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Sorption of Tetracycline Antibiotics on Hyper-Crosslinked Polystyrene from Aqueous and Aqueous-Organic Media

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Abstract—The sorption of tetracycline, oxytetracycline, chlortetracycline, and doxycycline on hyper-crosslinked polystyrene from aqueous and aqueous-organic solutions is studied under static and dynamic conditions in order to extend the range of the sorbents suitable for sorption isolation and the preconcentration of tetracycline antibiotics. Features of tetracycline sorption depending on the acidity of a solution and the nature and concentration of the compounds are explained. It is shown that hyper-crosslinked polystyrene can be used for the group sorption preconcentration of these compounds.

Keywords: tetracycline, oxytetracycline, chlortetracycline, doxycycline, hyper-crosslinked polystyrene, sorption.

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INTRODUCTION

The high antimicrobial activity and relatively low cost of tetracycline antibiotics (TCs) have led to their widespread use not only in medicine but also in animal husbandry for the prevention and treatment of infectious diseases and as promoters of animal growth, and in the food industry to extend the shelf life of food products. The scale of TC use still occupies a leading position among other veterinary antibiotics. The large-scale and often unauthorized use of these drugs in veterinary practice leads to their accumulation in foods of animal origin and in the environment, where they arrive with flush water from pharmaceutical companies and poultry and pig farms, and with the waste products of humans and animals. The residual amounts of TCs in these areas has a negative impact on human health and the ecological balance of the environment, leading to the development of antibiotic-resistant microorganisms [1].

The considerable interest in the sorption of TCs by different sorbents is related to the study of migration routes of TCs in the environment [2], the search for more efficient methods of removing these antibiotics from different types of water [3], and the development of procedures for their isolation and preconcentration before analytical determination [4, 5]. To assess the mobility and bioavailability of TCs in water, their sorption has been studied on such natural sorbents as soils [6–9], clays, and clay minerals [10, 11]. The sorption of TCs on oxides of iron [12], aluminum [13], and titanium [14] has been used to purify water of these antibiotics. A number of works have been devoted to studying the sorption of TCs on carbon sorbents, e.g., activated carbon [15], biochar [16, 17], graphene oxide [18], and carbon nanotubes [19–21]. Considerably fewer works have been devoted to studying the sorption of TCs on polymeric sorbents [22, 23].

In this work, we propose using hyper-crosslinked polystyrene (HPS) for the sorption preconcentration of TCs. HPS has recently found wide use in industrial sorption technologies and chromatography [24–26]. This sorbent has a number of advantages that distinguish it from other polymeric sorbents. These include high specific surface area, high mechanical stability, a combination of hydrophobicity and water wettability of the surfaces of sorbent particles, extremely high rigidity of the polymer structure, and pronounced affinity for polar organic compounds. In comparing the sorption behavior of oxytetracycline (the most frequently used TC) on various sorbents in [27], we showed that HPS is more efficient than such sorbents as Strata-X, Strata SDB-L, the carbon nanomaterial Taunit, and diethylaminoethyl cellulose. The aim of this work was to identify features of the sorption of tetracycline, oxytetracycline, chlortetracycline, and doxycycline on HPS from aqueous and aqueous-organic solutions under static and dynamic conditions, and to assess the possibility of using this sorbent for the group isolation and preconcentration of TCs.

EXPERIMENTAL

Reagents and Equipment

Hyper-crosslinked polystyrene (Diapak P-3, BioKhimMak, Russia) was used as a sorbent. The parameters of the porous structure of HPS were determined

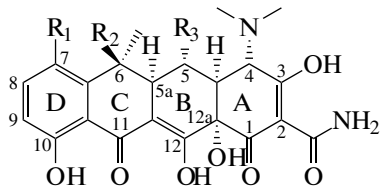
Table 1. Some physicochemical properties of the studied tetracyclines

Compound	R_1	R_2	R_3	M	$-\log P$	pK_{a1}	pK_{a2}	pK_{a3}
Tetracycline	H	OH	H	444	1.25	3.2	7.78	9.6
Oxytetracycline	H	OH	OH	460	1.12	3.2	7.46	8.9
Chlortetracycline	Cl	OH	H	478	0.62	3.3	7.55	9.3
Doxycycline	H	H	OH	444	0.54	3.5	7.7	9.5

For tetracycline, oxytetracycline, and chlortetracycline, the values of pK_{a1}, pK_{a2}, and pK_{a3} were taken from [2], along with hydrophobicity parameters ($\log P$). For doxycycline, they were taken from [29]. The values of hydrophobicity parameters ($\log P$) were calculated using the standard ACD software package (Toronto, Canada).

via low-temperature nitrogen adsorption using an ASAP 2010 N unit (Micromeritics, United States). The microstructure of the sorbent was studied on a LEO SUPRA 50 VP scanning electron microscope (Carl Zeiss, Germany) with a field emission source [28]. The HPS specific surface area was 912 m^2/g. The sorbent contained macro-, meso- and micropores, the fractions of which were 10, 79, and 11%, respectively. The sorbent particles had a regular spherical shape with diameters of ~60 μm. The HPS was activated with acetonitrile prior to use.

Tetracycline, oxytetracycline, chlortetracycline (Acros Organics, 99.0%), and doxycycline (Sigma, >98.0%) were selected as the objects of study:



Some physicochemical properties of the investigated substances are presented in Table 1. The initial solutions of tetracyclines (1×10^{-3} M) were prepared by dissolving their accurately weighed samples in methanol. The working solutions of the compounds were prepared by diluting the initial solutions before use.

The absorption spectra and optical density of the solutions were recorded on an SF-103 spectrophotometer (Akvilon, Russia); pH was monitored using an Expert 001 ionometer (Russia). Sorption in the dynamic mode was conducted using a vacuum setup for solid-phase extraction (an M6 manifold, Akvilon, Russia).

Procedure for Studying Tetracycline Sorption under Static Conditions

To study the sorption of TCs in the static mode, accurately weighed samples of HPS (0.010 ± 0.001 g) were placed into test tubes with ground stoppers. Then 5 mL of TC solution was added and shaken on an electromechanical vibromixer until sorption equilibrium was reached (20 min). The sorbents were then separated from the solution via decantation, and the concentration of TCs in the equilibrium aqueous phase was determined spectrophotometrically by measuring its characteristic absorption in the UV region (350–365 nm).

The values of the degree of sorption (R,%) were calculated using the equation

$$R, \% = \frac{c_0 - c}{c_0} \times 100,$$

where c_0 is the concentration of the compound in aqueous solution before sorption, and c is the concentration in the solution after sorption.

Procedure for Studying Tetracycline Sorption under Dynamic Conditions

To study the sorption of TCs in the dynamic mode, we used a concentrating microcolumn ($l = 6$ mm, $d = 10$ mm) filled with 30 mg of HPS and a vacuum setup for solid-phase extraction. The flow rate of the solution through the column was 1.0 mL/min. Before use, the column was washed repeatedly with water and acetonitrile. The column was also washed with a mixture of acetonitrile and methanol (1 : 1), and with water after sorption and desorption.

RESULTS AND DISCUSSION

The sorption of tetracycline, oxytetracycline, chlortetracycline, and doxycycline on HPS was studied under static conditions. These compounds, which are polyfunctional hydronaphtacene derivatives consisting of four cyclic structures, differ in their substituents, acidity constants, and hydrophobicity (i.e., Hansch parameters, logarithms of the distribution constants in *n*-octanol–water system) (Table 1). The conditions of sorption were optimized by varying the time of phase contact, the pH, and the composition of the aqueous phase. The results were interpreted by comparing the isotherms of TC sorption and the physicochemical parameters of sorption calculated from isotherms (Table 2).

It was found that the time required to reach sorption equilibrium for the investigated TCs did not exceed 20 min. The tetracyclines exist in solution in three forms: cationic (when $pH < pK_{a_1}$), anionic

Table 2. Calculated physicochemical parameters of tetracycline sorption on HPS

Compound	R, % ($n = 3$)	a_m, mmol/g	$K \times 10^{-3}$, L/mol	$-\Delta G^\circ_{298}$, kJ/mol
Tetracycline	98 ± 1	0.20	73	27.7
Oxytetracycline	98 ± 2	0.20	77	27.9
Chlortetracycline	98 ± 2	0.21	119	28.9
Doxycycline	99 ± 1	0.22	156	29.6

Table 3. Degrees of sorption (R, %) of oxytetracycline on a microcolumn filled with 0.030 g of HPS, depending on the volume fractions (α) of methanol (I), ethanol (II), and acetonitrile (III) in aqueous solution ($V = 25$ mL, $c_{OTC} = 5 \times 10^{-5}$ M, pH ~ 5)

α, vol %	I	II	III
0	100	100	100
5	100	99	99
10	100	98	86
15	100	90	30
20	100	75	9
30	99	40	0
40	81	11	0
50	64	0	0

(when $pH > pK_{a_2}$), and zwitterionic ($pK_{a_1} < pH < pK_{a_2}$), so one of the main factors influencing their sorption is the pH of the solution. The character of the dependence of the degree of sorption on pH (Fig. 1) indicates that tetracyclines are adsorbed on HPS in both zwitterionic and cationic forms: the maximum degree of sorption is observed in the pH range of 2–8, where these species predominate. In all subsequent investigations, sorption was conducted from solutions with pH ~ 3–6.

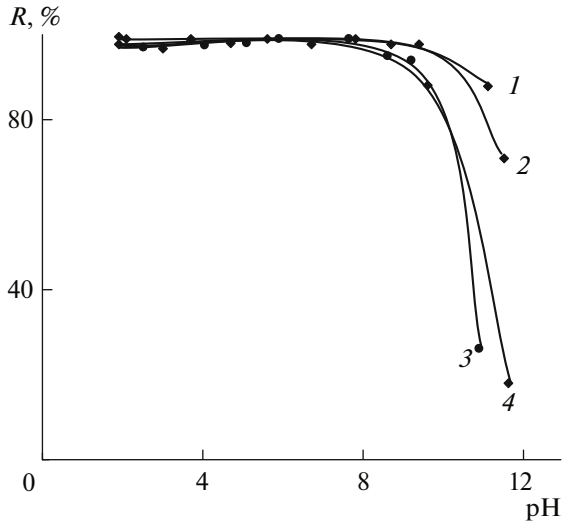


Fig. 1. Dependences of the degrees of sorption of (*1*) chlortetracycline, (*2*) tetracycline, (*3*) oxytetracycline, and (*4*) doxycycline on pH on the HPS sorbent: $c_{TC} = 5 \times 10^{-5}$ M; $V = 5$ mL; $m_{HPS} = 0.010 \pm 0.001$ g; $t = 20$ min.

Figure 2 shows the isotherms of tetracycline sorption from aqueous solutions on HPS. The isotherms are described by the Langmuir equation in the 0.001–0.17 mM range of equilibrium concentrations. Some physicochemical parameters were calculated using the equations of linear dependences constructed in coordinates $1/a$–$1/c$: sorption capacities of sorbents (a_m), sorption constants (K), the Gibbs energy changes (ΔG°_{298}) that are given in Table 2.

Comparison of the degrees of TC sorption indicates that HPS quantitatively sorbs these compounds (R = 98–99%). The values of the sorption constants increase along with the hydrophobicity parameters of TCs (shown in parentheses) in the series tetracycline (−1.25) < oxytetracycline (−1.12) < chlortetracycline (−0.62) < doxycycline (−0.54), indicating the occurrence of hydrophobic interactions. The increased affinity of the HPS surface for TCs could be due to π–π- and cation–π-interactions between the π-electron system of a TC and its protonated amino group and the π-electron system of the aromatic rings of the sorbent occurring on this sorbent along with hydrophobic interactions. The considerable contribution from π–π-interactions to the mechanism of the retention of other organic compounds on HPS was noted in [24, 25, 28].

The sorption of TCs under dynamic conditions was conducted on a microcolumn filled with HPS. The mass of sorbent, the volume of the solution, the concentration of TCs, and the nature and volume of the eluent was varied in optimizing the conditions of sorption. A microcolumn (10 × 6 mm) filled with 0.030 g of HPS proved to be suitable for minimizing the volume of the eluent. All of the investigated TCs were sorbed by 98–100% on such a column in the concentration range from 0.05 to 0.2 μg/mL from 25 mL of an aqueous solution. When selecting the conditions of desorption, acetonitrile, methanol, ethanol, and mixtures of these solvents with an aqueous solution of phosphoric acid, as well as a mixture of acetonitrile and methanol, were used as eluents. It was found that the pure solvents do not allow the quantitative desorbtion of TCs: when 5 mL of acetonitrile, methanol, or ethanol was passed through the column, the degrees of compound desorption were 66–85%. Quantitative desorption of TCs was achieved using 1–2 mL of the mixtures of these solvents with 0.1 M phosphoric acid or a mixture of acetonitrile and methanol in a ratio of 1 : 1. When the volume of the solution was raised to 100 mL, the degrees of TC sorption fell slightly to 94–98%.

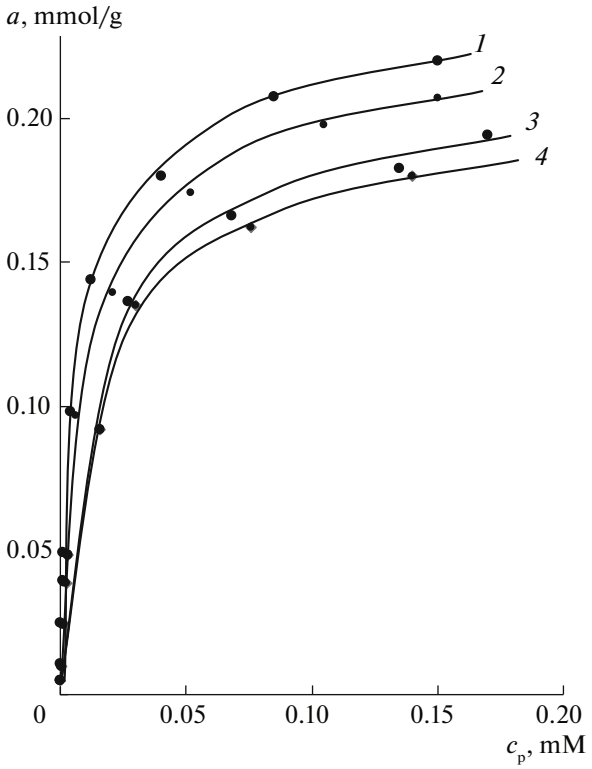


Fig. 2. Isotherms of doxycycline (*1*), chlortetracycline (*2*), oxytetracycline (*3*), and tetracycline (*4*) sorption from aqueous solutions on hyper-crosslinked polystyrene at pH ~ 5; $V = 5$ mL; $m_{HPS} = 0.010 \pm 0.001$ g; $t = 20$ min.

According to [4, 5], methanol, acetonitrile, or ethanol, and their mixtures with water are often used for recovery of TCs during the sample preparation of solids for analysis. We therefore studied the effect of additives of such solvents on the sorption of TCs. As an example, the experimental data for oxytetracycline are given in Table 3. A comparison of them shows that the presence of up to 5, 10, and 30 vol % of acetonitrile, methanol, and ethanol in aqueous solution has no effect on the degree of TC sorption. From the data shown in Table 4, it can be seen that not only oxytetracycline but other TCs were sorbed quantitatively ($R = 98–100\%$) from the solutions containing up to 30 vol % of methanol.

Table 4. Degrees of sorption (R, %) of tetracyclines on a microcolumn filled with 0.030 g of HPS, depending on volume fraction (α) of methanol in aqueous solution (V = 25 mL, $c_{TC} = 5 \times 10^{-5}$ M, pH ~ 5): (I) chlortetracycline, (II) doxycycline, (III) tetracycline, and (IV) oxytetracycline

α, vol %	I	II	III	IV
0	100	100	100	100
10	99	100	99	100
20	99	100	99	100
30	99	99	98	99
40	97	97	93	81
50	90	83	75	64

CONCLUSIONS

Our study showed that hyper-crosslinked polystyrene exhibits high affinity for the molecules of tetracyclines and can be used for group sorption preconcentration of this class of compounds from aqueous and aqueous-organic solutions.

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