Determination of Binding Capacity and Affinity Constants of Sevelamer Hydrochloride and its Market Preparation

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ABSTRACT: Sevelamer is an orally administered weakly basic anion exchange resin which consists of cross-linked polymeric amine that binds dietary phosphate in the gastrointestinal tract. This is approved for the treatment of hyperphosphatemia in adult patients with end-stage renal disease. The binding parameter constants of non-absorbable sevelamer in its hydrochloride form were determined using the Langmuir approximation for its active pharmaceutical ingredient and 400 mg generic tablet dosage form (Sevel) at pH 4.0, 5.5 and 7.0. The binding parameters were also compared with the innovator product Renagel 400 mg tablet. The results demonstrate the equivalency of the dosage form at each pH in terms of the *in vitro* binding parameters. The results also demonstrate a shift in the binding mechanism from pH 4.0 to 7.0.

Key words: Sevelamer hydrochloride; Ion chromatography; Langmuir approximation; affinity constant; Renagel, Sevel.

INTRODUCTION

Hyperphosphatemia and secondary hyperparathyroidism are common complications of end stage renal disease (ESRD) that, when untreated, may result in increased morbidity and mortality.[1] Hyperphosphatemia and hypercalcemia have been associated with increased coronary artery calcification. Achieving control of serum phosphorus without increasing serum calcium is an important goal for patients with ESRD.[2] Although calcium-based phosphate binders effectively reduce serum phosphorus and parathyroid hormone concentrations, these agents can lead to hypercalcemia and have been associated with increased vascular calcification. Aluminum hydroxide is a potent phosphate binder, but concern about skeletal, hematological, and neurological toxicity.[3]

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The phosphorus binder, sevelamer[4] was developed to overcome the limitations associated with the usual management of hyperphosphatemia and secondary hyperparathyroidism (i.e., mineral salts). Sevelamer, a non-absorbable hydrogel, is as efficacious as calcium-based phosphate binders for reducing serum phosphorus but does not cause hypercalcemia or other adverse metabolic effects. Sevelamer also exhibits beneficial effects on lipids, consistently and significantly decreasing LDL cholesterol and increasing HDL cholesterol in most studies.[5]

In patients with chronic kidney disease (CKD) stages 3-5D, there is need to avoid the long-term use of aluminum-containing phosphate binders and in patients with chronic kidney disease (CKD) stage 5D, avoiding dialysate aluminum contamination to prevent aluminum intoxication. Sevelamer hydrochloride is a well-tolerated alternative to calcium- or aluminum-containing phosphate binder in

the control of serum phosphate in CAPD (continuous ambulatory peritoneal dialysis) patients. Subsequently, the importance of Sevelamer hydrochloride is increasing for hyperphosphatemia.

Renagel tablet (sevelamer HCl) administered orally is the brand product of Genzyme Corporation, UK and is being used for the treatment of hyperphosphatemia. Pharmacology, pharmaco-kinetics study, side effects, contraindications, precautions, efficacy, effectiveness, bioequivalence, clinical study and others study of Renagel tablet were approved by the FDA.

This non-absorbable, non-systemic drug is formulated by ACME Laboratories Ltd. Bangladesh as generic drug with a product name Sevel, containing sevelamer hydrochloride 400 mg. The equivalency in terms of efficacy of Sevel tablet (400 mg) was taken into consideration to compare with Renagel (400 mg) tablet using in-vitro study. This in-vitro equivalence study was performed using Langmuir approximation equation approved by FDA.

Utilizing Langmuir equation the binding affinity constant (K_1) and capacity constant (K_2) of non-absorbable sevelamer HCl and its market preparations (Sevel 400 mg tablet) were determined and compared with innovator drug, Renagel 400mg tablet. Thus the present study was designed to evaluate the in-vitro equivalence of Sevelamer hydrochloride generic preparation available in our markets with the innovator product.

MATERIALS AND METHODS

Sevelamer hydrochloride powder (API) and Renagel 400 tablet manufactured by Genzyme Corporation, Ireland, UK, containing sevelamer HCl 400mg were from ACME Laboratories Ltd, The Sevel 400 (B 3001), Sevel 400 (B 3002). Sevel 400 (B 3003) tablet as test products manufactured by ACME Laboratories Ltd, Dhaka, Bangladesh was collected from local market. Potassium monobasic phosphate, N,N-Bis(Hydroxyetyl)-2-aminoethane-sulfonic acid (BES), Sodium chloride, Sodium hydroxide, Sodium carbonate, Sodium bicarbonate, Methanol are obtained from Sigma-Aldrich, USA and sulphuric acid was obtained from Merck, Germany. 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). Labline heated orbital shaker (Labline 3520 orbital shaker, Hyland scientific, USA) and Ion chromatography (Model no: 881 Compact IC pro 1, Metrohm Ltd., Switzerland), were used in conduction of the study.

Preparation of mobile phase:

1mM $NaHCO_3$ and 3.2 mM Na_2CO_3 solution. In a one liter volumetric flask 80 mg $NaHCO_3$ and 340 mg Na_2CO_3 were taken and dissolved them with deionized H_2O made up to one liter. Then the solution was sonicated and filtered through 0.22 micron vacuum filter paper.

Preparation of H_2SO_4. In a 1000 ml volumetric flask 5.5 ml conc. H_2SO_4 was and diluted with 994.5 ml deionized H_2O. The solution was sonicated and then filtered through 0.22 micron vacuum filter paper.

Preparation of standard phosphate solution. Three sets of 250 ml volumetric flask were taken. Each set was marked at 8 different phosphate concentration such as 38.7 mM, 30.0 mM, 14.5 mM, 10.0 mM, 7.5 mM, 5.0 mM, 2.5 mM, 1.0 mM. Required amount of KH_2PO_4 was taken appropriately by calculation for 8 different phosphate concentrations. NaCl 701.28 mg (80 mM) and N,N-Bis(hydroxiethyl)-2-aminoethanesulfonic acid (BES) 3198.75 mg (100 mM) was weighed and added for all the volumetric flasks so that all the solutions contain the same concentration of BES and NaCl for maintaining gastrointestinal tract (GIT) chemical environment in-vitro**.** The final solution was made up to 150 mL by addition of de-ionized water. Such sets of solutions were prepared for three different pH (4.0, 5.5 and 7.0) levels adjusted by 1.0 N hydrochloride acid and 1.0 N sodium hydroxide. Then all the solutions were placed in Labline Heated Orbital Shaker at 37ºC temperatures for 2 hours for maintaining GIT in-vitro**.** The sample flasks were removed from Orbital Shaker and filtered through a

25 mm, 0.2 micro meter nylon syringe filter. The solutions were fifty times diluted with deionized water. All the drug-free standard samples were prepared in duplicate for different pH levels.

Preparation of sample solution. As per above design to 150 ml phosphate solutions, Sevelamer HCl 400mg (API), Renagel 400mg tablet, Sevel 400 tablet (B 3001), Sevel 400 mg tablet (B 3002) and Sevel 400 mg tablet (B 3003) were added for pH 4.0, pH 5.5 and pH 7.0 accordingly. The pH levels of the solutions were adjusted using 1.0 N hydrochloride acid and 1.0 N sodium hydroxide accordingly. All the solutions were placed in Labline Heated Orbital Shaker at 37^0C temperatures for 2 hours for maintaining GIT condition in-vitro**.** The sample flasks were removed from Orbital Shaker and filtered through a 25 mm, 0.2 micro meter nylon syringe filter. The solutions were fifty times diluted with deionized water. All the test samples were prepared in duplicate for different pH levels. For all solutions the test was performed in triplicates.

Chromatographic conditions. Injector: Auto injector; Column: Polyvinyl alcohol with quaternary ammonium; 150 × 4.0 mm; Detector: Conductivity detector; Eluent composition: 1mM sodium bicarbonate and 3.2 mM sodium carbonate; Flow rate: 0.7 ml/min, Injection volume: 20 µl; Software: Magic Net. An ion chromatogram of standard phosphate solution has been shown in Fig. 1.

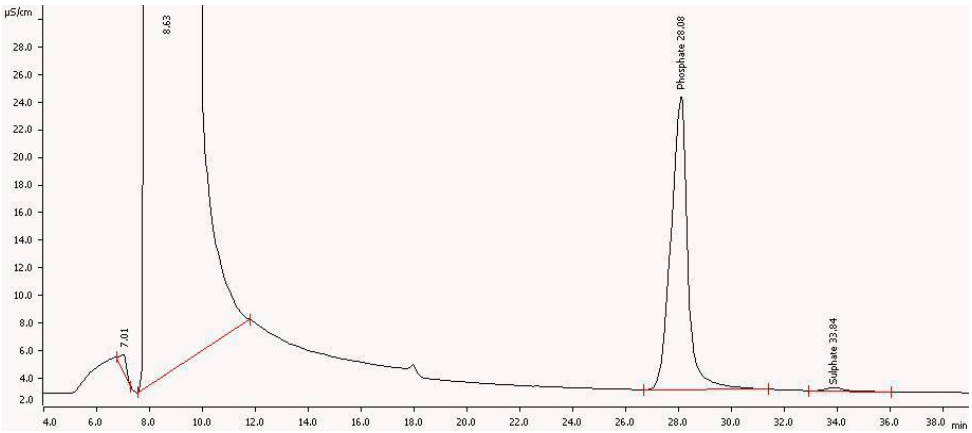

Fig. 1. Ion chromatogram of 2.5 mM standard phosphate

Calculations. The remaining unbound phosphate in terms of concentration in each sample was calculated from the linear regression line obtained from a plot of area of the phosphate peak versus the concentration of the phosphate (mM) using the following equation : [6]

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

From the known initial concentration of phosphate in each solution (i.e. 38.7, 30.0, 14.5, 10.0, 7.5, 5.0, 2.5, and 1.0 mM) before the addition of sevelamer hydrochloride, the bound concentration was calculated by subtracting the unbound concentration from the initial concentration. [6]

Bound phosphate concentration (mM) = initial concentration (mM) – unbound phosphate concentration (mM)

$$\text{Phosphate binding capacity } \left(\frac{\text{mmol}}{\text{g}}\right) = \frac{\text{bound phosphate concentration (mM)} \times \text{Vs(L)}}{\text{Weigh (g)}}$$

Where, Vs is the volume of the solution, approximately 150 ml or 0.15 L for the 400 mg tablet and powder. The weigh is the weight of the sevelamer hydrochloride expressed in (g).

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).[7] The process is described by the

$$\frac{C_{eq}}{\frac{x}{m}} = \frac{C_{eq}}{K_2} + \frac{1}{K_1 K_2}$$

Where C*eq* is the concentration of phosphate remaining in mM in solution at equilibrium or the unbound concentration. The x/m is the amount of phosphate bound per weight of polymer in mM/g**.** The constant K_1 is the affinity constant involved in binding and related to the magnitude of the forces. The constant K_2 is the Langmuir capacity constant and is the maximum amount of that can be bound per unit weight of sevelamer hydrochloride.[6]

The affinity constant and Langmuir capacity constants were calculated by performing linear regression on a plot of the unbound phosphate concentration (mM) / binding capacity (mM/g) versus unbound phosphate concentration (mM). The K_1 (= a/b) value is calculated by dividing the slope (a) of the regression line by the intercept (b), the K_2 (= 1/a) value is equal to the inverse of the slope.

RESULTS

The present study was designed to evaluate the *in vitro* equivalencey of sevelamer hydrochloride generic preparation available in our markets with the innovator product. The equivalency in terms of efficacy of Sevel tablet 400 mg of three different batches B 3001, B 3002 and B 3003 of the generic product designated as Sevel B 3001, Sevel B 3002 and Sevel B 3003, was compared with Renagel 400 mg tablet the innovator product using *in vitro* study. This *in vitro* equivalence study was performed using Langmuir approximation equation approved by the FDA.[6] In Langmuir equation the binding affinity constant (K_1) and capacity constant (K_2) of Sevelamer HCl and its market preparations (Sevel 400 tablet) were determined and compared with innovator drug Renagel 400 mg tablet.

Calibration curve of KH_2PO_4. Eight different concentration of KH_2PO_4 solution were prepared and obtained eight different types of peak areas through ion chromatography. Each solution contains 80 mM NaCland100 mM N,N-s(hydroxiethyl)-2-minoethanesulfonic acid (BES) as incubation media for maintaining the chemical environment of GIT in-vitro. All solutions were kept at Labline orbital shaker for 2.0 hours at 37ºC for maintaining the physical condition of the GIT. Calibration curves were obtained for pH 4.0 (Fig. 2), pH 5.5 (Fig. 3) andpH 7.0 (Fig. 4). These different pH levels are observed in different portions in the GIT and were adjusted either by adding 1.0 N HCl or 1.0 N NaOH in the experimental boratory. The RSQ values are 0.9999, 0.9998 and 0.9999, slopes 6.801, 7.070 and 7.109 and intercepts were -0.9542, -1.4086 and - 1.471 for pH 4.0, pH 5.5 and pH 7.0 respectively (Table 1). These values specially the slopes and intercepts were used in the calculation of unbound phosphate according to Langmuir approximation equation. The bound concentration was calculated by subtracting the unbound concentration from the initial phosphate concentration before the addition of sevelamer HCl API or its dosage forms.

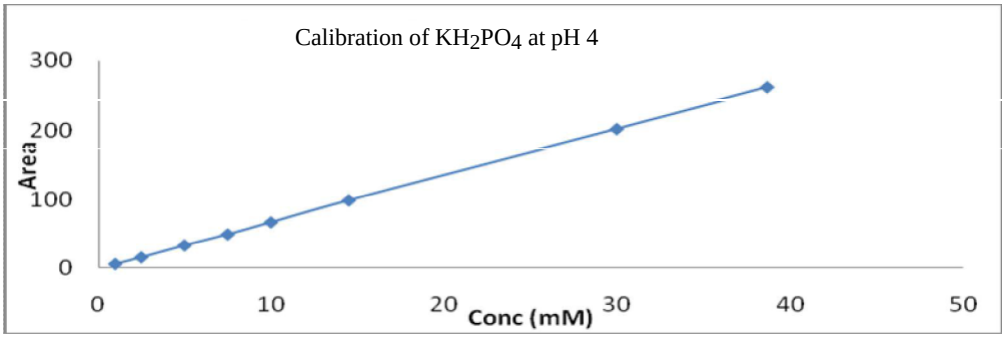

Fig. 2. Calibration curve of KH_2PO_4 at pH 4.0

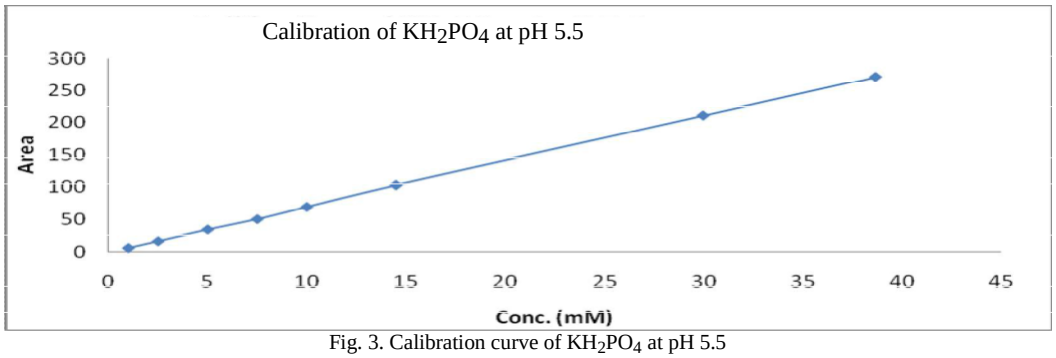

Fig. 3. Calibration curve of KH_2PO_4 at pH 5.5

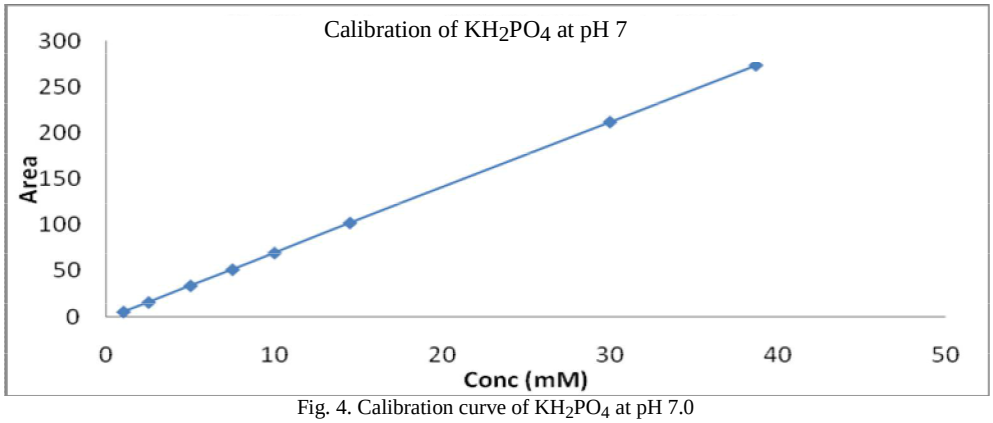

Fig. 4. Calibration curve of KH_2PO_4 at pH 7.0

Table 1. Data of the calibration curve at all pHs

	pH 4	pH 5.5	pH 7
RSQ	0.9999	0.9998	0.99999
Slope	6.801	7.070	7.109
Intercept	-0.9542	-1.4086	-1.471

Percentage of unbound and bound phosphate at different pH levels. The unbound phosphate concentration (mM) remaining in each sample was calculated from the linear regression generated plot of area of the phosphate peak against the initial concentration (mM) using intercept and slope of the respective pH according to Langmuir.[6] Thus bound phosphate concentration (mM) was also obtained by subtracting unbound phosphate concentration from initial phosphate concentration. At pH 4.0 using the active sevelamer HCl as binding agent the percentage of bound phosphate were higher in lower initial concentration as compared to the higher initial concentration of phosphate. At lower phosphate concentrations 1.0, 2.5, 5, 7.5, 10 and 14.5 mM the percentage of bound phosphate were 82.5, 70.02, 79.18, 78.20, 76.34 and 72.59%, respectively and at higher phosphate concentrations 30 and 38.7 mM, the percentage of bound phosphate concentrations were 52.42 and 44.61%, respectively (Table 2).

Table 2. Percentage of unbound and bound phosphate of Sevelamer HCl (API) at pH 4.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	17.49	82.50 ± 0.046
2.5	29.97	70.02 ± 0.231
5.0	20.81	79.18 ± 1.131
7.5	21.79	78.20 ± 0.369
10.0	23.65	76.34 ± 0.359
14.5	27.40	72.59 ± 0.227
30.0	47.57	52.42 ± 0.494
38.7	55.38	44.61 ± 0.719

Similarly at pH 4.0 using the reference product Renagel 400 containing sevelamer HCl as binding agent the percentage of bound phosphate at lower phosphate concentrations 1.0, 2.5, 5.0, 7.5, 10 and 14.5 mM were 75.15, 65.85, 60.20, 52.60, 49.87 and 47.50% and at higher phosphate concentrations 30 and 38.7 mM, the percentage of bound phosphate concentrations were 36.73 and 35.36%, respectively (Table 3).

Table 3. Percentage of unbound and bound phosphate of Renagel 400 mg tablets at pH 4.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	24.84 ±	75.15 ± 1.171
2.5	34.14 ±	65.85 ± 2.331
5.0	39.79 ±	60.20 ± 1.175
7.5	47.39 ±	52.60 ± 1.749
10.0	50.12 ±	49.87 ± 0.417
14.5	52.49 ±	47.50 ± 0.817
30.0	63.26 ±	36.73 ± 0.649
38.7	64.63 ±	35.36 ± 0.242

At pH 4.0 using the test preparation Sevel 400 (B 3001) containing sevelamer HCl as binding agent the percentage of bound phosphate at lower concentrations 1.0, 2.5, 5, 7.5, 10 and 14.5 mM were 74.15, 69.21, 63.97, 53.85, 52.43 and 48.54%, respectively and at higher phosphate concentrations 30 and 38.7 mM, the percentage of bound phosphate concentrations were 38.62 and 36.22%, respectively (Table 4). For the test preparation Sevel 400 (B 3002) the percentage of bound phosphate at concentrations were 75.15, 66.25, 59.34, 52.45, 50.31 and 47.49% and at higher phosphate concentrations the percentage of bound phosphate concentration were 32.88 and 28.27%, respectively (Table 5). For test preparation Sevel 400 (B 3003) the percentage of bound phosphate at lower concentrations were 73.87, 65.01, 60.09, 52.06, 50.35 and 47.83% and at higher phosphate concentrations the percentage of bound phosphate concentration were 36.67 and 33.45%, respectively (Table 6).

Table 4. Percentage of unbound and bound phosphate of Sevel B 3001 tablets at pH 4.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	25.84	74.15 ± 3.089
2.5	30.78	69.21 ± 1.256
5.0	36.02	63.97 ± 0.556
7.5	46.14	53.85 ± 0.419
10.0	47.56	52.43 ± 0.647
14.5	51.45	48.54 ± 1.513
30.0	61.37	38.62 ± 0.582
38.7	63.77	36.22 ± 0.836

At pH 5.5 using the active sevelamer HCl as binding agent the percentage of bound phosphate was higher in lower initial concentrations as compared to the higher initial concentrations of phosphate as that of pH 4.0. At lower phosphate concentrations 1.0, 2.5, 5.0, 7.5, 10 and 14.5 mM the percentage of bound phosphate were 79.92, 89.72, 88.92, 89.12, 87.15 and 83.30%, respectively and at higher phosphate concentrations 30 and 38.7 mM, the percentage of bound phosphate concentration were 61.33 and 50.11%, respectively (Table 7).

Table 5. Percentage of unbound and bound phosphate of Sevel B 3002 tablets at pH 4.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	24.84	75.15
2.5	33.74	66.25
5.0	40.65	59.34
7.5	47.54	52.45
10.0	49.68	50.31
14.5	52.50	47.49
30.0	67.11	32.88
38.7	71.72	28.27

Table 6. Percentage of unbound and bound phosphate of Sevel B 3003 tablets at pH 4.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	26.12	73.87
2.5	34.98	65.01
5.0	39.99	60.09
7.5	47.93	52.06
10.0	49.64	50.35
14.5	52.16	47.83
30.0	63.32	36.67
38.7	66.54	33.45

Table 7. Percentage of unbound and bound phosphate of Sevelamer HCl (API) at pH 5.5

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	20.07 ±	79.92 ± 0.860
2.5	10.27 ±	89.72 ± 0.403
5.0	11.07 ±	88.92 ± 0.245
7.5	10.87 ±	89.12 ± 0.205
10.0	12.84 ±	87.15 ± 0.135
14.5	16.69 ±	83.30 ± 0.098
30.0	38.66 ±	61.33 ± 0.079
38.7	49.88 ±	50.11 ± 1.675

Similarly at pH 5.5 using the reference product Renagel 400 containing sevelamer HCl as binding agent the percentage of bound phosphate at lower phosphate concentrations were 80.64, 91.90, 89.95,

89.14, 87.72 and 84.65% and at higher phosphate concentrations the percentage of bound phosphate concentration were 66.62 and 54.92% (Table 8). For the test preparation Sevel B 3001 containing sevelamer HCl as binding agent the percentage of bound phosphate at lower phosphate concentrations were 79.65, 85.07, 80.95, 83.27, 76.45 and 72.45% and at higher phosphate concentrations the percentage of bound phosphate concentration were 56.25 and 46.33% (Table 9). For the preparation Sevel B 3002 the percentage of bound phosphate at lower phosphate concentrations were 78.96, 85.81, 81.86, 83.54, 77.67 and 73.09% and at higher phosphate concentrations the percentage of bound

Table 8. Percentage of unbound and bound phosphate of Renagel 400 at pH 5.5

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	19.35	80.64
2.5	8.09	91.90
5.0	10.04	89.95
7.5	10.85	89.14
10.0	12.27	87.72
14.5	15.34	84.65
30.0	33.37	66.62
38.7	45.07	54.92

Table 9. Percentage of unbound and bound phosphate of Sevel B 3001 tablets at pH 5.5

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	20.34	79.65
2.5	14.92	85.07
5.0	19.04	80.95
7.5	16.72	83.27
10.0	23.54	76.45
14.5	27.54	72.45
30.0	43.74	56.25
38.7	53.66	46.33

Table 10. Percentage of unbound and bound phosphate of Sevel B 3002 tablets at pH 5.5

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	21.03	78.96
2.5	14.18	85.81
5.0	18.13	81.86
7.5	16.45	83.54
10.0	22.33	77.67
14.5	26.90	73.09
30.0	40.22	59.77
38.7	51.35	48.64

phosphate concentration were 59.77 and 48.64% (Table 10). Similarly the test product Sevel B 3003 the percentage of bound phosphate at lower phosphate concentrations were 79.75, 88.59, 83.17, 85.15, 77.97 and 75.47% and at higher phosphate concentrations the percentage of bound phosphate concentration were 59.29 and 49.75% (Table 11).

Table 11. Percentage of unbound and bound phosphate of Sevel B 3003 tablets at pH 5.5

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	20.24	79.75
2.5	11.40	88.59
5.0	16.82	83.17
7.5	14.84	85.15
10.0	22.02	77.97
14.5	24.52	75.47
30.0	40.70	59.29
38.7	50.24	49.75

At pH 7 using the active sevelamer HCl as binding agent the percentage of bound phosphate were higher in lower initial concentration as compared to the higher initial concentration of phosphate as that of pH 4 and pH 5.5. At lower phosphate concentrations 1.0, 2.5, 5.0, 7.5, 10 and 14.5 mM the percentage of bound phosphate were 79.29, 86.25, 84.26, 80.13, 78.21 and 63.80%, respectively and at higher phosphate concentrations 30 and 38.7 mM, the percentage of bound phosphate concentration were 40.24 and 46.15%, respectively (Table 12). Similarly at pH 7 using the reference product Renagel 400 the percentage of bound phosphate at lower phosphate concentrations were 78.98, 90.69, 90.99, 85.67, 84.65 and 78.37% and at higher phosphate concentrations the percentage of bound phosphate concentration were 50.10 and 40.29% (Table 13). For the test product Sevel B 3001 the percentage of bound phosphate at lower phosphate concentrations were 78.46, 91.87, 92.78, 90.22, 85.03 and 78.86% and at higher phosphate concentrations the percentage of bound phosphate concentration were 56.50 and 46.86% (Table 14). For the test product Sevel B 3002 the percentage of bound phosphate at lower phosphate concentrations were 78.35, 90.65, 91.63, 87.92, 83.57 and 76.87%

and at higher phosphate concentrations the percentage of bound phosphate concentration were 53.14 and 41.76% (Table 15). For Sevel B 3003 containing sevelamer HCl as binding agent the percentage of bound phosphate at lower phosphate concentrations were 78.96, 91.99, 92.61, 89.56, 84.95 and 79.16% and at higher phosphate concentrations the percentage of bound phosphate concentration were 54.99 and 44.29% (Table 16).

Table 12. Percentage of unbound and bound phosphate of working std. at pH 7.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	20.70	79.29
2.5	13.74	86.25
5.0	15.73	84.26
7.5	19.86	80.13
10.0	21.78	78.21
14.5	36.19	63.80
30.0	59.75	40.24
38.7	53.84	46.15

Table 13. Percentage of unbound and bound phosphate of Renagel 400 mg tablets at pH 7.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	21.01	78.98
2.5	9.30	90.69
5.0	9.00	90.99
7.5	14.32	85.67
10.0	15.34	84.65
14.5	21.62	78.37
30.0	49.89	50.10
38.7	59.70	40.29

Table 14. Percentage of bound and unbound phosphate of Sevel B 3001 tablets at pH 7.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	21.53	78.46
2.5	8.12	91.87
5.0	7.21	92.78
7.5	9.77	90.22
10.0	14.96	85.03
14.5	21.13	78.86
30.0	43.48	56.50
38.7	53.13	46.86

Langmuir approximation[6] plot at pH 4.0. At pH 4 using active sevelamer HCl as binding agent the affinity constant (K_1) and binding capacity constant (K_2) values were determined by plotting unbound phosphate concentration (mM)/binding capacity (mM/gm) versus unbound phosphate concentration (mM) at this pH (Table 17). The RSQ value, slope and intercept of the regressed plot were determined and the K_1 and K_2 values were obtained from these values respectively for samples, respectively.

Table 15. Percentage of bound and unbound phosphate of Sevel B 3002 tablets at pH 7.0

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	21.64	78.35
2.5	9.34	90.65
5.0	8.36	91.63
7.5	12.07	87.92
10.0	16.42	83.57
14.5	23.12	76.87
30.0	46.85	53.14
38.7	58.23	41.76

Table 16. Percentage of bound and unbound phosphate of Sevel 400 (B 3003) at pH 7

Total phosphate conc. (mM)	% Unbound PO_4	% Bound PO_4
1.0	21.03	78.96
2.5	8.00	91.99
5.0	7.38	92.61
7.5	10.43	89.56
10.0	15.04	84.95
14.5	20.83	79.16
30.0	45.01	54.99
38.7	55.70	44.29

The combined Langmuir plot of unbound phosphate verses unbound phosphate/binding capacity (Table 17) at pH 4 of Sevelamer HCl (API), Renagel 400 mg tablet, Sevel 400 mg tablet (B 3001), Sevel 400 mg tablet (B 3002) and Sevel 400 mg tablet B 3003 were shown in Fig. 5. The Langmuir affinity constant (K_1), binding capacity constant (K_2), RSQ, slope and intercept were very similar for all samples at this pH as shown in Table 18.

At pH 5.5 using active sevelamer HCl as binding agent the affinity constant (K_1) and binding capacity constant (K_2) values were also determined by plotting unbound phosphate concentration (mM)/binding capacity (mM/gm) versus unbound phosphate concentration (mM) at this pH (Table 19). The RSQ

value, slope and intercept of the regressed plot were determined and the K_1 and K_2 values were obtained from these values respectively for samples respectively.

The combined Langmuir plot of unbound phosphate verses unbound phosphate/binding capacity (Table 19) at pH 5.5 of Sevelamer HCl (API), Renagel 400 mg tablet, Sevel B 3001 400 mg tablet, Sevel B 3002 400 mg tablet and Sevel B 3003 400 mg tablet were shown in Fig. 6. The Langmuir affinity constant (K_1), binding capacity constant (K_2), RSQ, slope and intercept were very similar for all samples at this pH as shown in Table 20.

At pH 7.0 using active sevelamer HCl as binding agent the affinity constant (K_1) and binding capacity constant ($K_{2)}$ values were also determined by plotting unbound phosphate concentration (mM) / binding capacity (mM/gm) versus unbound phosphate concentration (mM) at this pH (Table 21). The RSQ value, slope and intercept of the regressed plot were determined and the K_1 and K_2 values were obtained from these values respectively for samples respectively.

The combined Langmuir plot of unbound phosphate verses unbound phosphate/binding capacity (Table 19) at pH 7.0 of Sevelamer HCl (API), Renagel 400 mg tablet, Sevel 400 mg tablet (B 3001), Sevel 400 mg tablet (B 3002) and Sevel 400 mg tablet B 3003 were shown in Fig. 7. The Langmuir affinity constant (K_1), binding capacity constant (K_2), RSQ, slope and intercept were very similar for all samples at this pH as shown in Table 22.

At each individual pH, **s**evelamer hydrochloride powder (working std.), Sevel 400 mg (B 3001), Sevel 400 mg (B 3002), Sevel 400 mg (B 3003) tablet, Renagel 400 mg tablet exhibited very similar binding properties. The affinity constant (K_1) of all samples increased gradually form pH 4.0 to 7.0.

Table 17. Unbound phosphate concentration and unbound PO_4/binding capacity of test and reference products at pH 4.0

Sevelamer (API)		Renagel 400		Sevel 400 (B 3001)		Sevel 400 (B 3002)		Sevel 400 (B 3003)	
Unbound PO4 Conc. mM	Unbound/B capacity	Unbound PO4 Conc. mM	Unbound/B capacity	Unbound PO4 Conc. mM	Unbound/B capacity	Unbound PO4 Conc. mM	Unbound/ B capacity	Unbound PO4 Conc. mM	Unbound/B capacity
0.174	0.565	0.248	0.881	0.258	0.929	0.248	0.881	0.261	0.943
0.749	1.141	0.853	1.382	0.769	1.185	0.843	1.358	0.874	1.434
1.040	0.700	1.989	1.762	1.801	1.501	2.032	1.826	1.999	1.777
1.634	0.742	3.554	2.402	3.460	2.284	3.565	2.416	3.595	2.455
2.365	0.826	5.012	2.679	4.756	2.418	4.968	2.633	4.964	2.629
3.973	1.006	7.611	2.946	7.460	2.826	7.613	2.948	7.564	2.908
14.273	2.420	18.978	4.591	18.413	4.237	20.135	5.443	18.997	4.604
21.434	3.310	25.014	4.874	24.681	4.695	27.758	6.765	25.754	5.305

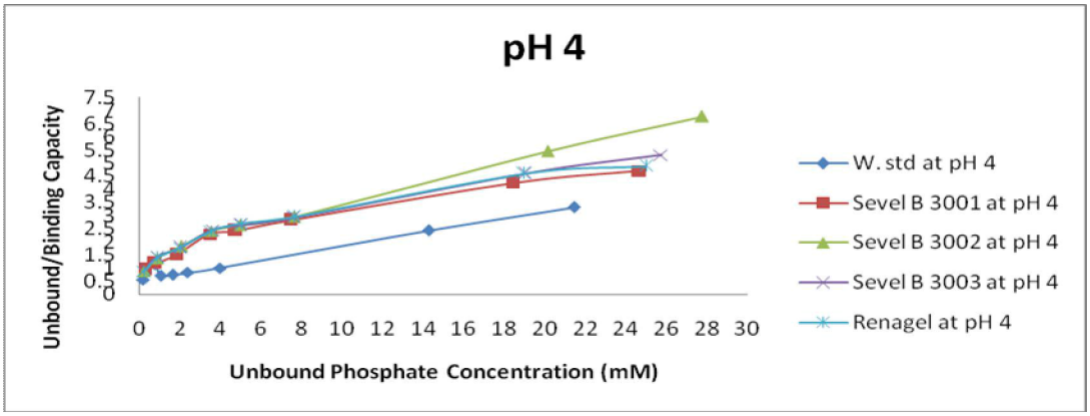

Fig. 5. Langmuir plot of working standard (Sevelamer HCl), Renagel 400 mg, Sevel 400 (B 3001), Sevel 400 (B 3002) and Sevel 400 (B 3003) at pH 4.0

Table 18. The Langmuir capacity and affinity constants of reference and test products calculated at pH 4.0

	Sevelamer (API)	Renagel 400	Sevel 400 (B 3001)	Sevel 400 (B 3002)	Sevel 400 (B 3003)
RSQ	0.9680	0.9230	0.9348	0.9816	0.9504
Slope	0.1242	0.1504	0.1472	0.2005	0.1578
Intercept	0.6305	1.5002	1.3760	1.3507	1.4941
k_2	8.0503	6.6457	6.7907	4.9874	6.3350
k_1	0.1969	0.1002	0.1070	0.1484	0.1054

Table 19. Unbound phosphate concentration and unbound PO_4/binding capacity of test and reference products at pH 5.5.

Sevelamer API		Renagel 400mg		Sevel B 3001 400mg		Sevel B 3002 400mg		Sevel B 3003 400mg	
Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity
0.200	0.668	0.193	0.640	0.203	0.681	0.210	0.710	0.202	0.676
0.256	0.305	0.202	0.234	0.373	0.467	0.354	0.440	0.285	0.343
0.553	0.332	0.502	0.297	0.952	0.627	0.906	0.590	0.841	0.539
0.815	0.325	0.813	0.324	1.254	0.535	1.234	0.525	1.113	0.464
1.284	0.393	1.228	0.373	2.354	0.821	2.234	0.766	2.202	0.753
2.420	0.534	2.225	0.483	3.993	1.013	3.901	0.981	3.556	0.866
11.598	1.680	10.012	1.335	13.123	2.073	12.067	1.794	12.215	1.830
19.305	2.654	17.443	2.188	20.769	3.088	19.875	2.815	19.445	2.693

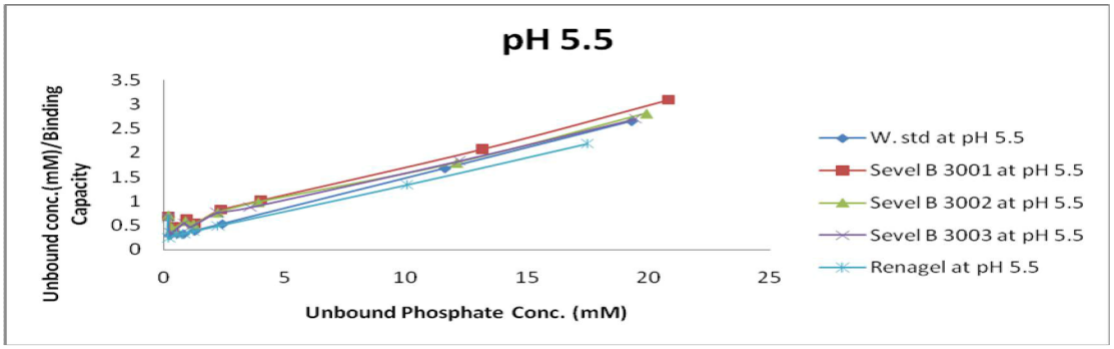

Fig. 6. Langmuir plot of working standard (Sevelamer HCl), Renagel 400 mg, Sevel 400 (B 3001), Sevel 400 (B 3002), and Sevel 400 (B 3003) at pH 5.5

Table 20. The Langmuir capacity and affinity constants calculated at pH 5.5

	Sevelamer API	Renagel 400	Sevel 400 (B 3001)	Sevel 400 (B 3002)	Sevel 400 (B 3003)
RSQ	0.974	0.962	0.991	0.985	0.983
Slope	0.119	0.106	0.123	0.114	0.114
Intercept	0.317	0.300	0.500	0.494	0.449
k_2	8.371	9.393	8.111	8.739	8.711
k_1	0.375	0.354	0.246	0.232	0.255

Table 21. Unbound phosphate concentration and unbound PO_4/binding capacity of test and reference products at pH 7.0

Sevelamer API		Renagel 400mg		Sevel 3001 400mg		Sevel 3002 400mg		Sevel 3003 400mg	
Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity	Unbound PO4 Conc.mM	Unbound/B capacity
0.207	0.696	0.210	0.709	0.215	0.731	0.216	0.736	0.210	0.710
0.343	0.424	0.232	0.273	0.203	0.235	0.233	0.274	0.201	0.232
0.786	0.498	0.450	0.263	0.360	0.207	0.418	0.243	0.369	0.212
1.490	0.661	1.074	0.446	0.732	0.288	0.905	0.366	0.782	0.310
2.178	0.742	1.534	0.483	1.496	0.469	1.642	0.524	1.504	0.472
5.247	1.512	3.135	0.735	3.064	0.714	3.352	0.801	3.020	0.701
17.925	3.959	14.967	2.655	13.046	2.0521	14.055	2.350	13.501	2.181
20.837	3.110	23.105	3.950	20.564	3.023	22.535	3.717	21.556	3.353

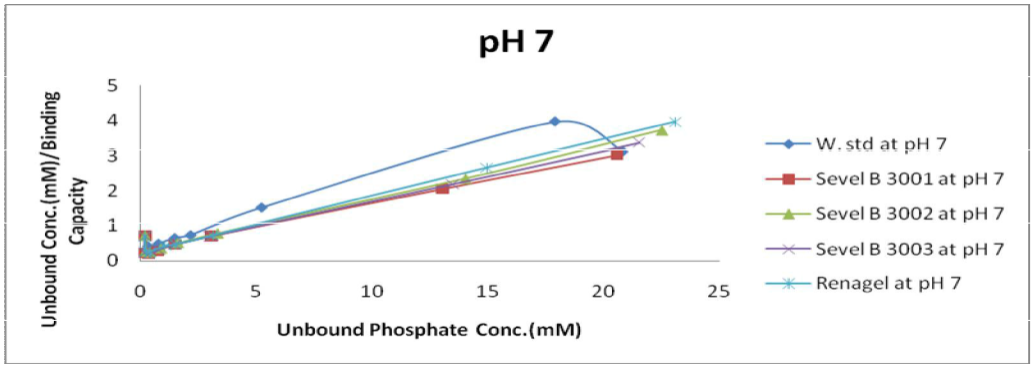

Fig. 7. Langmuir plot of working standard (Sevelamer HCl), Renagel 400 mg, Sevel B 3001, Sevel B 3002, and Sevel B 3003 at pH 7

Table 22. The Langmuir capacity and affinity constants of all sample calculated at pH 7.0

	Sevelamer API	Renagel 400mg	Sevel B 3001	Sevel B 3002	Sevel B 3003
RSQ	0.924	0.987	0.9722	0.9827	0.9794
Slope	0.154	0.156	0.1322	0.1486	0.1408
Intercept	0.502	0.314	0.3096	0.3212	0.2973
k_2	6.462	6.385	7.5635	6.7269	7.0992
k_1	0.307	0.497	0.4270	0.4627	0.4736

DISCUSSION

The *in vitro* equivalence study of Sevel 400 mg tablet was compared with reference brand product Renagel 400 mg tablet by maintaining GIT condition by the help of Langmuir approximation approved by FDA.[6] This study was performed to evaluate the efficacy of the generic drug Sevel 400 mg tablet with the innovator product Renagel 400 mg tablet.

The non-linearity of the Langmuir plot and the order of magnitude decrease in the affinity constants (K_1) 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 pKa of each phosphate ion and the equilibrium reaction.[6] At pH range of approximately 6 - 8, monobasic phosphate is in equilibrium with dibasic phosphate. It has been demonstrated that dibasic phosphate is predominately bound species at pH 7.0.[8]

At pH 5.5-6.0, the fraction of the monobasic ion increases. The small decreases in the binding affinity constant (K_1) demonstrates that the the binding force 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. At

pH 4.0, the monobasic phosphate ion is predominately present. The affinity constants (K_1) are an order of magnitude lower at pH 4.0 than the affinity constants (K_1) 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.[6]

The linearity of the Langmuir plots indicates mono-molecular binding. The relative non-linearity of the Langmuir plots at pH 4.0 may indicate non-monomolecular binding as a result of monobasic ion. The dibasic binding of phosphate at pH 7.0 and 5.5 of the test and reference were observed where the monobasic binding of phosphate was observed only at pH 4.0. The lower slope of the curve at pH 4.0 also demonstrates the lower binding affinity of monobasic ion.

A possible explanation for the similarities of the Langmuir plot at pH 7.0 and 5.5 is the apparent pKa values. 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 pKa 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.[6]

It shows a preference for phosphate over other intestinal anions, such as chloride and bicarbonate. The preference for phosphate is believed to be due to its dianion character, and may also involve hydrogen bonding. The absorption of phosphate *in vitro* is rapid (less than a minute) relative to the time of passage of such a drug through the small intestine (hours). It has also been found to prevent the absorption of dietary phosphate *in vivo* and in humans.[8]

CONCLUSION

Sevelamer hydrochloride (polyallylamine crosslinked with epichlorohydrin) is a polymeric hydrogel that has been developed as an oral pharmaceutical to prevent the absorption of dietary phosphate by kidney dialysis patients. It has been found to bind to phosphate *in vitro*, and to do so more effectively than a wide range of other cationic polymers. The results demonstrates that at each individual pH, Sevelamer hydrochloride (API), different batches of Sevel 400 mg tablets and Renagel 400 mg tablet exhibited very similar binding properties showing equivalent kinetic properties *in vitro*. So, it is evident that a good efficacy of generic drug Sevel 400 mg tablets manufactured by ACME Laboratories Ltd, Dhaka, Bangladesh was observed in terms of its Langmuir binding capacity constants (K_2) and affinity constants (K_1) as compared to innovator drug Renagel 400 mg tablets. So, Renagel 400 mg tablet manufactured by Genzyme Corporation, Ireland, UK can be substituted by generic product Sevel 400 mg tablet manufactured by ACME Laboratories Ltd, Dhaka, Bangladesh.

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