Adsorption Behavior of Penicillin G Sodium on Hydrophilic Gel Toyopearl HW-40F

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The adsorption of penicillin G sodium (PGS) on a hydrophilic gel, Toyopearl HW-40F, was investigated by the frontal analysis method. Effects of media, buffer concentration, and temperature on the adsorption equilibrium were examined in the buffer concentration range of (0.005 to 0.05) mol·L^{-1} and temperature range of (278.15 to 308.15) K. At the same pH value, the ionic strength of the mobile phase greatly influences the adsorption of PGS. The adsorption isotherms of PGS are comparable at different temperatures. The equilibrium data were modeled by the Freundlich equation with AARD in the range of (0.86 to 7.15) %.

Introduction

Penicillins are the earliest generation of β-lactam antibiotic agents and are still widely used as antibacterials on account of their bactericidal activity, broad spectrum, low toxicity, and excellent distribution throughout the body.[1] Nevertheless, penicillins are also the most common cause of severe allergic drug reactions. Allergic reactions were found to be elicited by macromolecular impurities such as penicilloylated proteins, penicilloylated peptides, or polymers that had at least two determinants per molecule.[2−4] Removal of the macromolecular impurities from drugs has been important for reducing the incidence of allergic reactions. The gel filtration chromatography method was usually used to separate the macromolecular impurities on the basis of their physical dimensions. Different gels have been utilized in previous studies.[5−11]

In our laboratory, when we intended to separate macromolecular impurities from penicillin G sodium (PGS) by gel filtration chromatography to examine the allergic substances in it carefully and thoroughly eliminate them from it, we found that the separation of macromolecular impurities from PGS was better on a column of Toyopearl HW-40F than on a column of Sephadex G-25 or Sephadex G-10. Toyopearl HW-40F would be a good media to separate the allergic macromolecular impurities. This could be attributed to the adsorption effect of PGS on the gel media. With the mobile phase of 0.02 mol·L^{-1} phosphate buffer (pH 7.0), the distribution coefficients of PGS, K_{av}, on the columns of Sephadex G-25, Sephadex G-10, and Toyopearl HW-40F were 0.92, 1.08, and 1.52, respectively. Adsorption data are necessary for optimization of the process and engineering design. Isotherms represent important information for understanding the interaction between a solute and an adsorbent. However, there is not any information in the literature concerning this. Therefore, the aim of this study is to investigate the adsorption equilibrium of PGS on Toyopearl HW-40F.

Materials and Methods

Chemicals and Reagents. PGS was purchased from the North China Pharmaceutical Group, Shijiazhuang, China. Toyopearl HW-40F was purchased from Tosoh, Tokyo, Japan. Blue dextran 2000 was purchased from Amersham Pharmacia Biotech, Uppsala, Sweden. All other reagents were of AR grade.

Apparatus. All breakthrough curves were obtained on a Waters HPLC system, which consisted of a 1525 binary pump, a 717 plus autosampler, and a 2487 dual λ adsorbance detector. The temperature of column was controlled by a thermostat water bath with the precision of ± 0.1 K.

Column. A stainless steel column with dimensions of (150·3.9) mm was used in all experiments. The column was slurry-packed with HW-40F gel. HW-40F is a hydroxylated methacrylic polymer with a spherical particle shape. Its particle size is in the range of (30 to 60) μm, and its pore size is 50 Å.

The void fraction of the column was determined by blue dextran 2000 (MW = 2·10^6 Da) with an injection volume of 5 μL. HW-40F was liable to shrink as a function of temperature and mobile phase, so the void volume of the column was determined every time before the breakthrough curve was measured. Gel volume, V_a, was calculated by subtracting the interparticle bed volume from the column volume.

Determination of the Adsorption Isotherm Data by Frontal Analysis. Because of the instability of PGS,[12] a static method was not suitable for studying this adsorption equilibrium system. The adsorption equilibrium data of PGS on HW-40F was acquired by a frontal analysis method.[13] The column was equilibrated by flowing buffer solution until a stable baseline was achieved. For the determination of the breakthrough curve, the column was loaded with PGS solution until the PGS concentration in the outlet stream was equal to that in the inlet stream. The concentration of solute in the eluent increased stepwise. The amount of PGS absorbed on HW-40F per unit volume of gel was determined from the breakthrough times of step changes in the feed concentration. The adsorption isotherms of PGS were measured over the concentration range of (0 to 25) mg·mL^{-1}. Prepared buffers were used as the mobile phases, and all the experiments were run at a mobile phase flow rate of 0.5 mL·min^{-1}. A wavelength of 270 nm was used for detection. Relatively low temperatures of (278.15, 288.15, 298.15, and 308.15) K and neutral mobile phases were chosen in the experiments to maintain the stability of PGS.

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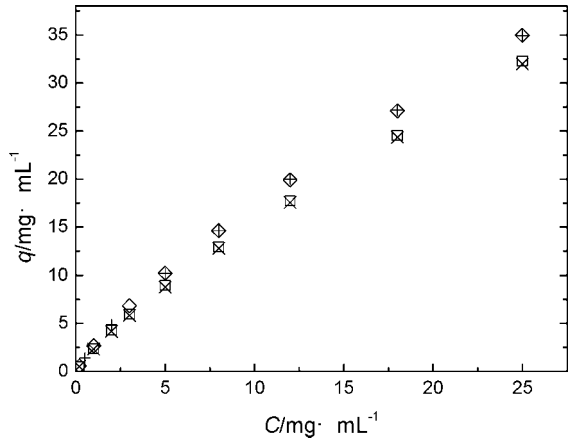

Figure 1. Adsorption amount, q, of PGS on HW-40F as a function of PGS concentration, C, at $T = 278.15$ K in (0.03 and 0.05) mol·L^{-1} citrate buffers (pH 7.0): +, 0.05 mol·L^{-1}; ◇, 0.05 mol·L^{-1}; ×, 0.03 mol·L^{-1}; □, 0.03 mol·L^{-1}.

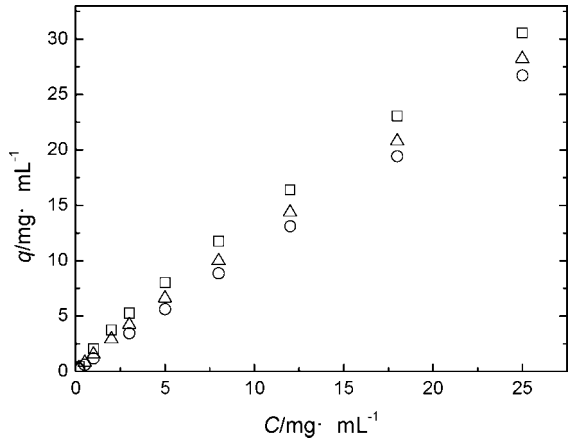

Figure 2. Adsorption amount, q, of PGS on HW-40F as a function of PGS concentration, C, at $T = 278.15$ K in 0.02 mol·L^{-1} buffers (pH 7.0): □, citrate buffer; △, phosphate buffer; ○, acetate buffer.

Results and Discussion

Reproducibility of the Frontal Analysis Data. Frontal analysis experiments were duplicated under defined conditions to check the reproducibility of the obtained adsorption equilibrium data. Figure 1 shows the adsorption isotherms of PGS on HW-40F at 278.15 K when (0.03 and 0.05) mol·L^{-1} citrate buffers were used as mobile phases. The error in the adsorption amount measured by the frontal analysis method was caused by fluctuations in the experimental parameters and measurement errors made on the intermediate data needed to calculate the isotherm data points.[14] In the experiments, temperature fluctuations were controlled within ± 0.1 K, and flow rate precision was less than 0.1 %, so the error caused by the fluctuations in experimental parameters was small. In our experiments, the error in the obtained adsorption equilibrium data mainly came from the measurement errors, such as the determination of breakthrough time and the void volume of the column. The deviation of the amount of PGS adsorbed by HW-40F was less than 3.0 %. Data obtained by the frontal analysis method are repeatable and credible.

Effect of Buffers on the Adsorption. The adsorption behavior of PGS on HW-40F was studied in 0.02 mol·L^{-1} acetate, phosphate, and citrate buffers. As shown in Figure 2, buffers influence the adsorption isotherm of PGS differently. The nonideal gel filtration chromatography of PGS on HW-40F is mainly caused by hydrophobic interactions and electrostatic interactions. It has been found that aromatic and carboxylic groups have a strong influence on the elution volume of the solute,[15,16] and PGS contains both of them. On one hand, the aromatic group of PGS might induce an adsorption effect with the hydrophobic sites of the gel; on the other hand, the carboxylic group of PGS might induce electrostatic repulsive interactions with the weakly negatively charged sites of the gel, which are induced by the ether bonds and hydroxyl groups in the gel skeleton. Hydrophobic interactions and electrostatic repulsive interactions are two opposing effects. Suppression of electrostatic effects enhances hydrophobic interactions and vice versa.[17] When buffers are used as mobile phases, the electrolyte can competitively interact with the negatively charged sites of the gel and thus suppress the electrostatic repulsive interactions of PGS and HW-40F. As a result, hydrophobic interactions prevail in the process of gel filtration chromatography. The difference in the buffer ionic strength should be a significant reason for the different adsorption equilibrium data of PGS in various buffers. The calculated ionic strength of citrate, phosphate, and acetate buffers are (0.11, 0.036, and 0.020) mol·L^{-1}, respectively. Citrate buffer has the highest ionic strength so that it can suppress the electrostatic repulsive interactions more effectively and thus results in a higher adsorption amount.

The adsorption of PGS on HW-40F was enhanced when the ionic strength of acetate and phosphate buffer was adjusted by NaCl to be same as that of the citrate buffer. The distribution coefficients of PGS, K_{av}, on the HW-40F column were calculated as

$$K_{av} = \frac{V_e - V_0}{V_t - V_0} \quad (1)$$

where V_e was the elution volume of PGS, V_0 was the void volume determined by blue dextran 2000, and V_t was the column volume. The K_{av} values of PGS were 2.26, 1.66, 1.89, 1.15, and 1.77 in citrate, phosphate, phosphate−NaCl, acetate, and acetate−NaCl buffers, respectively. The K_{av} values of PGS greatly increased after NaCl was added to the mobile phase, indicating that the ionic strength of the buffer could affect the adsorption behavior of PGS.

Effect of Buffer Concentration, pH, and Temperature on the Adsorption. The effects of buffer concentration on the adsorption equilibrium of PGS were investigated in the concentration range of (0.005 to 0.05) mol·L^{-1} and (0 to 25) mg·mL^{-1} for the citrate buffer and PGS, respectively. As shown in Figure 3, the adsorption isotherms of PGS are greatly influenced by buffer concentration. The amount of PGS adsorbed on the gel increases as the buffer concentration increases. The buffer with the higher concentration has a higher ionic strength so that the adsorption effect of PGS on the gel matrix is larger in high concentration citrate buffer.

We further confirmed the hydrophobic interactions between PGS and HW-40F by studying the effect of pH on the chromatographic behavior of PGS. PGS samples with a concentration of 2 mg·mL^{-1} were injected into the column at 288.15 K when citrate buffers with different pH values were used as mobile phases. The results in Figure 4 show that when the pH values of the mobile phase were larger than 5, the distribution coefficient of PGS on the column was about 2.67 and did not change with the pH. When the pH values were smaller than 5, the distribution coefficient of PGS increased as the pH value decreased. This can be attributed to the ionization of PGS and the gel matrix. The pK_a of PGS was about 2.7, so

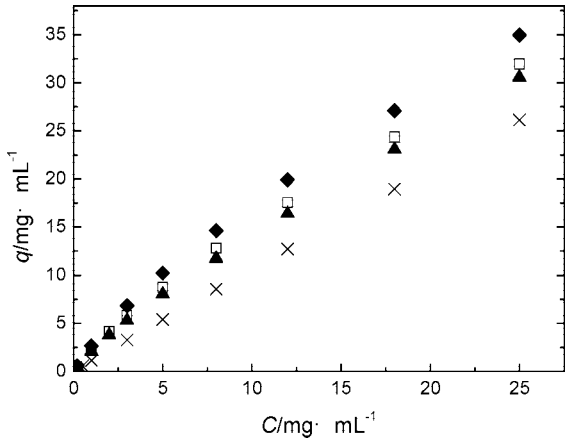

Figure 3. Adsorption amount, q, of PGS on HW-40F as a function of PGS concentration, C, at $T = 278.15$ K in citrate buffers (pH 7.0) with concentration from (0.005 to 0.05) mol·L^{-1}: ◆, 0.05 mol·L^{-1}; □, 0.03 mol·L^{-1}; ▲, 0.02 mol·L^{-1}; ×, 0.005 mol·L^{-1}.

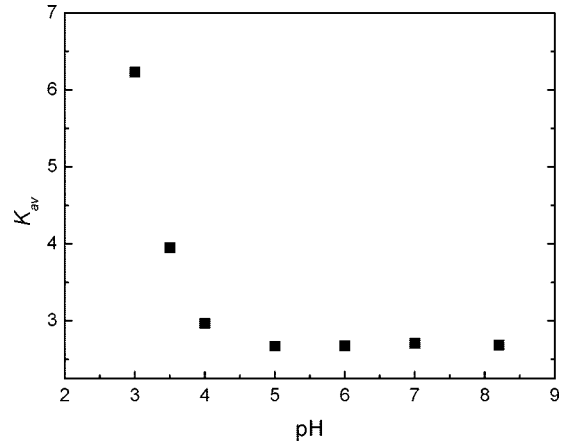

Figure 4. Distribution coefficient, K_{av}, of PGS on HW-40F in citrate buffer as a function of pH.

most of the PGS was ionized under neutral or slightly acidic circumstances. When pH values were lower than 5, the ionization of both PGS and the stationary phase surface was suppressed, and thus their electrostatic repulsive interactions were suppressed. The enhanced hydrophobic interactions caused a retardation of PGS on the column.

Figure 5 shows the adsorption isotherms of PGS on HW-40F in 0.05 mol·L^{-1} citrate buffer at four temperatures, (278.15, 288.15, 298.15, and 308.15) K. The adsorption isotherm of PGS is almost identical from (278.15 to 308.15) K. This is probably attributed to the change in the interactions between PGS and gel and the gel volume. In general, adsorption is an exothermic process and decreases with increasing temperature. However, HW-40F gel shrinks with increasing temperature. This made the calculated adsorption equilibrium data tend to be increased. These two effects counteract and lead to a small difference in adsorption isotherms at different temperatures.

Adsorption Equilibrium Modeling. The adsorption data of PGS on HW-40F were fitted by the Freundlich equation expressed as

$$q = K_F C^{(1/n_F)} \quad (2)$$

where q is the amount of PGS adsorbed per unit volume of adsorbent at equilibrium, C is the equilibrium PGS concentration in the mobile phase, and K_F and n_F are the Freundlich constants.

The calculated parameters are listed in Table 1. Freundlich parameters K_F and n_F increase with increasing buffer concentration, indicating an increase in adsorption tendency and intensity. The heterogeneity factors, $1/n_F$, are all found to be less than unity, indicating that the PGS is favorably adsorbed by the gel HW-40F. The average absolute relative deviation (AARD) values were in range of (0.86 to 7.15) %.

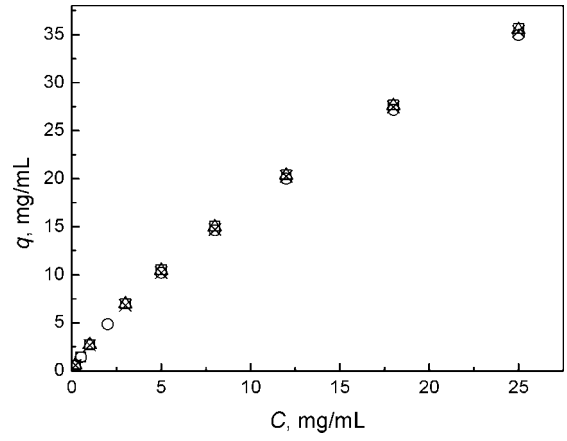

Figure 5. Adsorption amount, q, of PGS on HW-40F as a function of PGS concentration, C, in 0.05 mol·L^{-1} citrate buffer (pH 7.0) from $T = (278.15$ to 308.15) K: ○, 278.15 K; □, 288.15 K; △, 298.15 K; ×, 308.15 K.

Table 1. Freundlich Isotherm Parameters for PGS Adsorption on HW-40F at $T = 278.15$ K

C_b[a]	$K_F^{(1-n_F^{-1})}$			AARD[b]
mol·L^{-1}	mg·mL^{-1}	n_F	R^2	%
0.005	1.115 ± 0.004	1.020 ± 0.001	1	0.86
0.02	2.076 ± 0.023	1.198 ± 0.006	0.99993	2.64
0.03	2.381 ± 0.026	1.241 ± 0.006	0.99993	3.94
0.05	2.932 ± 0.053	1.298 ± 0.011	0.99983	7.15

[a] C_b is citrate buffer concentration. [b] AARD = $100/N$ ($\sum_{i=1}^{N}$ |($q_{exptl} - q_{calcd}$)/q_{exptl}|); subscripts "calcd" and "exptl" are the calculated and experimental values, respectively; N is the number of measurements.

Conclusions

PGS can be adsorbed on the hydrophilic gel HW-40F in buffers. The adsorption behavior of PGS is greatly influenced by the ionic strength of the buffer. Among the buffers examined in this work, citrate has the most potential to enhance the adsorption of PGS on HW-40F. Higher buffer concentration is helpful in suppressing the electrostatic repulsive interactions and thus results in larger adsorption equilibrium data. The adsorption isotherms of PGS are comparable in the experimental temperature range. The Freundlich isotherm was used for mathematical modeling in the citrate buffer concentration of (0.005 to 0.05) mol·L^{-1} with AARD values in range of (0.86 to 7.15) %. The equilibrium adsorption amounts are up to 35 mg·mL^{-1} in the PGS concentration range of (0 to 25) mg·mL^{-1}, which is quite large considering such a low concentration.

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