行政院國家科學委員會補助專題研究計畫成果報告

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※ 輸送眼藥用之原位成膠高分子混合溶液流變行為 ※

※ 及活體外、活體內藥物釋放之研究 ※

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計畫主持人：林 鴻 儒

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執行單位：嘉南藥理科技大學醫藥化學系

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行政院國家科學委員會專題研究計畫成果報告

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摘要

本研究主要目的為製備一系列以 Carbopol 及 Pluronic 為主的溶液做為輸送眼藥用之原位成膠載體。評估各種高分子溶液包含 Carbopol、Pluronic 及 Carbopol/Pluronic 混合溶液的流變性質、活體外藥物釋放及活體內之藥理反應。結果顯示 Carbopol 溶液做為輸送眼藥之原位成膠系統的最適濃度為 0.3%(w/w)，而 Pluronic 溶液則為 14%(w/w)。結果也顯示 0.3% Carbopol 與 14% Pluronic 混合溶液的凝膠強度在生理條件下有顯著的增強，並發現此凝膠混合溶液在 pH 4.0 及 25℃時可自由流動，而且 Carbopol/Pluronic 混合溶液之流變行為不受添加 Pilocarpine hydrochloride 之影響。由活體外藥物釋放及活體內藥理研究顯示 Carbopol/Pluronic 混合溶液對於延長藥物在眼角膜滯留時間之能力比單獨的 Carbopol 或 Pluronic 溶液強。研究結果證明 Carbopol/Pluronic 混合溶液可做為原位成膠載體，以增強眼睛的生體可用率。

關鍵詞：眼藥輸送系統、原位成膠、相轉變、藥物釋放、眼睛的生體可用率

Abstract

The major purpose of this study is to develop and characterize a series of carbopol and pluronic based solutions as the *in situ* gelling vehicles for ophthalmic drug delivery. The rheological properties, *in vitro* release as well as *in vivo* pharmacological response of various polymer solutions, including carbopol, pluronic and carbopol/pluronic solution, were evaluated. It was found that the optimum concentration of carbopol solution for the *in situ* gel forming delivery systems was 0.3% (w/w), and that for pluronic solution was 14% (w/w). The mixture of 0.3% carbopol and 14% pluronic solutions showed a significant enhancement in gel strength in the physiological condition ; this gel mixture was also found to be free flowing at pH 4.0 and 25℃. The rheological behaviors of carbopol/pluronic solution were not affected by the incorporation of pilocarpine hydrochloride. Both the *in vitro* release and *in vivo* pharmacological studies indicated that the carbopol/pluronic solution had the better ability to retain drug than the carbopol or pluronic solutions alone. The results demonstrated that the carbopol/pluronic mixture can be used as an *in situ* gelling vehicle to enhance the ocular bioavailability.

Keywords: Ophthalmic delivery system; *In situ* gelling; Phase transition; Drug release; Ocular bioavailability.

Introduction

In order to reduce the total polymer content and improve the gelling properties, Joshi et al. [1] first used the combination of polymers in the delivery system. The main idea is that aqueous compositions reversibly gelled in response to simultaneous variations in at least two physical parameters, such as pH, temperature, and ionic strength can be formed by using a combination of polymers which exhibit reversible gelation properties. Although the above results demonstrate the advantages of employing various *in situ* gelling polymers in the ophthalmic drug delivery system, the use of combined *in situ* gelling polymers with various phase transition mechanisms in delivery vehicle is not extensively explored. In the present study, aqueous solutions of different compositions containing either carbopol or pluronic were prepared to identify compositions suitable to be used as *in situ* gel forming systems. An alternative *in situ* gelling system prepared by the combination of carbopol and pluronic was also developed. The rheological behaviors of various aqueous polymer solutions under controlled shear conditions of varying magnitude were evaluated. In addition, the *in vitro* pilocarpine release and *in vivo* pilocarpine pharmacological response of various drug-containing polymer solutions were characterized to evaluate the use of *in situ* gelling polymer solutions for ophthalmic drug delivery.

Methods and Materials

The carbopol solutions (0.1 to 0.6% (w/w), 1.0 and 2.0% (w/w)) were prepared by dispersing the required amount in distilled, deionized water with continuous stirring until completely dissolved. For preparation of pluronic solutions (10 to 25% (w/w)), the required amount of polymer was dispersed in distilled, deionized water with continuous stirring for 1 h. The

partially dissolved pluronic solutions were stored in the refrigerator until the entire polymer was completely dissolved (approximately 24 h). The carbopol/pluronic solutions were prepared by dispersing the required amount of pluronic in the desired concentration of carbopol with continuous stirring for 1 h. The partially dissolved solutions were then refrigerated until solutions were thoroughly mixed (approximately 24 h). The reported composition of carbopol/pluronic mixture was the final concentration of carbopol and pluronic content in the mixture. All the sample solutions were adjusted to pH 4.0 ± 0.1 or 7.4 ± 0.1 by 0.5 M sodium hydroxide solution and then stored in the refrigerator prior to the evaluation of their rheological properties.

The rheological studies were carried out on a cone (4°) and plate geometry viscometer (Brookfield RVCP DV-III). The viscosity and shear stress of the sample solutions were measured at various shear rates at 25℃ and 37℃, respectively.

The *in vitro* drug release from various polymer solutions was first carried out by filling 3 g of pilocarpine-containing polymer solution into small, circular plastic containers (2.5 cm i. d. and 1.5 cm in depth) in triplicate and placing each container in a 1000- mL beaker. The beaker was then filled with 1000 mL simulated tear fluid. The temperature and stirring rate were maintained at 37℃ and 75 rpm, respectively. Aliquots (1mL) were withdrawn from the release mediums at each sampling time. The samples were filtered through 0.45-mm syringe filters and subjected to HPLC analysis to determine the pilocarpine concentrations.

New Zealand albino rabbits were used as the model animals in the *in vivo* experiments. Fifty microliters of polymer solutions or simulated tear fluid (STF), each with 1% of pilocarpine hydrochloride, were dosed from a micropipet. After administration of both the control vehicle and the drug-containing polymer solutions, pupil diameters of both eyes were measured according to the following time schedule: 1, 15, 30, 45, 60, 90, 120, 150, 180, 240; 300 and 360 minutes.

Results and Discussion

Figure 1 shows the shear stress versus shear rate flow curves of carbopol solution (0.3% (w/w)), pluronic solution (14% (w/w)) as well as the mixture of carbopol (0.3% (w/w)) /pluronic (14% (w/w)) solution at non-physiological and physiological conditions. For carbopol solution at physiological condition, the medium resisted the initial rotatory motion and a sudden increase in the shear stress was observed at higher shear rate (Figure 1). The solution began to flow after the shear stress reached its yield point. Accordingly, the flow curve for carbopol solution at physiological condition demonstrated a pseudoplastic behavior. Figure 1 also shows that, for carbopol solution at non-physiological condition as well as for pluronic solution at either non-physiological or physiological conditions, the shear stress increased linearly with an increase in shear rate, demonstrating a Newtonian flow behavior. Despite the flow curve of carbopol/pluronic solution at pH 4.0 and 25℃ shows a Newtonian flow behavior (Figure 1), nevertheless, a pseudoplastic flow behavior with a hysteresis was observed for carbopol/pluronic solution at pH 7.4 and 37℃. For all the polymer systems studied, the shear stresses at pH 7.4 and 37℃ were higher than those at pH 4.0 and 25℃. For instance, at shear rate of 100 s^{-1}, the shear stresses of carbopol and carbopol/pluronic solutions at physiological condition were approximately five and 18 times greater than those at non-physiological condition, respectively, suggesting the occurrence of phase transition between these two conditions for both systems. Only slightly higher shear stresses were observed for the pluronic solutions at physiological condition comparing to those at non-physiological condition for the shear rates ranging from 0 to 200 s^{-1}. Although the shear stress of carbopol solution increased significantly at physiological condition, a stronger gel can be formed by combining pluronic with carbopol solutions. Figure 1 shows that, at pH 4.0 and 25℃, the shear stress of carbopol/pluronic solution was higher than that of pluronic solution and slightly lower than that of carbopol solution at each shear rate. However, at pH 7.4 and 37℃, the shear stress of carbopol/pluronic solution was significantly greater than that of individual carbopol and pluronic solutions at each shear rate.

In order to investigate the effects of pilocarpine hydrochloride on the rheological behaviors of the polymer solutions, the rheological studies on the pilocarpine-containing polymer solutions at pH 7.4 and 37℃ were performed and the results are shown in Figure 2. These results show that the shear stress of the carbopol as well as pluronic solutions were affected by the incorporation of drug into solutions, therefore, the viscosities of the solutions and their abilities to withstand the shear forces in the cul-de-sac were influenced. The untoward effect may be minimized by combining those two individual polymer solutions. Figure 2 demonstrates that the pilocarpine-containing 0.3% carbopol/14% pluronic solution had similar flow behaviors as the 0.3% carbopol/14% pluronic solution, suggesting that the incorporation of pilocarpine did not disrupt the strong three-dimensional gel network formed at physiological condition.

Figure 3 shows the cumulative amount of pilocarpine released versus time profiles for various drug–containing polymer solutions and the drug-containing STF (simulated tear fluid). All the polymer solutions and the STF contained 0.1% (w/w) pilocarpine hydrochloride. For the drug-containing STF, almost all the pilocarpine released immediately after the start of release experiment. In the case of pilocarpine-containing 0.3% carbopol solution, the drug released about 19 % to the medium after one minute and then the drug gradually released afterwards. Approximately 78% of pilocarpine released from the

carbopol solution after 6 hours. The pilocarpine-containing pluronic solution had similar release trend as the carbopol solution, which may be attributed to the comparative viscosity of both solutions (as shown in Fig. 2) at pH 7.4 and 37℃. For the pilocarpine-containing 0.3% carbopol/14% pluronic solution, significant lower drug release rates were observed. There was only 1.3% pilocarpine released in the first minute , approximately 76% released after 6 hours, and the release profile was still climbing hereafter. The results indicated that the 0.3% carbopol/14% pluronic mixture had better ability to retain drugs than the individual polymer solution and pure STF. The results also suggest that the carbopol/pluronic aqueous system can be utilized as an *in situ* gel-forming system for ophthalmic drug delivery system.

Figure 4 shows the pharmacological response (the decrease in pupil diameter , Δpupil diameter) versus time profiles for the various pilocarpine-containing polymer solutions and the pilocarpine-containing STF. Since some variations of the *in vivo* pharmacological responses were observed, the following results and discussion focus on reporting the general trends of experimental data. For the carbopol/pluronic formulation, the pharmacological responses were significantly higher than the STF between 5 minutes and 240 minutes of experimental times. A similar phenomenon was also observed for the pluronic solution, however, the Δpupil diameters were slightly lower than those of the carbopol/pluronic formulation between 90 minutes and 240minutes of experimental times. As compared to the pilocarpine-containing STF, the carbopol formulation had higher Δpupil diameters between 5 minutes and 90 minutes of experimental times and a similar profile can be observed afterwards. These results indicate that the overall miotic responses were greater for the drug-containing polymer formulations than the drug-containing STF.

Conclusions

From the sample preparation, it was found that the optimum concentration of carbopol solution for *in situ* gel forming delivery systems was 0.3 % (w/w), and that for pluronic solution was 14% (w/w). When these two individual solutions (0.3% carbopol/14 % pluronic) were combined, the gel strength in the physiological condition was significantly enhanced. It was also found that the 0.3% carbopol/14% pluronic solution can flow freely at non-physiological condition and its rheological properties were not affected by the incorporation of drug. Both the *in vitro* and *in vivo* results indicated that the combined polymer systems performed better in retaining drugs than the individual solution. Accordingly, the results demonstrated that, without increasing the concentration of individual polymer solutions, the carbopol/pluronic solution mixture may be reproducibly administered into the eye as drops and form strong gel following the phase transition to withstand the shear force in the cul-de-sac. Therefore, the combined carbopol/pluronic system can be used as the *in situ* gelling vehicle for ophthalmic drug delivery.

References

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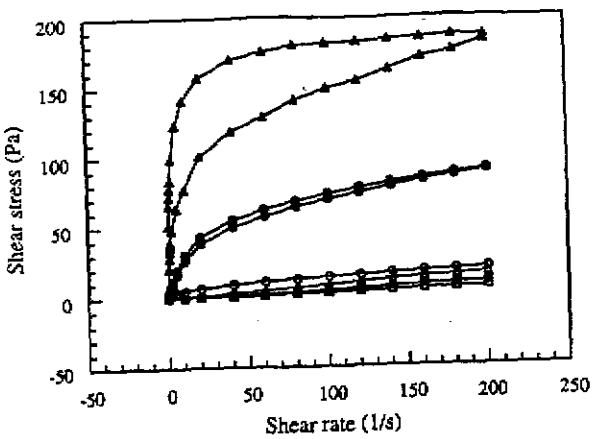

Fig. 1. Shear stress vs. shear rate flow curves of different aqueous polymer solutions. ○: 0.3% carbopol solution measured at pH 4.0 and 25°C; ●: 0.3% carbopol solution measured at pH 7.4 and 37°C; □: 14% pluronic solution measured at pH 4.0 and 25°C; ■: 14% pluronic solution measured at pH 7.4 and 37°C; △: 0.3% carbopol/14% pluronic solution measured at pH 4.0 and 25°C; ▲: 0.3% carbopol/14% pluronic solution measured at pH 7.4 and 37°C. All the measurements were performed in triplicate and the standard deviations were all within 3%.

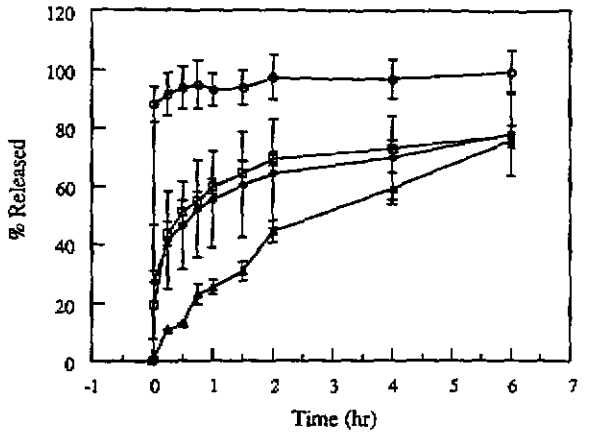

Fig. 3. Cumulative amount of pilocarpine released as a function of time from various pilocarpine-containing solutions. □: pilocarpine-containing 0.3% carbopol solution, ♦: pilocarpine-containing 14% pluronic solution, ▲: pilocarpine-containing 0.3% carbopol/14% pluronic solution, and ○: pilocarpine-containing STF.

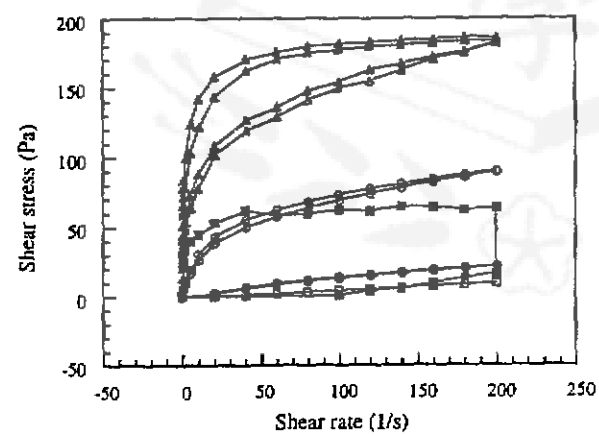

Fig. 2. Effect of drug on the shear stress vs. shear rate flow curves of different aqueous polymer solutions at pH 7.4 and 37°C. ○: 0.3% carbopol solution; ●: pilocarpine-containing 0.3% carbopol solution; □: 14% pluronic solution; ■: pilocarpine-containing 14% pluronic solution; △: 0.3% carbopol/14% pluronic solution; ▲: pilocarpine-containing 0.3% carbopol/14% pluronic solution. All the measurements were performed in triplicate and the standard deviations were all within 3%.

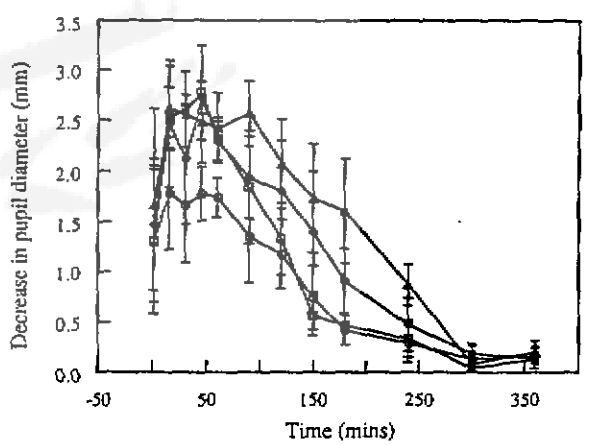

Fig. 4. The decrease in pupil diameter vs. time profiles for various pilocarpine-containing solutions. □: pilocarpine-containing 0.3% carbopol solution, ♦: pilocarpine-containing 14% pluronic solution, ▲: pilocarpine-containing 0.3% carbopol/14% pluronic solution, and ○: pilocarpine-containing STF.