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Investigation of Furosemide Dialysis Rate in the Presence of Anionic Polymers

Parvin Zakei-Milani[1,2], Ziba Islambolchilar[3,4], Shole Ebrahimpoor[1,5], Mohammad Barzegar-Jalali[1], and Hadi Valizadeh[1,6,*]

e-mail: valizadeh@tbzmed.ac.ir

[1]*Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*

[2]*Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[3]*Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*

[4]*Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[5]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

[6]*Research Center for Pharmaceutical Nanotechnology, Department of Pharmaceutics, Faculty of Pharmacy, Tabriz Universi ty of Medical Sciences, Tabriz, Iran 51664*

ABSTRACT

The aim of the present study was to determine the in vitro dialysis of furosemide, an anionic drug, in the presence of anionic macromolecules. The polymers (sodium carboxymethylcellulose, sodium alginate, and methylcellulose) were used at different concentrations, and the drug concentration in the dialysis bag was 250 µg/mL. The entire process was performed in a receptor chamber in which a dialysis bag was placed as a donor medium. The samples were collected at different times up to 4 h at 37 °C. Finally, the samples were analyzed by UV spectrophotometry at 277.5 nm. Methylcellulose was used as a neutral macromolecule to show the influence of polymer viscosity in the dialysis process. The dialysis rate increased with increasing macromolecule concentration up to a certain limit. This effect could be explained by Donnan equilibrium. Polyelectrolytes (e.g., sodium alginate and sodium carboxymethylcellulose) enhance the transport rate of ionic drugs with the same electrical charge. However, the increased medium viscosity in the dialysis process limits the Donnan effect. It is concluded that anionic macromolecules in specific concentrations could be used as transport-enhancer excipients for anionic drugs such as furosemide.

INTRODUCTION

Although oral delivery is the preferred method of drug administration, this is not possible for roughly 50% of currently marketed drug compounds due to their low solubility in water and low oral bioavailability (*1*). Drug absorption from the gastrointestinal (GI) tract is complex and can be influenced by several variables. Of all the possible factors, drug solubility and intestinal permeability are generally recognized to be critical determinants of the rate and extent of absorption. Most pharmaceutical excipients currently on the market are considered inert and thus will not affect bioequivalence, although certain active excipients in the formulation have been reported to alter the drug absorption rate, extent, or both (*2*). One of the recent approaches to the problem of poor oral bioavailability of drugs includes administration of drug components with excipients that can alter drug absorption potential. Pharmaceutical dosage forms contain both pharmacologically active compounds and excipients that aid the formulation and manufacture of the subsequent dosage form for patient administration (*3*). In recent years, the biomedical and pharmaceutical industries have shown increased interest in the use of macromolecules as excipients. They have long been used as thickening, gel-forming, and colloidal stabilizing agents (*4*–*7*). They are also used as binders and disintegrants in tablet manufacture. In addition, some of them possess several characteristics that make them suitable for the development of controlled-release systems (*7*). The effect of polymeric excipients on the bioavailability of drugs is of great interest, especially when the drug molecule is electrically charged and the macromolecule has the same charge. In these cases, several aspects of the interaction between drug and macromolecule can be studied including complex formation, the effect of viscosity changes, Donnan equilibrium, drug adsorption on polymer surfaces, and electrical interactions between drug and macromolecule. According to Higuchi et al. (*8*), although there is evidence that nondiffusible polyelectrolytes associate with oppositely charged drug ions and thus retard their absorption, the converse phenomenon of enhancement of drug absorption by such ionic substances may take place. These phenomena are largely explicable on the basis of the Donnan membrane theory in which there is an unequal distribution of diffusible ions between two ionic solutions separated by a membrane that is impermeable to at least one of the ionic species present (e.g., because they are too large to pass through the pores of the membrane). According to this theory, some charged polyelectrolytes may enhance the dialysis and absorption rate of charged drugs (*9*, *10*). Furosemide, an acidic drug and sulfonamide derivative, is a well-known diuretic that is indicated in adults and pediatric patients for the treatment of edema associated with congestive heart failure, cirrhosis of the liver, and renal disease including

**Corresponding author.*

nephrotic syndrome. Furosemide belongs to Class IV of the Biopharmaceutics Classification System (BCS) (low solubility and permeability), so finding appropriate methods for improving its oral absorption is of interest (*11*). The aim of this project was to determine the dialysis rate of furosemide in the presence of different macromolecules. The macromolecules were selected according to their electrical charge and the charge of furosemide ions. The anionic polymers sodium carboxymethylcellulose (NaCMC) and sodium alginate (NaAlg) were used as candidate enhancers for the anionic drug, furosemide. Methylcellulose (MC), an electrically neutral macromolecule, was also used to investigate the influence of viscosity. The present study used the equilibrium dialysis method, which is capable of determining the dialysis rate of furosemide and the influence of macromolecules on it as well as the probability of complex formation between furosemide and macromolecules (*12*).

MATERIALS AND METHODS

Chemicals

Furosemide was purchased from Shasun Chemicals and Drugs Ltd. (Pondicherry, India). Methylcellulose (MW 14000) was obtained from Sigma Chemical Company (St. Louis, MO, USA). Sodium carboxymethylcellulose (MW 90000) was purchased from Fluka (Finland). Sodium alginate (MW 16000) was from BOH Chemicals Ltd (Poole, England); NaOH and KH_2PO_4 were obtained from Merck (Darmstadt, Germany).

Preparation of Macromolecule Solutions

Sodium alginate stock solution (0.4 g/mL) was prepared by adding 75 mL of pH 6.8 phosphate buffer to 4 g sodium alginate and allowing it to remain in contact with water overnight to hydrate sufficiently. Then 0.1%, 0.5%, 1%, and 2% working solutions were prepared by serial dilution of the stock solution with monobasic potassium phosphate buffer. Working solutions of 1%, 2%, 4%, 6%, and 8% sodium carboxymethylcellulose were prepared from a 1.2 g/mL stock solution, and a 1.6 g/mL stock solution of methylcellulose (1.6 g/mL) was diluted to prepare 1% and 8% working solutions.

Equilibrium Dialysis Procedure

In dialysis studies, the experimental procedure previously employed for other studies on the binding effects of macromolecular substances on drugs was used (*8*, *13*, *14*). The method consisted of bringing two solutions, one containing the macromolecular component and the other the drug under study, into equilibrium across a semipermeable membrane. The membrane was so chosen that it permitted the low molecular weight drug to pass freely through the barrier and achieve equilibrium in both solution phases, yet at the same time it acted as an impermeable barrier toward the polymeric substance. Equilibrium dialysis was performed using 20-cm strips of dialysis tubing (3-cm diameter, 4.8-nm pore diameter with a molecular weight cutoff of not greater than 12,000). Dialysis bags of the same size were prepared and soaked in each of distilled water and buffer solution for 24 h before use. The tubing was tied with a double knot at one end and filled with 5 mL of furosemide solution (500 µg/mL) and 5 mL of macromolecule solution; the other end was then sealed closely with a knot. The dialysis membrane was immersed in 225 mL of buffer solution in a beaker that served as the receiver; sink conditions were maintained. A stir bar was used at a mixing speed of 330 rpm to enable mixing inside the beaker, and a hot plate was used to maintain the temperature inside the beaker at 37 °C. Two-milliliter samples were withdrawn from the outside compartment at specified time intervals. The total volume was kept constant by the addition of 2 mL of phosphate buffer to the outside compartment. Since furosemide is susceptible to photodegradation, all solutions were protected from light with aluminum foil (*15*, *16*). To study the possible complex formation between polymers and drug molecules, certain concentrations of each polymer were selected. Dialysis bags were prepared as mentioned above. Five milliliters of polymer solution and 5 mL of furosemide solution were dropped into each dialysis bag, and the bags were then sealed by knotting the ends and immersed in a beaker containing the dialysis environment. The beakers were placed on a magnetic heater–stirrer and equilibrated at 37 °C for 24 h. After equilibration, aliquots of the external solution were withdrawn, and the concentrations of furosemide were determined spectrophotometrically. The complex between drug and polymer was formed if the measured concentration was less than theoretical concentration. All experiments were performed in triplicate for the mean calculation.

Method of Analysis

A Shimadzu ultraviolet spectrophotometer, Model 160, was used for the analysis of furosemide in samples at a wavelength of 277.5 nm.

UV Spectroscopy

A polymer-free furosemide solution was prepared in phosphate buffer at 10 mg/mL, and the absorption spectrum was recorded. The UV spectra of 10 mg/mL furosemide solutions containing 0.05% NaCMC and 0.05% methylcellulose were recorded separately and compared with the spectrum of the macromolecule-free solution.

Data Analysis

Because the transport of drug across the dialysis membrane is considered a process of passive diffusion, the in vitro flux was determined by Fick's law of diffusion. Flux ($mg/cm^2/h$) was determined from the slope of the linear portion of the cumulative amount permeated per unit area versus the time plot.

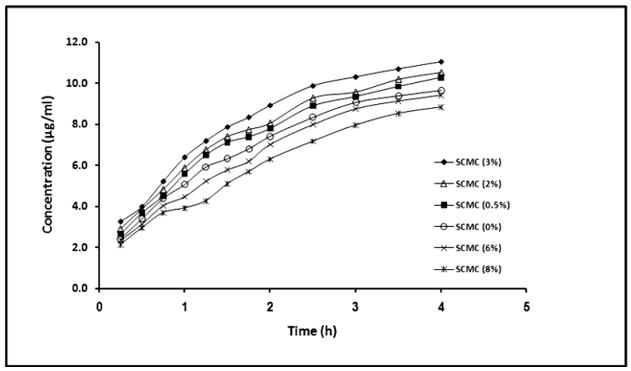

Figure 1. The effect of different concentrations of NaCMC on dialysis rate of furosemide.

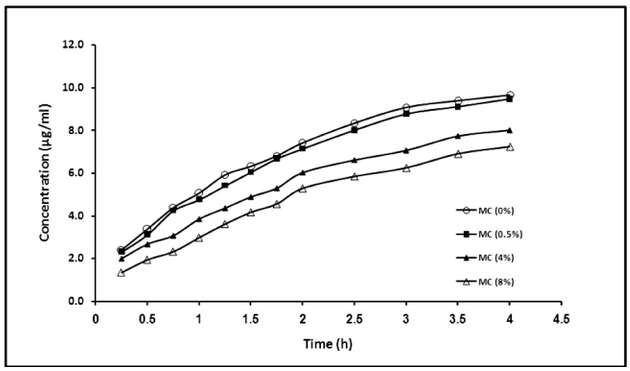

Figure 3. The effect of different concentrations of MC on dialysis rate of furosemide.

Statistical Analysis

The statistical difference between the dialysis rates of furosemide in the presence of different macromolecules was evaluated by two-tailed Student's *t*-test (SPSS, version 13, IBM Inc., New York, USA) at $p < 0.05$.

RESULTS AND DISCUSSION

The influence of the presence of polymers (methylcellulose, sodium carboxymethylcellulose, and sodium alginate) in different percentages on the dialysis rate of furosemide is illustrated in Figures 1–3. The data indicate that the dialysis rate increased by increasing the amount of anionic polymers up to a certain concentration. This is apparently due to the influence of polyelectrolytes on the ionic drug as a result of the Donnan effect on the process. The interactions between an anionic macromolecule and furosemide are influenced by viscosity, the macromolecule's electrical charge, and the complexation between drug and macromolecule; the resultant determines the rate of dialysis. As indicated in Figures 1–3, the dialysis rate of furosemide increased significantly ($p < 0.01$) with an increase in polyelectrolyte concentration up to 3% of NaCMC and 0.25% of NaAlg. This trend was confirmed by calculating the flux (µg/cm^2/h) for each solution transport.

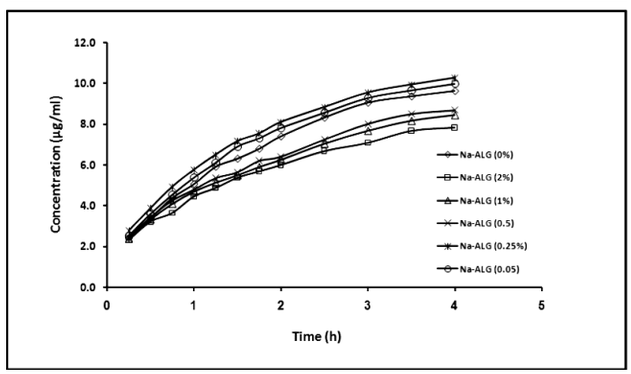

Figure 2. The effect of different concentrations of NaAlg on dialysis rate of furosemide.

The highest and lowest values are 18.7 and 11.3 µg/cm^2/h for drug solutions containing 3% NaCMC and 8% MC, respectively. The flux values, calculated as µg/cm^2/h for all drug solutions, are shown in Table 1. Statistical analysis shows significant differences ($p < 0.01$) between each drug solution flux and the other one. Previous reports (*8*, *18*) have demonstrated the influence of some macromolecular excipients on drug dissolution, dialysis, and absorption; for example, Higuchi et al. (*8*) reported that sodium carboxymethylcellulose and sodium polyacrylate enhanced the dialysis rate of sodium salicylate and potassium benzylpenicillinate through cellophane. Wurster and Taylor (*18*) reported that an increase in viscosity results in a decrease in the rate of dissolution and absorption of a drug molecule. Another study by Redman et al. (*19*) showed that the dialysis rate of radioactive

Table 1. Calculated Fluxes (µg/cm^2/h) for Furosemide in Solutions Containing Different Concentrations of Macromolecules

Solutions		Furosemide Flux (µg/cm^2/h)
NaCMC	0.5% w/w	16.69 (±0.09)
	2% w/w	17.18 (±0.13)
	3% w/w	18.73 (±0.04)
	6% w/w	14.22 (±0.34)
	8% w/w	12.58 (±0.18)
NaAlg	0.05% w/w	16.58 (±0.04)
	0.25% w/w	16.92 (±0.06)
	0.5% w/w	13.12 (±0.09)
	1% w/w	12.63 (±0.14)
	2% w/w	12.22 (±0.06)
MC	0.5% w/w	15.03 (±0.09)
	4% w/w	12.26 (±0.24)
	8% w/w	11.30 (±0.25)
Macromolecule-free		15.49 (±0.45)

Table 2. Results of Equilibrium Dialysis of Furosemide in the Presence of Different Macromolecules

	Measured Concentrations in Dialysis Environment (µg/mL)					
Polymer (% w/v)	**Series no.1**	**Series no.2**	**Series no.3**	**Mean (µg/mL)**	**SD (µg/mL)**	**RSD (%)**
NaCMC (3%)	11.47	11.59	11.64	11.57	0.09	0.77
NaAlg (0.25%)	11.35	11.49	11.44	11.42	0.07	0.62
MC (0. 5%)	10.66	10.38	10.47	10.50	0.14	1.36

iodide was retarded in the presence of suspending agents. Lamy et al. (*20*) reported that the viscosity of sodium carboxymethylcellulose solutions accounted for the depressed rate of drug transfer in an in vitro model. Hewitt and Levy (*21*) observed that the rate and extent of thiamin and riboflavin absorption were not significantly altered by methylcellulose. Levy and Rao (*22*) reported that the oral absorption of riboflavin-5′-phosphate increased significantly if 2% sodium alginate was included in the aqueous solution. Davison et al. (*23*) demonstrated that 2% methylcellulose delayed the oral absorption of sodium salicylate. Levy and Jusko (*17*) reported that methylcellulose reduced the absorption of ethanol and salicylic acid in rats. In our study, the observed increase in dialysis at low concentrations of polyelectrolyte was attributed to the Donnan membrane effect. Beyond these concentrations, the dialysis rate of the drug decreased because of the dominance of the viscosity to the Donnan effect. The results obtained are agree with the results of a previous study on the effect of anionic macromolecules, sodium alginate, and sodium carboxymethylcellulose on the dialysis rate of sodium diclofenac (*24*). Methylcellulose, an electrically neutral polymer, was used to investigate the influence of viscosity on the dialysis rate, since the Donnan effect was eliminated. As shown in Figure 3, methylcellulose at the two higher concentrations of 4% and 8% reduced the dialysis rate of furosemide compared with the control (polymer-free) samples. In the dialysis of furosemide in the presence of anionic macromolecules, viscosity acts as an opposing factor in contrast with the Donnan membrane effect and would limit it, but there would be a concentration threshold for the dialysis rate decreasing the effect of the polyelectrolyte. In lower concentrations, anionic macromolecules are capable of enhancing the absorption of furosemide and other anionic drugs. To study the influence of complexation between drug and macromolecule, two methods were used. First, the ultraviolet spectra of drug in the presence and absence of the macromolecules were obtained and compared with each other. If the complex between drug and polymer molecule was formed, the UV spectrum of the solution would be changed (*8*, *25*). The UV spectra of furosemide in the present and absence of each NaAlg and NaCMC showed no difference between them, so it seems that there was no complex formation between furosemide and these macromolecules. The equilibrium dialysis method was then used to prove the results of the spectroscopic studies. In brief, the method consisted of bringing two solutions, one containing the macromolecular component and the other the drug under study, into equilibrium across a semipermeable membrane. If the drug under study does not complex with the polymer, the concentration of the drug on each side of the barrier will be equal. In such a case, the total drug concentration in the solution containing the polymer and the concentration of the unbound drug in the external solution would be the same. If, however, complexation does occur, the total drug concentration in the polymer side of the barrier will be higher. The difference between the total drug concentration in the internal solution and the concentration of the unbound drug in the external solution is the amount of drug that is complexed (*13*, *14*, *26*, *27*). So the theoretical concentration of furosemide (made by dissolving the same amount of furosemide in a volume of buffer equal to the total volume of the dialysis environment) was calculated and compared with the practical concentrations obtained from the equilibrium dialysis studies. According to Table 2, while the amount of drug in dialysate was greater than the theoretical value (10.6 µg/mL) for anionic macromolecules, the practical and theoretical amounts were the same for methylcellulose. These results confirmed that there were no complex formation between furosemide and the macromolecules.

CONCLUSION

The results show that at certain concentrations, anionic polymers may be used as absorption enhancers for an anionic drug like furosemide because in high concentrations of macromolecules, viscosity acts as an opposing factor and lowers the Donnan membrane effect.

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