ELSEVIER

Journal of Controlled Release 68 (2000) 273–282

journal of controlled release

www.elsevier.com/locate/jconrel

Evaluation of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) gels as a release vehicle for percutaneous fentanyl

Jiahorng Liaw[a,*], Yen-Chao Lin[b]

[a]*Department of Pharmaceutics, School of Pharmacy, Taipei Medical College, 250 Wu Hsing St., Taipei 110, Taiwan*
[b]*Graduate Institute of Pharmaceutical Science, Taipei Medical College, 250 Wu Hsing St., Taipei 110, Taiwan*

Received 31 January 2000; accepted 26 April 2000

Abstract

The primary objective of this study was to investigate the feasibility of PEO–PPO–PEO copolymer gel as a release vehicle for percutaneous administration of fentanyl in vitro and in vivo. A cellulose membrane and nude mouse skin with series concentrations of PEO–PPO–PEO block copolymers were used to examine the sustained-release pattern and permeation of fentanyl. The in vivo percutaneous absorption was examined using rabbits to evaluate the preliminary pharmacokinetics of fentanyl with 46% PEO–PPO–PEO copolymer formulation patches. The micelle formation ability of this block copolymer and the penetration ability of PEO–PPO–PEO copolymer over time were also studied by pyrene fluorescence probe methods and the dynamic light scattering test. At a concentration of 46% at 37°C, PEO–PPO–PEO copolymers formed a gel and showed a pseudo-zero-order sustained-release profile. With increasing concentration of copolymer in the cellulose membrane transport, the apparent release flux of fentanyl (200 μg/ml) decreased to 1.09±0.19 μg cm^{-2} h^{-1}. Assessment of the effect of the copolymer on nude mouse skin also showed a decrease in the apparent permeability coefficient [$(P_{H_2O}) = 2.24\pm0.47\times10^{-6}$ cm s^{-1} vs. $(P_{46\%\ \text{block copolymer}}) = 0.93\pm0.23\times10^{-7}$ cm s^{-1}]. The preliminary pharmacokinetics of the fentanyl patch was shown to be in steady state within 24 h, and this was maintained for at least 72 h with an elimination half-life ($t_{1/2}$) of 10.5±3.4 h. A fluorescence experiment showed polymeric micelle formation of PEO–PPO–PEO copolymers at 0.1% (w/w) within 50 nm micelle size and the PEO–PPO–PEO copolymers were able to penetrate nude mouse skin within 24 h. Thus, it appears that fentanyl preparations based on PEO–PPO–PEO copolymer gel might be practical for percutaneous delivery.

Keywords: PEO–PPO–PEO; Fentanyl; Percutaneous delivery; Fluorescence probe

1. Introduction

Fentanyl, a synthetic opioid, is used clinically as both an analgesic and an anesthetic agent preoperatively [1] for its potent narcotic analgesic property. However, repeated intravenous bolus doses or continuous intravenous infusion is required to sustain analgesic plasma levels because of its short duration of action with high liver metabolism [2]. Alternately, fentanyl can be delivered transdermally

*Corresponding author. Tel.: +886-2-2377-9873.
E-mail address: jhorng@tmc.edu.tw (J. Liaw).

0168-3659/00/$ – see front matter
PII: S0168-3659(00)00268-6

to sustain analgesia for longer periods because of its suitable physicochemical properties for skin transport [3,4]. In addition, percutaneous delivery of fentanyl can offer several advantages over conventional dosage forms [5] and the pulsed nature of delivery from discrete dosages can be eliminated [6].

Recently, PEO–PPO–PEO copolymer with an average molecular weight of 8400, a group of triblock copolymers derived from propylene oxide (20%) and ethylene oxide (80%) [7], has been widely used in medical, pharmaceutical, and cosmetic systems as solubilizing, wetting, and emulsifying agents [8–10]. With its relatively low toxicity and ability to form clear gels in aqueous media, the use of PEO–PPO–PEO copolymers in human skin wound cleanser has also been reported [11], and has been approved by the Food and Drug Administration. The unique characteristic of this copolymer is its reverse thermal gelation behavior; concentrated solutions (46% w/w) of the copolymer are fluid at refrigerator temperature (4–5°C), but are soft gels at body temperature. In addition, a PEO–PPO–PEO copolymer micelle-containing formulation has been evaluated as an antibiotic carrier in wound treatment [12] as well as in an indomethacin percutaneous formulation [13]. Thus, the copolymer gels appear to have good potential for use as topical drug delivery carriers since they exhibit reverse thermal gelation behavior and have good drug-release characteristics.

Furthermore, there are many nano-polymeric-micelle drug systems which, having hydrophilic PEO chains as palisade regions, can prohibit protein absorption, liver cellular interaction, and increase stability in the blood stream [14]. This PEO type of AB block copolymer carrier not only leads to enhanced passive transport, but can also avoid liver degradation. Thus, the primary objective of this work was to develop gel formulations for percutaneous controlled delivery of fentanyl based on the ABA type of PEO–PPO–PEO block copolymer. The release profiles for fentanyl from a series of concentrations of this copolymer were evaluated, and the results of these in vitro studies were utilized to guide further in vivo sustained pharmacokinetic evaluations in New Zealand rabbits.

2. Materials and methods

2.1. Materials

Fentanyl citrate, amine-, carboxylate-modified fluorescent latexes and pyrene were purchased from Sigma (St. Louis, MO, USA). PEO–PPO–PEO copolymer with average molecular weight 8400 was obtained from BASF (Ludwigshafen, Germany). The cellulose membrane (molecular weight cutoff 3500) used in this study was a Cell.Sep® T1 from MFPI (Membrane Filtration Products, San Antonio, TX, USA). Radiolabelled [^{14}C]estradiol and [^{14}C]mannitol were obtained from NEN research products (Du Pont, Wilmington, DE, USA). All other chemicals used in the study were of analytical reagent grade and were used as such without further purification.

2.2. Animals

The nude mouse (BALB/c-nu) used in the in vitro permeation study was aged 6 to 8 weeks and was purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Male albino New Zealand rabbits (Animal Center of National Taiwan University), weighing between 2 and 3 kg, were used in the in vivo pharmacokinetics studies.

2.3. Preparation of PEO–PPO–PEO copolymer formulation with fentanyl

All PEO–PPO–PEO copolymer formulations of fentanyl citrate used in these studies were prepared on a weight percentage basis using the cold method described by Schmolka [15]. A weighed amount of copolymer was slowly added to different concentrations of cold fentanyl solutions in a vial containing a magnetic stirring bar with gentle mixing. These dispersions were stored in a refrigerator for at least 12 h to ensure complete dissolution. Eventually, a clear and viscous gel or solution formed.

2.4. In vitro membrane release of fentanyl

In order to study the effect of PEO–PPO–PEO

copolymer concentration on fentanyl release, a Franz cell with a cellulose membrane (active diffusion area 0.627 cm^2) was used for the in vitro release studies. An aqueous or gel formulation (0.5 ml) was placed in the donor compartment and 6 ml of pH 7.4 phosphate buffer solution (PBS) in the receiver compartment. The diffusion cells were maintained at 37°C by a water bath (SR70, Shimaden, Tokyo, Japan), and stirring was set at 700 rpm throughout the experiment. Samples (0.6 ml) were withdrawn from the receiver compartments at fixed intervals and replaced with an equal volume of previously warmed PBS. The fentanyl samples were assayed by the HPLC–UV method. The initial concentration of fentanyl in the vehicles was held at 1000 ng/ml, while the concentration of the PEO–PPO–PEO copolymer was varied (0.01, 0.1, 1, 10, 20 and 46% w/w). Alternatively, a pure fentanyl solution was also used to compare the release effect. The release profile of fentanyl was obtained by plotting the cumulative amount of fentanyl released from each copolymer formulation against time.

2.5. In vitro nude mouse skin permeation of fentanyl, estradiol, mannitol, and fluorescent latexes

Fresh samples of whole nude mouse skin were removed from the abdomen of cadavers immediately after postmortem and mounted carefully between the two compartments of the Franz cell with a rigid clamp. The receiver compartments were filled with PBS (pH 7.4) and were stirred throughout the permeation studies. Samples (0.6 ml) were taken from the receiver compartments at fixed intervals and replaced with an equal volume of previously warmed PBS. The fentanyl samples were assayed by the HPLC–UV method. Receiver samples of [^{14}C]estradiol and [^{14}C]mannitol were diluted in 2 ml scintillation cocktail (Biosafe II, RPI, Mount Prospect, IL, USA) and analyzed by evaluation of the total radioactivity (dpm) in a liquid scintillation counter (Tric Cab 460 CD, Packard Instruments, Downers Grove, IL, USA). The fluorescent emission spectra of two fluorescent latexes were obtained using a fluorescence spectrophotometer F-4500 (Hitachi, Tokyo, Japan). Experiments with aqueous latex were performed with excitation and emission wavelengths of 470 and 505 nm, respectively. The excitation bandwidth was set at 5 nm and the emission bandwidth at 3 nm. All fluorescence experiments were carried out at 25°C.

2.6. Drug assay

The fentanyl samples were determined chromatographically by the method of Dewell et al. [16] with slight modification. Samples (0.6 ml) were centrifuged at 3000 rpm for 15 min, and 50 μl of the supernatant was injected into a LiChrospher 100 RP-18 column (5 mm, 250–4 mm, Merck); acetonitrile/water (35:65, pH 3.0) was used as mobile phase. The flow rate was 1 ml/min with the detector set at 210 nm, and an EZChromTM Chromatography Data System (USA) integrated the peak height. Calibration curves were obtained by plotting the peak height of the authentic drug as a function of drug concentration. The inter- and intra-day coefficients of variation of each assay were both less than 10%. The lower limit of quantitation of fentanyl was 3 ng/ml. Fentanyl concentrations in plasma samples were determined by a similar HPLC–UV procedure.

2.7. Preparation of fentanyl patch

One milligram freshly dispensed fentanyl in 46% PEO–PPO–PEO copolymer gel was sandwiched between an impermeable backing and a cellulose membrane and the edges of the backing and membrane were immediately heat-sealed. The active (diffusion) area of the patch was 10 cm^2.

2.8. In vivo preliminary percutaneous absorption experiments

Male New Zealand rabbits were fasted for 12 h before the experiment but were allowed free access to water. After the hair on the dorsal surface of the male New Zealand rabbits was removed by clipping, a fentanyl gel patch of known area was attached to the dorsal site of the skin and secured with 3M Transpore™ tape. The fentanyl gel patch was removed after 72 h. At predetermined intervals, blood samples (1 ml) were collected from an ear vein and

centrifuged at 3000 rpm for 10 min for HPLC analysis.

2.9. Data analysis

The in vitro skin permeation of fentanyl was determined according to a previous description by Liaw [17]. The apparent permeability coefficient (P) was calculated according to the following equation:

$$P = [dC/dt]V/A \times C_0$$

where $V \times [dC/dt]$ is the steady-state rate of appearance of the percutaneously applied fentanyl in the receiver chamber after an initial lag time, C_0 is the initial fentanyl concentration in the donor chamber, and A is the area of the membrane/skin in the penetration experiment, 0.627 cm^2. The cumulative amount of fentanyl percutaneous transport was calculated by multiplying the volume of the receiver chamber during a 24-h incubation.

2.10. Characterization of PEO–PPO–PEO copolymer micelle formation using a pyrene fluorescence probe and dynamic light scattering

The formation of PEO–PPO–PEO copolymer micelles was confirmed by a fluorescence probe technique using pyrene and the partitioning of pyrene into the micellar phase can be determined using the ratio peak I_1/peak I_3 of the pyrene spectrum as previously reported [18,19]. The fluorescence emission spectrum of pyrene in the PEO–PPO–PEO copolymer micelle solutions was measured from 350 to 500 nm using a fixed excitation wavelength of 339 nm with a constant pyrene concentration of 6×10^{-7} M. The PEO–PPO–PEO block copolymer varied from 0.001 to 46% (w/w). Spectral data were acquired using an Hitachi F-4500 Fluorescence Spectrophotometer (Hitachi, Tokyo, Japan). All fluorescence experiments were carried out at 25°C.

The sizes of PEO–PPO–PEO copolymer micelles or latexes were analyzed by dynamic light scattering using a Malvern Zetasizer 3000 (Malvern Instrument, Malvern, Worcs, UK) with a helium laser light source operating at a wavelength of 632 nm with an assumed refractive index ratio of 1.33 and reference of viscosity [20]. The sample cell was cleaned before each measurement by flushing with double distilled water and drying with air. All measurements were performed at 25°C at a measurement angle of 90°.

2.11. In vitro skin permeation of PEO–PPO–PEO copolymer gel

Fresh samples of whole nude mouse abdominal skin were used in the Franz cell experiment. Forty-six percent PEO–PPO–PEO copolymer gel (0.5 ml) was applied to the donor chambers, and samples (6 ml) were taken from the receiver compartments at fixed intervals. A known amount and final concentration of pyrene (6×10^{-7} M) was added to the samples and measured by fluorescence spectrophotometry as described previously.

3. Results

3.1. Effect of PEO–PPO–PEO copolymer concentration on drug release

The release rates of fentanyl from gels or solutions containing different PEO–PPO–PEO copolymer concentrations were determined. Fig. 1 shows the cumulative amount of fentanyl versus time in hours with a series of copolymer concentrations. The release patterns indicate that increased concentrations of block copolymer decrease the release rates of

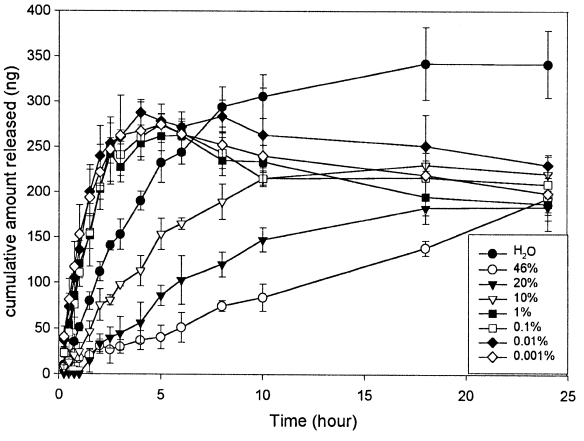

Fig. 1. Effect of PEO–PPO–PEO copolymer concentration on fentanyl release from a cellulose membrane (each value represents the mean±S.D. of $n = 3$).

fentanyl. Under a concentration of 1% copolymer, the fentanyl release profiles have similar patterns with rapid release. With increasing concentration of PEO–PPO–PEO copolymer in the formulation, a corresponding decrease in the apparent release rate of the drug occurred. At a concentration of 46% copolymer, fentanyl showed a pseudo-zero-order release profile and calculation of the release rate of fentanyl by the least-squares Higuchi method ($M_t/M_\infty = k\sqrt{t}$) [3] yielded $1.21\pm0.13\times10^{-2}$ μg cm^{-2} $\sqrt{h}^{-1}$. As expected, excellent linearity ($r > 0.99$) was only observed at 46% copolymer when the amount of fentanyl release from the gel was plotted against time.

3.2. Effect of drug concentration on release

The effect of initial drug concentration on the release pattern (interval flux) was tested at four drug concentrations (500, 1000, 20,000, and 200,000 ng/ml) with 46% copolymer gel formulations and the results are shown in Fig. 2. A steady-state flux with an initial burst followed by a pseudo-zero-order release of drug was observed after 2 h. These results indicate that a 46% copolymer gel formulation provides a sustained-release effect with variation of the initial drug release in the vehicle. The fluxes of these four different fentanyl concentrations (500, 1000, 20,000, and 200,000 ng/ml) were $8.39\pm1.32\times10^{-3}$, $1.21\pm0.13\times10^{-2}$, $1.52\pm0.29\times10^{-1}$, and 1.09 ± 0.19 μg cm^{-2} h^{-1}, respectively.

3.3. In vitro nude mouse skin permeation of fentanyl, estradiol, and mannitol with PEO–PPO–PEO copolymer and fluorescent latexes

A series of concentrations of PEO–PPO–PEO copolymer with nude mouse skin transport of fentanyl were also investigated. The effects of PEO–PPO–PEO copolymer concentrations on fentanyl skin permeability are shown in Fig. 3. The cumulative amount of fentanyl decreased as the concentration of the block copolymer increased. The apparent permeability coefficients (cm s^{-1}) decreased with increasing copolymer concentration (without, 0.01, 0.1, 1, 10, and 46%) to 2.24 ± 0.47, 1.41 ± 0.49, 1.31 ± 0.38, 1.30 ± 0.25, 0.74 ± 0.06, and $0.13\pm0.02\times10^{-6}$, respectively. It was reported that the higher the PEO–PPO–PEO copolymer concentration, the greater the yield or viscosity [20]. Thus, the reasons for the decreased release rate may be a reduction in the size of the water channels and an increase in the micro-viscosity of the water channels of the gel as well as the affinity of the drug for the copolymer environment.

Table 1 shows that the effect of 46% PEO–PPO–PEO copolymer on fentanyl transport through nude mouse skin and the apparent permeability coefficients (cm s^{-1}) for fentanyl, estradiol, and mannitol

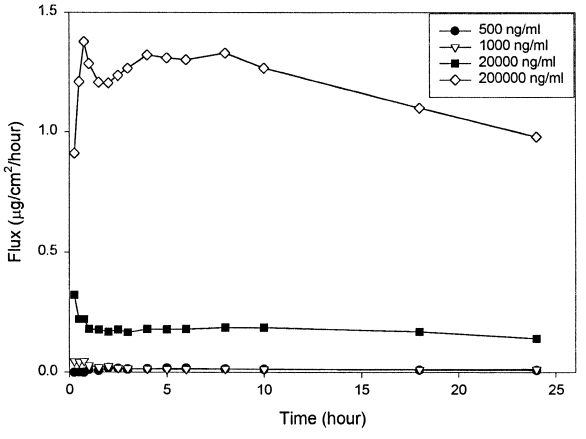

Fig. 2. Effect of drug loading on fentanyl release from a cellulose membrane with 46% PEO–PPO–PEO copolymer (each value represents the mean±S.D. of $n = 6$).

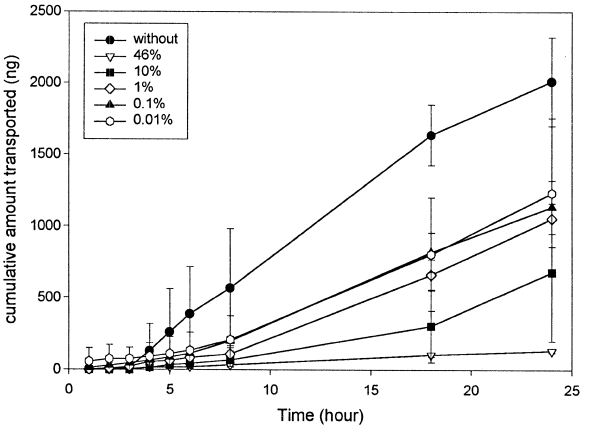

Fig. 3. Permeation profiles of fentanyl (20,000 ng/ml) with five PEO–PPO–PEO copolymer concentrations in nude mouse skin (each value represents the mean±S.D. of $n = 6$).

Table 1
Apparent permeability coefficients of fentanyl, estradiol and mannitol with and without 46% PEO–PPO–PEO copolymer gels and two different charge latex beads

	Apparent permeability coefficient $P \pm$S.D. (cm s^{-1})	Particle size by dynamic light scattering (nm)
Fentanyl without 46% PEO–PPO–PEO	$2.24 \pm 0.47 \times 10^{-6}$	–
Fentanyl with 46% PEO–PPO–PEO	$0.13 \pm 0.02 \times 10^{-6}$ [a]	53
Estradiol without 46% PEO–PPO–PEO	$4.41 \pm 0.43 \times 10^{-6}$	–
Estradiol with 46% PEO–PPO–PEO	$0.23 \pm 0.06 \times 10^{-6}$ [a]	57
Mannitol without 46% PEO–PPO–PEO	$2.40 \pm 0.48 \times 10^{-7}$	–
Mannitol with 46% PEO–PPO–PEO	$1.03 \pm 0.30 \times 10^{-7}$	56
Carboxylate-modified latex beads	$1.75 \pm 0.11 \times 10^{-8}$ [b]	46
Amine-modified latex beads	$4.33 \pm 1.15 \times 10^{-8}$ [b]	46

[a] Statistically significant difference at $P < 0.001$ between with or without 46% PEO–PPO–PEO.
[b] Statistically significant difference at $P < 0.01$ as compared to fentanyl transport.

were 0.13 ± 0.02, $0.23 \pm 0.06 \times 10^{-6}$, and $1.03 \pm 0.48 \times 10^{-7}$, respectively. In all cases, a steady-state flux of the three drugs was attained within 4 h of application of the formulation (linearity > 0.99). The permeability coefficients of two drugs (estradiol and fentanyl citrate) on nude mouse skin were significantly decreased by 46% copolymer. In addition, two non-flexible nano-particles of polystyrene latex with average bead size 46 nm were also applied on the nude mouse skin. The uptake and flux of the two different charges of latex decreased significantly ($P = 4.33 \pm 1.15$ and $1.75 \pm 0.11 \times 10^{-8}$ cm s^{-1}) as compared to the 46% PEO–PPO–PEO copolymer micelles (~50 nm by dynamic light scattering test).

3.4. In vivo preliminary percutaneous absorption experiments

Fig. 4 shows the preliminary pharmacokinetics of the fentanyl patch in six rabbits. The steady-state drug concentration was attained within 24 h after application of the patch, and could be maintained for at least 72 h. This experimental result suggests that the fentanyl patch formulated with 46% PEO–PPO–PEO copolymer could be a good release vehicle for

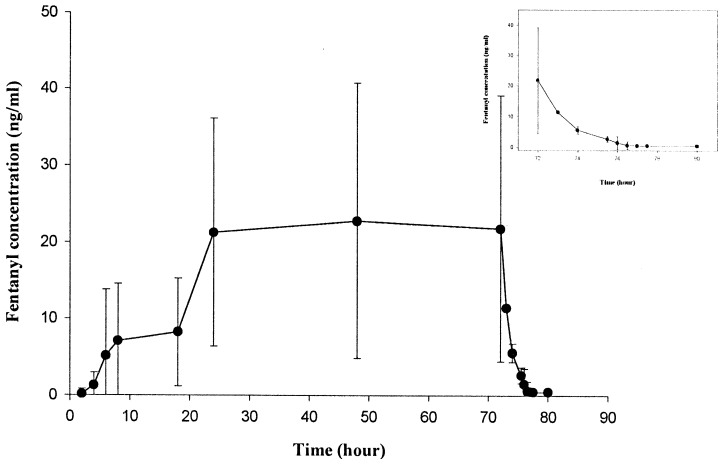

Fig. 4. Plasma concentration profile of fentanyl in six individual rabbits (each value represents the mean±S.D. of six rabbits.). Insert: elimination profile of fentanyl after detachment of the patch.

transdermal delivery. The insert of the figure represents the fentanyl elimination profile after detachment of the patch from the rabbits. The fentanyl elimination half-life ($t_{1/2}$) was calculated with the last four data points (non-compartment analysis), and the average was 10.5±3.4 h.

3.5. Characterization of micelle formation of PEO–PPO–PEO copolymer

Fluorescence spectra of the PEO–PPO–PEO copolymer samples at various concentrations in the presence of 6×10^{-7} M pyrene (with excitation at 339 nm) are shown in Fig. 5. The intensity increases with increasing copolymer, which indicates micelle formation [17]. In addition, by measuring the intensity ratios of the first to the third vibrational bands of pyrene, it was found that the I_1/I_3 peak height ratio decreased above 0.1% w/w copolymer (Fig. 6, open triangles). The magnitude of I_1/I_3 in the high concentration range (here 1.55) is somewhat higher than that for pyrene in toluene solution (1.04), but significantly lower than that in water (1.9). This ratio of fluorescence intensities was correlated with the hydrophobicity of the molecular environment of the pyrene probe. Taken together, above 0.1% PEO–PPO–PEO copolymer, pyrene was transferred into the hydrophobic domain of the micelles. By dynamic light scattering particle size measurement, a concentration of PEO–PPO–PEO copolymer above 0.1% (w/w) also exhibited a single modular population of particle distribution within the 50 nm range (Table 1).

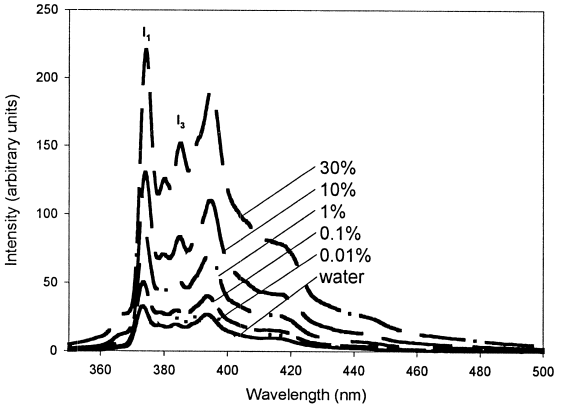

Fig. 5. Fluorescence emission spectra of pyrene in the presence of different concentrations of PEO–PPO–PEO copolymer with a fixed excitation wavelength of 339 nm.

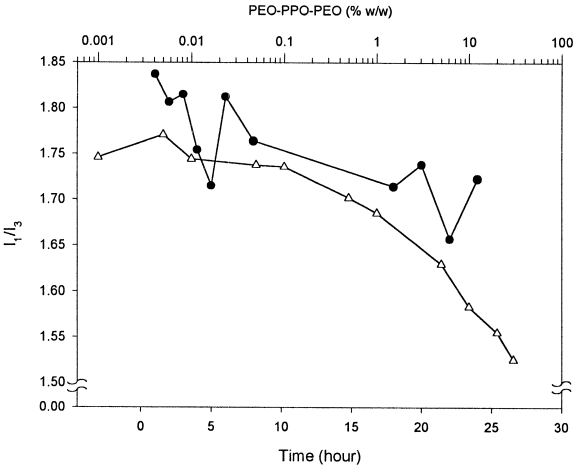

Fig. 6. Comparison of the intensity of the I_1/I_3 ratio of vibrational bands in the pyrene fluorescence spectrum with 46% PEO–PPO–PEO copolymer gel transport through nude mouse skin (●) and as a function of PEO–PPO–PEO copolymer concentration (△) in pyrene solution.

3.6. In vitro PEO–PPO–PEO copolymer gel percutaneous experiments

Fig. 6 (filled circles) shows the I_1/I_3 peak height ratio of the pyrene spectrum within 24-h profiles of 46% PEO–PPO–PEO copolymer gel transport through nude mouse skin. The I_1/I_3 ratio of the pyrene peak height was found to be <1.70 at 10–15 h after collecting samples from the receiver chamber compared with the 1.80 after collecting samples without 46% copolymer. The I_1/I_3 ratio decreased with time, which also indicates pyrene was transferred into the hydrophobic environment of the micelle [17].

4. Discussion

PEO–PPO–PEO copolymer exhibits gelation, low toxicity, and a pseudo-zero-order release rate, which make it attractive for use in sustained-release carrier systems. Its excellent solubilization characteristics make it useful as a vehicle for incorporating water-

insoluble drugs. In this work, we studied the release of fentanyl citrate from aqueous gels of PEO–PPO–PEO copolymer by an in vitro release method using a cellulose membrane, and a sustained-release pattern was achieved with a concentration of 46% (w/w). In addition, 46% copolymer only influenced the lipophilic estradiol release pattern, not the hydrophilic pattern of mannitol (Table 1). This may indicate that the micro-reservoir of PEO–PPO–PEO did not incorporate the hydrophilic drug to affect release and penetration into the skin. Thus, PEO–PPO–PEO may be used as a reservoir from which lipophilic drugs are released and controlled when placed percutaneously for systemic treatment, since these copolymers form a soft reverse thermal gel at body temperature.

In addition, Duragesic®, a commercial transdermal fentanyl patch, is designed to release fentanyl continuously for 72 h upon application to intact skin. It was reported that a rate-controlling membrane determines the delivery rate of fentanyl from the system to the skin surface with a flux of 2.5 $\mu g\ cm^{-2}\ h^{-1}$. Our 46% PEO–PPO–PEO copolymer controlling gel with 200 $\mu g/ml$ fentanyl had a release flux of around $1.09 \pm 0.19\ \mu g\ cm^{-2}\ h^{-1}$, which is similar to the commercial product. Moreover, the apparent permeability coefficient of fentanyl through human cadaver skin has been reported to be $3.6 \pm 0.8 \times 10^{-6}$ cm s^{-1} at pH 7.4 [4]. Our in vitro apparent permeability coefficient of pure fentanyl on nude mouse skin also had a similar range ($2.24 \pm 0.47 \times 10^{-6}$ cm s^{-1}) and this result agrees well with estimates for human skin. Furthermore, in in vivo percutaneous absorption of the fentanyl patch, a steady-state drug concentration was attained within 24 h of application of the patch, and could be maintained for at least 72 h with an exceptional increase of the elimination rate to 10.5 h. In addition, Miyazaki et al. [13] succeeded in delivering indomethacin with a 30% pluronic copolymer with good drug release for topical administration. Thus, with this in vitro and in vivo experimental evidence, PEO–PPO–PEO copolymer gel has the potential for sustained delivery in fentanyl percutaneous therapy.

In the fluorescence experiments, two noteworthy features of the spectra are that the intensity increases with increasing polymer concentration and that there are small changes in the I_1/I_3 intensity ratio. In general, pyrene is a widely used fluorescence probe because its vibrational structure is sensitive to the polarity of the microenvironment [18]. The major contribution to the intensity change is a shift in the absorption and excitation spectra of pyrene. The I_1/I_3 peak height ratio was shown to be a function of a decreasing exponent of the PEO–PPO–PEO copolymer concentration, and it dramatically decreased above a concentration of 0.1% w/w (Fig. 6) due to the formation of micelles and the transfer of pyrene into the hydrophobic domain of the micelle. In addition, the 339/334 nm intensity ratio in the excitation spectrum increased dramatically above that of the 0.1–0.5% concentration (data not shown). This also indicates that, at low concentrations, this ratio takes the value characteristic of pyrene in water and, at high concentrations, it takes the value of pyrene in a hydrophobic environment. Therefore, taken together, this concentration can be defined as the critical micelle concentration (cmc), and this result is very similar to the value reported by Saski and Shah [21] using surface tension and dye spectral absorption methods.

Regarding the surface charge and the particle size of the particles, a significant reduction in the percentage transport was seen after incubation with non-flexible amine- or carboxylate-modified polystyrene latexes (46 nm). However, the 46% PEO–PPO–PEO copolymer percutaneous transport experiment showed a reduced I_1/I_3 peak height ratio for pyrene, and the value was even less than the I_1/I_3 peak height ratio of the critical micelle concentration (1.74) or without 46% copolymer after collecting sample at 10–15 h (I_1/I_3 peak height ratio, 1.80). This shows that the 46% PEO–PPO–PEO copolymer (53 nm) could penetrate through nude mouse skin and the amount of transported polymer formed was above the critical micelle concentration. Because the dimensions of the pores and gaps between the cells in the skin are presumably small ($<$70 nm range) [22–24], 46% copolymer micelle vesicles may be able to permeate through this pathway without deformation of the micelles. Recently, Cevc et al. [25], using non-invasive delivery of insulin by ultraflexible transfersomes, showed that not only insulin could lower the blood glucose concentration by at least 50% through the skin, but the carrier could also be transported through intact skin. How-

ever, after collection from the receiver chamber, it is not easy to identify whether single monomers passed through the skin layers and formed the micelles or if the micelles penetrated the skin directly due to the dynamic/flexible movement of the micelles and the dilution factor in the receiver chamber. Flexible penetrating mechanisms are under further examination.

By coating with PEO or poloaxmer, a number of studies have demonstrated 'sheath' behavior of decreased uptake or degradation by the reticuloendothelial system [26]. In our preliminary in vivo pharmacokinetic data of the fentanyl patch (insert of Fig. 4), we found the elimination half-life ($t_{1/2}$) of fentanyl to be 10.5 h (range 7–14 h). By comparison with the intravenous administration of fentanyl in the rabbit, the intravenous elimination half-life ($t_{1/2}$) of fentanyl was reported to be around 40 min to 2 h [27,28], which is almost five times faster than our data. Thus, this may suggest that PEO–PPO–PEO gel, which contains PEO in the outer shell of the micelles, may serve not only as a drug carrier in percutaneous administration, but also has the advantage of providing a further sustained-release effect of drugs with sheath protection. On the other hand, the depot effect in the skin could not be excluded due to the high lipophilic nature of the drug. Thus, micelle permeation and formation in an in vivo skin dynamic system still requires further examination.

5. Conclusions

PEO–PPO–PEO copolymer has the potential for use as a gel vehicle for percutaneous formulations. Although attempts were not made in this study to optimize the formulations, it is clear from the results that PEO–PPO–PEO copolymer has the potential to increase the therapeutic efficacy of fentanyl citrate or other lipophilic drug by prolonging percutaneous input into the systemic circulation in the rabbit.

Acknowledgements

This work was supported by a grant from the National Science Council (NSC89-2623-D038-001).

References

[1] W.R. Martin, Pharmacology of opioids, Pharmacol. Rev. 35 (1983) 283–323.
[2] L.E. Mather, Clinical pharmacokinetics of fentanyl and its newer derivatives, Clin. Pharmacokinet. 8 (1983) 422–446.
[3] S.D. Roy, M. Gutierrez, G.L. Flynn, G.W. Cleary, Controlled transdermal delivery of fentanyl: characterizations of pressure-sensitive adhesives for matrix patch design, J. Pharm. Sci. 85 (1996) 491–495.
[4] S.D. Roy, S.E. Hou, S.L. Witham, G.L. Flynn, Transdermal delivery of narcotic analgesics: comparative metabolism and permeability of human cadaver skin and hairless mouse skin, J. Pharm. Sci. 83 (1994) 1723–1728.
[5] R.H. Guy, J. Hadgraft, Transdermal drug delivery: a perspective, J. Control. Release 4 (1987) 237–251.
[6] G.W. Cleary, Transdermal controlled release systems, in: R.S. Langer, D.L. Wise (Eds.), Medical Applications of Controlled Release, Vol. I, CRC Press, Boca Raton, FL, 1984, p. 205.
[7] I.R. Schmolka, Poloxamers in the pharmaceutical industry, in: P.J. Tarcha (Ed.), Polymers for Controlled Drug Delivery, CRC Press, Boca Raton, FL, 1991, pp. 189–214, Chapter 10.
[8] M. Yalin, F. Oner, L. Oner, A.A. Hincal, Preparation and properties of a stable intravenous lorazepam emulsion, J. Clin. Pharm. Ther. 22 (1997) 39–44.
[9] J.K. Yoon, D.J. Burgess, Interfacial properties as stability predictors of lecithin-stabilized perfluorocarbon emulsions, Pharm. Dev. Technol. 1 (1996) 333–341.
[10] O. Schubert, A. Wretlind, Intravenous infusion of fat emulsions, phosphatides and emulsifying agents, Acta Chir. Scand. 1 (Suppl. 278) (1983).
[11] G.T. Rodeheaver, L. Kurtz, B.J. Kircher, R.F. Edlich, Pluronic F-68: a promising new skin wound cleanser, Ann. Emerg. Med. 9 (1980) 572–576.
[12] D.M. Faulkner, S.T. Sutton, J.D. Hesford, B.C. Faulkner, D.A. Major, T.B. Hellewell, M.M. Laughon, G.T. Rodeheaver, R.F. Edlich, A new stable pluronic F68 gel carrier for antibiotics in contaminated wound treatment, Am. J. Emerg. Med. 15 (1997) 20–24.
[13] S. Miyazki, T. Tobiyama, M. Takada, D. Attwood, Percutaneous absorption of indomethacin from Pluronic F127 gels in rats, J. Pharm. Pharmacol. 47 (1995) 455–457.
[14] G. Kwon, M. Naito, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Physical entrapment of adriamycin in AB block copolymer micelles, Pharm. Res. 12 (1995) 192–195.
[15] I.R. Schmolka, Artificial skin I. Preparation and properties of Pluronic F-127 gels for treatment of burns, J. Biomed. Mater. Res. 6 (1972) 571–582.
[16] W.M. Dewell, M.K. Martin, H.M. Solomon, High-performance liquid chromatographic determination of fentanyl and sufentanil returned from the operating room, Am. J. Hosp. Pharm. 50 (1993) 2374–2375.
[17] J. Liaw, T. Aoyagi, K. Kataoka, Y. Sakurai, T. Okano, Permeation of PEO-PBLA-FITC polymeric micelles in aortic endothelial cells, Pharm. Res. 16 (1999) 213–220.
[18] K. Kalyanasundaram, J.K. Thomas, Environmental effects on

vibronic band intensities in pyrene monomer fluorescence and their application in studies of micellar systems, J. Am. Chem. Soc. 99 (1977) 2039–2044.
[19] K.N. Prasad, T.T. Luong, A.T. Florence, J. Paris, C. Vautin, M. Seiller, F. Puisieux, Surface activity and association of ABA polyoxyethylene–polyoxypropylene block copolymers in aqueous solution, J. Colloid Interface Sci. 90 (1982) 303–309.
[20] S.C. Miller, B.R. Drabik, Rheological properties of poloxamer vehicles, Int. J. Pharm. 18 (1984) 269–276.
[21] W. Saski, S.G. Shah, Availability of drugs in the presence of surface-active agents I. Critical micelles concentrations of some oxyethylene oxypropylene polymers, J. Pharm. Sci. 54 (1965) 71–76.
[22] H.M. Reinl, A. Hartinger, P. Dettmar, T.M. Bayerl, Time-resolved infrared ATR measurements of liposome transport kinetics in human keratinocyte cultures and skin reveals a dependence on liposome size and phase state, J. Invest. Dermatol. 105 (1995) 291–295.
[23] J.S. Schwartz, M.R. Weisspapir, D.I. Friedman, Enhanced transdermal delivery of diazepam by submicron emulsion creams, Pharm. Res. 12 (1995) 687–697.
[24] E. Marly, V. Kuijk-Meuwissen, L. Mougin, H. Junginger, J. Bouwstra, Application of vesicles to rat skin in vivo: a confocal laser scanning microscopy study, J. Control. Release 56 (1998) 189–196.
[25] G. Cevc, D. Gebauer, J. Stieber, A. Schatzlein, G. Blume, Ultrafexible vesicles, transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin, Biochim. Biophys. Acta 1368 (1998) 201–215.
[26] S.E. Dunn, A.G.A. Coombes, M.C. Garnett, S.S. Davis, M.C. Davis, L. Illum, In vitro cell interaction and in vivo biodistribution of poly(lactide-co-glycolide) nanospheres surface modified by poloxamer and poloxamine copolymer, J. Control. Release 44 (1997) 65–76.
[27] R. Hess, G. Stiebler, A. Herz, Pharmacokinetic of fentanyl in man and rabbit, Eur. J. Clin. Pharmcol. 4 (1972) 137–141.
[28] J. Irazuzta, U. Ahmed, A. Gancayco, S. Ahmed, J. Zhang, K. Anand, Intracheal administration of fentanyl: pharmacokinetics and local tissue effects, Intensive Care Med. 22 (1996) 129–133.