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Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 753–760
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of the binding parameter constants of Renagel® capsules and tablets at pH 7 by high performance capillary electrophoresis

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Received 31 January 2003; received in revised form 12 December 2003; accepted 13 February 2004

Available online 12 April 2004

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 (ESRD). The binding parameter constants of sevelamer hydrochloride were determined using high performance capillary electrophoresis (HPCE) and the Langmuir approximation for three different dosage forms at pH 7.0. The three dosage forms were Renagel® 403 mg capsules, Renagel® 400 mg tablets and Renagel® 800 mg tablets. The results demonstrate the in vitro bioequivalence of all three dosage forms at pH 7.0. These results are in very good agreement with previously published results obtained by ion chromatography.

Keywords: Sevelamer hydrochloride; Capillary electrophoresis; 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) [1–6]. The advantage of sevelamer hydrochloride for end-stage renal disease over existing therapies, such as calcium or aluminum supplementation, is that it is non-absorbed, leading to an improved safety profile. There is evidence that treatment with sevelamer hydrochloride leads to the attenuation of the progression of coronary artery and aortic calcification as well as improved control of parathyroid hormone levels relative to calcium salts [7]. The structure of sevelamer hydrochloride is shown in Fig. 1.

This paper describes the methodology and procedures for the determination of the binding constants by

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doi:10.1016/j.jpba.2004.02.021

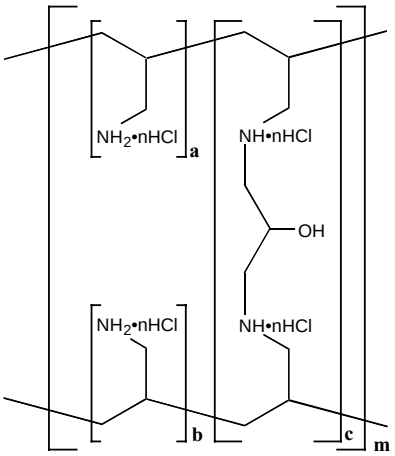

Fig. 1. Structure of sevelamer hydrochloride; a, b: number of primary amine groups (a + b = 9); c: number of cross-linking groups (c = 1); *n*: fraction of protonated amines (*n* = 0.4); *m*: large number to indicate extended polymer network.

high performance capillary electrophoresis (HPCE) at pH 7.0 utilizing the Langmuir approximation. HPCE offers the advantage of rapid, efficient separations due to the flat flow profile that is inherent with electro-driven techniques. Due to the small volumes required (nl to μl) there is little solvent consumption or waste generation. Also the HPCE columns have small inner diameters, typically 50 μm, and are equilibrated in less than 10 min, allowing for quicker analysis times. Utilizing HPCE methodology will also validate the previously published results obtained by IC and confirm the in vitro bioequivalence of all three dosage forms of Renagel® by an additional technique [4].

A comparison of these binding constants, as determined by HPCE, demonstrates the in vitro bioequivalence of the tablet dosage forms to the capsule dosage form. These results are in very good agreement with previously published results obtained by ion chromatography [4].

2. Materials and methods

2.1. Chemicals

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

2.2. Apparatus

A Hewlett Packard (Agilent Technologies, Palo Alto, CA, USA) ^{3D}CE system, equipped with HP Chemstation software (Rev. A.06.03), was used for phosphate analysis. Separations were performed using a bare silica capillary with an extended light path, 50 μm × 42.5 cm effective length (Agilent Technologies P/N G1600-6132). Samples were shaken using a Labline Heated Orbital Shaker Model# 4628 (Labline Instruments, Melrose Park, IL, USA).

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, and 5.0 mM. The phosphate solutions were prepared so that a final pH of 7.0 ± 0.2 was obtained after the addition of Renagel® capsules and tablets, as described below. All solutions contained 100 mM BES and 80 mM NaCl.

The solutions were prepared by adjusting the pH of each solution to pH 7.0 ± 0.2 with 1N NaOH [5]. Approximately 50 ml of 1N NaOH was volumetrically added per liter of solution. After the addition of the capsules and tablets, the pH of the solution was pH 7.0 ± 0.2. 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. It has been demonstrated that BES, in concentrations from 60 to 120 mM, does not affect the phosphate binding (Swearingen et al., unpublished results).

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.

All samples were then placed on a Labline Orbital Shaker at 37 °C for 2 h to ensure the establishment of binding equilibrium. Previous studies have indicated that 15 min of shaking time is sufficient to establish binding equilibrium [5]. The samples were removed, filtered through a 25 mm, 0.2 μm nylon syringe filter (Pall Corp., Ann Arbor, MI). The filtrates were diluted with deionized water in the appropriate ratios. The dilutions ranged from 1:100 to 1:10.

The phosphate standards were prepared by diluting the phosphate solutions, 1:50 with deionized water to generate a six point calibration curve. This dilution was performed prior to the addition of capsules and tablets. A calibration curve was generated by plotting the area of the phosphate peak versus the concentration of phosphate. The slope of the calibration curve was 296.9 with an intercept of 2.69. The coefficient of determination was greater than 0.995. The average of the two phosphate binding values was used to generate the phosphate binding isotherms and Langmuir plots.

2.4. HPCE conditions

Standards and samples were analyzed for phosphate levels by HPCE using the column described above. The HPCE conditions, use of run buffers and pH has been described in detail [8]. The run buffer consisted of 2.25 mM, 1,2,4,5-tetracarboxylic acid (pyromelletic acid), 0.75 mM hexamethonium hydroxide, 1.6 mM triethanolamine in 6.5 mM NaOH, pH 7.7.

Pyromelletic acid was utilized because its electrophoretic mobility closes matches that of many inorganic ions and it has a high molar absorptivity. A carrier ion with a high molar absorptivity is advantageous because a signal is generated in indirect-UV detection when an analyte ion displaces the carrier

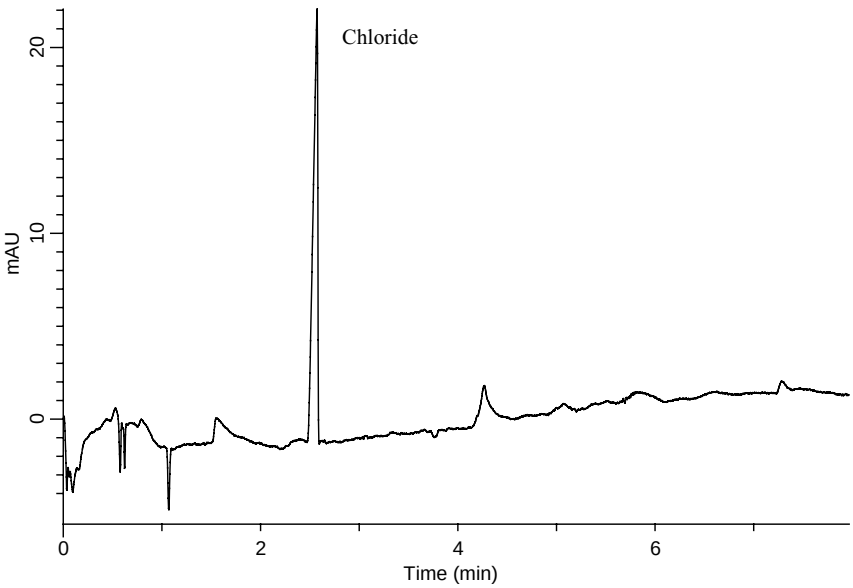

Fig. 2. Typical electropherogram of a blank solution. The chloride peak and the peak at approximately 4.3 min is from the binding media (80 mM NaCl, 100 mM BES diluted 1:50 with water). Indirect UV at 350 nm with a reference of 245 nm.

ion, thus creating a decrease in absorbance. This decrease in absorbance is maximized when a carrier ion has a high molar absorptivity thus giving a larger signal response and improving the limit of detection for the method. Detection was performed by indirect UV with a signal of 350 nm and a reference of 245 nm. The decrease in absorbance at 245 nm produces a positive peak at 350 nm during indirect UV detection.

Hexamethonioum hydroxide, 1,6-bis-trimethylammonium hexane, was used as a flow modifier to reverse the electroosmotic flow (EOF) and reduce analysis time. The samples were injected at 50 mbar for 4 s in the negative polarity mode with an applied voltage of (−) 30 kV. The negative or reverse polarity mode is set up with the cathode at the inlet and the anode at the detector. Anions may migrate to the detector in this mode, however they will go migrating against the EOF, resulting in longer migration times. Therefore, hexamethonioum hydroxide was used to reverse the EOF and reduce the analysis time. Triethanolamine was used to buffer the solutions at pH 7.7.

To ensure reproducibility, the capillary was flushed for 1.5 min with 0.1N NaOH followed by an inlet dip in water and then flushed with run buffer for 2.0 min [9]. This flushing sequence was repeated after every injection. A vial replenishing step was also performed after at least five injections to ensure a fresh supply of buffer and maintain a constant level of buffer in the vials. A typical blank electropherogram and phosphate standard electropherogram are shown in Figs. 2 and 3.

2.5. Calculations

The determination of the unbound phosphate concentration (mM) remaining in each sample and the phosphate binding capacity (mmol/g) has been previously described [4,5]. 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) [10]. This process is described by the Langmuir equation:

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

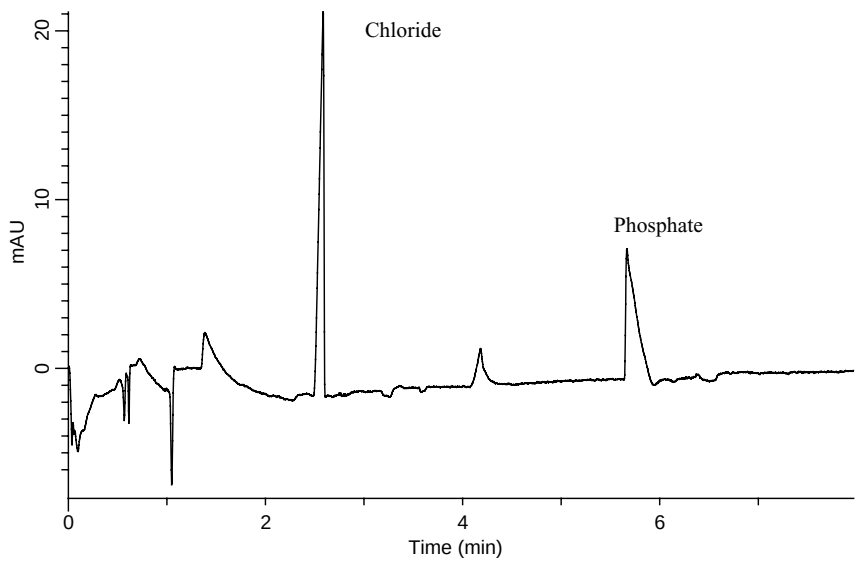

Fig. 3. Typical electropherogram of a 10 mM phosphate standard. The chloride peak and the peak at approximately 4.3 min is from the binding media (80 mM NaCl, 100 mM BES diluted 1:50 with water). Indirect UV at 350 nm with a reference of 245 nm.

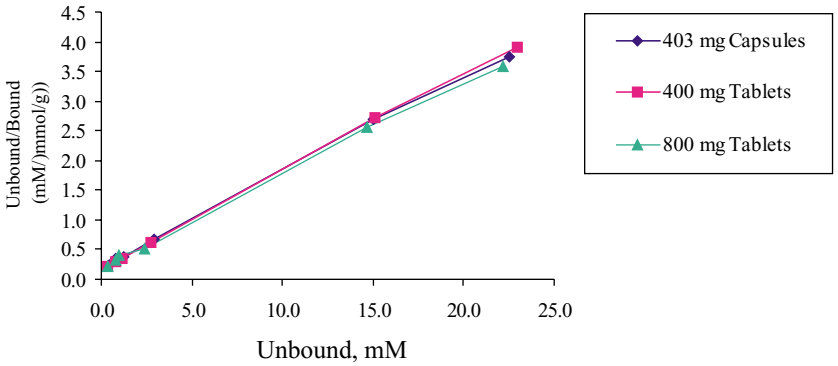

Fig. 4. Langmuir plot of Renagel® 403 mg capsules, 400 and 800 mg tablets at pH 7.

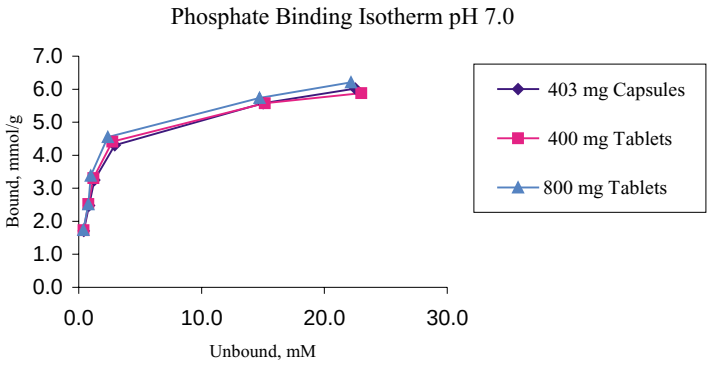

Fig. 5. Phosphate binding isotherm of Renagel® 403 mg capsules, 400 and 800 mg tablets at pH 7.0.

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 capacity 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) concentrations versus the unbound (mM)/bound (mmol/g). The affinity constant (k_1) value is calculated by dividing the slope of the regression line by the intercept, the capacity constant (k_2) value is equal to the inverse of the slope. The results are listed in Figs. 4–11 and in Tables 1 and 2.

3. Results and discussion

An overlay of the Langmuir plots, generated from the HPCE data, demonstrates the in vitro bioequiva-

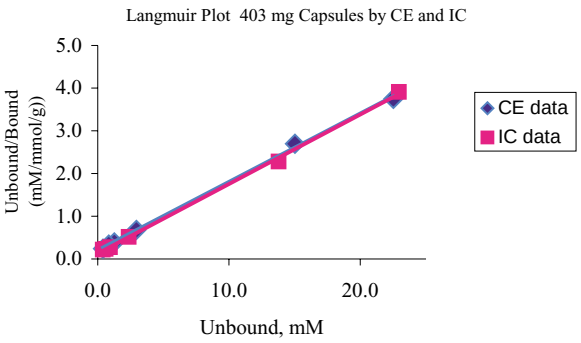

Fig. 6. Langmuir plot of Renagel® 403 mg capsules by HPCE and IC.

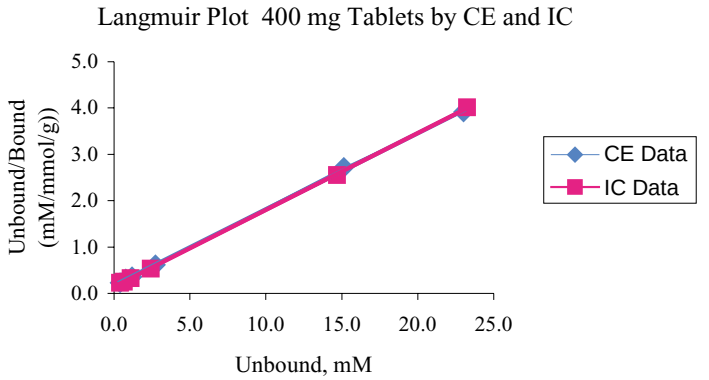

Fig. 7. Langmuir plot of Renagel® 400 mg tablets by HPCE and IC.

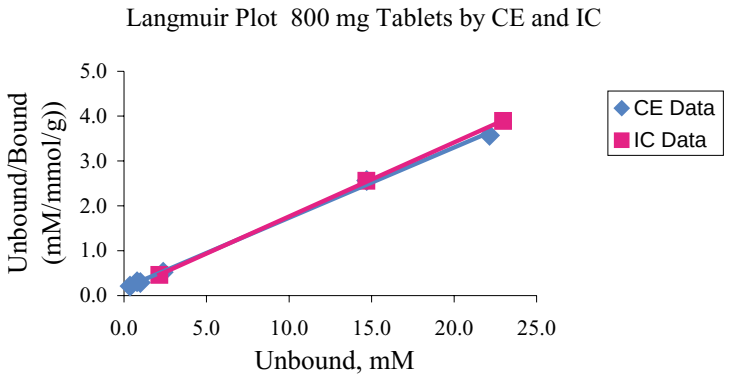

Fig. 8. Langmuir plot of Renagel® 800 mg tablets by HPCE and IC.

lence of all three dosage forms. An overlay of the phosphate binding isotherms, generated from the HPCE data, demonstrates that all three dosage forms have equivalent phosphate binding properties. The affinity and capacity constants for the 800 mg tablets obtained by IC were calculated from a 3 point Langmuir plot at 38.7, 30 and 14.5 mM. These results are in excellent agreement with the results obtained by HPCE.

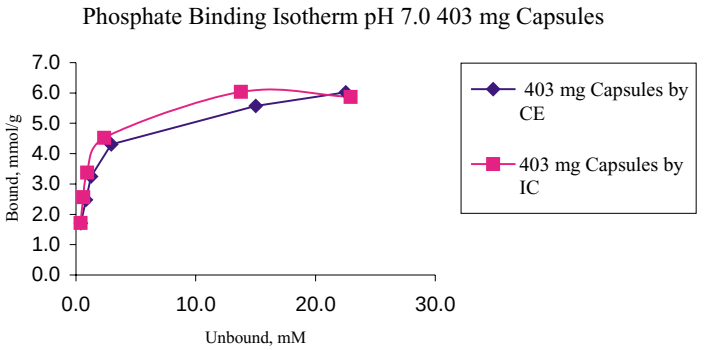

Fig. 9. Phosphate binding isotherm of Renagel® 403 mg capsules by HPCE and IC.

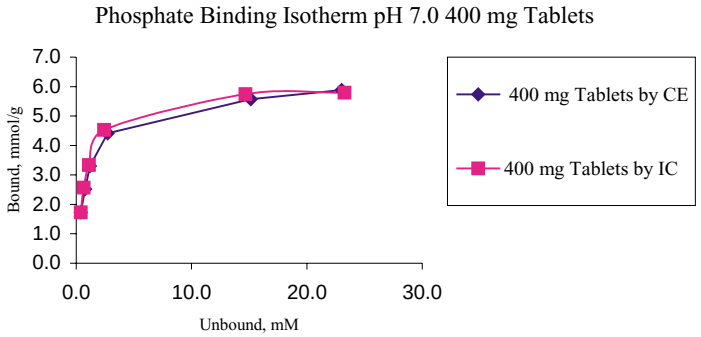

Fig. 10. Phosphate binding isotherm of Renagel® 400 mg tablets by HPCE and IC.

The HPCE results were also compared to the previously published IC results by overlaying the Langmuir plots and phosphate binding isotherms of all three dosage forms obtained by each technique. The results are listed in Figs. 6–11 and in Tables 1 and 2.

The %R.S.D. of the affinity constant (k_1) and capacity constant (k_2) values obtained by HPCE for all three dosage forms are 9.4 and 2.3%, respectively, and demonstrate their in vitro bioequivalence. The %R.S.D. of the affinity constant (k_1) and capacity constant (k_2) values obtained by IC for all three dosage forms are 11.0 and 1.0%, respectively, and demonstrate their in vitro bioequivalence [4].

The %R.S.D. of the affinity constant (k_1) and capacity constant (k_2) values obtained by both techniques for all three dosage forms are 21.4 and 2.2%, respectively, and demonstrate very good agreement between the results obtained by HPCE and IC. The affinity constant values (k_1) are expected to have a higher %R.S.D. than the capacity constants values (k_2) because the k_1 values are more species dependent on the type of species bound (i.e. monobasic or dibasic phosphate) than the capacity constants. At pH 7 monobasic and dibasic phosphate ions are present in approximately equal quantities. Previously published results suggest that the dibasic ion is the predominately bound species at pH 7 [4,6]. However some monobasic phosphate may be bound at this pH, leading to a higher variation in the affinity constants (k_1) as compared to the capacity constants. The results are still in very good agreement.

Also the affinity constants (k_1) are calculated from the intercept of the plot of the unbound (mM) concentrations versus the unbound (mM)/bound (mmol/g). The intercept of this graph can change slightly and have a large impact on the affinity constant (k_2) values.

The Langmuir plots obtained by HPCE are in very good agreement with the Langmuir plots obtained by IC and demonstrate the accuracy of the HPCE methodology.

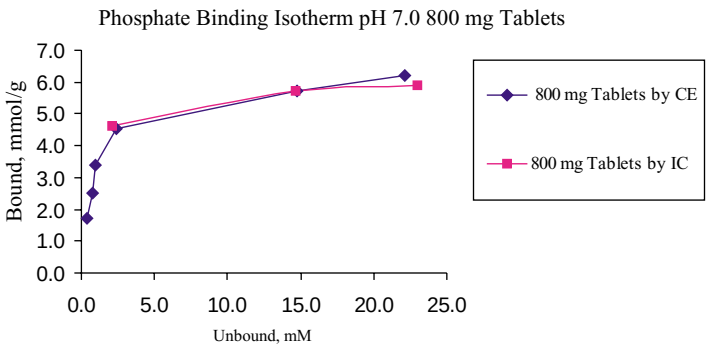

Fig. 11. Phosphate binding isotherm of Renagel® 800 mg tablets by HPCE and IC.

Table 1
The affinity and Langmuir capacity constants calculated at pH 7 by HPCE and IC for all three dosage forms of Renagel®

	Capsules 403 mg	Tablets 400 mg	Tablets 800 mg
Results by HPCE			
Intercept	0.20	0.18	0.17
Slope	0.16	0.16	0.16
R^2	0.999	1.00	0.998
k_1 (l/mmol)	0.79	0.93	0.94
k_2 (mmol/g)	6.2	6.1	6.4
Results by IC			
Intercept	0.13	0.15	0.12
Slope	0.16	0.17	0.16
R^2	0.999	0.999	0.999
k_1 (l/mmol)	1.2	1.2	1.4
k_2 (mmol/g)	6.2	6.0	6.1

Table 2
Average and %R.S.D. of k_1 and k_2 values for all three dosage forms of Renagel® as determined by HPCE and IC

	HPCE results ($n = 3$)	IC results ($n = 3$)	Combined results ($n = 6$)
Average k_1 (l/mmol)	0.89	1.3	1.1
%R.S.D. k_1	9.4	11.0	21.4
Average k_2 (mmol/g)	6.2	6.1	6.2
%R.S.D. k_2	2.3	1.0	2.2

Similarly the phosphate binding isotherms of all three dosage forms obtained by HPCE are in very good agreement with the results obtained by IC.

4. Conclusion

The phosphate binding constants, affinity constant (k_1) and capacity constant (k_2), were determined at pH 7.0 by capillary electrophoresis for the following dosage forms of Renagel®: 403 mg capsules, 400 and 800 mg tablets. The results demonstrate that the binding constants and affinity constants at pH 7.0, as calculated by HPCE are equivalent. These results are in very good agreement with previously published results obtained by ion chromatography and demonstrate the in vitro bioequivalence of all three dosage forms of Renagel® as determined by two different techniques.

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