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Transdermal delivery of selegiline from alginate–Pluronic composite thermogels

Chih-Chieh Chen[a], Chia-Lang Fang[b], Saleh A. Al-Suwayeh[c], Yann-Lii Leu[d], Jia-You Fang[a,c,e,*]

[a] Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan
[b] Department of Pathology, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[d] Natural Products Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan
[e] Department of Cosmetic Science, Chang Gung Technology University, Kweishan, Taoyuan, Taiwan

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ABSTRACT

The present work was carried out to design a practical, controlled-release transdermal system for selegiline using thermosensitive hydrogels. The copolymers of alginate and Pluronic F127 (PF127) were used to design thermogels by either physical blending (A + P) or chemical grafting (AP). The thermogels were characterized in terms of the sol–gel temperature, scanning electron microscopy (SEM), degradation ratio, and skin permeation behavior. The chemical grafting of alginate to PF127 could delay the sol–gel temperature from 24.1 to 30.4 °C, which is near the temperature of the skin surface. The gelling temperature of the physical mixture of alginate and PF127 (A + P) did not significantly differ. The porosity of the A + P structure was greater compared to that of the AP structure. AP thermogels were regularly degraded, with 60% of the gel matrix remaining after a 48-h incubation. PF127 and A + P hydrogels showed almost no degradation. The results of skin permeation across porcine skin and nude mouse skin suggested that the thermogels could produce sustained selegiline release, with AP showing the most-sustained permeation. AP hydrogels exhibited linear permeation properties for the transdermal delivery of selegiline. Inter-subject variations in skin permeation were reduced by incorporation of the thermogel. Such a thermosensitive hydrogel can be advantageous as a topical therapeutic formulation for selegiline.

1. Introduction

Comparative studies found selegiline, a monoamine oxidase inhibitor (MAOI) drug, to be more effective than other antidepressant agents in treating atypical depression and bipolar depression (Howland, 2006). Selegiline also shows potential indications for treating Parkinson's disease, Alzheimer's disease, and cocaine addiction (Nyholm, 2006; Fernandez and Chen, 2007). Selegiline was the first selective, irreversible MAOI approved for use in Parkinson's disease in the US. A selegiline transdermal system (STS, Emsam®) was approved for treating major depressive disorders which avoids the first-pass effect and provides greater and more-prolonged levels of selegiline compared to the oral regimen (Small and Dubois, 2007). The STS permits required antidepressant concentrations to the brain while maintaining the intestinal barrier to dietary tyramine, thus avoiding the risk of a tyramine-induced hypertensive crisis (Kolli et al., 2010). An important concern for transdermal selegiline patches is local dermal reactions. The most frequent adverse effect is an application-site skin reaction (40%) to the STS (Feiger et al., 2006). These skin reactions can occur due to components of the patch. STS is a matrix-type transdermal system composed of three layers. The predominant ingredients in the three layers are acrylic adhesive, polyester, polyurethane, and silicone. Hence there is still a need to develop novel systems for selegiline delivery.

Thermosensitive hydrogels may be a good choice to reduce local irritation. A thermosensitive approach can be advantageous for particular applications as it is transformed from a liquid to a gel when administered topically. Pluronics/Poloxamers are a series of synthetic block copolymers of poly(ethylene oxide–b-propylene oxide–b-ethylene oxide) (PEO-PPO-PEO). They are nonionic surfactants possessing excellent wetting, antifoaming, and solubilizing properties. Pluronic F127 (PF127) is an ABA-type triblock copolymer consisting of PEO units (A = 70%) and PPO units (B = 30%), which is transformed from a low-viscosity solution to a semisolid gel upon heating to body temperature at concentrations of >20% (Moebus et al., 2009). Potential drawbacks of Pluronics include their weak mechanical strength and the non-biodegradability of PEO–PPO–PEO (Ruel-Gariépy and Leroux, 2004; Gong et al., 2009). To circumvent these problems, Pluronics can be physically or chemically modified by other polymers (Xiong et al., 2006; Hsu et al., 2009). Alginate is a natural polysaccharide composed of

* Corresponding author at: Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, 259 Wen-Hwa 1st Road, Kweishan, Taoyuan 333, Taiwan. Tel.: +886 3 2118800x5521; fax: +886 3 2118236.
E-mail address: fajy@mail.cgu.edu.tw (J.-Y. Fang).

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1,4-linked-β-D-mannuronic acid and α-L-guluronic acid residues. Alginate is highly hydrophilic, biocompatible, and relatively economical, and it is widely utilized in drug delivery (Lin et al., 2004; Kulkarni et al., 2010). We previously developed thermosensitive alginate–Pluronic (AP) graft hydrogels for cisplatin delivery by injection (Fang et al., 2009). This hydrogel exhibited controlled drug release and increased cisplatin deposition in tumors. However, this hydrogel showed a similar sol–gel temperature (26.6 °C) as the PF127 system which gels at room temperature, especially in summer or in the subtropical and tropical environments. To overcome this disadvantage, we modified the feeding ratio of PF127 and alginate to delay the sol–gel temperature in this study.

In the present work, we attempted to develop temperature-sensitive systems based on an alginate/PF127 graft copolymer and a polymeric blend of alginate and PF127, which can serve as transdermal selegiline delivery systems. We also attempted to develop a delivery system with controlled and sustained release of selegiline since the inter- and intra-individual absorption of selegiline is highly variable (Lombardi Borgia et al., 2005; Pae et al., 2007). These thermogels were characterized using Fourier-transformed infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), viscosity, and the degradation ratio. Permeation of selegiline from hydrogels through porcine skin and nude mouse skin was examined by an in vitro method.

2. Materials and methods

2.1. Materials

Selegiline hydrochloride (molecular weight (MW) = 187.3; octanol/water partition coefficient, log P = 3.4), Pluronic F127 (PF127, MW = 1.26×10^4), sodium alginate (MW = 1.2×10^5), methylene chloride, triethylamine (TEA), ethylenediamine, petroleum ether, 4-nitrophenyl chloroformate, and potassium bromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxylsuccinimide (NHS) were obtained from Acros (Geel, Belgium).

2.2. Alginate–PF127 (AP) composite synthesis

The AP copolymer was synthesized using an EDC/NHS cross-linking method. Alginate, as a backbone, was incorporated with monoamine-terminated PF127 (MATP), which was first prepared by a two-step reaction (Cho et al., 2003). PF127 (0.8 mM) was reacted with 8 mM 4-nitrophenyl chloroformate dissolved in methylene chloride in the presence of 0.3% TEA at room temperature for 4 h to yield a 4-nitrophenyl formate-derived intermediate. After recovery using petroleum ether extraction three times, this intermediate was reacted with 2% ethylenediamine in methylene chloride at room temperature. After a 12-h reaction, the mixture was extracted by petroleum ether three times, then dialyzed against double-distilled water (ddH_2O) using a membrane with an MW cutoff of 3500 for 3 days. The dialysis product was then lyophilized to obtain MATP. MATP and alginate (at a weight ratio of 32:1) were dissolved in ddH_2O. EDC (0.05 M) and NHS (0.25 M) were added to the above solution for the cross-linking reaction for 24 h. The mixture was dialyzed against ddH_2O using a membrane with an MW cutoff of 12,000–14,000 for 3 days. The mixture was then lyophilized to obtain the final product.

2.3. Measurements of the grafting ratio and efficiency of grafting

The grafting ratio was calculated as $(W_{AP} - W_{alginate})/MW_{MATP}$ divided by $W_{alginate}/MW_{alginate}$, where W_{AP} is the weight of the freeze-dried graft copolymer, and $W_{alginate}$ is the weight of alginate in the feed. MW_{MATP} and $MW_{alginate}$ are the MWs of MATP and alginate, respectively. The efficiency of grafting (%) of AP was calculated from the relationship $(W_{AP} - W_{alginate})/W_{MATP} \times 100$, where W_{MATP} is the weight of MATP in the feed.

2.4. Fourier-transformed infrared (FTIR) absorption spectroscopy

The chemical structure of the AP copolymer was verified by FTIR. Tablets of PF127, MATP, alginate, and the AP copolymer were obtained using potassium bromide disc pellets. The measurement was carried out on an FTIR spectrometer (FT/IR-4100, Jasco, Tokyo, Japan).

2.5. Preparation of hydrogels

PF127 and AP copolymers were added to ddH_2O to yield concentrations of 20% (w/v) and 15%, respectively. These concentrations were determined by the minimum amounts of these copolymers which formed semisolid hydrogels at the sol–gel temperature. The physical mixture of the alginate/PF127 system (A + P) was also prepared based on the weight ratio the same as with AP synthesis (32:1). The percentages of alginate and PF127 in the A + P hydrogels were 20% and 0.63%, respectively. The copolymers were added to ddH_2O at 4 °C with gentle stirring by a magnetic bar for 2 h. A homogenous solution with a clear appearance was then obtained. The mixtures were allowed to reach room temperature before the experiments. The selegiline dose in the hydrogels was 0.07% (w/v), which was incorporated in the vehicles with the copolymers simultaneously. The total volume of the prepared hydrogels was 10 ml.

2.6. Sol–gel phase transition behavior

The thermosensitive sol–gel phase transition of the copolymers in ddH_2O was first monitored using a test tube with a 25-ml vial with a tight screw cap and an inner diameter of 20 mm (Gong et al., 2009). The volume of the systems was maintained at 10 ml. The sol–gel transition was visually observed by tilting the vials, and conditions of the sol and gel were respectively defined as “flow” and “no flow” at 1 min.

2.7. Sol–gel transition temperature

Viscosity measurements were used to assess the gelation temperature of the thermogels. The viscosity values of these hydrogels were determined using a rheometer (AR-G2, TA Instruments, New Castle, DE, USA). Each sample was rotated between the parallel plates, the diameter of which was 40 mm. The viscosity was determined at 15–40 °C.

2.8. Scanning electron microscopy (SEM)

Hydrogels were frozen at −80 °C and then lyophilized by a freeze-drying method (Bhattari et al., 2005). Samples were fractured in liquid nitrogen and sputter-coated with gold. The resulting dried samples were examined using an SEM (S-2400, Hitachi, Tokyo, Japan).

2.9. Degradation ratio

The degradation ratio was determined by a volumetric method with some modification (Van Tomme et al., 2006). Hydrogel samples were prepared as described in Section 2.5 and then a 1.1-ml (V_0) sample was transferred into a 1.5-ml vial and centrifuged at $10^4 \times g$ for 10 min. The hydrogel was subsequently equilibrated at 4 °C. After 12 h, 200 μl ddH_2O was added, and the sample was then placed in an oven at 32 °C. The volume of the hydrogel (V_t)

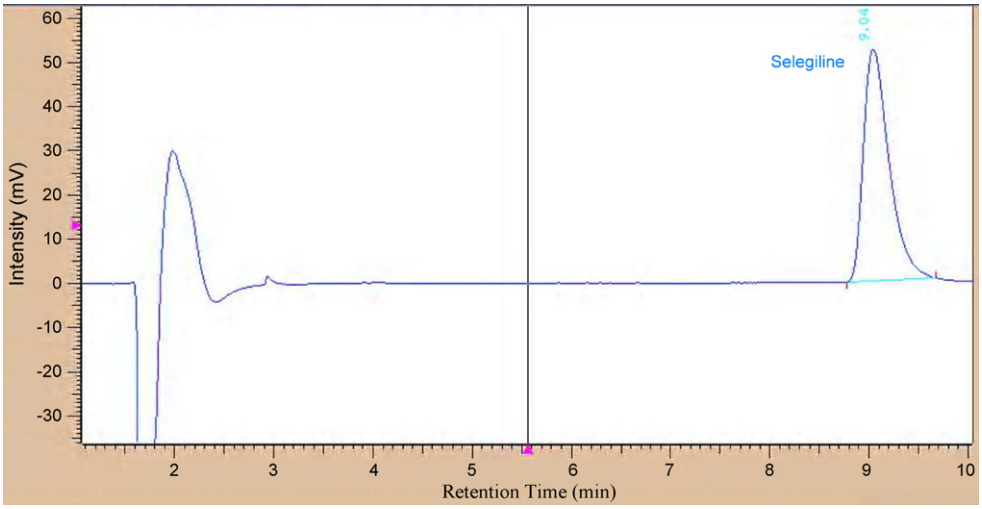

Fig. 1. A representative HPLC chromatogram of selegiline dissolved in water. The concentration of selegiline in this chromatogram was 20 μg/ml.

was recorded at appropriate intervals. The degradation ratio was calculated as: $R_d = V_t/V_0 \times 100\%$.

2.10. Skin preparation

Specific pathogen-free (SPF) pigs (1 week old) were supplied by the Animal Technology Institute Taiwan (Miaoli, Taiwan). Female nude mice (8 weeks old) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University. Animals were housed and handled according to institutional guidelines. Full-thickness skin was excised from the dorsal region of the pigs and mice. The subcutaneous fat, tissue, blood vessels, and epidermal hairs were carefully removed before use.

2.11. In vitro permeation of selegiline from the thermogels

Drug permeation from the thermogels was measured using a Franz diffusion cell. Porcine skin, nude mouse skin, or a cellulose membrane (SpectraPor® 3, with an MW cutoff of 3500) was mounted between the donor and receptor compartments with the skin stratum corneum side facing upward into the donor compartment. The donor was filled with 0.5 ml of a hydrogel. pH 7.4 buffer (5.5 ml) was used as the receptor medium. The available diffusion area between the cells was 0.785 cm^2. The stirring rate and temperature were maintained at 600 rpm and 37 °C, respectively. At appropriate intervals, 300-μl aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh buffer. The amount of selegiline in the receptor was determined by high-performance liquid chromatography (HPLC).

2.12. HPLC analytical method

The HPLC system for selegiline included a Hitachi (Tokyo, Japan) L-7110 pump, a Hitachi L-7200 sample processor, and a Hitachi L-7400 ultraviolet (UV)/visible detector. A 25-cm-long, 4-mm inner diameter stainless-steel RP-18 column (Merck, Darmstadt, Germany) was used. The mobile phase was acetonitrile: pH 7.0 buffer (65:35) at a flow rate of 1.0 ml/min. The UV/visible detector was set to 205 nm. The retention time of selegiline was 9 min. The representative HPLC chromatogram is shown in Fig. 1.

2.13. Statistical analysis

Statistical analyses of differences between the various treatments were performed using an unpaired Student's *t*-test. A 0.05 level of probability ($p < 0.05$) was taken as the level of significance. An analysis of variance (ANOVA) test was also used if necessary.

3. Results

3.1. Alginate–PF127 (AP) composite synthesis

The graft copolymer was prepared by coupling MATP with an alginate backbone using EDC and NHS as coupling agents. The amide bonds were formed through the reaction between the amino groups of MATP and carboxyl groups of alginate as shown in Fig. 2. The grafting ratio and efficiency of grafting for the AP composite were 17.7 and 62.6%, respectively, as shown in Table 1. The estimated molecular mass of AP was approximately 2.33×10^3 kDa as determined by the end group analysis (Stevens, 1990). Fig. 3 shows the FTIR spectra of PF127, alginate, and the grafted AP. An absorbance band at 1610 cm^{-1} was assigned to the COOH stretching of alginate. In the FTIR spectrum of the grafted copolymer, the carbonyl absorption band of carboxylate sodium salt in the alginate had disappeared, and a new characteristic amide I band had appeared at 1633 cm^{-1}. It was confirmed that PF127 had been grafted to the molecular chains of alginate.

3.2. Sol–gel phase transition behavior

With the aim of developing a drug delivery system suitable for topical applications, the thermosensitive properties of the prepared

Table 1
The grafting ratio, efficiency of grafting, and molecular mass of alginate–PF127 (AP) copolymer.

Parameter	Value
Grafting ratio	17.7
Efficiency of grafting (%)	62.6
Molecular mass (kDa)	2.33×10^3

Grafting ratio = $[(W_{AP} - W_{alginate})/MW_{MATP}]/(W_{alginate}/MW_{alginate})$; the efficiency of grafting = $(W_{AP} - W_{alginate})/W_{MATP}$, where W_{AP} is the weight of freeze-dried graft copolymer, MW_{AP} and MW_{MATP} are the molecular weights of AP and MATP, respectively, and $W_{alginate}$ and W_{MATP} are the weights of alginate and MATP in the feed, respectively.

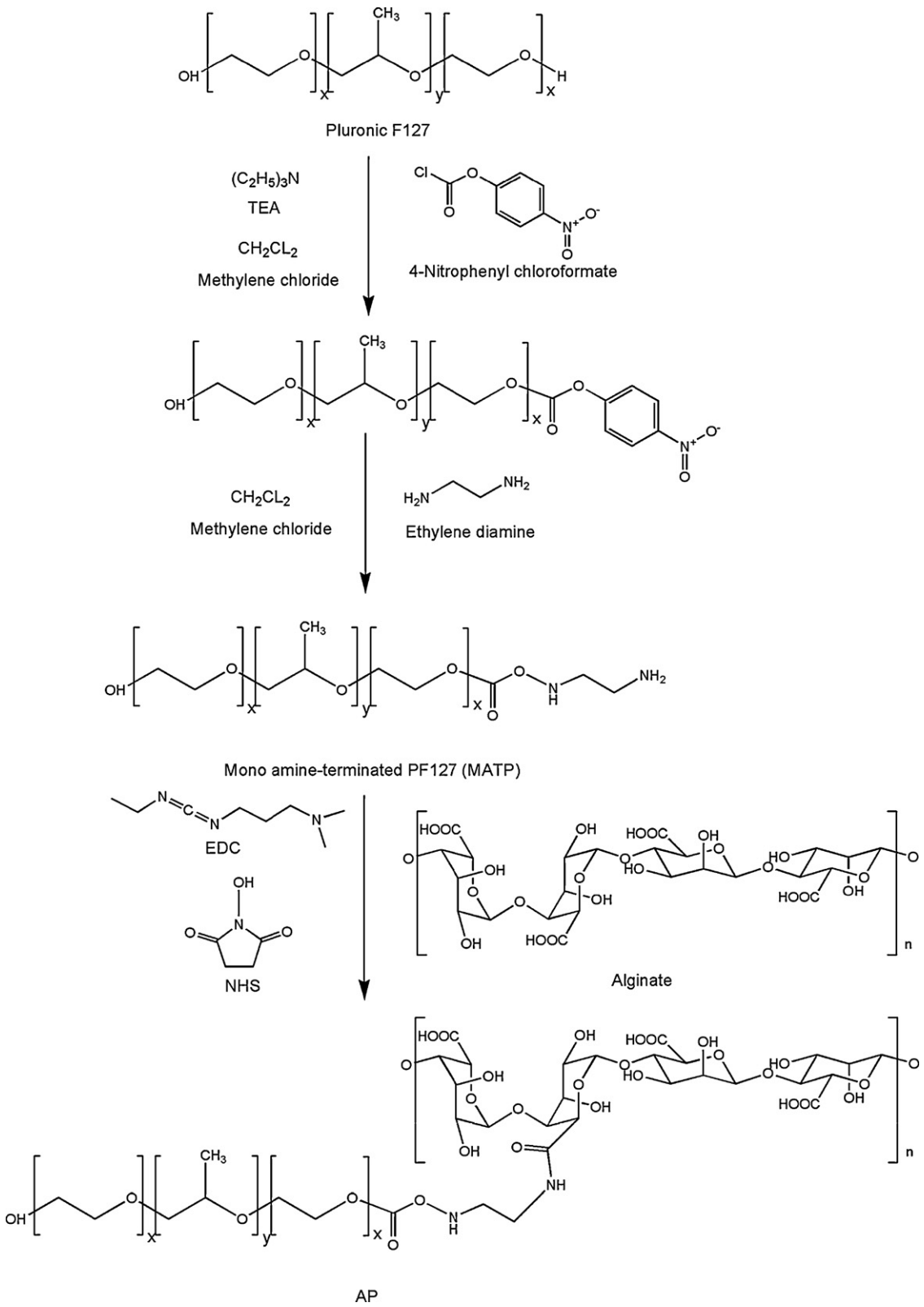

Fig. 2. Chemical reaction scheme for synthesizing the alginate–Pluronic F127 composite copolymer (AP).

hydrogels were tested. An ideal gelling delivery system should be a free-flowing liquid with low viscosity under non-physiological conditions to allow reproducible administration onto the skin surface as drops; it should also undergo a rapid phase transition to form a strong gel capable of withstanding shear forces under physiological conditions (Lin et al., 2004). The sol–gel transition was first characterized by monitoring the flow property as a function of temperature as shown in Fig. 4. The effect of temperature was evaluated at 15–35 °C. In Fig. 4A, at a low temperature of 15 °C, all systems including PF127, A + P, and AP were flowing solutions.

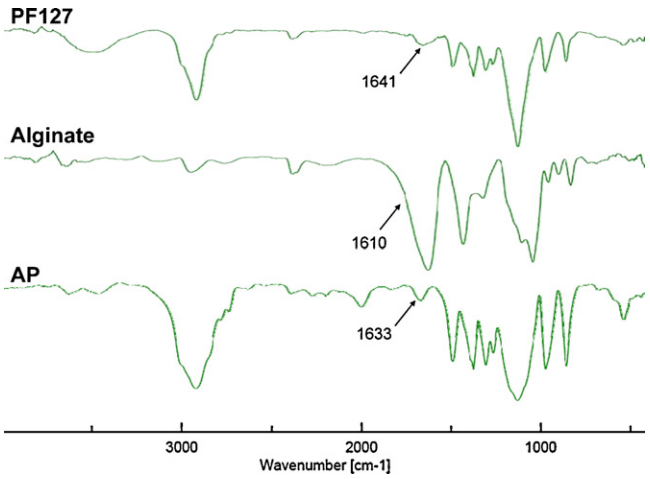

Fig. 3. Fourier-transformed infrared (FTIR) spectra of Pluronic F127, alginate, and the alginate–Pluronic F127 composite copolymer (AP).

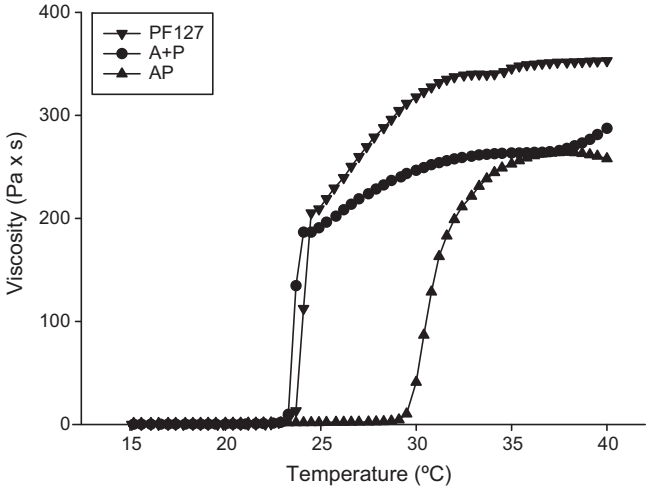

Fig. 5. Effects of temperatures of 15–40 °C on the viscosities of PF127 (20%), alginate and PF127 blended (A + P, 20% + 0.63%), and alginate–Pluronic F127 composite copolymer (AP, 15%) systems determined by an AR-G2 rheometer. Each sample was rotated between the parallel plates, which had a diameter of 40 mm. (▼) PF127; (●), A + P; (▲) AP.

Since Pluronics exhibit reverse thermal behavior, their viscosity can increase as the temperature increases. It can be seen in Fig. 4B that PF127 and the A + P polymer blend began to form a gel-like system at 20 °C. Complete gelation was observed for both systems at 25 °C as shown in Fig. 4C. The AP graft copolymer still exhibited the solution type at the same time. The AP system began to be sticky at 30 °C. This formulation could be completely gelled from the solution type within 20 s. Hence a long waiting for gelation is not necessary when applied on the skin. Fig. 5E displays a representative visual inspection of the AP gels at 35 °C. The visual consistency of the thermogel was good, with no sliding or shifting of the gel mass when the vial was flipped over or shaken. The gels obtained were transparent and non-gritty. It should be noted that a concentration of 15% was sufficient for AP to exhibit sol–gel transition behavior. PF127 and A + P should form viscoelastic gels upon heating at concentrations of >20%.

3.3. Sol–gel transition temperatures

The gelation of a thermogel is frequently concurrent with the variation in viscosity. Hence viscometry is widely used to determine sol–gel transition temperatures. The viscosity of the copolymer systems was examined as a function of temperature as shown in Fig. 5. The gelation kinetics were dependent on the type of copolymers used. An abrupt increase in viscosity at 24.1 °C marked the onset of the gelation process of PF127 (20%). Then a steep increase in viscosity occurred until 30 °C. The physical mixture of alginate and PF127 (A + P) showed an onset of gelation at 23.7 °C, which was approximately that of the PF127 system. The critical gel temperature of the AP system was considerably higher than those of the other systems. The AP transition from liquid-like behavior to elastic gel-like behavior occurred at 30.4 °C.

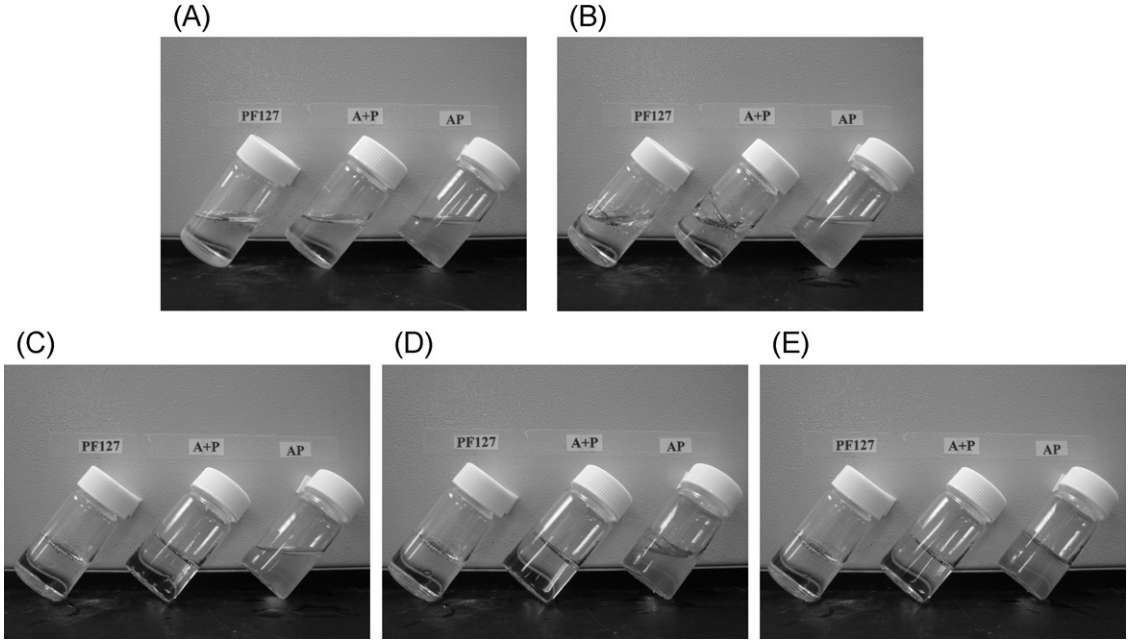

Fig. 4. Representative visual inspection of the aqueous copolymer system at 15 °C (A), 20 °C (B), 25 °C (C), 30 °C (D), and 35 °C (E) (left to right: PF127 (20%), alginate and PF127 blending (A + P, 20% + 0.63%), and the alginate–Pluronic F127 composite copolymer (AP, 15%)).

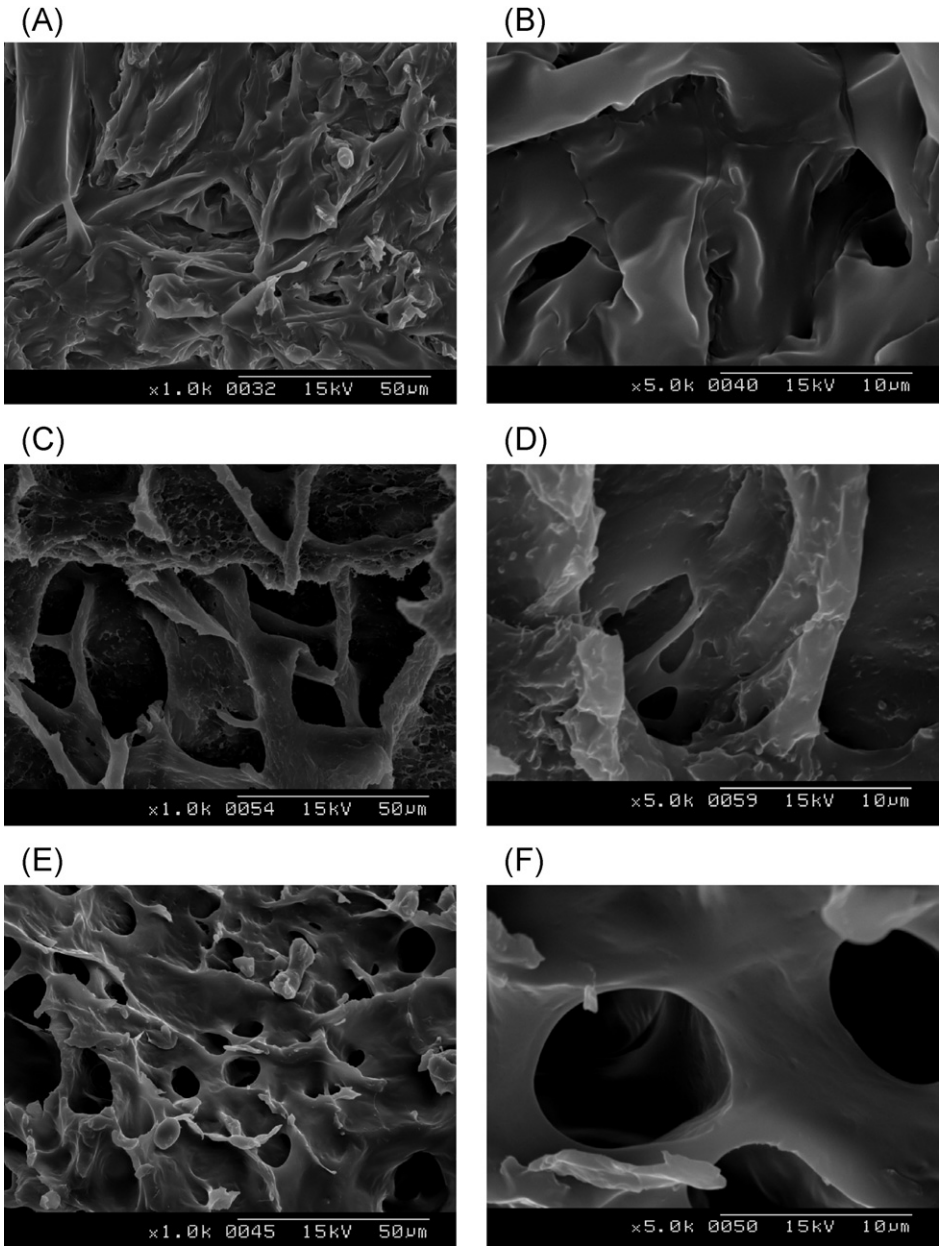

Fig. 6. Cross-sectional scanning electron microscopic (SEM) images of hydrogels after the freeze-drying process composed of PF127 at a magnification of 1000× (A), PF127 at a magnification of 5000× (B), the alginate and PF127 blend (A + P) at a magnification of 1000× (C), A + P at a magnification of 5000× (D), the alginate–Pluronic F127 composite copolymer (AP) at a magnification of 1000× (E), and AP at a magnification of 5000× (F).

3.4. SEM

Fig. 6 shows the cross-sectional morphologies of the thermogels. The PF127 nanostructure in the hydrogels assembled into a transient polymeric network as illustrated in Fig. 6A. PF127 exhibited a fibrous structure in cross-sectional view. Enlarging the magnification from 1000× to 5000× revealed some pores among the fibrous structures (Fig. 6B). SEM images of the dehydrated A + P hydrogels show an irregular texture with pores as observed in Fig. 6C and D at various magnifications. It can be seen that the inner pores of the A + P systems are interconnected and have irregular shapes and highly porous structures, with pores ranging 10–20 μm in diameter. Micrographs of the AP composite thermogels show that the systems are a network with sponge-like structures as shown in Fig. 6E. The spherical pores are well interconnected throughout the scaffold matrix (Fig. 6F). The network structure of the AP systems was more condensed than that of A + P systems, with pore sizes of 5–10 μm.

3.5. Degradation ratio

The degradation of thermogels composed of PF127, A + P, or AP was studied by swelling experiments at 32 °C as shown in Fig. 7. After taking up water, the gels began to lose weight and ultimately dissolved. There was some chemical disintegration of PF127 and A + P hydrogels produced by water during a 48-h period. Degradation of 10% was detected for PF127 hydrogels. The degradation kinetics of the A + P systems were similar to those of PF127. AP hydrogels exhibited more-significant degradation compared to the others ($p < 0.05$). A 40% reduction in volume was determined for AP systems at the end of the experiment (48 h).

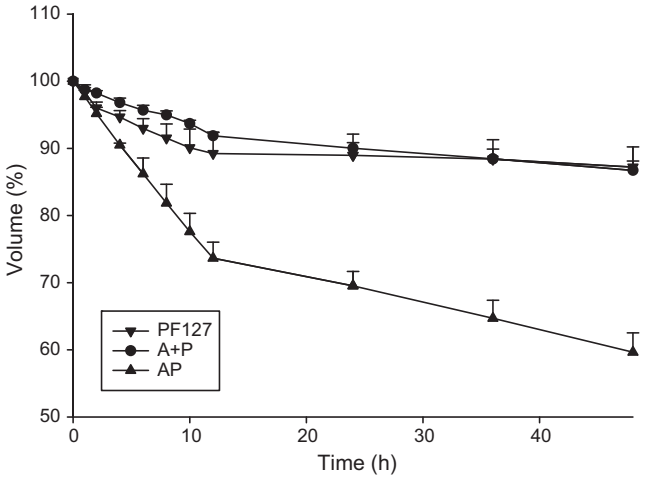

Fig. 7. Degradation ratios of the PF127 (20%), alginate and PF127 blended (A + P, 20% + 0.63%), and alginate–Pluronic F127 composite copolymer (AP, 15%) systems determined by the change in total volume (%). (▼) PF127; (●) A + P; (▲) AP.

3.6. *In vitro permeation of selegiline from the thermogels*

Vehicles used in the topically applied formulations can greatly influence the rate and extent of drug permeation across the skin. The transdermal systems of selegiline were studied to establish the in vitro permeation kinetics from the hydrogels to the skin. The cumulative amounts of selegiline permeated across porcine skin as a function of time are shown in Fig. 8A. ddH_2O was used as the control vehicle. All hydrogels decreased the permeation of selegiline across porcine skin compared to the control. The thermogels notably inhibited the burst release of selegiline from the control group. The cumulative amounts of the drug at 48 h from PF127, A + P, and AP thermogels were 44, 32, and 14 μg/cm^2, respectively. A wide inter-subject variation was observed for the control vehicle according to the standard deviation bar shown in Fig. 8A. This extent of variation can be explained by actual biological and morphological differences among the skin tissues of different subjects. The variation in selegiline permeation was substantially reduced after incorporation in the hydrogels. Permeation was more sustained with thermogels containing the AP graft copolymer. It practically matched zero-order release kinetics (correlation coefficient, $r = 0.98$).

Nude mouse skin was also used as a barrier for selegiline permeation as demonstrated in Fig. 7B. Drug permeation into the receptor was highest for the aqueous control, followed by the PF127, A + P, and AP systems. This trend was the same as that when using porcine skin as the barrier. Discrepancies in selegiline delivery among formulations were attenuated by using nude mouse skin instead of porcine skin. Curves of the cumulative amount-time profiles of the control, PF127, and A + P ascended in the late stage of permeation after 24 h. This indicates non-sustained permeation behavior for these vehicles. Drug permeation from the AP thermogels followed zero-order kinetics ($r = 0.99$).

Selegiline diffusion across the cellulose membrane was determined to evaluate release rates as shown in Fig. 7C. The results indicated that the release of selegiline from the aqueous control was faster than from the hydrogels. The control vehicle showed an initial burst (0–4 h), followed by a sustained burst (4–8 h), and then a plateau (8–48 h). The same release behavior was observed for PF127 thermogels although the drug in that system was released much more slowly than from the control. The release profiles indicated that A + P and AP were best able to retain selegiline. The AP system showed a release profile quite superimposable to that of A + P hydrogels ($p > 0.05$). Both systems produced a zero-order release rate ($r = 0.99$).

4. Discussion

Transdermal drug delivery continues to be an ideal treatment option for depressive and neurodegenerative disorders. However, conventional vehicles fail to provide stable and safe exposure to the skin. Thermosensitive hydrogels may provide some potential benefits for selegiline permeation into the skin. In the past decade, sol–gel reversible gels have garnered increasing attention for practical biomedical and pharmaceutical applications since their administration is convenient, and no organic solvents or toxic cross-linkers are involved during gelation (Liu et al., 2004). The copolymer systems described here, including PF127, A + P, and AP, undergo a thermodynamically reversible transition. They were flowing solutions at low temperatures and turned into non-flowing gels at skin temperature (32 °C). PF127 should gel at a concentration of >20%. In the present work, a 15% concentration of the AP composite copolymer was sufficient to produce a semisolid gel. This is beneficial for practical applications in terms of cost and toxicity.

Selegiline is a weak base with a low MW of 187.3 and a log *P* of 3.4 (Pae et al., 2007). These characteristics are optimal for transdermal delivery. As expected, higher skin permeation and faster release were observed for free selegiline from the aqueous control. Since episodes of depression require longer and more-sustained pharmacological therapy (Howland, 2006), thermogel systems for transdermal selegiline delivery were developed with the aim of prolonged and controlled release but not enhanced permeation. Burst release is a major problem for most drugs because the concentration of the drug decreases rapidly (Xiong et al., 2006). It was seen that selegiline exhibited slower permeation via porcine skin in the late stage of the in vitro experiments. In vitro permeation profiles clearly suggested that selegiline permeation was sustained and controlled by the stable and entangled networks of the hydrogels, especially the AP graft copolymer.

The viscosity of the formulation is important as it is the property that enables adhesives to flow into the adherend and attach securely (Rippon et al., 2007). A feasible viscosity of the thermogels can guarantee the practicability to skin administration. Aqueous PF127 solutions clearly indicated a micellar mode of association. Micelle formation occurs at the critical micellization temperature as a result of PPO block dehydration (Ruel-Gariépy and Leroux, 2004). With increasing temperatures, micellization becomes more important, and at a definite point, micelles come into contact and no longer move. In addition, the formation of highly ordered structures, such as a cubic crystalline phase, was proposed as the driving force in gel formation (Park et al., 2009). Hence, the packing of micelles and micellar entanglements are mechanisms of PF127 solution gelation at increased temperatures. Our results suggest that gelation was dependent on the type of copolymer used. The critical gel concentration of AP (15%) was considerably lower than that of the parent PF127 (20%). The hydrophilic property of alginate may interfere with the hydrophobic interactions of PF127 (Fang et al., 2009). Grafting hydrophilic polymers increases the hydrophilicity of the system. This leads to an increase in the sol–gel temperature, since the hydrophobic interactions, which increase with temperature, are compensated for at higher temperatures by an increase in polymer–water interactions (Meyer et al., 2001; Fang et al., 2008b).

The addition of alginate to PF127 as a physical blend (A + P) slightly lowered the sol–gel temperature compared to PF127 alone. This is because the addition of sodium alginate exerts a strong influence on the structural process, by hindering both the initial caging and subsequent structural rearrangement of micelles. The

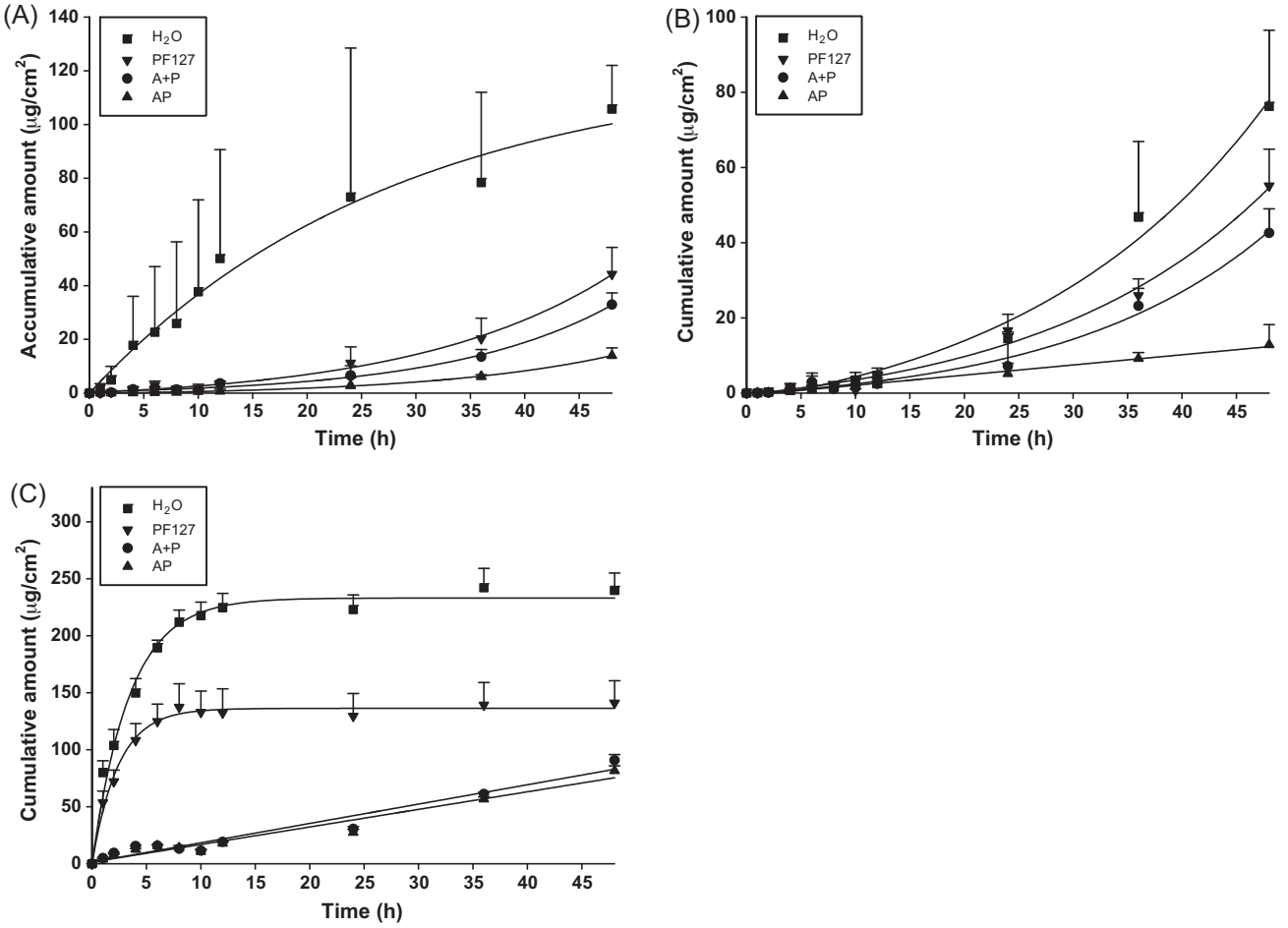

Fig. 8. In vitro cumulative amount (μg/cm²)–time profiles of the permeation of selegiline from hydrogels across porcine skin (A), nude mouse skin (B), and a cellulose membrane (C). Each value represents the mean ± S.D. ($n = 4$). (■) H_2O; (▼) PF127; (●) A + P; (▲) AP.

same phenomenon was observed in a previous study (Grassi et al., 2006). Although a little discrepancy existed between the transition temperatures of PF127 and A + P, the sol–gel transitions of both hydrogels were very close to each other. This indicates that PF127 continued to dictate the transition behavior of the thermogels. A previous study (Shin and Cho, 2006) suggested that the relationship between the viscosity of PF127 gel systems and bioadhesion may facilitate optimization of the mucoadhesive performance, leading to the development of more-effective skin-dosage forms. It was shown that PF127 exists in a solution state at refrigerator temperatures and in a gel state on warming to 25 °C. It is possible that the PF127 system forms a gel under non-physiological conditions, which is unfavorable for in vivo skin positioning. This problem can be resolved by using AP composite hydrogels because of their ability delaying the sol–gel temperature.

PF127 micelles in aqueous solution undergo thermally induced swelling and desolvation. This induces the creation of a cross-linked network (Escobar-Chávez et al., 2006), which was confirmed by SEM images. The conjugation of alginate to PF127 (AP) produced a different microstructure compared to the parent hydrogels. The chain entanglement further enhanced the formation of hydrogel networks. The A + P polymer blend also formed a cross-linked morphology in its hydrogel form. This observation can be explained by the construction of cross-links between the two polymers. The water molecules may act as a cross-linking agent to form hydrogen bonds between the carboxyl groups of alginate and ether groups of PF127, which may lead to a three-dimensional network (Lin et al., 2004).

PF127 showed some disintegration according to the determination of the degradation ratio. This was due to the dissociation of packed PF127 micelles in an excess of water (Moebus et al., 2009). This degradation was further promoted by grafting alginate (AP). In the process of hydration of polymer materials, water binds to hydrophilic groups (Van Tomme et al., 2006). Water molecules were absorbed in the pores of the AP hydrogel. Hydrolysis and decomposition of the amide linkage between the alginate backbone and PF127 resulted in degradation of the AP gels. On the other hand, the physical incorporation of alginate into PF127 (A + P) maintained the swelling behavior of PF127 alone. This is because the carboxyl group which is rich in the A + P mixture is still more water-resistant compared to the amide group (Feil et al., 1993; Hsu et al., 2009).

A previous study (Rohatagi et al., 1997) indicated the feasibility of using porcine skin as an animal model of human skin for selegiline delivery. Due to its availability and easy handling, rodent skin is also commonly used for skin permeation experiments. There are a number of hairless species (nude mice and hairless rats) in which the absence of a coat of hair mimics human skin better than hairy skin (Godin and Touitou, 2007; Zhang et al., 2010). Hence, nude mice were also used in the present work for both the in vitro and in vivo experiments. The same trend of selegiline permeation from various vehicles between porcine skin and nude mouse skin was determined in this study. It was noted that the cumulative selegiline delivered via nude mouse skin quickly increased in the late stage of the in vitro permeation experiment. This may have been partly associated with the effects of hydration, which reduce the barrier properties of rodent skin (Rohatagi et al., 1997). The hydrogels are

expected to reduce selegiline permeation across the skin. The composition of the thermogels significantly affected drug delivery, and the permeation decreased in the order of PF127 > A + P > AP according to the cumulative amount–time profiles of skin permeation studies. The dissociation state of selegiline in the vehicles may be an important factor governing skin permeation. We examined the pH of the copolymer vehicles. pH values of the hydrogels composed of PF127, A + P, and AP were 7.21, 7.13, and 3.80, respectively. Since the pK_a of selegiline is 7.5, the ionization of selegiline was almost complete in AP systems. The undissociated drug can more easily permeate through the skin compared to dissociated drug because of the lipophilicity of the stratum corneum. Moreover, partitioning of the drug between the skin and Pluronic hydrogel matrix is considered to play an important role in the permeation process (Escobar-Chávez et al., 2006). Ionized selegiline may preferentially be retained within hydrophilic AP systems, thus reducing partitioning from the gel to the skin. Another possibility is the electrostatic interaction of the positively charged selegiline in AP systems and the negatively charged alginate (carboxylic group). This interaction may retard the drug release from this hydrogel.

There was a correlation between selegiline permeation via the skin and its release across a cellulose membrane. This demonstrates that the release rate of selegiline from the vehicles is the permeation-determining step. Both the aqueous control and PF127 thermogels exhibited a burst release of selegiline. As observed in the SEM images, the PF127 system had a soft and amorphous appearance. Xiong et al. (2006) also suggested that the strength of hydrogels formed from PF127 copolymers is weak. The burst release can be attributed to the more-permeable polymeric networks, and thus the drug being loosely entrapped in the PF127 hydrogel matrix. No time lag or burst effect was observed for thermogels containing alginate (A + P and AP). A zero-order release kinetic was observed for both gels, indicating a sustained and controlled release of selegiline was obtained. Hence a prolonged therapeutic activity may be achieved. Selegiline was slowly released from the polymeric blend and graft copolymer of alginate and PF127. This suggests that alginate can act as a diffusion barrier for entrapped drug molecules. The entrapped drug always occupies the water channels of the hydrogel from which it is released by diffusing along a concentration gradient (Shin et al., 1999; Fang et al., 2008a). High cross-linking in gels typically indicates slower diffusion (Josef et al., 2010). A considerable decrease in the diffusion of selegiline is believed to have occurred when the strong cross-linkage was formed by A + P and AP as seen on SEM. The decrease in pore size would increase the tortuosity and polymeric density of the structure (Alvarez-Lorenzo et al., 2005; Zhan et al., 2006). The smaller pore size of AP composite hydrogels may have led to lower skin permeation and release of selegiline compared to the A + P system, although AP only exhibited a slightly lower drug release than did A + P.

All selegiline permeation and release results showed zero-order kinetics for the AP hydrogels. The quicker degradation of the AP system did not accelerate drug permeation or release in the late stage of the experiments, suggesting that the gel degradation/erosion might not be the predominant factor governing drug delivery. PF127, although widely accepted as a pharmaceutical excipient with a thermoreversible property, was found to ensure drug release for only a few hours (Giovagoli et al., 2010). A combination of alginate and PF127 can improve this drawback by producing sustained permeation behavior.

Transdermal drug therapy is confounded by poor and erratic drug absorption. High inter- and intra-individual absorption rates limit the transdermal delivery of some drugs (Lombardi Borgia et al., 2005). Pae et al. (2007) demonstrated that selegiline absorption largely depends on the condition of a patient's skin. Rohatagi et al. (1997) also suggested variability in selegiline plasma levels following transdermal administration. The skin is a greatly variable organ, species differences are prominent, and a multitude of possible penetration routes exist (Baert et al., 2007). The thermogels successfully minimized the variability of selegiline permeation among individuals. The low variation in drug delivery following transdermal application may have been due to the uniformity and control capability of the hydrogels, which were effective in all subjects.

5. Conclusions

The combination of alginate and PF127 provides a new possibility to develop hydrogels that simultaneously exhibit the properties of both polymers. The graft copolymer of alginate and PF127 retains the temperature sensitivity of PF127 and improves the biocompatibility. Each copolymer system tested in this study had distinct characteristics as a transdermal carrier for selegiline. The sol–gel temperature was delayed by the graft composite of alginate and PF127 (AP), which helps achieve the goal of maintaining the solution form before application to the skin surface. The in vitro skin delivery experiments indicated that the thermogels can serve as a rate-controlling barrier and are useful as a vehicle for topically administered sustained-release preparations of selegiline. The AP composite showed the highest sustained delivery, followed by the polymeric blend of alginate and PF127 (A + P) and PF127 alone. Although the hydrogels of A + P could also produce a selegiline permeation with sustainability and minimized variability among individuals, the earlier transformation temperature from solution to gel form may limit its use. The hydrogel structure and the affinity between the drug and hydrogel backbone may have contributed to the main mechanisms determining selegiline permeation. We concluded that selegiline transdermal delivery systems can be developed by employing thermogels composed of both alginate and PF127. To the best of our knowledge, this is the first report to describe the use of thermogels for the purpose of selegiline skin permeation.

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