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A phosphate binding assay for sevelamer hydrochloride by ion chromatography

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Abstract

Sevelamer hydrochloride is a cross-linked polymeric amine; it is the active ingredient of Renagel® capsules. Renagel® is indicated for the control of hyperphosphatemia in patients with end-stage renal disease. An in vitro phosphate-binding assay is required to measure the drug's efficacy. The assay developed for this purpose involves mixing the drug (polymer) with a solution of known phosphate concentration, filtering off the polymer–phosphate complex, and quantitating the unbound phosphate concentration by ion chromatography. The binding capacity, reported as mmol of phosphate bound g of polymer^{-1}, is calculated from the calculated amount of bound phosphate and the weight of polymer used. The method has been validated for accuracy, precision, linearity, range, and ruggedness.

Keywords: Sevelamer hydrochloride; Ion chromatography; Phosphate binding; Hydrogel; Renagel®

1. Introduction

Sevelamer hydrochloride, cross-linked poly (allylamine hydrochloride), is a potent phosphate binder used for the reduction of serum phosphate levels in patients with end-stage renal disease (ESRD) [1–4]. The structure of sevelamer hydrochloride is shown in Fig. 1. The protonated amines in sevelamer hydrochloride bind phosphate ionically. Phosphate is also bound by hydrogen bonding.

Sevelamer hydrochloride is the active ingredient in Renagel® capsules. The advantage of Renagel® for ESRD over existing therapies, calcium or aluminium supplementation, is that it is non-absorbed, leading to an improved safety profile. An important aspect of the analytical characterization of sevelamer hydrochloride is an in vitro phosphate binding assay. This paper describes the method to perform this assay, and provides validation results.

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2. Materials and methods

2.1. Chemicals

Sevelamer hydrochloride was obtained from GelTex Pharmaceuticals (Waltham, MA). *N*, *N*-Bis(hydroxyethyl)-2-aminoethanesulfonic acid (BES) was obtained from Sigma (St. Louis, MO). Potassium phosphate, monobasic (KH_2PO_4) and 1 M aqueous sodium hydroxide were obtained from Aldrich (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, Dubuque, IA).

2.2. Apparatus

A Dionex (Dionex, Sunnyvale, CA) DX-500 IC system was used for phosphate analysis. This system consists of an AS3500 autosampler, GP40 quaternary gradient pump, ED40 conductivity detector, and PeakNet software control and data acquisition. Separations were performed using a Dionex AS-11 analytical column (4×250 mm) and an AG-11 guard column (4×40 mm). Suppression was achieved with an ASRS-I anion suppressor from Dionex. Samples were shaken using a Burrell Model 25 Wrist-Action Shaker (Burrell Scientific, Pittsburgh, PA). Injection loop volume was 100 μl. Loss on drying was determined using a TA Instruments Model 2950 Thermogravimetric Analyzer (TGA) (TA Instruments, New Castle, DE).

2.3. Sample preparation

A stock phosphate solution containing 20.0 mM KH_2PO_4, 100 mM BES, and 80 mM NaCl was prepared. The pH of the solution was adjusted to 6.95–7.05 with 1 N sodium hydroxide. Separate 1 l and 200 ml portions of the 20 mM phosphate solution were prepared. The 1 l portion was used for binding experiments and to prepare the 14 mM chromatography standard. The 200 ml portion was used to prepare a 14 mM check standard. Both the chromatographic and check standards were diluted 1:100 by two successive 5:50 dilutions.

To perform the binding step, duplicate samples of sevelamer hydrochloride (135–141 mg) were weighed into a tared, 125 ml wide-mouthed plastic bottle, to which 100 ml of 20 mM phosphate stock solution was added. The bottle was then capped and shaken with a Burrell wrist-action shaker at an angle of 10° from horizontal for 15 min. Approximately 6–7 ml of the resultant slurry was filtered using a Uniprep syringeless filtration device (Whatman, Clifton, NJ). The resulting filtrate was diluted 1:100 by two successive 5:50 dilutions in 50 ml volumetric flasks using 5 ml glass pipettes. Binding capacities for the duplicate samples were required to agree within 3%.

2.4. Chromatographic conditions

Standards and samples were analyzed for phosphate levels by ion chromatography using the columns described above. Conditions were adapted from the column manual. The mobile phase was 25 mM NaOH pumped at 1 ml min^{-1}.

Suppression was performed in the recycle mode, with an applied current of 100 mA. The injection volume was 100 μl. A typical phosphate standard chromatogram is shown in Fig. 2.

Five injections of the working standard were made for system suitability, with a phosphate peak area RSD requirement of $\leq 2.0\%$. One injection of the check standard was then performed, with an agreement requirement between the test standard area and the average standard area of $\leq 2\%$. After every three samples, an injection of a standard was performed to confirm system suitability.

2.5. Calculations

The unbound phosphate concentration remaining in a sample was calculated with the following equation:

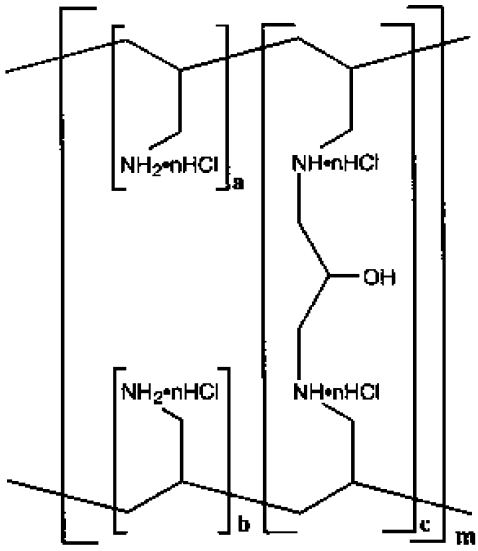

Fig. 1. Structure of sevelamer hydrochloride; a, b = number of primary amine groups a + b = 9; c = number of cross-linking groups c = 1; n = fraction of protonated amines n = 0.4; m = large number to indicate extended polymer network.

$$\frac{area\ sample * 14\ \text{mM}}{avg.\ area\ std.}$$

where *area sample* is the phosphate peak area in the sample, *avg. area std.* is the average phosphate peak area from five injections of the phosphate working standard, and 14 mM is the standard concentration before dilution. Precision of the method will not be affected by the two significant figures used for the 14 mM standard and 20 mM binding solution, because the 14 mM standard is prepared from the 20 mM solution.

The phosphate binding capacity, in mmol of phosphate g of Renagel®$^{-1}$, was calculated as follows:

$$\frac{(20\ \text{mM} - unbound\ phosphate\ concentration) * 0.1\text{l}}{g\ of\ polymer * [1 - (LOD/100)]}$$

where 20 mM is the phosphate concentration applied, *unbound phosphate concentration* is the unbound phosphate concentration, in mM, determined by IC, 0.1 l is the volume of 20 mM phosphate solution applied, *g of polymer* is the weight of sevelamer hydrochloride used, and *LOD* is the loss on drying of the sample obtained by thermogravimetric analysis.

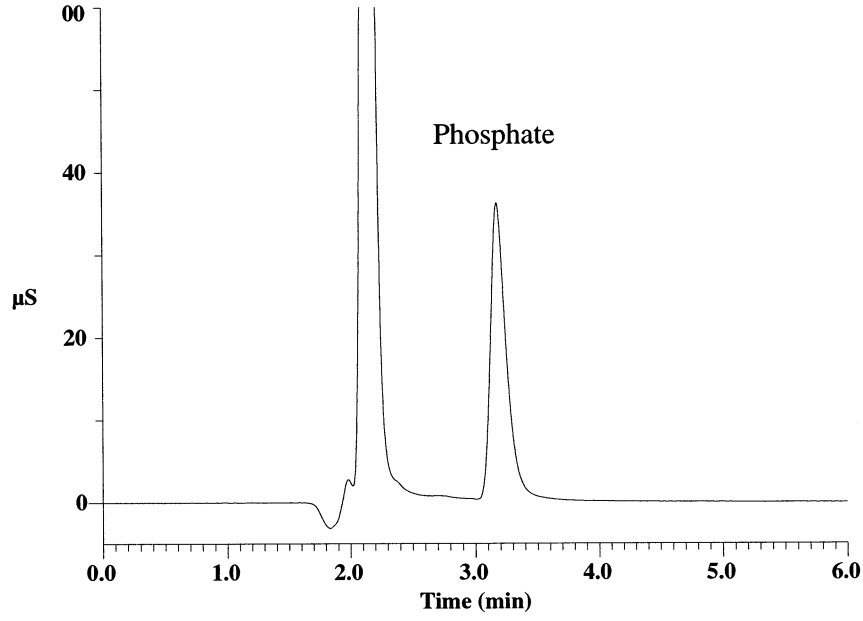

Fig. 2. Chromatogram of typical phosphate standard (see text for conditions).

Table 1
Peak area repeatability for 1 day and linearity results for 2 days

Concentration (mM)	Peak area	Average	% RSD
10	2487936		
10	2493998	2489615	0.2
10	2486910		
12	2967583		
12	2964896	2966980	0.1
12	2968461		
14	3432280		
14	3426502	3430244	0.1
14	3431949		
16	3934891		
16	3943429	3936996	0.1
16	3932668		
18	4415814		
18	4406064	4409374	0.1
18	4406245		
Day	R^2	Slope	Intercept
1	0.9998	240477	79967
2	0.9983	246348	−18939

2.6. Validation

Linearity of phosphate peak area was determined by preparing and analyzing phosphate solutions at concentrations of 10, 12, 14, 16, and 18 mM. Standards were diluted 1:100 prior to IC analysis. On one day, each solution was injected three times, while on a second day each solution was injected once. As seen in Table 1, the RSDs of the average peak areas ranged from 0.1 to 0.2% on day 1. Linear regression results for both days are also given in Table 1. Good linearity was obtained on both days.

Accuracy of phosphate determination was measured by preparing phosphate samples at 11, 14, and 17 mM, and analyzing them in triplicate versus a phosphate standard. Accuracy was expressed as percent recovery. Results are in Table 2; average recoveries ranged from 99.0 to 100.6%. Robustness was measured by assaying triplicate samples at the normal assay amount, 138 mg, and at 110 and 166 mg (80 and 120% of the normal assay amount). The average binding capacity at 110 and 166 mg was compared to the average at 138 mg. Results are shown in Table 3. The averages at 110 and 166 mg were 102 and 100%, respectively, of the average at 138 mg. System precision was measured by injecting the phosphate standard ten times and determining the RSD of peak area. The RSD was 0.4%. Repeatability and range were determined from the RSDs at each polymer weight in the robustness study, Table 3. RSDs at all three levels were 1.7%.

Two analysts assaying triplicate samples on different days determined intermediate precision. Results are in Table 4. The overall RSD was 0.7%, indicating excellent precision. Inter-day error was determined by comparing the average for each day with the overall average. On each day the binding capacity was 5.8 mmol g^{-1}, leading to a percent error of 0% for the two day evaluation.

3. Results and discussion

Designing a phosphate binding assay for a polyamine binder requires careful consideration

Table 2
Accuracy of phosphate quantitation

Actual concentration	Found concentration	Percent of recovery
10.98	11.03	100.5
10.98	11.03	100.5
10.98	11.06	100.7
	Average	100.6
	SD	0.1
	% RSD	0.1
13.98	13.85	99.1
13.98	13.85	99.1
13.98	13.83	98.9
	Average	99.0
	SD	0.1
	% RSD	0.1
16.97	16.82	99.1
16.97	16.87	99.4
16.97	16.89	99.5
	Average	99.3
	SD	0.2
	% RSD	0.2

Table 3
Robustness with respect to sample weight

Weight of sevelamer hydrochloride (mg)	Binding capacity (mmol g^{-1})
110	5.8
110	5.9
110	5.9
Average	5.9
SD	0.1
% RSD	1.7
138	5.8
138	5.9
138	5.8
Average	5.8
SD	0.1
% RSD	1.7
166	5.8
166	5.7
166	5.8
Average	5.8
SD	0.1
% RSD	1.7

of the binding conditions. The influence of the polymer on phosphate ions equilibrium and hence on solution pH must be considered. This assay was designed for a pH of 7.0, where phosphate exists in equilibrium between the mono and divalent anion (pK_a 7.2). If either of the two forms are bound preferentially, solution pH will change. Furthermore, solution pH increases when sevelamer hydrochloride is added if the system is unbuffered. Additionally, cationic polyamines will bind other anions. Because of these issues, 100 mM BES, pK_a 7.1, was chosen to hold the pH at 7.0. BES is a zwitterion and therefore is not bound by Renagel®. Using this buffer, addition of polymer led to a negligible change in solution pH. Furthermore, after completion of the binding step, solution pH was unchanged.

In the case of a hydrogel, another important consideration is the phosphate to polymer ratio. Sevelamer hydrochloride is a hydrogel, absorbing $\approx 6–9$ times its weight of water. Therefore, the ratio of solution volume to polymer mass must be quite high to avoid changes to solution concentrations. Ideally, one would like to work under conditions where the amount of phosphate bound is solely dependent on the mass of polymer added. An investigation of different phosphate concentrations at fixed polymer mass and volume of solution was performed (J. Petersen and X. Chen, unpublished results). Optimum conditions were 14 mg of polymer with 10 ml of 20 mM KH_2PO_4. These conditions were scaled-up to 138 mg with 100 ml to improve precision. Under these conditions, the phosphate binding capacity was independent of sample amount from 110 to 166 mg (80–120% of assay amount), Table 3.

In summary, a phosphate binding assay for sevelamer hydrochloride was developed and validated. The method demonstrates excellent accuracy and precision and is being used at several contractors' sites to measure the phosphate binding of Renagel®. Over the range of phosphate concentrations expected in the assay (10–18 mM), the IC method was linear and showed recoveries of 99–101%. Repeatability of injection for the working standard was 0.4% RSD. Overall, the method gave an intermediate precision of 0.7%.

Table 4
Intermediate precision

Analyst 1	Analyst 2
5.8	5.8
5.9	5.8
5.8	5.8
Overall average	5.8
Overall SD	0.04
Overall % RSD	0.7

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