function, $g^2(\tau)$ using the forced single-exponential fit (equation 1):

$$g^2(\tau) = Ae^{-2\Gamma\tau} + B \quad [1]$$

$$\Gamma = Dq^2 \quad [2]$$

$$q = (4\pi n/\lambda_0)\sin(\theta/2) \quad [3]$$

where τ is the delay time, both A and B are constant, D is the translational diffusion coefficient, q is the scattering vector, n is the refractive index of pure solvent, λ_0 is the wavelength of incident light in vacuum, and θ is the scattering angle. The hydrodynamic radius, R_H, was calculated using the following Stokes-Einstein equation:

$$R_H = k_B T/6\pi\eta D_0 \quad [4]$$

where k_B, T, and η are the Boltzmann constant, the absolute temperature, and the solvent viscosity, respectively.

Release Studies of the Drug from the Micelles and Determination of Drug Content

The drug release from the micelles was monitored by an apparatus consisting of a release cell containing 2 ml of the micelle suspension in PBS separated by a dialysis membrane (Mw cut off = 14.000 Daltons) from a receving compartment containing 10 ml PBS, replaced after time intervals suitable to guarantee sink conditions throughout the runs. The apparatus was thermostated at 37°C for 24 hr. The drug was analyzed in the receiving phase by HPLC.

Amount of Drug Placed on Skin in the Permeation Studies

The amount of drug available for skin absorption in skin permeation studies, starting from 2 ml of all the suspensions analyzed, was determined supplementing these systems with 23 ml of N-methylpyrrolidone to solubilize the polymer and provide drug release from the micelles. The same procedure also was used with the retinyl palmitate dispersion. The solutions were subsequently filtered and the drug amount determined by HPLC.

Skin Permeation Studies In Vitro

Porcine ears were obtained from a local slaughterhouse. The skin was separated into circular segments of 36-mm diameter and hydrated in phosphate saline buffer at 4 ± 1°C for 24 hr. Only the segments with thickness of 1.50 ± 0.05 mm were selected for the present study. The permeation study was conducted in a Franz-type permeation cell with a diffusional area of 10.7 cm^2. The skin sample was mounted horizontally between donor and receptor compartments of the cell and clamped with the dermal side in contact with the receptor medium. To avoid drying the donor sample, the donor compartment was closed with a glass stopper. At time zero, 2 ml of the micelle suspension and of the drug dispersion were placed on the skin in the donor compartment. The receiver phase (100 ml of an isotonic PBS, pH 7.4, maintained at 37°C by means of surrounding jacket) was stirred constantly. Six permeation cells used for each suspension analyzed to obtain the amount of drug in the skin at 1, 2, 3, 4, 5, and 6 hr after suspension application. At each time interval, the skin segment used in the cell was rinsed with water, gently dried with a cotton swab, and weighed. Following the addition of 5 ml acetone, the skin sample was subjected to Ultraturrax (10000 rev/min, 5 min) and an ultrasonic (Vibracell VCX, Danbury, CT, USA) treatment at elevated temperatures (50 ± 5°C, 5 min). Subsequently, the suspension was centrifuged at 15000 rev/min (ALC 4239R, Milan, Italy). Then 2.5 ml from the supernatant was evaporated by vacuum rotation and the remainder was redissolved in 1 ml acetone. The drug in the solvent 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 computerized integration system, ChromatoPlus (Shimadzu, Kyoto, Japan). Manual injections were made using a Rheodyne 7125 injector with a 20-μl sample loop.

Separations (Jenning et al. 2000) were obtained on a C18 Phenomenex Luna (3 μm, 150 $\times$ 4.60 mm i.d.) (Chemtek Analitica, Bologna, Italy) column at room temperature using 100% acetonitrile at a flow rate of 0.8 ml/min. Ultraviolet absorption was read at 325 nm and the retention time was 4.8 min. The limit of detection (signal-to-noise ratio 3:1) was 45.3 ng/ml retinyl palmitate. Reproducibility was 3.2%.

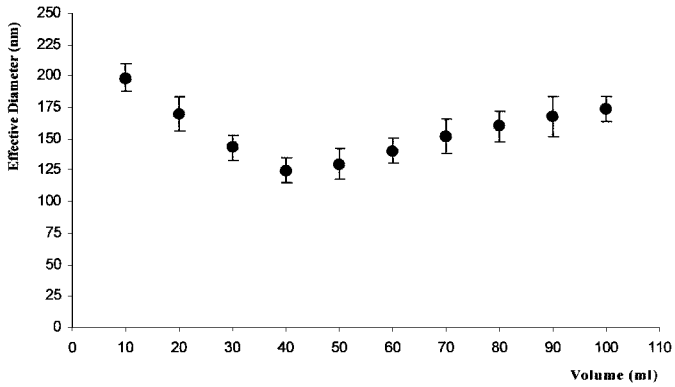

FIG. 2. Correlation between the micelle size (mean $\pm$ SD, $n = 5$) and the volume of the external aqueous phase used in the preparative method.

RESULTS AND DISCUSSION

Characterization of the Substituted PVA

Elemental analysis revealed that the substitution degree (substituent:hydroxyvinyl-monomer-mol e ratio) in the substituted PVA corresponded to 0.05:1 for the polymers prepared from a substituent:hydroxyvinyl-monomer-mol e ratio of 0.05:1. By H^1-NMR analysis, the substitution degree was obtained by comparing the signal of the oleoyl proton (2) at 5.10 ppm to that of the acyl proton (1) at 1.95 ppm present at 20% in the polyvinylalcohol (Figure 1). The substitution degree calculated from the spectra was 4.8%.

DLS Measurements of Micelles in Solution

The dilution of the micelles in PBS did not modify their size, whereas change in water volume during their preparation did. The smallest size of the micelles was obtained by injecting the polymer glyme solution in 40 ml PBS (Figure 2). At this dilution, the presence of the drug increased the size of the micelles even after filtration (Table 1). The size of the loaded micelles, before and after filtration, monitored for 6 hr at 37°C, was constant, indicating that no aggregation occurs over time.

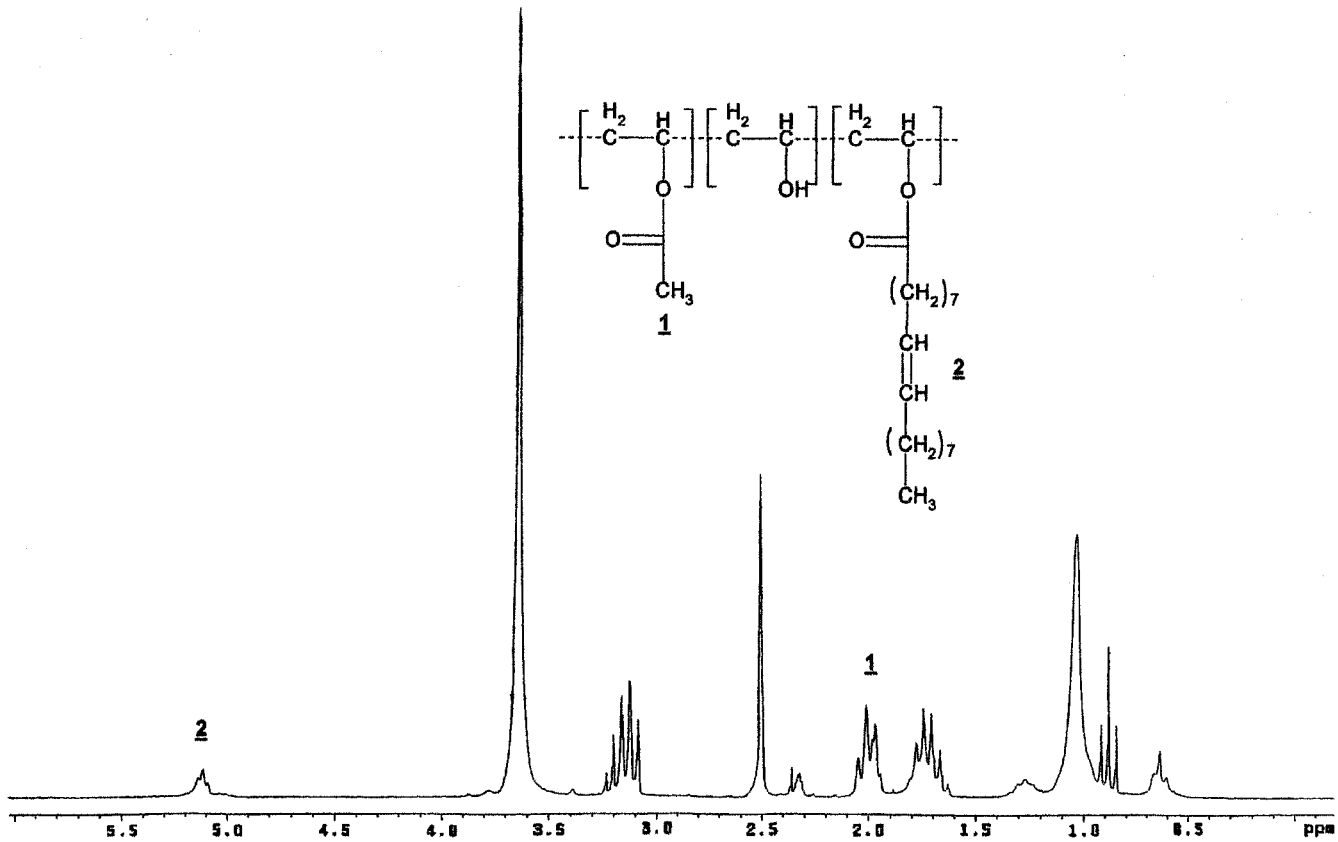

FIG. 1. Structural analysis of PVA substituted with oleic acid by H^1-NMR spectroscopy.

TABLE 1
Diameters (mean ± SD, $n = 5$) of micelles and drug dispersion 3 hr after preparation

	Diameter (nm)	
Type of system	Before filtration	After filtration
PVA_{OL}-RetP20	228.2 ± 6.1	129.7 ± 4.2
PVA_{OL}-RetP40	243.9 ± 5.1	150.7 ± 2.3
PVA_{OL}-RetP60	254.0 ± 5.6	166.6 ± 3.5
PVA_{OL}-RetP80	291.2 ± 6.2	201.4 ± 4.3
PVA_{OL}-RetP100	372.6 ± 5.9	210.4 ± 5.0
RetP20	299.5 ± 4.6	109.1 ± 3.9

TABLE 2
Amount of drug (mean ± SD, $n = 5$) placed on the skin in the permeation studies

	Amount of drug (mg)	
Type of system	Before filtration	After filtration
PVA_{OL}-RetP20	0.96 ± 0.01	0.38 ± 0.03
PVA_{OL}-RetP40	1.85 ± 0.03	0.77 ± 0.05
PVA_{OL}-RetP60	2.91 ± 0.03	1.15 ± 0.10
PVA_{OL}-RetP80	3.90 ± 0.08	1.54 ± 0.09
PVA_{OL}-RetP100	4.94 ± 0.05	1.92 ± 0.07
RetP20	0.98 ± 0.02	0.86 ± 0.07

Release Studies of Drug from the Micelles and Determination of Drug Content

As retinyl palmitate was not released from the micelles (not filtered and filtered) over time, the drug content in the micelles approached 100% of the amount used in the preparative mixture, indicating a high ability of this polymer to load lipophilic molecules.

Amount of Drug Placed on Skin in the Permeation Studies

Retinyl palmitate concentration in the 2-ml not-filtered suspensions placed on the skin was identical to that obtained during micelle preparation. In contrast, after micelle filtration the amount of drug in the same volume suspensions placed on the skin, decreases as the filter can retain larger sized micelles (Table 2).

Skin Permeation Studies In Vitro

The transcutaneous permeation increased with decreasing micelle size (Figure 3), indicating the micelle's ability to deliver the loaded drug into the stratum corneum. This hypothesis is confirmed by the dependence of the amount of drug absorbed (and recovered in the skin) on the effective diameter of micelles both before and after filtration. The mechanism allowing drug release after entrapment probably can be attributed to the destabilization of the polymeric micelles in the presence of the stratum corneum components (Barry 1987). This was confirmed by a fluorescent probe (4′,5′-dibromofluorescein) released in the stratum corneum from the same polymeric micelles after their application on the skin. The interaction of 4′,5′-dibromofluorescein with the stratum corneum components produced an evident color change of the probe indicating its release from polymeric micelles. Moreover, the transcutaneous permeation is not characterized by a lag time indicative of drug diffusion from a releasing system to the skin, but it presents a linear profile from the start followed by a plateau indicating achievement of a lower drug concentration susceptible to permeation (Figures 4a and 4b). This behavior may be interpreted as a release because of the destabilization of the micelles establishing a constant concentration gradient through the skin that remains constant until the drug content decreases below certain values.

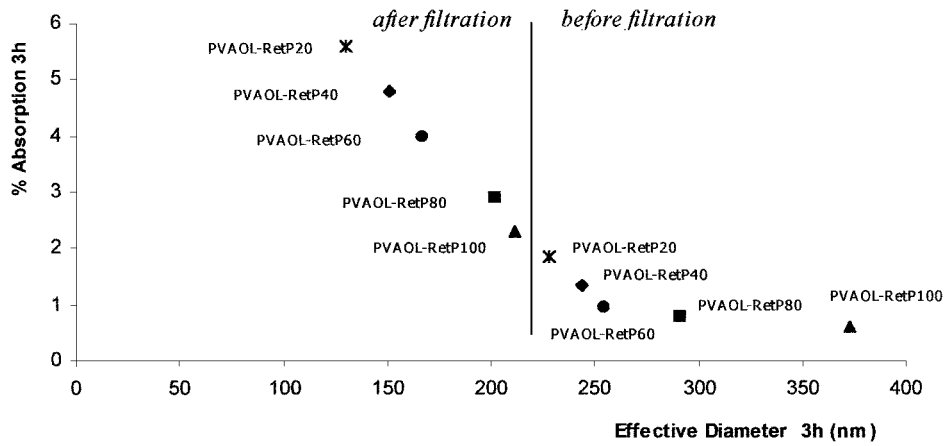

FIG. 3. Correlation between the micelle size and the percent absorption (mean ± SD, $n = 3$) of retinyl palmitate [(mg absorbed/mg placed on the skin/g skin) × 100] 3 hr after application on the skin.

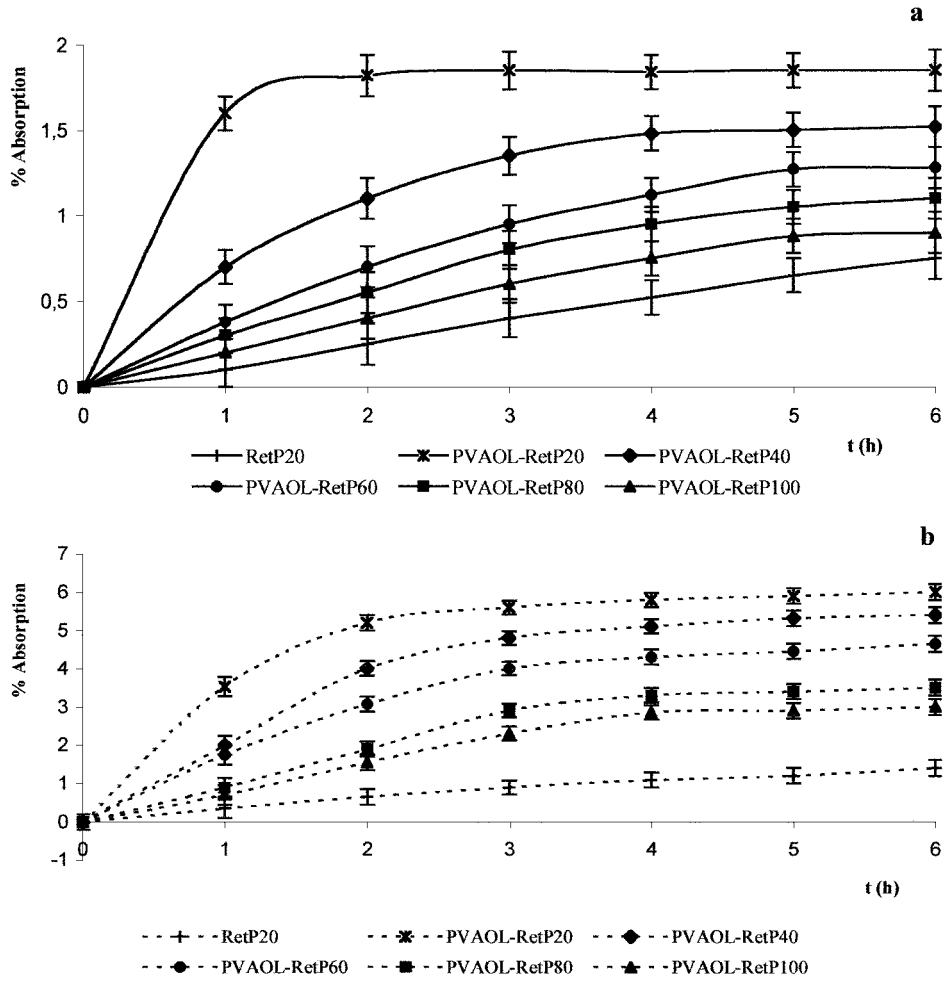

FIG. 4. Percent absorption (mean ± SD, $n = 3$) profiles of retinyl palmitate [(mg absorbed/mg placed on the skin/g skin) × 100] obtained from all the systems before (**a**) and after (**b**) filtration.

CONCLUSION

PVA substituted with oleic acid is an interesting material for the formulation of polymeric micelles able to entrap retinyl palmitate. The amphiphilic nature of this substituted polymer yields micellar systems characterized by high loading ability toward lipophilic drugs and good stability in an aqueous environment over time. Such properties suggest the use of these systems as drug carriers for different administration routes. When used for the transcutaneous route, the absorption of the active principle loaded in the micelles is enhanced with respect to the corresponding drug dispersion obtained in the same preparative method. This effect seems to be linked to an interaction of the amphiphilic polymer with the stratum corneum components, enhancing drug absorption.

Moreover, the drug concentration in the preparative mixture influences micelle size and consequently drug absorption. The filtration procedure after micelle preparation allows a smaller micelle size to be selected, thus improving the functional properties of these systems and providing maximum drug absorption.

REFERENCES

Abdel-Galil, A. M., Wrba, H., and El-Mofty, M. M. 1984. Prevention of 3-methylcholanthrene-induce d skin tumors in simultaneous application of 13-cis-retinoic acid and retinyl palmitate (vitamin A palmitate). *Exp. Pathol.* 25:97–102.

Barry, B. W. 1987. Mode of action of penetration enhancers in human skin. *J. Controlled Release* 6:85–97.

Giménez, V., Reina, J. A., Mantecòn, A., and Càdiz, V. 1999. Unsaturated modified poly(vinyl alcohol). Crosslinking through double bonds. *Polymer* 40:2759–2767.

La, S. B., Okano, T., and Kataoka, K. 1996. Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporate d poly(ethylene oxide)-poly(beta-benzyl L-aspartate) block copolymer micelles. *J. Pharm. Sci.* 85:85–90.

Lian, J., and Lin, Y. 2000. Evaluation of poly(ethylene oxide)-poly(propylene oxide)-polyethylene oxide) (PEO-PPO-PEO) gels as a release vehicle. *J. Controlled Release* 68:273–282.

Jenning, V., Gysler, A., Schäfer-Korting, M., and Gohla, S. H. 2000. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *Eur. J. Pharm. Biopharm.* 49:211–218.

Jones, M. C., and Leroux, J. C. 1999. Polymeric micelles—a new generation of colloidal drug carriers. *Eur. J. Pharm. Biopharm.* 48:101–111.

Orienti, I., Gentilomi, G., Bigucci, F., Luppi, B., and Zecchi, V. 1998. Substituted poly(methyl vinyl ether-alt-maleic anhydride) for the release control and targeting of methotrexate. *Arch. der Pharmazie—Pharm. Med. Chem.* 331:347–351.

Yokoyama, M. 1998. Novel passive targetable drug delivery with polymeric micelles. In *Biorelated polymers and gels, ed.* T. Okano, 193–229. San Diego: Academic Press.

Yu, B. G., Okano, T., Kataoka, K., and Kwon, G. 1998. Polymeric micelles for drug delivery: solubilization and hemolytic activity of amphotericin B. *J. Controlled Release* 53:131–136.