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QUANTITATIVE CRITERION FOR EVALUATION OF HYDROPHOBIC SORBENTS

A. BREIER*

Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava (Czechoslovakia)

P. GEMEINER

Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, Dúbravská cesta 9, 842 38 Bratislava (Czechoslovakia)

A. ZIEGELHÖFFER and R. MONOŠÍKOVÁ

Institute of Experimental Surgery, Centre of Physiological Sciences, Dúbravská cesta 9, 842 33 Bratislava (Czechoslovakia)

and

E. SEDLÁKOVÁ

Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, Dúbravská cesta 9, 842 38 Bratislava (Czechoslovakia)

SUMMARY

Study of sorption of perphenazine onto bead O-(3-phenoxy-2-hydroxypropyl)cellulose revealed that in equilibrium the partition law is the ruling one. Partition coefficient $\overline{p}$ proved to be independent of bulk concentration of sorbate. Similar results were obtained with bovine serum albumin applied as sorbate. As an easily available measure of hydrophobicity of polymer matrices, coefficient $\overline{p}$ proved to be particularly useful in screening the properties of carriers applied as stationary phase for hydrophobic interaction chromatography.

INTRODUCTION

Hydrophobic effects play a dominant role in a variety of technological

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processes, in the stabilization of supramolecular biological structures and in the mechanisms associated with biosynthesis, metabolism and several physiological functions [1]. Hydrophobic interactions have been found to take part in regulation of certain methods for phase separation, such as affinity partition [2] and hydrophobic interaction chromatography [3]. Sorption of amphiphilic biopolymers onto the amphiphilic carriers, i.e. polysaccharide derivatized by hydrophobic agents, plays a dominant role in the latter type of phase separation. The portion of hydrophobic bonds participating in the interaction between sorbent and sorbate is given by the hydrophobicity of both sorbent and sorbate. As a sorbate in modelling of hydrophobic sorption, it is advantageous to apply low-molecular-weight substances with expressive amphiphilic character. The hydrophobicity of such substances may be suitably characterized by the partition coefficient and/or by the Hansch parameter π [4]. However, the hydrophobicity of sorbents may be evaluated using more criteria: the sorption capacity of hydrophobic probes [5–9], the solubility of sorbate in internal gel water [10], the partition coefficient of sorbate between the internal gel water and external water [11], the distribution coefficient of sorbate [12] and the contact angle of non-polar solute on the sorbent surface during drenching with water [13]. Unfortunately, none of these criteria is fully accepted.

In a preliminary study of hydrophobic sorption [14], we pointed out the fact that the ratio between the amount of adsorbed substance and its concentration in water under equilibrium conditions is constant and independent of bulk concentration. Hence, hydrophobicity of sorbent may be adequately characterized by the partition coefficient of the amphiphilic probe in the system amphiphilic sorbent–water. The equilibrium parameters may be established from recently derived eqn. 1:

$$B = B_e \frac{t}{t + t_{0.5}} \tag{1}$$

where B is the sorption in time t, B_e is the equilibrium sorption when $t \to \infty$, and $t_{0.5}$ is the half-time of sorption [15].

The concentration of sorbate in solution at time t (c_t) may be expressed from eqn. 1 as:

$$c_t = c_o - c_c B_e \frac{t}{t + t_{0.5}} \tag{2}$$

where c_o and c_c are bulk concentrations of sorbate and sorbent, respectively. The partition coefficient of sorbate in the system amphiphilic sorbent–water may then be established from eqn. 3, obtained by dividing eqn. 1 by eqn. 2 [16]:

$$p = \overline{p} \frac{t}{t + t_{0.5p}} \tag{3}$$

where p represents the ratio B/c_t; $\overline{p}$ is the partition coefficient defined under equilibrium conditions as $\overline{p} = B_e(c_o - c_c B_e)^{-1}$; $t_{0.5p}$ is the time interval when $p = 0.5\overline{p}$, defined as $t_{0.5p} = c_o t_{0.5}(c_o - c_c B_e)^{-1}$.

The present paper offers experimental evidence for the validity of the above considerations, using the hydrophobic sorption of perphenazine onto phenoxy-hydroxypropyl porous-bead cellulose as a model system.

EXPERIMENTAL

Chemicals

Applied celluloses: porous-bead cellulose (I) with particle diameter 50–350 μm and 18.84% dry weight, provided by Dr. J. Štamberg from the Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences (Prague, Czechoslovakia); two derivatives of the same cellulose, prepared by cross-linking with epichlorohydrin [17], with one interglucose bridge per sixteen (II) and per ten (III) glucose units; cross-linked porous-bead cellulose, Ostsorb C (IV), with particle diameter 50—290 μm and 25.30% dry weight (Spolchemie, Ústí nad Labem, Czechoslovakia).

1-(2-Hydroxyethyl)-4-[3-(2-chloro-10-phenothiazinyl)propyl] piperazine (perphenazine) with a purity of 99.3% was supplied by Léčiva (Prague, Czechoslovakia). All other chemicals were obtained from Serva (Heidelberg, F.R.G.) or Lachema (Brno, Czechoslovakia) and were of analytical grade.

Hydrophobization of bead celluloses

All four types of bead celluloses (I—IV) were treated in a first step by alkylation with epichlorohydrin [18]. The products obtained, O-(3-chloro-2-hydroxypropyl)celluloses (CHPC), were equilibrated and resuspended in dry dioxane followed by alkylation with sodium phenolate. Alkylation was done by boiling in dioxane under reflux. The molar ratio of phenolate and chlorohydroxypropyl group (CHP) in the celluloses was 1—10:1. Products were subsequently washed with ethanol, 0.1 *M* hydrochloric acid in 50% ethanol–water solution

TABLE I

DEGREE OF CROSS-LINKING, PHP CONTENT OF THE INVESTIGATED BEAD CELLULOSES AND VALUES OF THE PERPHENAZINE PARTITION COEFFICIENT

No.	Cross-linking*	PHP group content	$\bar{p}$
I	—	—	—**
II	+	—	—**
III	++	—	—**
IV	+++	—	—**
V	—	126.9	388.1 ± 22.6
VI	—	297.2	657.5 ± 48.7
VII	—	453.4	809.9 ± 71.0
VIII	+	596.5	1767.6 ± 237.9
IX	++	652.1	967.1 ± 56.4
X	++	749.3	1350.2 ± 192.4
XI	++	813.0	2106.2 ± 184.1
XII	+++	938.2	2412.1 ± 185.0

*Cross-linking: (+) one interglucosic bridge per sixteen glucose units; (++) one interglucosic bridge per ten glucose units; (+++) commercially cross-linked sample, Ostsorb C.

**Indeterminably low sorption of perphenazine onto respective cellulose.

and water. By this procedure, eight O-(3-phenoxy-2-hydroxypropyl) derivatives (PHPC) of porous-bead celluloses (V—XII) having different contents of phenoxyhydroxypropyl (PHP) group and degrees of cross-linking were obtained (Table I).

The degree of substitution with CHP groups was characterized according to the chlorine content, established with the aid of a Perkin-Elmer automatic analyser Type 240. The content of PHP groups in the PHPC celluloses was ascertained spectrophotometrically by absorbance measurements at 495 nm of 2.5% (w/v) solutions of PHPC in concentrated sulphuric acid against the respective CHPC solution with the same concentration. At these measurements, a similarly prepared solution of Phenyl-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) with known PHP content was applied as a standard.

Measurement of sorption kinetics

Perphenazine. Hydrophobic-bead cellulose (50 mg) was left to swell in 100 ml of borate buffer (50 m*M*, pH 9.0) for 2 h. Sorption was started by addition of perphenazine up to final concentrations of 10—50 mg/l with constant stirring. The concentration of free perphenazine in the suspension was checked in 0.5-ml aliquots, taken in different time intervals between 5 and 120 min by means of a double-beam Beckman DB-6 spectrometer at a wavelength of 257 nm.

Bovine serum albumin (BSA). Hydrophobic-bead cellulose (20 mg) was left to swell for 2 h in 0.9 ml of citrate—phosphate buffer (20 m*M*, pH 4.9) with intense shaking. Sorption was started by adding 100 μl of BSA solution (1—2 g/l) in the same buffer. The concentration of free BSA was checked in 0.5-ml aliquots taken in various time intervals between 5 and 120 min, according to Bradford [19].

Processing of data

Equilibrium sorption as well as partition coefficients were established from the intercepts in double-reciprocal plots of eqns. 1 and 3. The $\bar{p}$ values obtained were characterized by a confidence interval of 95% [15]. The possible differences between $\bar{p}$ values established during sorption at different bulk concentrations of perphenazine and BSA onto the same derivate of PHPC were evaluated by means of the Student's *t*-test.

RESULTS AND DISCUSSION

Commercially available Sepharose, derivatized with 3-phenoxy-2-hydroxypropyl moiety (Phenyl-Sepharose CL-4B), has been found to be a suitable compound for application as an amphiphilic carrier for hydrophobic interaction chromatography [5, 20]. All eight of the amphiphilic derivatives of bead cellulose (Table I) used in the present study contained 3-phenoxy-2-hydroxypropyl groups. The presence of the above hydrophobic moiety in the cellulose structure allows perphenazine to accumulate from the applied buffered aqueous solution (pH 9.0). A pH value of 9 prevents the protonization of perphenazine (pK_a 8.65—9.00) [21]. The kinetics of sorption of perphenazine onto all the PHPC derivatives investigated followed the recently derived eqn. 1

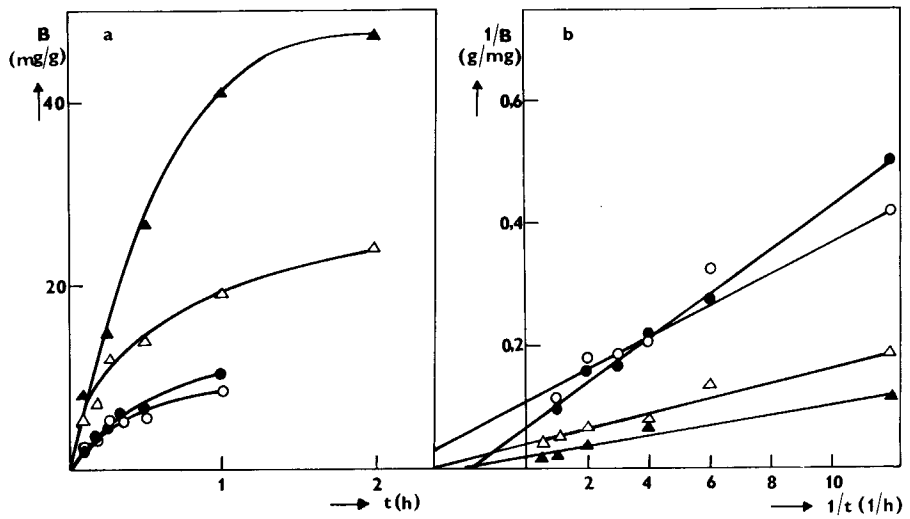

Fig. 1. Sorption kinetics of perphenazine onto PHPC XII (a) and linearization of kinetic dependence on the double-reciprocal system (b). Intercepts on the ordinate in the double-reciprocal plot represent the respective B_e^{-1} values. Bulk concentrations of perphenazin (PFN): (○) 10 mg/l; (●) 15 mg/l; (△) 25 mg/l; (▲) 50 mg/l.

[15], as shown in the example of cellulose XII (Fig. 1). The same experimental data, when plotted according to eqn. 3, pointed to a constant and on the bulk concentration-independent distribution* of perphenazine (indicated as $\overline{p}$) between the cellulose and the sorbate solution (Fig. 2). Similar results were observed in the case of sorption of phenylhydrazonopropanedinitrile derivatives onto PHPC [14]. This clearly confirms the partition law to be a dominating principle in the sorption of amphiphilic agents onto the carriers investigated. A similarly dominant role of the partition law was also investigated in experiments with BSA sorption onto each PHPC as shown for cellulose XII (Fig. 3). Contrary to hydrophobic sorption, the process of chemisorption is directed by a gradual reaction of sorbate with the reactive groups of sorbent [22, 23]. This difference may be used to distinguish hydrophobic sorption from chemisorption [24]. The ability of different PHPC to bind perphenazine was dependent on the amount of PHP residues in their structure. This fact was manifested in the relatively narrow correlation relationship (eqn. 4), with $r = 0.893$ ($P < 0.05$):

$$\overline{p} = 2.385 \cdot \text{PHP content} + 71.949 \quad (4)$$

The respective deviations from the above relationship may be explained partially by different supramolecular structure, namely by different degrees of cross-linking of the celluloses tested (Table I).

It may be concluded from our results that the partition coefficient $\overline{p}$ of

*The difference in $\overline{p}$ values at various bulk concentrations of perphenazine, evaluated using the Student's t-test, were insignificant for all the celluloses studied ($t \leq 1.052$, ten degrees of freedom).

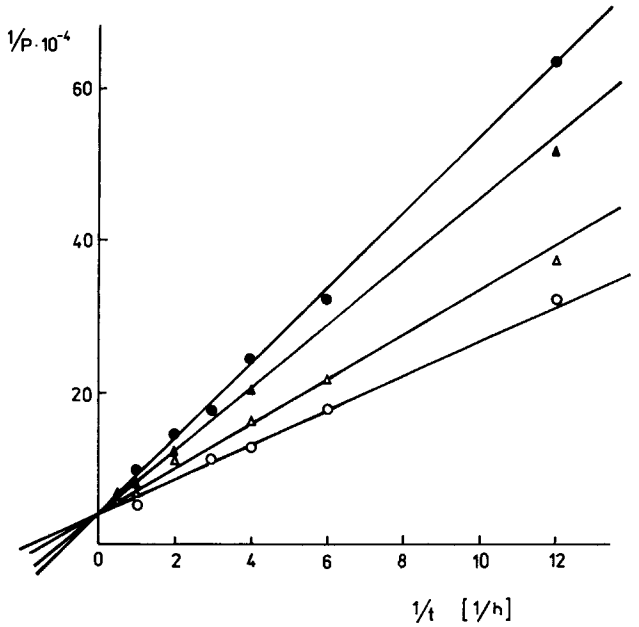

Fig. 2. Double-reciprocal plot of time dependence of the p value for perphenazine sorption onto PHPC XII. Intercept on the ordinate represents the p^{-1} value. Bulk concentrations: (○) 10 mg/l; (●) 15 mg/l; (△) 25 mg/l and (▲) 50 mg/l.

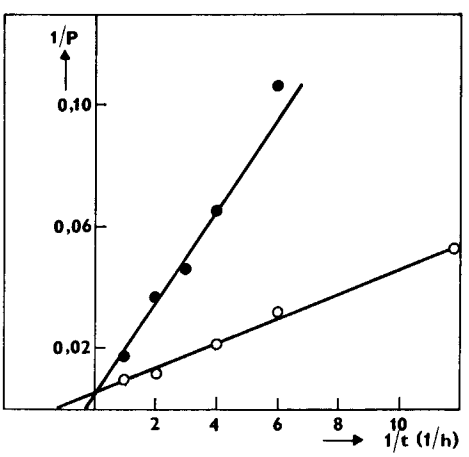

Fig. 3. Double-reciprocal plot of time dependence of the p value for BSA sorption onto PHPC XII. (○) 100 mg/l BSA; (●) 200 mg/l BSA.

perphenazine may be applied as a criterion for the characterization of amphiphilic properties of sorbents. A successfull application of the latter criterion may already be documented in the case of development of new carriers for hydrophobic interaction chromatography of calmodulin [25, 26].

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