Chiang Mai J. Sci. 2014; 41(1) : 166-173
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Methocel E4M: Preparation and Properties as a Vehicle for the Ocular Drug Delivery of Vancomycin

Anutra Khangtragool
Division of Pharmacy, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.
*Author for correspondence; e-mail: akhangtr@mail.med.cmu.ac.th

Received: 22 November 2012
Accepted: 15 April 2013

ABSTRACT

In this study, hydroxypropyl methylcellulose (HPMC, Methocel™ E4M) was chosen in 0.3% and 0.4% w/v concentrations at pH 7.1 as an ocular drug delivery vehicle for vancomycin and its *in vitro* characteristics and stability determined. The viscosity, osmolality, clarity, pH and contamination of each of the formulations were determined. From stability assessments, Methocel™ E4M 0.3% and 0.4% w/v pH 7.1 could be stored at 2-8°C and 30°C for 360 days. Osmolalities and pH were acceptable for use in the eyes. No contamination was found in both 0.3% and 0.4% w/v Methocel™ E4M pH 7.1. All solutions remained clear throughout the study. The kinematic viscosity of Methocel™ E4M 0.3% w/v relative to artificial tears indicated that it was suitable for the ocular drug delivery of vancomycin 50 mg/ml.

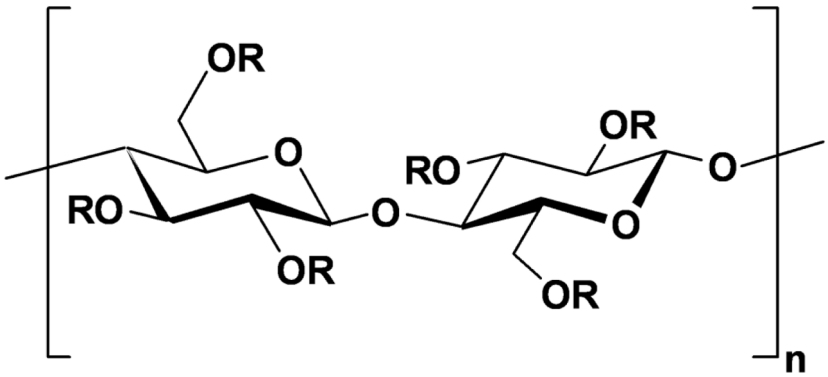
Keywords: methocel, hydroxypropyl methylcellulose, vancomycin, ocular drug delivery

1. INTRODUCTION

Topical ophthalmic eye drops have a bioavailability of less than 5% [1,2]. In extemporaneous eye drops, vancomycin (50 mg/ml) is made from a commercially available injection form of vancomycin and diluted in vehicles. Vancomycin is a glycopeptide antibiotic [3]. It has been used for its antibacterial activity against gram positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and resistant enterococci [4]. For ocular infection, Fleischer et al. [5] used topical vancomycin 50 mg/ml in sterile water to treat patients with severe *Staphylococcus epidermidis* blepharoconjuctivitis. In the literature, many ways have been described to prepare vancomycin 50 mg/ml eye drops such as by dilution in sterile water, artificial tears and chitosan solution [5-8]. In the Maharaj Nakorn Chiang Mai Hospital, Thailand, vancomycin 50 mg/ml topical extemporaneous eye drops are prepared following the guidelines of Reynolds and Closson [8] and Barbault et al [9]. Khangtragool et al. [10] found that vancomycin 50 mg/ml diluted in artificial tears, 0.9% sodium chloride solution and chitosan solution was rather acidic (pH 3.19-4.03). The selected pH should be the optimum value for the stability of the active ingredient [11]. Vancomycin is stable over the pH range

of 3-5 [12]. Mathew et al. reported that the maximum stability region of vancomycin is pH 3.0-5.7 [13]. Ophthalmic solutions are generally formulated in the range of pH 4-8 [14], while the pH value of normal tears is about 7.4 [15]. Eye irritation may occur outside the physiological pH range.

Aqueous solutions topically used with the eye undergo rapid drainage within the first 15-30 secs after application due to reflex tearing and drainage via the nasolacrimal duct [16]. Chitosan is a mucoadhesive cationic polymer [15] which is currently undergoing clinical trials in ophthalmology in Thailand. In this present work, another cellulose derivative, hydroxypropyl methylcellulose (HPMC, Methocel™ E4M), was chosen as a vehicle for the delivery of vancomycin. The chemical structure of Methocel™ E4M is shown in Figure 1.



$R = -H, -CH_3, -CH_2-CHOH-CH_3$

CH_3 = 28-30%
CH_2-CHOH-CH_3 = 7-12%
Figure 1. Chemical structure of Methocel™ E4M.

HPMC is a water-soluble polymer and mucilages of HPMC have greater clarity and usually contain fewer undispersed fibres. Due to its clarity and viscosity, it is often used as a vehicle for eye drops since it prolongs contact of the medication with the eye. Other uses may include as a "wetting solution" for contact lenses, as a lubricant for inserting artificial eyes, as well as other diagnostic uses. It is widely used clinically in ophthalmic solutions and is available over the counter as eye drops. HPMC is also used either alone or with other viscosity-increasing agents in artificial tear preparations for the management of dry eye. Solutions containing 0.3% to 1% w/v of HPMC are commonly used [17]. Ludwig et al. reported that HPMC non-ionic charged polymers demonstrated lower mucoadhesive capacity than both anionic and cationic polymers [15]. HPMC was selected as the most appropriate polymer for this current study due to its common use in ophthalmic formulations, reliability and non-ionic structure with no reports of its incompatibility.

Polymers are used in ocular drug delivery in order to increase the residence time on the ocular surface, thereby increasing bioavailability, and HPMC has been studied in ophthalmic solutions for this purpose [17-20]. Several grades of HPMC are available. Recently, Khangtragool *et al*. [21]

developed 0.3% and 0.4% w/v HPMC (Methocel™ E4M with 28-30% methoxy groups and 7-12% hydroxypropyl groups) in pH 7.1 to deliver vancomycin 50 mg/ml in extemporaneous eye drops and found that both formulations were stable for 14 days. Thus, 0.3% and 0.4% w/v Methocel™ E4M in pH 7.1 were chosen in this study in order to compare their stabilities under various conditions, osmolality, pH, clarity and contamination.

2. MATERIALS AND METHODS

2.1 Materials

Hydroxypropyl methylcellulose (Methocel™ E4M, Dow Chemical Co., USA) was used as supplied. Boric acid and sodium borate decahydrate were purchased from Prolabo and Ajax Finechem. Methylparaben and propylparaben preservatives of British Pharmacopeia (BP) grade were purchased from Sharon-Laboratories Ltd., Israel.

2.2 METHODS

2.2.1 Viscosity, Clarity and Stability

Preparation of vehicles

Two different concentrations of Methocel™ E4M (0.3%, 0.4% w/v) were used. To prepare the vehicles, 0.02% methylparaben and 0.01% propylparaben were dissolved in 1/3 of the required amount of Feldman's buffer for ophthalmic preparation of pH 7.1 and heated to 90°C in order to dissolve the preservatives. When the preservatives had completely dissolved, the Methocel™ E4M (0.3%, 0.4% w/v) was added to the solution and stirred until wetted and evenly dispersed. The remaining cold Feldman's buffer pH 7.1 was added and stirring continued until the Methocel™ E4M had completely dissolved. The Methocel™ E4M 0.3% and 0.4% w/v solutions were then placed into injection vials and sterilized by autoclaving at 121°C and 15 psi for 15 mins. The Feldman's buffer used for the ophthalmic preparations was composed of both acid and alkaline stock solutions. The acid stock solution in purified water 1,000 ml contained boric acid 12.368 gm and sodium chloride 2.925 gm, while the alkaline stock solution, also in purified water 1,000 ml, contained sodium borate decahydrate 19.07 gm. Feldman's buffer of pH 7.1 was prepared by mixing the boric acid solution 94 ml with the sodium borate solution 5 ml.

Kinematic viscosity measurements

The kinematic viscosities of each vehicle were determined by viscometry. For flow-time measurements, 10 ml of each solution were accurately pipetted into a calibrated Ostwald-type size C viscometer and clamped vertically in a water bath at a constant temperature of 25.0 ± 0.1°C. At least 15 minutes were allowed for temperature equilibration before flow-time measurements were made. The kinematic viscosity, ν, was calculated from the equation for Newtonian liquids: $\nu = kt$ where ν is the kinematic viscosity (mm^2/s), k is the viscometer constant (in this case, $k = 0.027505366\ mm^2/s^2$) and t is the flow-time (s). Flow-times were determined as the average of at least 3 readings, all of which agreed to within ± 0.2% of their average value.

Stability studies and clarity

The samples were divided into 2 groups: Group I was stored at 2-8°C in a refrigerator and Group II at 30°C in an incubator. Stabilities were determined by measuring the kinematic viscosity, ν, over a period of 12 months. Clarity was determined against a black and white background for particulate matter.

2.2.2 Measurements of osmolality and pH

To determine the osmolality of the 0.3% and 0.4% w/v Methocel™ E4M at pH 7.1, each sample was analyzed in duplicate. For each analysis, 50 µl of the sample were transferred to a vessel (50 µl volume) and placed in the osmometer (Osmomat 030). The pH values of the Methocel™ E4M (0.3%, 0.4% w/v) stored at 2-8°C and 30°C were determined by a pH meter at days 0, 3, 7, 10, 14, 21 and 30. Osmolality was determined on the day of preparation.

2.2.3 Contamination

Determinations of bacteria and fungi were carried out using Tryptic Soy Broth (TSB) and Sabouraud Dextrose Broth (SDB) prepared by suspending 30 gm in 1 litre of water and autoclaving for 15 minutes at 121°C. Determinations of bacteria and fungi contamination were carried out by culturing the samples in TSB for bacteria and SDB for fungi. Signs of the growth of bacteria and fungi were observed after incubation for 2 days at 35°C and 7 days at 25°C respectively.

3. RESULTS AND DISCUSSION

3.1 Viscosity, Clarity and Stability

Vehicles with different viscosities were prepared with the purpose of increasing contact of the vancomycin with the eye in extemporaneous eye drops. Two concentrations were used (0.3% and 0.4% w/v Methocel™ E4M), based on Methocel™ E4M 0.3% w/v in Tears Naturale II™ which also contains dextran 70 0.1% and HPMC 0.3% as active ingredients. This present formulation also contained 0.02% methylparaben and 0.01% propylparaben as preservatives. Vehicles for the delivery of extemporaneous eye drops needed to be sterilized by autoclaving for 15 mins at 121°C. However, this autoclave sterilization also caused viscosity reductions in the Methocel™ E4M (0.3%, 0.4% w/v) solutions. This type of viscosity loss had previously been observed with sodium carboxymethylcellulose [22]. To determine the susceptibility of Methocel™ E4M to hydrolytic degradation during autoclave sterilization, the two concentrations of Methocel™ E4M (0.3%, 0.4% w/v) before and after autoclave sterilization were assessed. Table 1 shows the observed decreases in kinematic viscosity with that of the Methocel™ E4M 0.4% w/v solution having decreased more than that of the Methocel™ E4M 0.3% w/v solution. This viscosity decrease was due to partial hydrolytic degradation of the glucosidic bonds in the polymer backbone. There was little difference between the two temperatures of 2-8°C and 30°C with both solutions showing similar stability over the 12 months period. In previous work, the effect of autoclaving on chitosan solutions for potential use in ocular drug delivery showed that the decrease in viscosity was biphasic with an initial rapid decrease during the sterilization phase followed by a much slower decrease during the storage phase [23]. Both Methocel™ E4M solutions (0.3%, 0.4% w/v) remained clear throughout the 12 months evaluation period.

Table 1. The effect of autoclaving on kinematic viscosity.

Vehicle	Kinematic Viscosity (mm^2/s)	
	Before Autoclaving	After Autoclaving
0.3% w/v Methocel™ E4M pH 7.1	5.6	5.3
0.4% w/v Methocel™ E4M pH 7.1	11.0	9.2

3.2 Measurements of Osmolality and pH

Figure 2 shows that the pH of the solutions remained constant within the range of 6.8-7.0 for 30 days at both 2-8 and 30°C. Methocel™ E4M solutions have been reported to be stable at pH 3-11 [24]. Because they are non-ionic, the viscosity of their solutions is generally stable over a wider pH range than ionic cellulose derivatives. The pH values of vancomycin 50 mg/ml in Methocel™ E4M (0.3%, 0.4% w/v pH 7.1) were in the range of 5.5-6.0 [21]. The pH range of 3.5 to 10.5 is usually tolerated by the eye [25]. The maximum stability region of vancomycin has been reported as pH 3.0-5.7 [13] with degradation being principally by deamidation [12]. The osmolalities of the Methocel™ E4M (0.3%, 0.4% w/v) in pH 7.1 were 288 and 291 mOsmol/kg respectively. The osmolality range which can be tolerated by the eye is 160-670 mOsmol/kg [6].

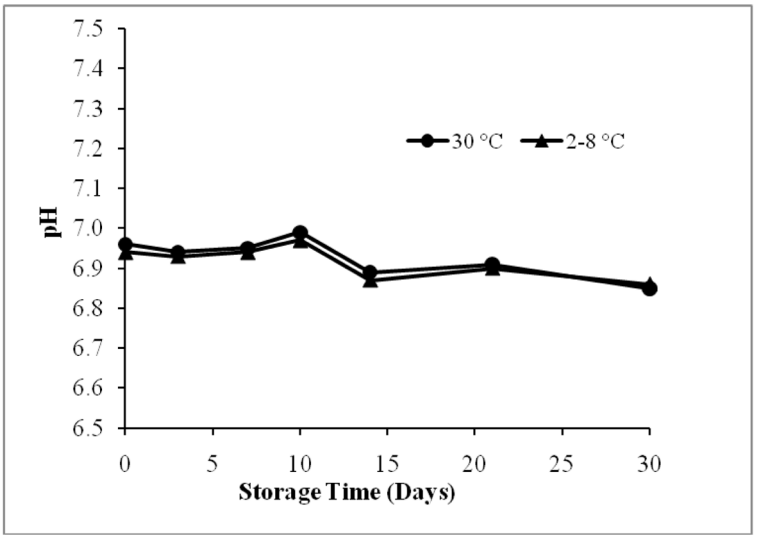


Figure 2. Changes in pH of 0.3% w/v Methocel™ E4M at 2-8°C and 30°C over a storage time of 30 days.

3.3 Contamination

Ophthalmic preparations should be prepared in a suitable clean air environment using an aseptic technique [26]. The most important factor in the preparation of ocular eye drops is that there should be no contamination. Determination of bacteria and fungus contamination in Methocel™ E4M (0.3%, 0.4% w/v) found no bacterial growth after 2 days and no fungal growth after 7 days.

Improving bioavailability by using a viscous vehicle for the dissolved drug is one way to prolong drug retention in the eye. Preparing extemporaneous topical eye drops by using commercially available artificial tears as a vehicle is convenient but some

preparations are unsuitable for some patients. For example, Khangtragool et al. [10, 21] reported that vancomycin 50 mg/ml solutions in artificial tears and 0.9 % w/v sodium chloride solution were rather acidic and unstable. Vancomycin solutions 5 mg/ml in injection form (10 times lower concentration than extemporaneous eye drops) were stable for 17 days at 24°C and for 63 days at 5°C and -10°C [27]. Thus, the vehicle used for the delivery of vancomycin 50 mg/ml should be chosen carefully with regard to compatibility, pH, sterility, clarity, osmolality and storage stability. Although chitosan 0.3% w/v has been found to be stable for 30 days at 2-8°C and its pharmacokinetics are similar to artificial tears, it cannot be used in hospitals at the present time because it is still undergoing clinical trials [7, 21]. Methocel™ E4M 0.3% w/v is an interesting alternative to chitosan since its viscosity is stable for 12 months at 30°C and 2-8°C, while its pH, clarity and osmolality are acceptable for use in eye drops. Furthermore, it can be sterilized by autoclaving and prolongs drug retention compared with 0.9% sodium chloride solution [21]. Methocel™ E4M 0.3% w/v pH 7.1 is also more similar to artificial tears (Tears Naturale II™) than Methocel™ E4M 0.4% w/v pH 7.1 [21]. This study has focused on Methocel™ E4M as a non-ionic cellulose derivative because of concerns about potential interactions between charged cellulose derivatives such as chitosan and other ingredients. Even though its mucoadhesive effect is less than an ionic derivative, its viscosity effect could still help to prolong drug delivery in topical eye drops.

4. CONCLUSIONS

Vehicles prepared from 0.3% w/v and 0.4% w/v Methocel™ E4M pH 7.1 have been compared for the ocular drug delivery of vancomycin 50 mg/ml. Methocel™ E4M 0.3% w/v pH 7.1 showed good stability when stored at 2-8°C and 30°C for 12 months while its kinematic viscosity was comparable with artificial tears (Tears Naturale II™). Osmolalities, clarity and pH were all acceptable for use in the eyes and no contamination was found.

ACKNOWLEDGEMENTS

The author would like to acknowledge the financial support of the Biomedical Engineering Center, Chiang Mai University, Thailand. I also thank Dr. Robert Molloy for his valuable suggestions and useful comments.

REFERENCES

[1] Urtti A., Challenges and obstacles of ocular pharmacokinetics and drug delivery, *Adv. Drug. Deliv. Rev.*, 2006; **58(11)**: 1131-1135.

[2] Urtti A. and Salminen L., Minimizing systemic absorption of topically administered ophthalmic drugs, *Surv. Ophthalmol.*, 1993; **37(6)**: 435-456.

[3] Barna J.C., Williams D.H., The structure and mode of action of glycopeptide antibiotics of the vancomycin group, *Annu. Rev. Microbiol.*, 1984; **38**: 339-357.

[4] Katzung B.G., *Basic & Clinical Pharmacology*, 10th Edn., Boston, McGraw-Hill, 2007.

[5] Fleischer A.B., Hoover D.L., Khan J.A., Parisi J.T. and Burns R.P., Topical vancomycin formulation for methicillin-resistant *Staphylococcus epidermidis* blepharoconjunctivitis, *Am. J. Ophthalmol.*, 1986; **101(3)**: 283-287.

[6] Charlton J.F., Dalla K.P. and Kniska A., Storage of extemporaneously prepared ophthalmic antimicrobial solutions, *Am. J. Health. Syst. Pharm.*, 1998; **55(5)**: 463-466.

[7] Khangtragool A., Ausayakhun S., Leesawat P., Laokul C. and Molloy R., Chitosan as an ocular drug delivery vehicle for vancomycin, *J. Appl. Polym. Sci.*, 2011; **122(5)**: 3160-3167.

[8] Reynolds L.A., Closson R.G., *Extemporaneous Ophthalmic Preparations*, Vancouver, WA, Applied Therapeutics, 1993.

[9] Barbault S., Aymard G., Feldman D., Pointereau-Bellanger A. and Thuillier A., Stability of vancomycin eye drops, *J. Pharm. Clin-Series.*, 1999; **18(2)**: 183-189.

[10] Khangtragool A., Ausayakhun S., Leesawat P., Molloy R. and Laokul C., Evaluation of the use of chitosan in ocular drug delivery of vancomycin, *CMU. J. Nat. Sci.*, 2009; **8(1)**: 1-9.

[11] Remington J.P. and Troy D.B., *Remington: The Science and Practice of Pharmacy*, 21st Edn., Philadelphia, PA., Lippincott, Williams & Wilkins, 2006.

[12] Trissel L.A., *Handbook on Injectable Drugs*, 14th Edn., Bethesda, MD.: American Society of Health-System Pharmacists, 2007.

[13] Mathew M. and Gupta V.D., Stability of vancomycin hydrochloride solutions at various pH values as determined by high-performance liquid chromatography, *Drug. Dev. Ind. Pharm.*, 1995; **21(2)**: 257-264.

[14] Ali Y. and Lehmussaari K., Industrial perspective in ocular drug delivery, *Adv. Drug. Deliv. Rev.*, 2006; **58(11)**: 1258-1268.

[15] Ludwig A., The use of mucoadhesive polymers in ocular drug delivery, *Adv. Drug. Deliv. Rev.*, 2005; **57(11)**: 1595-1639.

[16] Kaur I.P. and Kanwar M., Ocular preparations: The formulation approach, *Drug. Dev. Ind. Pharm.*, 2002; **28(5)**: 473-493.

[17] Martindale W. and Sweetman S.C., *Martindale: The Extra Pharmacopoeia*, 30th Edn., Singapore, Info Access & Distribution, 2007.

[18] Liu .Z., Li J., Nie S., Liu H., Ding P. and Pan W., Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin, *Int. J. Pharm.*, 2006; **315(1-2)**: 12-17.

[19] Wu C., Qi H., Chen W., Huang C., Su C., Li W. and Hou S., Preparation and evaluation of a carbopol/HPMC-based *in situ* gelling ophthalmic system for puerarin, *Yakugaku, Zasshi.*, 2007; **127(1)**: 183-191.

[20] Bucolo C., Melilli B., Piazza C., Zurria M. and Drago F., Ocular pharmacokinetics profile of different indomethacin topical formulations, *J. Ocul. Pharmacol. Ther.*, 2011; **27(6)**: 571-576.

[21] Khangtragool A., Comparison of vehicles for vancomycin drug delivery in extemporaneously prepared ophthalmic solutions, *Adv. Mat. Res.*, 2012; **506**: 481-484.

[22] Chu P.I. and Doyle D., Development and evaluation of a laboratory-scale apparatus to simulate the scale-up of a sterile semisolid and effects of manufacturing parameters on product viscosity, *Pharm. Dev. Technol.*, 1999; **4(4)**: 553-559.

[23] Khangtragool A., Ausayakhun S., Leesawat P., Molloy R. and Laokul C., Stability of chitosan solutions for potential use in ocular drug delivery, *CMU. J. Nat. Sci.*, 2008; **7(2);** 209-217.

[24] Rowe R.C., Sheskey P.J. and Weller P.J., *Handbook of Pharmaceutical Excipients*, 4th Edn., London: Pharmaceutical Press, 2003.

[25] Lund W., Royal Pharmaceutical Society of Great Britain, *The Pharmaceutical Codex: Principles and Practice of Pharmaceutics*, 12th Edn., Singapore: Info Access, 1994.

[26] Trissel L.A., *Trissel's Stability of Compounded Formulations*, 4th Edn., American Pharmacists Association, 2009.

[27] Gupta V., Stewart K. and Nohria S., Stability of vancomycin hydrochloride in 5% dextrose and 0.9% sodium chloride injections, *Am. J. Health-Syst. Pharm*. 1986; **43(7)**: 1729-1731