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Determination of the binding parameter constants of Renagel® capsules and tablets utilizing the Langmuir approximation at various pH by ion chromatography

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Abstract

Sevelamer hydrochloride is a cross-linked polymeric amine; it is the active ingredient in Renagel® capsules and tablets. Sevelamer hydrochloride is indicated for the control of hyperphosphatemia in patients with end-stage renal disease. The binding parameter constants of sevelamer hydrochloride were determined using the Langmuir approximation for three different dosage forms at pH 4.0, 5.5 and 7.0. The three dosage forms were Renagel® 403 mg capsules, Renagel® 400 mg tablets and Renagel® 800 mg tablets. The results demonstrate the equivalency of all three dosage forms at each pH. The results also demonstrate a shift in the binding mechanism from pH 4.0 to 7.0.

Keywords: Sevelamer hydrochloride; Ion chromatography; Langmuir; Quaternary amines; Hydrogel; Renagel®

1. Introduction

Sevelamer hydrochloride is the active ingredient in Renagel® capsules and tablets. Sevelamer hydrochloride, a cross-linked poly(allylamine hydrochloride), is a novel phosphate binder used for the reduction of serum phosphate levels in patients with end-stage renal disease (ESRD) [1–4]. The advantage of sevelamer hydrochloride for ESRD over existing therapies, such as calcium or aluminum supplementation, is that it is non-absorbed, leading to an improved safety profile.

An important aspect of the analytical characterization of sevelamer hydrochloride tablets is to demonstrate equivalency to the capsule dosage form. The structure of sevelamer hydrochloride is shown in Fig. 1. The amines in sevelamer hydrochloride may bind phosphate ionically and through hydrogen bonding.

This paper describes the methodology and procedures for the determination of the binding constants at three different pH levels utilizing the Langmuir approximation. A comparison of these binding constants demonstrates the equivalency of

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the tablet dosage form to the capsule dosage form at each pH studied. The binding of the dibasic phosphate anion and monobasic phosphate anion is also discussed.

2. Materials and methods

2.1. Chemicals

Sevelamer hydrochloride was obtained from GelTex Pharmaceuticals, Inc. (Waltham, MA). *N*,*N*-Bis(hydroxyethyl)-2-aminoethanesulfonic acid (BES) was obtained from Sigma Chemical Company (St. Louis, MO). Potassium phosphate, monobasic (KH_2PO_4) and 1 N aqueous sodium hydroxide were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Sodium chloride and sodium hydroxide pellets were from VWR Scientific Products (West Chester, PA). All chemicals were of ACS grade or higher and were used without further purification. Deionized water was obtained from an in-house Barnstead Nanopure System (Barnstead/Thermolyne Corporation, Dubuque, IA).

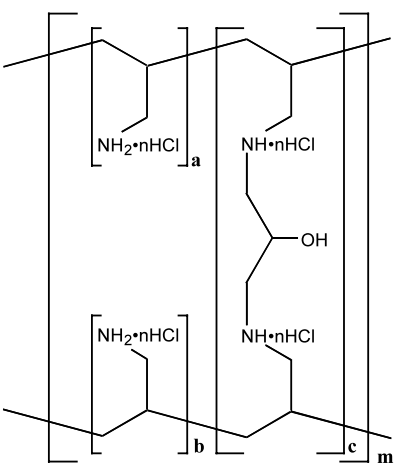

Fig. 1. Structure of sevelamer hydrochloride; (a, b = number of primary amine groups) $a + b = 9$; (c = number of cross-linking groups) $c = 1$; (n = fraction of protonated amines) $n = 0.4$; (m = large number to indicate extended polymer network).

2.2. Apparatus

A Dionex (Dionex Corporation, Sunnyvale, CA) DX-500 IC system was used for phosphate analysis. This system consists of an AS50 autosampler, GP50 quaternary gradient pump, CD20 conductivity detector, and PeakNet software control and data acquisition (version 5.10d). Separations were performed using a Dionex AS-11 analytical column (4×250 mm^2) and an AG-11 guard column (4×40 mm^2). Suppression was achieved with an ASRS-II anion self regenerating suppressor from Dionex. Samples were shaken using a Labline Heated Orbital Shaker Model No. 4628 (Labline Instruments, Melrose Park, IL). Injection loop volume was 100 μl with a full injection setting.

2.3. Sample preparation

Three individual sets of aqueous phosphate solutions were prepared at the following concentrations: 38.7, 30.0, 14.5, 10.0, 7.5, 5.0, 2.5 and 1.0 mM. Each set of phosphate solutions were prepared so that a final pH of 4.0, 5.5 and 7.0 ± 0.3 was obtained after the addition of Renagel® capsules and tablets, as described below. All solutions contained 100 mM BES and 80 mM NaCl.

For the 403 mg capsule and 400 mg tablet, one unit dose was placed into 150 ml of each phosphate solution. For the 800 mg tablet, one unit dose was placed into 300 ml of each phosphate solution in order to keep the phosphate to polymer ratio constant.

The solutions at pH 4.0 were prepared by adding the capsules and tablets to a set of phosphate solutions, which had no prior pH adjustments. Upon their disintegration, approximately 3 ml of 1 N HCl was added to all solutions with the exception of 300 ml solutions (800 mg tablets) at 38.7 and 30.0 mM phosphate in which approximately 6 and 5 ml were added, respectively. The pH of the final solutions was approximately pH 4.0. All of the samples were prepared in duplicate.

The solutions at pH 5.5 were prepared by adjusting the pH of a set of phosphate solutions to pH 4.0, volumetrically, with 1 N NaOH. Approximately 2–4 ml of 1 N NaOH was added per liter

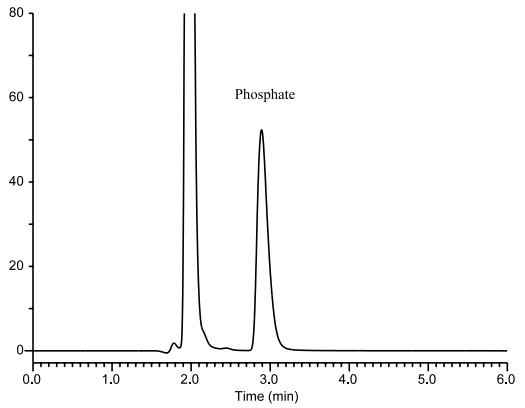

Fig. 2. Chromatogram of typical phosphate standard (see text for conditions).

of solution. After the addition of the capsules and tablets, the pH of all solutions was approximately 5.5. The samples were prepared in duplicate.

The solutions at pH 7.0 were prepared by adjusting the pH of each solution to pH 7.0 with 1 N NaOH [5]. Approximately 50 ml of 1 N NaOH was volumetrically added per liter of solution. After the addition of the capsules and tablets, the pH of the solution was approximately pH 7.0. The pH of this solution does not change because the pK_a of BES is 7.1 and thus provides excellent buffering capacity in this pH range. BES was utilized throughout this experiment so that a direct comparison of all results is possible. It has been demonstrated that BES, in concentrations from 60 to 120 mM, does not affect the phosphate binding (R. Swearingen et. al., unpublished results).

All samples were then placed on a Labline Orbital Shaker at 37 °C for 2 h. The samples were removed, filtered through a 25 mm, 0.2 μm nylon syringe filter (Pall Corp., Ann Arbor, MI). The samples were diluted with deionized water in the appropriate ratios. The dilutions ranged from 1:100 to undiluted for the 1 mM phosphate concentration.

The phosphate standards were prepared by diluting each set of phosphate solutions, 1:50 with deionized water to generate an eight point calibration curve. This dilution was performed prior to the addition of capsules and tablets. A calibration curve was generated at each pH to produce a total of three separate 8 point calibration curves for quantitation at pH 4.0, 5.5 and 7.0. The area of the phosphate peak versus the concentration was plotted and the coefficient of determination values for each curve was greater than 0.998.

The average of the two phosphate binding values was used to generate the phosphate binding isotherms and Langmuir plots.

2.4. Chromatographic conditions

Standards and samples were analyzed for phosphate levels by ion chromatography using the columns described above [5]. The mobile phase was 25 mM NaOH pumped at 1 ml/min. Suppression was performed in the recycle mode, with an applied current of 100 mA. The injection volume was 100 μl with a full injection setting. A typical phosphate standard chromatogram is shown in Fig. 2.

2.5. Calculations

The unbound phosphate concentration remaining in each sample was calculated from the linear regression generated from a plot of the area of the phosphate peak versus the concentration of phosphate (mM) using the following equation:

$$\text{Unbound phosphate concentration (mM)} = \frac{\text{area phosphate} - \text{intercept}}{\text{slope}}$$

From the known initial concentration of phosphate in each solution before the addition of sevelamer hydrochloride (i.e. 38.7, 30.0, 14.5, 10.0, 7.5, 5.0, 2.5 and 1.0 mM), the bound concentration was calculated by subtracting the unbound concentration from the initial concentration.

$$\text{Bound phosphate concentration (mM)} = \text{initial concentration} - \text{unbound phosphate concentration (mM)}$$

The phosphate binding capacity, in mmol of phosphate/g of polymer, was calculated as follows:

$$\text{Phosphate binding capacity (mmol/g)} = \frac{\text{bound phosphate concentration (mM)} \times V_s}{\text{weight (g)}}$$

where V_s is the volume of solution, approximately 0.15 l for the 403 mg capsules and the 400 mg tablets and approximately 0.30 l for the 800 mg tablets. The weight (g) is the weight of sevelamer hydrochloride.

The phosphate binding constants were calculated from the Langmuir approximation. The Langmuir approximation describes the monomolecular adsorption of an adsorbate (phosphate) from solution, at constant temperature, onto an adsorbent (sevelamer hydrochloride) [6]. This process is described by the Langmuir equation:

$$\frac{C_{eq}}{x/m} = \frac{1}{k_1 k_2} + \frac{C_{eq}}{k_2}$$

where C_{eq} is the concentration, in mM, of phosphate remaining in solution at equilibrium or the unbound concentration. x/m is the amount of phosphate bound per weight of polymer in mmol/g. The constant k_1 is the affinity constant and is related to the magnitude of the forces, which are involved in binding. The constant k_2 is the Langmuir capacity constant and is the maximum amount of phosphate that can be bound per unit weight of sevelamer hydrochloride.

The affinity and Langmuir capacity constants were calculated by performing linear regression on a plot of the unbound (mM)/bound (mmol/g) versus the unbound (mM) concentrations. The k_1 value is calculated by dividing the slope of the regression line by the intercept, the k_2 value is equal to the inverse of the slope. The results are listed in Figs. 3–5 and in Table 1.

3. Results and discussion

The results demonstrate that at each individual pH, all three dosage forms exhibit very similar binding properties. The increase in the k_2 values from pH 7.0 to 5.5 may be attributed to the decrease in the ionic strength of the solutions as prepared at pH 5.5. The non-linearity of the

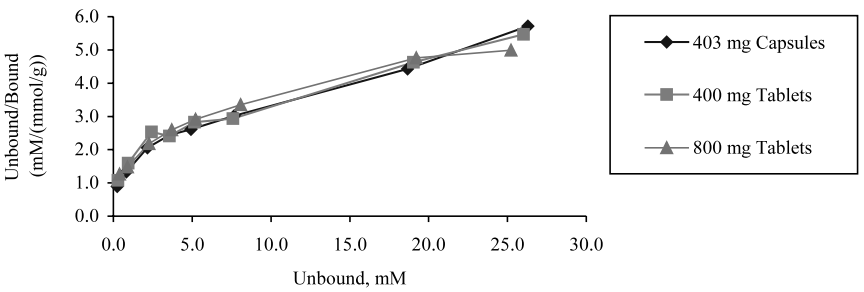

Fig. 3. Langmuir plot of Renagel® 403 mg capsules, 400 mg tablets and 800 mg tablets at pH 4.0.

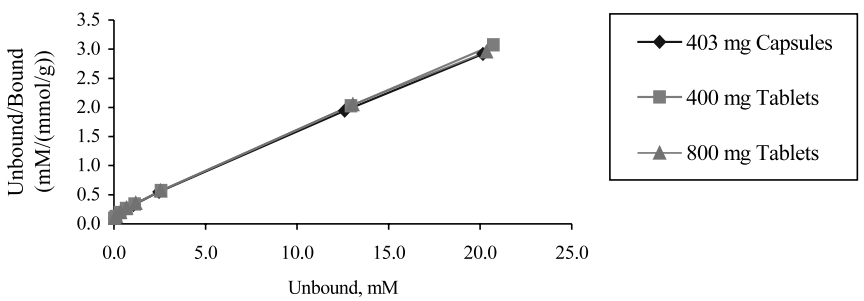

Fig. 4. Langmuir plot of Renagel® 403 mg capsules, 400 mg tablets and 800 mg tablets at pH 5.5.

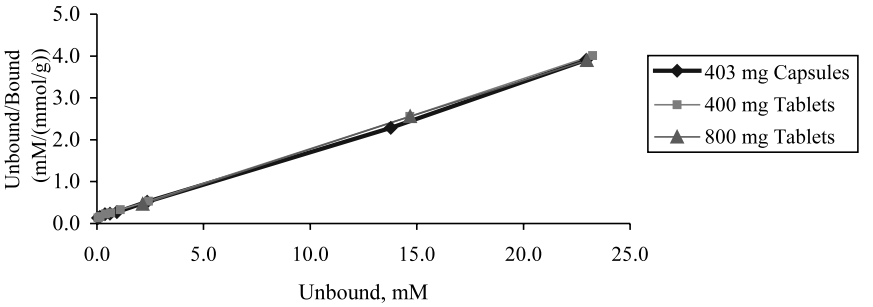

Fig. 5. Langmuir plot of Renagel® 403 mg capsules, 400 mg tablets and 800 mg tablets at pH 7.0.

Langmuir plot and the order of magnitude decrease in the affinity constants at pH 4.0 can be explained by examining the fraction of each phosphate ion present as a function of pH in dilute solution. This is accomplished by taking into account the hydronium ion concentration at each pH, the pK_a of each phosphate ion and the equilibrium reaction. The results are shown in Fig. 6. The value at which one fraction of an ion is equal to another, corresponds to the pK_a value of the phosphoric acid ions in dilute aqueous solution (i.e. 2.1, 7.2 and 12.4).

At a pH range of approximately 6–8, monobasic phosphate is in equilibrium with dibasic phosphate. It has been demonstrated that at pH 7, the molar ratio of amine to bound phosphate is approximately 2. The results are listed in Table 2. This may indicate that the dibasic phosphate is the predominately bound species at pH 7, as has been previously suggested [7].

At pH 5.5–6.0, the fraction of monobasic ion increases. The small decrease in the binding affinity constants (k_1) demonstrates that the binding forces are weaker at pH 5.5–6.0 This is due to the decrease in the amount of dibasic phosphate bound and increase in the amount of the monobasic bound. The linearity of the Langmuir plots indicates monomolecular binding.

At pH 4, the monobasic phosphate ion is predominately present. The affinity constants are an order of magnitude lower than the affinity constants at pH 7.0. These results suggest that the monobasic ion, which has only one site for binding, is more weakly bound than the dibasic ion, which has two sites for binding. The relative

Table 1
The affinity and Langmuir capacity constants calculated at three pH levels

	Capsules 403 mg	Tablets 400 mg	Tablets 800 mg
pH 7.0			
Intercept	0.13	0.14	0.12
Slope	0.16	0.17	0.16
R^2	0.999	0.999	0.999
k_1	1.2	1.2	1.4
k_2	6.2	6.0	6.1
pH 5.5			
Intercept	0.15	0.15	0.16
Slope	0.14	0.14	0.14
R^2	0.998	0.999	0.998
k_1	0.94	0.95	0.89
k_2	7.2	7.0	7.1
pH 4.0			
Intercept	1.5	1.7	1.7
Slope	0.16	0.15	0.14
R^2	0.946	0.939	0.915
k_1	0.11	0.09	0.08
k_2	6.1	6.6	7.0

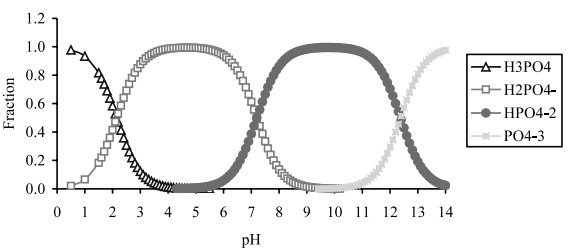

Fig. 6. Fraction of phosphate ion present as a function of pH.

Table 2
Molar amine to phosphate ratio

Sevelamer sample number	Phosphate (mmol/g)	Total amines (mmol/g)	Amine/Phosphate ratio
1	5.5	11.74	2.13
2	5.5	11.56	2.10
3	5.7	11.52	2.02
4	5.6	11.69	2.09
5	5.7	11.63	2.04
6	5.5	11.79	2.14
7	5.5	11.54	2.10
8	5.5	11.63	2.11
Average			2.1
S.D.			0.04
%R.S.D.			2.0

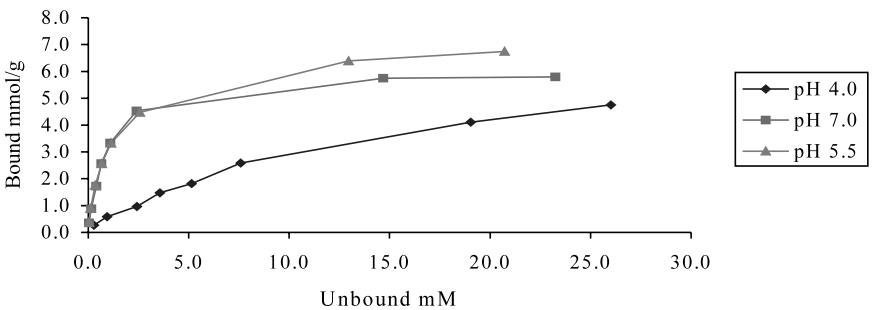

Fig. 7. Phosphate binding as a function of pH.

non-linearity of the Langmuir plots at pH 4.0 may indicate non-monomolecular binding as a result of the monobasic ion. The change in the binding of the dibasic anion to the monobasic anion can be seen by plotting the phosphate binding isotherms as a function of pH. The isotherm are presented in Fig. 7.

The graph shows the dibasic binding of phosphate at pH 7.0 and 5.5, and the monobasic binding of phosphate at pH 4.0. The lower slope of the curve at pH 4.0 also demonstrates the lower binding affinity of the monobasic ion. These results show the differences in the binding of the dibasic and monobasic ion. The lower affinity constants values at pH 4.0 are not pharmacologically significant as binding occurs in the intestine where the pH range is typically 6–7.

A possible explanation for the similarities of the isotherms at pH 7.0 and 5.5 is the apparent pK_a values. Fig. 6 shows the fraction of each ion at various pH in dilute solution with only phosphoric acid present in water. Sevelamer hydrochloride has an internal charge and hence its own internal ionic strength due to the amines, which are present. This intermolecular charge of sevelamer hydrochloride may shift the pK_a of the dibasic anion from 7.2 to a slightly lower value when in solution and in contact with sevelamer hydrochloride. This would cause the fraction of the dibasic ion, at pH 5.5, to be substantially more than predicted by Fig. 6. It has also been shown that there are more protoanated amines at pH 5.5 than at pH 7.0 [7].

4. Conclusion

The phosphate binding constants, k_1 and k_2, were determined at pH 7.0, 5.5 and 4.0 for the following dosage forms of sevelamer hydrochlo-

ride: 403 mg capsules, 400 mg tablets, and 800 mg tablets. The results demonstrate that the binding constants and affinity constants at pH 7.0, 5.5 and 4.0 for all three dosage forms exhibit similar binding properties at each pH.

The differences in the affinity constants at pH 4.0, as compared to the affinity constants at pH 7.0, are due to the fact that at pH 4.0 the monobasic ion is predominately bound and at pH 7.0 the dibasic ion is predominately bound. The relative non-linearity of the Langmuir plot at pH 4 and the linearity of the Langmuir plot at pH 7 indicate a shift in the binding mechanism from pH 4 to 7.

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