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Report

In Vitro Adsorption of Bile Salts by Colestipol Hydrochloride

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The acid–base titration of colestipol hydrochloride exhibits no sharp inflection points, indicating a weakly basic anion-exchange copolymer. The swelling of colestipol hydrochloride in water and the adsorption of cholate anion are inversely related to pH and are, therefore, related to the ionization state of the copolymer. The Langmuir adsorption parameters at pH 7.5 and 37°C are similar for cholate, glycocholate, and taurocholate anions. Adsorption capacity was not related to particle size and exceeded the adsorptive capacity of the external surface by three orders of magnitude. Therefore, it is believed that the swelling of colestipol hydrochloride makes extensive internal surface area available for adsorption of bile salts. The rate of adsorption depends on the concentration of sodium cholate to which the colestipol hydrochloride is exposed. Adsorption was complete within 5 min when the concentration was below the adsorptive capacity. In contrast, adsorption at levels of sodium cholate at or above the adsorptive capacity was not complete within a 3-hr test period.

KEY WORDS: colestipol hydrochloride; bile salt adsorption; internal surface area; anion-exchange copolymer.

INTRODUCTION

Colestipol hydrochloride is an antihyperlipidemic agent which is effective in decreasing serum cholesterol levels (1). It is a high molecular weight anion-exchange copolymer which binds bile salts in the intestine, forming a complex that is excreted in the feces. Serum cholesterol levels are consequently reduced. The binding of taurocholate anion (2,3), glycocholate anion (2,3), and cholate anion (4) by colestipol hydrochloride has been demonstrated. Adsorption was found to depend on pH and exposure time (4). The adsorption of drugs by colestipol hydrochloride has also been studied and the effect of ionic strength, pH, and type of competing ion has been demonstrated (5–7). The adsorption of oxalate anion (8) by colestipol hydrochloride has been studied as well. This research was undertaken to characterize further the *in vitro* adsorption of bile salts by colestipol hydrochloride in order to optimize its antihyperlipidemic effect.

EXPERIMENTAL

Colestipol hydrochloride was obtained commercially (Upjohn). In addition, a sample having a smaller mean particle size than the commercial sample was provided by The Upjohn Company. Commercial colestipol hydrochloride was also dry milled to a fine powder (Wig L Bug, Crescent Dental Mfg. Co.). The mean particle size of all three samples was determined by optical microscopy. The surface area was determined by krypton adsorption (DigiSorb 2600, Micromeritics).

Bile salts were quantified by high-pressure liquid chromatography (Model M-45, Waters) using a UV detector at 214 nm (Model 441, Waters) and a C-18 reverse-phase column (μBondapak C18, Waters) by modification of a published method (9). The mobile phase used for analysis of cholate or glycocholate anion consisted of 0.01 *M* monobasic potassium phosphate adjusted to pH 3 with phosphoric acid and then diluted with an equal volume of acetonitrile. The mobile phase used for analysis of taurocholate anion consisted of 75% acetonitrile and 25% distilled deionized water. All standard curves had an R^2 of 0.99 or greater.

The acid–base titration curve of colestipol hydrochloride was determined after treating a sample with three successive washings of 1 *N* NaOH (10). The sample was then washed with distilled, deionized water until the rinse water was neutral. The sample was dried in a vacuum oven at 60°C. Immediately after drying, a suspension of 1 g of sodium hydroxide-washed colestipol hydrochloride in 100 ml of 1 *M* KCl was prepared and titrated with 1 *N* HCl containing 1 *N* KCl (PHM 62, TTT 60, ABU 12, REA 160, Radiometer).

The swelling of colestipol hydrochloride was measured by modifying a standard technique (11). A 4.5-g sample was placed in a 100-ml graduated cylinder. The sample was tapped and the equilibrium volume of the powder determined. One hundred milliliters of distilled, deionized water

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adjusted to the desired pH with 1 *N* HCl or 1 *N* NaOH was added to the cylinder. The suspension was allowed to equilibrate at 37°C for 24 hr. The equilibrium pH was measured and the volume of expansion determined by the following equation:

$$\text{volume of expansion} = \frac{V_F - V_0}{4.5} \quad (1)$$

where V_F is the final volume occupied by the hydrated resin, and V_0 is the volume occupied by 4.5 g of dry colestipol hydrochloride.

Adsorption isotherms at pH 7.5 and 37°C were determined by combining 100 mg of colestipol hydrochloride and 100 ml of the appropriate bile salt solution (ranging from 0.0001 to 0.01 *M*) in a 150-ml beaker which was covered and placed in a water jacket at 37°C. The mixture was magnetically stirred and the pH maintained using a pH-stat titrator (PHM 62, TTT 60, ABU 12, REA 160, Radiometer) with 0.05 *N* HCl. The volume of titrant added was directly related to the concentration of the bile salt solution but was less than 4.1 ml (range, 0.1 to 4.1 ml). Appropriate corrections were made in the calculations to adjust for the dilution which occurred in order to maintain pH 7.5. The sample was judged to be at equilibrium when no more titrant was required. The stirring was stopped and the colestipol hydrochloride was allowed to settle. Approximately 20 ml of the supernatant was removed and centrifuged to separate any remaining colestipol hydrochloride. The supernatant was used for HPLC analysis. The amount of bile salt which was adsorbed was determined by difference. The Langmuir equation was used to calculate the adsorption coefficient and the adsorptive capacity.

The effect of pH on the adsorption of cholate anion by colestipol hydrochloride was characterized by the equilibrium distribution coefficient, *D* (12).

$$D = \frac{\text{amount of cholate anion adsorbed at equilibrium}}{\text{amount of cholate anion in solution at equilibrium}} \quad (2)$$

The equilibrium distribution coefficient was determined under two conditions: 100 ml of 0.006 *M* sodium cholate was equilibrated with 0.1 g of colestipol hydrochloride at 37°C and pH conditions ranging from 7.0 to 9.0, and 100 ml of 0.02 *M* sodium cholate was equilibrated with 1.0 g of colestipol hydrochloride at 37°C and pH conditions ranging from 6.15 to 9.45. The suspension was maintained at the desired pH by a pH-stat titrator (PHM 62, TTT 60, ABU 12, REA 160, Radiometer). The volume of titrant added to control the pH was less than 2 ml. After the system equilibrated, i.e., no additional titrant was required to control the pH, the supernatant was separated and the cholate anion concentration determined by HPLC analysis. The amount of cholate anion adsorbed was determined by difference and the equilibrium distribution coefficient was calculated.

The rate of adsorption was studied by exposing 100 mg of colestipol hydrochloride to 100 ml of the appropriate sodium cholate solution (0.0024, 0.0050, or 0.0071 *M*). The suspension was maintained at pH 7.5 and 37°C using a pH-stat titrator (PHM 62, TTT 60, ABU 12, REA 160, Radiometer). Aliquots removed at 5, 10, 15, 30, 60, or 180 min were centrifuged and the supernatant was analyzed for cholate anion by HPLC. The amount of cholate anion adsorbed at various sampling times was determined by difference.

RESULTS AND DISCUSSION

The adsorption mechanism was investigated by titrating the sodium hydroxide-washed sample of colestipol hydrochloride to determine the ionization characteristics of the copolymer. As shown in Fig. 1, the titration curve exhibits no sharp inflection points, indicating a gradual ionization of the secondary and tertiary amines. This behavior is typical of that obtained with a weakly basic anion exchange resin (10,13).

Colestipol hydrochloride swells in water (Fig. 2). Above pH 4, the swelling is inversely related to the pH. This reflects the decreased protonation of the secondary and tertiary amines in the copolymer as the pH is increased.

The reversibility of the swelling was tested by hydrating colestipol hydrochloride in water. The equilibrium pH was 5.9. When the pH was lowered to 4.2 by the addition of 1 *N* HCl, the volume of expansion was 7.6 ml/g. The pH was then increased to 8.5 and 11.6 by the addition of 1 *N* NaOH. The volume of expansion decreased to 5.6 and 4.4 ml/g, respectively. These values agree with the results shown in Fig. 2 indicating that the pH-dependent swelling of colestipol hydrochloride is reversible.

The adsorption of cholate anion by colestipol hydrochloride is accompanied by an increase in pH. For example, 0.01 *M* sodium cholate has a pH of 6.9 and a 0.1% colestipol hydrochloride suspension in water has an equilibrium pH of 5.9. However, the equilibrium pH of 0.1% colestipol hydrochloride in 0.01 *M* sodium cholate was 8.8. This behavior suggests that cholate anions are exchanging with adsorbed hydroxyl anions and is consistent with adsorption by anion exchange.

Since the adsorption of cholate anion by colestipol hydrochloride is due to anion exchange (1), an inverse relationship between pH and adsorption is expected. As shown in Fig. 3, the equilibrium distribution coefficient decreases as the pH increases from 6.15 to 9.45. The adsorption behavior

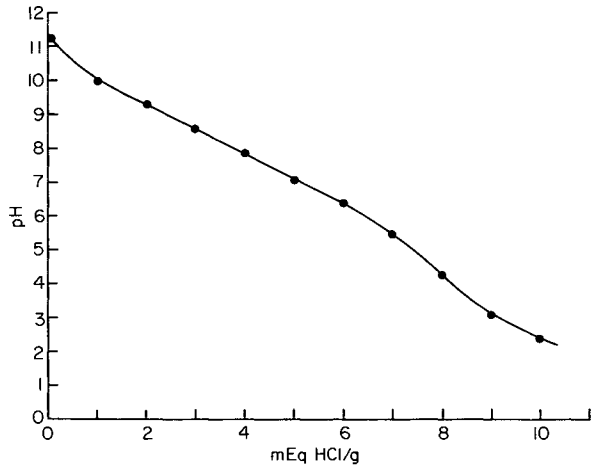

Fig. 1. Titration of sodium hydroxide-washed colestipol hydrochloride with 1 *N* HCl containing 1 *M* KCl.

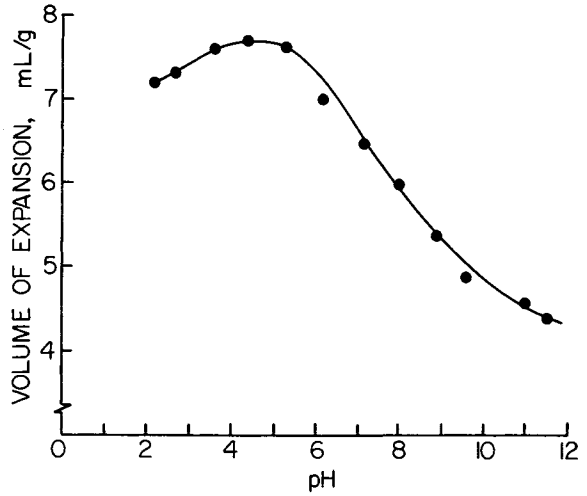

Fig. 2. Effect of pH on volume of expansion of colestipol hydrochloride in water.

reflects the ionization state of the secondary and tertiary amines of the colestipol hydrochloride. It is interesting to note that the effect of pH on both adsorption of cholate anion (Fig. 3) and swelling (Fig. 2) is very similar, suggesting that both properties depend on the ionization state of the copolymer.

DeSimone *et al.* (4) studied the adsorption of cholate anion by three anionic exchange resins including colestipol hydrochloride and concluded that "the binding capacity of all three resins was scarcely modified by the pH of the buffer solution." This discrepancy with the results reported in Fig. 3 may be due to the use of a phosphate buffer in the earlier study. The phosphate anions are likely to compete with cholate anion for adsorption sites (6), and thus, the results

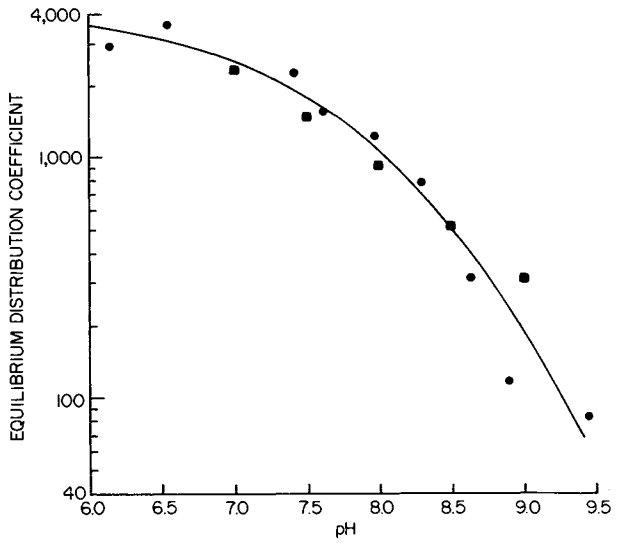

Fig. 3. Effect of pH on the adsorption of cholate anion by colestipol hydrochloride. (●) One hundred milliliters of 0.02 *M* sodium cholate equilibrated with 1 g of colestipol hydrochloride at 37°C; (■) 100 ml of 0.006 *M* sodium cholate equilibrated with 0.1 g of colestipol hydrochloride at 37°C.

may be confounded by competitive adsorption. Since the data in Fig. 3 were obtained without the use of a buffer, i.e., a pH-stat titrator was used to control the pH, it is believed that it more accurately represents the effect of pH on the adsorption of cholate anion.

The adsorption isotherms of cholate, glycocholate, and taurocholate anions by colestipol hydrochloride were determined at pH 7.5 and 37°C using the Langmuir equation. The Langmuir equation has been found to approximate the ion-exchange isotherm when the solute approaches the maximum capacity of the resin (14) and was used by Johns and Bates (15) to study the binding of conjugated and unconjugated bile salts to cholestyramine. Adsorption at pH 7.5 is of interest because it represents the pH expected at the distal portion of the ileum, where bile salt absorption is believed to occur (6). As shown in Table I, these bile salts exhibit very similar adsorption parameters.

It is interesting to note the adsorption parameters for cholate anion which were obtained when the pH was not controlled. As shown in Table I, the adsorptive capacity was reduced by approximately 50% when the pH was uncontrolled. This is because the pH equilibrated at 8.7. The data in Fig. 3 indicate that the equilibrium distribution coefficient decreased substantially when the pH was increased from 7.5 to 8.7. Thus, pH control is essential for the determination of the Langmuir adsorption parameters.

The effect of particle size of colestipol hydrochloride on the adsorption of cholate anion at pH 7.5 and 37°C was also studied. Three samples were compared: (A) commercial material having a mean diameter of 130 μm; (B) a specially prepared sample having a mean diameter of 65 μm; and (C) sample A dry milled to a mean diameter of 17 microns. Table II shows that all three samples adsorbed approximately 3.4 mmol of the cholate anion when 4 mmol/100 ml was exposed per g of colestipol hydrochloride. The three samples also adsorbed approximately 3.6 mmol of the cholate anion when 8 mmol/100 ml was exposed per g of colestipol hydrochloride. Thus, approximately 3.5 mmol of cholate anion was adsorbed per g of colestipol hydrochloride regardless of particle size in the range of 17–130 μm or cholate anion concentrations ranging from 4 to 8 mmol/100 ml. This result was unexpected, as adsorption is usually related to particle size.

Since adsorption was not related to particle size, the

Table I. Adsorption Constants[a] for Adsorption of Bile Salts by Colestipol Hydrochloride at 37°C

Sample	pH	Adsorption Coefficient (L/mol)	Adsorption Capacity (mmol/g)
Sodium cholate[b]	7.5	1.60×10^3	4.72
Sodium glycocholate[b]	7.5	1.90×10^3	4.28
Sodium taurocholate[b]	7.5	1.15×10^3	4.72
Sodium cholate[c]	No control	1.68×10^3	2.30

[a] All isotherms had $R^2 > 0.99$.
[b] Seven points.
[c] Four points.

Table II. Effect of Particle Size on Adsorption of Cholate Anion by Colestipol Hydrochloride at pH 7.5, 37°C

Sample	Mean Diameter (μm)	mmol/g adsorbed (% Bound)[a] 4 mmol Exposed/g	8 mmol Exposed/g
A	130	3.42 (86)	3.58 (45)
B	65	3.34 (84)	3.76 (47)
C	17	3.42 (86)	3.71 (46)

[a] One gram of colestipol hydrochloride exposed to 100 ml containing the indicated amount of sodium cholate.

surface area of the dry samples was determined by gas adsorption and compared to the calculated surface area required to adsorb a quantity equal to the adsorptive capacity, assuming monolayer adsorption. The surface area of the 130-μm colestipol hydrochloride (sample A) was 0.31 ± 0.003 m^2/g. The surface area increased to 0.52 ± 0.002 m^2/g when the sample was dry milled (sample C). The dimensions of the cholate anion were determined by constructing a molecular model. The dimensions and surface area which would be covered by a single molecule in either a horizontal or a vertical orientation are given in Table III. If the adsorptive capacity of cholate anion (4.72 mmol/g) from Table I is used and it is assumed that monolayer adsorption occurs by either a horizontal or a vertical orientation, then the surface area needed for the observed adsorptive capacity can be calculated. Based on this model, a surface area of 2600 or 900 m^2/g is required for adsorption in a horizontal or vertical orientation, respectively. These surface areas exceed the surface area determined by krypton adsorption by three orders of magnitude. Thus, the adsorptive capacity of colestipol hydrochloride for bile salts cannot be explained by adsorption on the same surface area which adsorbed the krypton gas. The surface area responsible for adsorption of the krypton gas is termed the external surface area. It is believed that the swelling of colestipol hydrochloride particles in water makes additional surface area available for adsorption of cholate anions from solution. The surface area which arises upon swelling is termed the internal surface area. This comparison suggests that the swelling which occurs when colestipol hydrochloride is dispersed in water makes extensive internal surface area available for adsorption. The major role played by the internal surface is consistent with observation that adsorption is not related to the particle size.

Colestipol hydrochloride has a limited amount of time to exert its therapeutic action within the gastrointestinal tract. Therefore, the rate at which anion exchange occurs is of interest. The equilibration times reported in the literature for the adsorption of bile salts by colestipol hydrochloride vary considerably. Equilibration times of 20, 60, and 180 min have been reported (5,6,8). As shown in Fig. 4, the rate of adsorption depends on the concentration of sodium cholate to which the colestipol hydrochloride is exposed. Adsorption was complete within 5 min at the lowest sodium cholate concentration. In contrast, adsorption at higher levels of sodium cholate was not complete within the 3-hr test period. The cholate anion concentrations of 2.4, 5.0, and 7.1 m*M* to which the colestipol hydrochloride was exposed represent approximately 50, 106, and 150% of the adsorptive capacity of colestipol hydrochloride for cholate anion (Table I).

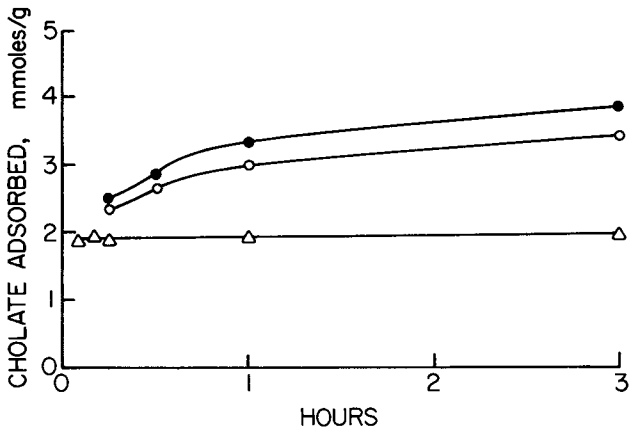

Fig. 4. Rate of adsorption of cholate anion by colestipol hydrochloride at pH 7.5, 37°C. (△) Cholate anion equal to 50% of adsorptive capacity/g; (○) cholate anion equal to 106% of adsorptive capacity/g; (●) cholate anion equal to 150% of adsorptive capacity/g. The adsorptive capacity was 4.72 mmol/g.

It is hypothesized that the relationship between the rate of adsorption and the concentration of cholate anion shown in Fig. 4 arises because most of the adsorption occurs on the internal surface of the colestipol hydrochloride particles. At sodium cholate concentrations below the adsorptive capacity, adsorption requires only a fraction of the total surface area. Thus, adsorption occurs at the external surface and the most accessible internal exchange sites and equilibration is rapid. As the sodium cholate concentration approaches or exceeds the adsorptive capacity, all of the internal surface is required for adsorption. The cholate anions must diffuse through the pores to the less accessible exchange sites, thereby resulting in longer equilibration times. An increased sodium cholate concentration, above the adsorptive capacity, appears to enhance this diffusion process, as a greater amount of cholate anion was adsorbed at each time point when the cholate anion concentration was 150% of the adsorptive capacity in comparison to 106%.

Table III. Dimensions of Cholate Anion[a]

Orientation	Length (m)	Width (m)	Surface Area (m^2)
Horizontal	15×10^{-10}	6×10^{-10}	9×10^{-19}
Vertical	6×10^{-10}	5×10^{-10}	3×10^{-19}

[a] Based upon the construction of a molecular model.

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