Drug Development and Industrial Pharmacy, 34:330–335, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040701662644

informa
healthcare

Development of the Ambroxol Gels for Enhanced Transdermal Delivery

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Ambroxol is an expectoration improver and mucolytic agent that has been used to treat acute and chronic disorders. However, ambroxol needs to be administered percutaneously in order to avoid systemic adverse effects, such as headache, drowsiness, dizziness, and insomnia, which can occur after oral administration. The aim of this study was to develop a gel preparation containing a permeation enhancer to enhance the delivery of ambroxol. The ambroxol gels were prepared using hydroxypropyl methylcellulose (HPMC) and poloxamer 407. The release characteristics of the drug from the gels were examined according to the receptor medium, drug concentration, and temperature. The rate of drug permeation into the skin was enhanced by incorporating various enhancers such as the ethylene glycols, the propylene glycols, the glycerides, the nonionic surfactants, and the fatty acids into the gels. The permeation study through mouse skin was examined at 37°C. The rate of drug release increased with increasing drug concentration and temperature. Among the enhancers used, propylene glycol mono caprylate showed the best enhancing effects. The estimated activation energy of release (Ea), which was calculated from the slope of a log P versus 1000/T plot, was 14.80, 14.22, 13.91, and 12.46 kcal/mol for ambroxol loading doses of 2, 3, 4, and 5%, respectively. The results of this study show that the gel preparation of ambroxol containing a permeation enhancer could be developed for the enhanced transdermal delivery of ambroxol.

Keywords ambroxol; gels; permeation enhancer; drug delivery; activation energy of permeation

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INTRODUCTION

Ambroxol is an expectorant and an active metabolite of the mucolytic agent, bromhexine. It is used in bronchitis treatments to improve expectoration (Dariusz, Antczak, & Król, 1994; Diaz et al., 1984; Ericsson, Juhasz, Johnson, & Mossberg, 1987; Guyatt, Townsend, Kazim, & Newhouse, 1987; Olivieri et al., 1987; Ratjen et al., 1985; Severina et al., 2000; Vergin et. al., 1985). However, the oral administration of ambroxol, due to transient high blood concentration, can cause side effects, such as headache, drowsiness, dizziness, insomnia, and languor. To avoid the systemic side effects and gastric disorders that can occur after oral administration, alternative routes of administration have been considered. To reduce these side effects in oral administration, a transdermal drug delivery system of ambroxol could be considered.

The percutaneous administration of bioadhesive gels has good accessibility and can be applied, localized, and easily removed. Because of its excellent accessibility, the self-administration of a dose form is possible and drug administration can be stopped at any time. In recent years, the use of hydrophilic polymers, in particular cellulose derivatives, has attracted considerable attention for the development of controlled release technology in the formulation of pharmaceutical products due to hydrophilic polymers' ability to form gels in aqueous medium. The percutaneous delivery of ambroxol has advantages in avoiding the hepatic first pass effect and delivering the drug at a sustained level over an extended period of time. However, it is very difficult for the drug to penetrate the skin on account of the intrinsic barrier function of the skin. The use of penetration enhancers is a logical approach to increasing the drug flux across the epithelium. It has been shown that dermal penetration can be improved using compounds that have been proven to be effective enhancers on skin. The effects of various transdermal penetration enhancers such as bile salts, surfactants,

glycols and their derivatives as well as chelators on the diffusion properties of drugs in semisolid vehicles, particularly when the release of the drug at the application site is likely to be diffusion-controlled, have been reported (Aungst & Rogers, 1989; Coutel-Egro et al., 1992; Ishida et al., 1981; Shin & Kim, 2000; Shin et al., 2002). Although most topical formulations consist of rather simple components, the ability of a vehicle to release the drug at the local site is limited by several factors such as the drug-vehicle, drug-skin, and vehicle-skin interactions (Kartz & Poulsen, 1971).

The aim of this study was to develop a gel preparation containing a permeation enhancer to enhance the transdermal delivery of ambroxol. This study examined the amounts of drug permeated and evaluated the mechanism of drug release from the HPMC-poloxamer gels. Various enhancers were used in an attempt to improve the drug permeability, including non-ionic surfactants, glycols, fatty acids, and propylene glycols. The effects of temperature and the drug concentration on the release rate were evaluated.

MATERIALS AND METHODS

Materials

The ambroxol hydrochloride (ambroxol) was obtained from Handok Pharm. Co., Ltd. (Korea). The hydroxypropyl methylcellulose (HPMC) was obtained from DOW Chemical Co. Ltd. (USA). The Poloxamer 407 was purchased from BASF Co. (Germany). The non-ionic surfactants such as polyoxyethylene 2-stearyl ether, polyoxyethylene 2-oleyl ether, polyoxyethylene 23-lauryl ether, and the glycols, such as tetraethylene glycol (TEG) and diethylene glycol (DEG), were supplied by Sigma Chemical Co. (USA). The fatty acids, such as octanoic acid and oleic acid, were obtained from Tokyo Kasei Kogyo Co., Ltd. (Japan). The propylene glycols, such as propylene glycol mono caprylate, propylene glycol mono laurate, and propylene glycol laurate, and the glycerides, such as oleoyl macrogol-6 glycerides and caprylocaproyl macrogol-8 glycerides, were kind gifts from Gattefosse (France). The acetonitrile and anhydrous ethyl alcohol used in this study was of HPLC grade and was obtained from J. T. Baker Inc. (USA). All other chemicals were of reagent grade and were used without further purification. A HPLC system (Waters, USA) with a RESTEK C_{18} column (250 × 4.6mm, 5 μm) was used.

Preparation of HPMC-Poloxamer Gels Containing Ambroxol

Two grams of HPMC was dissolved in hot water at water bath to make a 20 g solution. Twenty grams of poloxamer 407 was dissolved in 5°C water to make a 40 g solution. The solution was left overnight in a refrigerator to complete dissolution. 0.1% ambroxol and water were added to the above combined two polymer solutions with vigorous stirring to make a 100 g solution.

Permeation Study of Ambroxol from the HPMC-Poloxamer Gels

The ambroxol flux from the HPMC-poloxamer gels was determined using various polyethylene glycol 400 (PEG, a receptor) concentrations ranging from 0% (v/v) to 60%. A synthetic cellulose membrane was mounted on the receptor compartment of the diffusion cell. Two grams of the prepared HPMC-poloxamer gels containing the ambroxol was placed in intimate contact with the cellulose membrane and the donor cap was covered with a parafilm and clamped. The sampling port was sealed with parafilm to prevent evaporation from the receptor medium. A 40% PEG 400 solution was used as the receptor solution. The receptor solution was maintained at 37°C with a circulating water bath and stirred with a magnetic stirring bar. The donor compartment was maintained at ambient temperature of 37 ± 1°C. The effect of the drug concentration on its release from the gels was examined as a function of the drug concentration (2, 3, 4, and 5% (w/w) temperature (27, 32, 37, and 42°C in a thermostated water bath). The total samples (20 ml) from the receptor compartment were withdrawn at predetermined times to maintain a sink condition, and immediately replaced by the same volume of fresh 40% PEG solution.

HPLC Determination of Ambroxol

The HPLC system consisted of a pump (Waters 501, USA), an ultraviolet detector, a RESTEK C_{18} column (250 × 4.6 mm, 5 μm), and an integrator (D520A, Youngin Scientific Co., Ltd., Korea). The mobile phase was composed of a mixture (70:30, v/v) of acetonitrile and 0.01 M of a diammonium phosphate buffer, which was adjusted with $H_3PO_4$to pH = 6. A flow rate of 1.5 ml/min yielded an operating pressure of ~1000 psi. The UV detector was operated at the wavelength of 252 *nm*. Under these conditions, the retention time of ambroxol peak was 4.7 min.

Data Treatment for Drug-Release Studies

Higuchi proposed two mathematical equations to describe the kinetics of drug release based on the state of the drug in the vehicle: Release from solutions and release from suspensions. In this study, the drug release rates were evaluated according to the simplified Higuchi diffusion Equation (1), which depicted the drug release from one side of a semisolid layer in which the drug was dissolved.

$$q = 2C_0 (D_t/\pi)^{1/2} \quad (1)$$

where q is the amount of drug released into the receptor medium per unit area of exposure, C_0 is the initial drug concentration in the vehicle, D is the apparent diffusion coefficient of the drug, and t is the time elapsed since the beginning of drug release.

In the case of passive diffusion, Fick's law can be used to model the steady-state flux through unit area of a membrane,

$$J = P \bullet (C_d - C_r) \quad (2)$$

where J is the flux per unit area, P is the permeability coefficient, and C_d and C_r are the concentrations in the donor and receptor solutions, respectively. In the case where the sink conditions are maintained on the receptor side, ($C_d - C_r$) is replaced by C_d.

$$J = P \bullet C_d \quad (3)$$

The permeability coefficient, P, is constant for a given drug under the same experimental conditions. There should be a linear relationship between the flux and the donor concentration.

Skin Preparation

A male rat (Sprague Dawley rat strain) was sacrificed by snapping the spinal cord at the neck. The hair of the abdominal area was carefully removed with electric clippers. A square section of the abdominal skin was excised. After excision, the adhering fats and other visceral debris in the skin were carefully removed from the undersurface with tweezers (Durrhein, Flynn, Higuchi, & Behl, 1980). The excised skin was used immediately.

Effects of Enhancers on the Permeation of Ambroxol from the HPMC-Poloxamer Gels Through Rat Skin

The excised abdominal skin was mounted in a diffusion cell. The other conditions were the same as reported above. (A 0.1% ambroxol gel was mixed with 5% (w/v) of the enhancer.) The following enhancers were used: glycols, such as diethylene glycol and tetraethylene glycol; non-ionic surfactants, such as polyoxyethylene-2-oleyl ether, polyoxyethylene-2-stearyl ether, and polyoxyethylene-23-lauryl ether; fatty acids, such as octanoic acid and oleic acid; propylene glycols, such as propylene glycol mono caprylate, propylene glycol mono laurate, and propylene glycol laurate; and glycerides, such as oleoyl macrogol-6 glycerides and caprylocaproyl macrogol-8 glycerides. The enhancer might affect the fluidity of the stratum corneum structure and the drugs can permeate better through the rat skin. The amount of drug permeated was determined by HPLC.

Analysis of Permeation Data

The cumulative amount of the permeated drug from the mucosal to serosal side was plotted as a function of time, and the flux was calculated from the steady-state part of the curve. The efficiency of the penetration enhancer was determined by comparing the flux of the ambroxol in the presence and absence of the enhancer, and was defined as the enhancement factor (EF). The EF was calculated using the following equation:

$$EF = (\text{flux of the samples containing an enhancer}) / (\text{flux of the control sample})$$

RESULTS AND DISCUSSION

Effect of Ambroxol Concentration on the Drug Release

The effect of the ambroxol concentration on drug release through the synthetic cellulose membrane was examined using the prepared HPMC-poloxamer gels at 37 ± 0.5°C. Figure 1 shows the ambroxol flux from the gel formulations through the synthetic cellulose membranes (SPECTRA/POR, MW 12–14,000) for 24 hr. The level of drug permeation increased with increasing drug concentration in the gels up to 5%, with a slight increase being observed at higher concentrations.

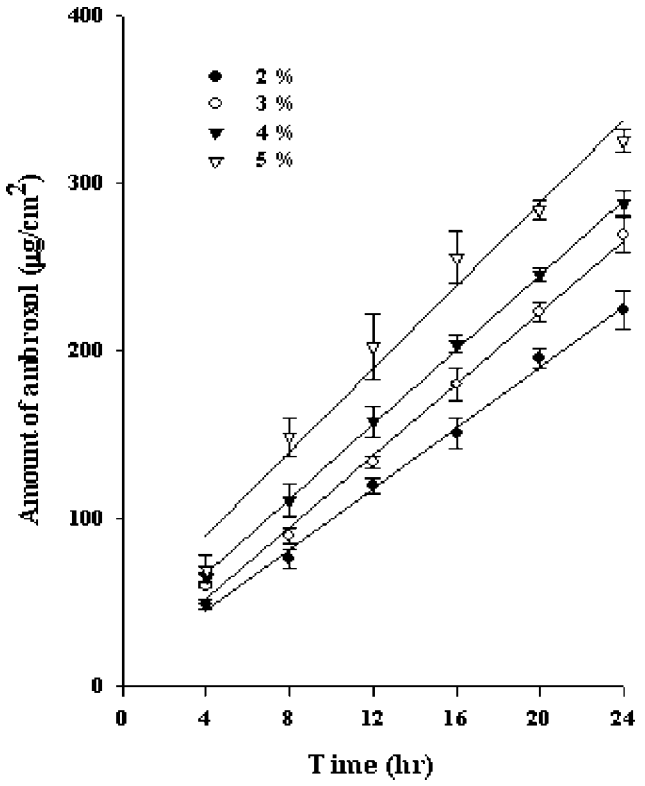

FIGURE 1. Effects of the ambroxol concentration on rate of drug from the HPMC-poloxamer gels through a cellulose membrane.

Effect of Temperature on Drug Release

The effect of temperature on the release of ambroxol from the gel formulations was evaluated at 27, 32, 37, and 42°C. All the experiments were carried out at least in triplicate. Figure 2 shows the temperature dependency of drug release as a function of time. The apparent diffusion coefficient increases with increasing temperature. However, for the practical use, 37°C, was chosen to reflect the temperature of the stratum corneum (Chien & Lau, 1976). The relationship between the diffusion coefficient and the temperature is as follows:

$$D = D_0\, e^{-Ea/RT} \tag{4}$$

The slope of the line of best fit was used to estimate the activation energy (Ea) for drug diffusion. The intercept was used to estimate the pre-exponential term.

The permeability coefficient was then defined as follows:

$$P = \text{flux} / \text{solubility} \tag{5}$$

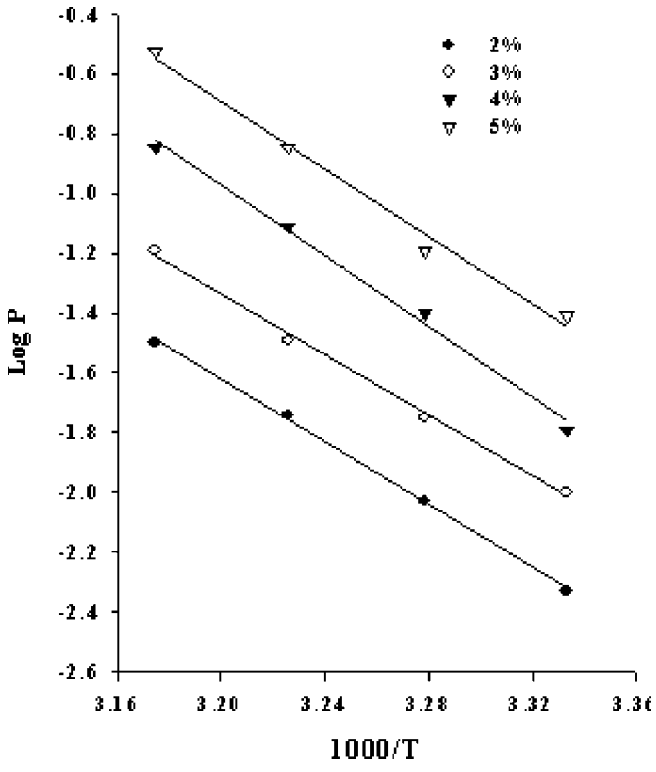

FIGURE 2. Effect of temperature on the rate of drug release from the HPMC-poloxamer gels containing various drug doses through the cellulose membrane.

$$P = Po\, e^{-Ea/RT} \tag{6}$$

$$\log P = \log Po - Ea / R \bullet 2.303 \bullet 1000 \times 1000/T \tag{7}$$

As expected from Equation 7, a plot of log P versus 1000/T yielded a straight line (Figure 2). Ea was determined from the slope of log P versus 1000/T plots (Equation 9) as follows:

$$\text{Slope} = -Ea / R \bullet 2.303 \bullet 1000 \tag{8}$$

$$\begin{aligned} Ea &= -\text{slope} \times R \times 2.303 \times 1000 \text{ cal} \\ &= -\text{slope} \times 1.987 \times 2.303 \text{ kcal} \end{aligned} \tag{9}$$

The rate of drug release increased with increasing temperature. The activation energy (Ea) of release at loading dose of 2, 3, 4, and 5% was 14.80, 14.22, 13.91, and 12.46 kcal/mol, respectively. This observation clearly shows that the release of drug from the gels is an energy-linked process (Miyazaki, Takeuchi, Yokouchi, & Takada, 1984).

In addition, the results show that an increase in drug concentration reduces the activation energy for diffusion from the gel through the other side of the membrane. However, it should be noted that the overall process involves the release of the drug from the gel followed by its diffusion through the membrane. It is believed that the increase in drug concentration may lower the barrier for drug release from the gel but it is not expected to affect the mechanism for drug diffusion through the membrane.

Permeation of Ambroxol from the HPMC-Poloxamer Gels Containing Various Enhancers Through the Rat Skin

The effect of the enhancer on the permeation of ambroxol across the rat skin was examined. Enhancers such as ethylene glycols, propylene glycols, glycerides, the non-ionic surfactants, and the fatty acids, were used at a concentration of 5%. Figure 3 shows the permeation profile of ambroxol from the HPMC-poloxamer gel including enhancers, such as propylene mono caprylate, propylene mono laurate, and propylene laurate. Among them, propylene mono caprylate was found to be the best.

Table 1 shows the permeation data of ambroxol with or without the enhancers. The permeation of ambroxol from the gels containing an enhancer showed better enhancing effect than that without the enhancer. Among the enhancers used, the propylene glycol mono caprylate showed the best enhancement considering the enhancement factor.

The mechanism of the penetration enhancers is unclear. In a previous study (Chien & Lau, 1976; Shin & Kim, 2000) on the effects of a penetration enhancer via thermal analysis and

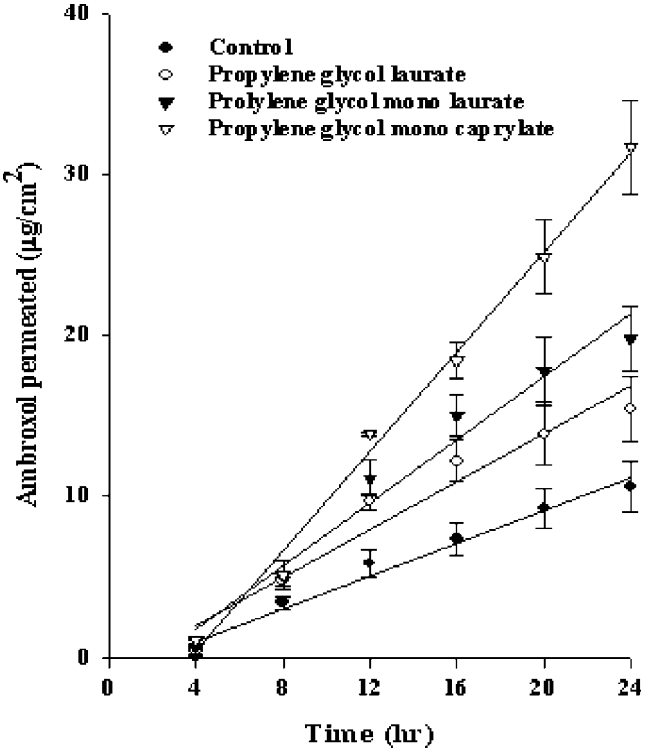

FIGURE 3. Effects of glycerides on drug permeation from the ambroxol gels through rat skin.

histological examinations, it was suggested that the incorporation of a penetration enhancer decreases the lipid order and has a fluidizing effect on the lipids of the stratum corneum. The role of the penetration enhancer might be explained as an interfacial saturation phenomenon. For the stratum corneum lipids to be dissolved, it is most likely that enhancers, such as a surfactant, accumulate at the lipid/liquid interface after penetrating into the tissue. The enhancer might affect the fluidity of the stratum corneum structure, which can allow drugs to permeate better through the rat skin.

TABLE 1
Enhancement Factor of Various Enhancers from Ambroxol Gels

Enhancer	Flux ($\mu g/cm^2/hr$)	EF
Control	0.51 ± 0.04	1
Polyoxyethylene-23-lauryl ether	0.95 ± 0.07	1.86
Polyoxyethylene-2-stearyl ether	1.04 ± 0.08	2.04
Polyoxyethylene-2-oleyl ether	1.17 ± 0.09	2.29
Octanoic acid	1.11 ± 1.10	2.18
Oleic acid	1.37 ± 1.10	2.69
Diethylene glycol	0.86 ± 0.06	1.69
Tetraethylene glycol	1.30 ± 0.09	2.55
Caprylocaproyl macrogol-8 glycerides	0.64 ± 0.03	1.25
Oleoyl macrogol-6 glycerides	1.09 ± 0.09	2.14
Propylene glycol mono caprylate	1.55 ± 0.10	3.04
Propylene glycol mono laurate	0.98 ± 0.09	1.92
Propylene glycol laurate	0.75 ± 0.03	1.47

CONCLUSION

The rate of drug release increased with increasing drug concentration. In addition, the rate of drug release increased with increasing temperature, showing an Arrhenius-type relationship. The activation energy of drug release for the 2, 3, 4, and 5% concentration was 14.80, 14.22, 13.91, and 12.46 kcal/mol respectively. Among the enhancers used, propylene glycol mono caprylate had the most enhancing effect. These results suggest that the gel preparation containing a permeation enhancer could be developed for the enhanced transdermal delivery of ambroxol.

ACKNOWLEDGMENTS

This study was financially supported by research fund of Chonnam National University in 2006.

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