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Surface affinity chromatographic separation of blood cells

VII. Relationship between capacity factors of human peripheral blood cells and the rate of penetration of liquids into xerogel column packings

USHIHO MATSUMOTO, YŌICHI SHIBUSAWA* and MASAAKI YAMASHITA
Division of Analytical Chemistry, Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-03 (Japan)
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SUMMARY

Eleven kinds of column packing gels which bonded poly(ethylene glycol) (PEG) to Sepharose 6B (PEG–C_{10}–Sepharose) were prepared. Human peripheral blood cells were chromatographed on these gel columns by eluting with 0.09 *M* phosphate-buffered 2% (w/w) dextran T40 solution at the pH of the respective isoelectric points of the blood cells. The rate of penetration of water or the mobile phases into the PEG–C_{10}–Sepharose xerogels as a measure of the hydrophobicity of the gels depended on both the oxyethylene residue content and the number of oxyethylene units of the packing gels. The capacity factors of granulocytes and lymphocytes were increased on the columns packed with gels having a slower rate of penetration of the liquids into the xerogels.

INTRODUCTION

In previous papers[1–6], the chromatographic behaviour of human peripheral blood cells was studied by the use of bisoxirane-coupled poly(ethylene glycol) 400, 4000, 6000 and 20 000 (PEG 400, 4000, 6000 and 20M)–Sepharose 6B (PEG–C_{10}–Sepharose) columns and poly(propylene glycol) 200, 400 and 950 (PPG 200, 400 and 950)-coupled agarose (PPG–C_3–Chromagel A4) columns. Sodium phosphate-buffered solutions containing dextran T40 or T500 were used as the mobile phase at pH 7.4 or the pH of the respective isoelectric point of blood cells ($pH_{c.p.}$). The retention behaviour of blood cells on PEG 20M–C_{10}–Sepharose columns depended on the molecular weight and concentration of dextrans and on the neutral salts, such as sodium chloride, in the mobile phase at pH 7.4[1,2].

Further, the retention volumes of granulocytes and lymphocytes increased with increasing number of oxyethylene units in the range of 9–450 on PEG 400–, 4000–,

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6000– and 20M–C_{10}–Sepharose columns eluted with 0.09 *M* sodium phosphate-buffered solution containing 2% (w/w) dextran T40[3].

The hydrophobicities ($\Delta\log K$) of four kinds of blood cells were determined by the hydrophobic affinity partition method[7] using PEG 6000 monopalmitate as a hydrophobic ligand. A linear correlation between the retention volumes of platelets, granulocytes and lymphocytes on a PEG 20M–C_{10}–Sepharose column and the $\Delta\log K$ values of these cells was observed[4]. Similarly, an approximately linear relationship was found between the $\Delta\log K$ values of platelets granulocytes and lymphocytes and the retention volumes of these cells on PPG 200–, 400– and 950–C_3–, PPG 400–C_{10}– and PEG 20M–C_{10}–agarose columns[5].

In the preceding study[6], the $\Delta\log K$ values of four kinds of blood cells and the PPG 200–, 400– and 950–C_3–Chromagel beads were determined by the hydrophobic affinity partition method using Pluronic P84, a block polymer of PEG and PPG, as a hydrophobic ligand. The $\Delta\log K$ values of blood cells increased in the order erythrocytes, platelets, granulocytes and lymphocytes, which coincided with the elution order of these cells from PPG–C_3–Chromagel columns, except in a few instances. A linear relationship was found between the capacity factors [$k' = (V_R - V_m)/V_m$, where V_R is the retention volume of the cells and V_m the interstitial volume of the column determined from the elution volume of native dextran] of granulocytes on the various PPG–C_3–Chromagel columns and the $\Delta\log K$ values of the PPG-bonded gel beads. The packing beads with $\Delta\log K > 0.5$ caused a considerable increase in the retention of lymphocytes. The k' values of platelets increased slightly on the PPG-bonded gel beads that have the highest $\Delta\log K$ values. On the other hand, the retention of erythrocytes on the columns was not dependent on the $\Delta\log K$ values of the gel beads. The $\Delta\log K$ values of the column packing beads determined by the hydrophobic affinity partition method merely indicate the affinity between the hydrophobic ligand, such as PEG monopalmitate or Pluronic P84, and the gel beads. It is necessary to measure the affinity between the column packing beads and a liquid, such as water or aqueous mobile phase, and to evaluate the degree of hydrophobicity of the gel beads.

The affinity between a solid and a liquid is determined by the wettability of the solid as the contact angle. However, in order to measure the contact angle of powder particles, the particles must be moulded into the plate and the liquid dropped on it for measurement. The other method for measurement of wettability is known as the ascending capillary method[8], that is, the liquid rises in a column which is packed with powder particles, the ascending rate of penetration of the liquids is measured and the affinity between the powder particles and liquid is determined.

In this study, we measured the rate of penetration of water or the mobile phase used for chromatography of blood cells into PEG–C_{10}–Sepharose xerogels with different oxyethylene residue contents and with different numbers of oxyethylene units for the determination of the hydrophobicity of the packing gel beads. The relationship between the k' values of four kinds of human peripheral blood cells and the rate of penetration of the liquid into the column packing xerogels is discussed.

EXPERIMENTAL

Materials

Epoxy-activated Sepharose 6B and dextran T40 (weight-average molecular weight $M_w = 40\,000$) were obtained from Pharmacia LKB (Uppsala, Sweden). PEG 400 (number-average molecular weight $M_n = 400$), 4000 ($M_n = 3000$), 6000 ($M_n =$ 6000-7500) and 20M ($M_n = 15\,000$–$20\,000$) were purchased from Wako Junyaku (Osaka, Japan). Other reagents were of analytical-reagent grade.

Instruments

A JNM-FX 200 NMR spectrometer (JEOL, Tokyo, Japan) operating at 199.5 MHz in the pulsed Fourier transform mode was used for the determination of oxyethylene residue content of PEG–C_{10}–Sepharose 6B. A Model 5100C osmometer (Wescor, Logan, UT, U.S.A.) was used for the measurement of the osmotic pressure of the mobile phase. An ISCO Model UA-5 absorbance monitor (Instrumentation Specialties, Lincoln, NE, U.S.A.) was used for detecting the absorbance of eluates at 254 and 405 nm simultaneously. An LKB 2112 Varioperpex II or 2132 Microperpex peristaltic pump and a RediRac fraction collector (LKB, Bromma, Sweden) were employed for chromatographing blood cells and for fractionation of the eluates. A Coulter counter Model D (Coulter Electronics, Harpenden, U.K.) was used for counting the number of blood cells. A FACE type PHW immersion wetting measurement apparatus (Kyowa Kaimenkagaku, Tokyo, Japan) was used for the determination of the rate of penetration of both water and mobile phase into PEG–C_{10}–Sepharose 6B xerogels.

Preparation of PEG–C_{10}–Sepharose 6B

Oxirane coupling of PEG 400, 4000, 6000 and 20M to epoxy-activated Sepharose 6B was performed at 40°C for 16 h in a solution of pH 12.0 as described previously[1]. PEG–C_{10}–Sepharose 6B gels having lower oxyethylene residue contents were prepared by oxiran coupling of PEG to epoxy-activated Sepharose 6B for shorter times. The residual epoxy groups on the PEG–C_{10}–Sepharose 6B were hydrolysed with 0.1 *M* perchloric acid for 1 h at room temperature[3].

Oxyethylene residue content in PEG–C_{10}–Sepharose 6B

Visible absorbance photometric determination. PEG–C_{10}–Sepharose 6B column packings were carefully washed with acetone on a glass filter-funnel (G3) and about 5 g of the packings were lyophilized. A 20-ml volume of 88% (v/v) formic acid was added to about 100 mg of the PEG–C_{10}–Sepharose xerogels and was heated for about 4 h on a boiling water-bath for hydrolysis and then evaporated to dryness under reduced pressure at 70°C. The hydrolysed product was dissolved in 10 ml of water. To 1-ml volume of this solution, 1 ml of dilute hydrochloric acid (1:4), 1 ml of 10% (w/v) barium chloride and 1 ml of 10% (w/v) phosphomolybdic acid were added. This mixture was allowed to stand for at least 1 h, during which a flocculent greenish precipitate formed from the phosphomolybdic acid and any PEG present. The precipitate was washed with 3 ml of 0.1 *M* hydrochloric acid and then with 7 ml of water. After centrifugation, the precipitate was dissolved in 3 ml of sulphuric acid, then 1 ml each of nitric acid and 70% (v/v) perchloric acid were added. The oxyethylene

residue content in the solution was determined according to the method of Shaffer and Critchfield[9] as follows. The solution was diluted with water, neutralized with sodium hydroxide and then diluted to 100 ml. To a 10-ml aliquot, phenylhydrazine in dilute sulphuric acid was added and the mixture was heated on water-bath. The absorbance was measured at 490 nm.

Determination by ^1H NMR. In a similar manner to the above, PEG–C_{10}–Sepharose 6B was hydrolysed with formic acid and the PEG present in this product was precipitated with phosphomolybdic acid. The precipitate was dissolved in 0.5 ml of [$^2H_2$]sulphuric acid for ^{1}H NMR measurements. A signal from the PEG–phosphomolybdic acid complex was observed at about 8.5 ppm as a single peak. The content of oxyethylene residues in the PEG-bonded gels (mg/g dry powder) was obtained from the integrated peak intensity originating from the PEG complex using calibration graphs (0–0.5 mg PEG/ml). The mean values of the oxyethylene residue contents (mg/g dry gel) in PEG 400–, 4000–, 6000– and 20M–C_{10}–Sepharose 6B column gels are given in Table I. The values obtained by ^{1}H NMR are in good agreement with those determined by visible absorbance photometry. The procedure for the determination of the oxyethylene residue content by ^{1}H NMR is simpler than that by absorbance photometry. The quantitative values obtained by the NMR method were used as the oxyethylene residue contents of PEG–C_{10}–Sepharose column packings.

Determination of rate of penetration of water or mobile phase solution into PEG–C_{10}–Sepharose xerogels

About 100 mg of PEG–C_{10}–Sepharose 6B xerogels were packed into a Teflon column (10 × 0.6 cm I.D.) by vertical tapping 200 times with the aid of a mechanical packer. The column was hung vertically with its lower end dipping into a liquid. The increase in weight of the packed column as a result of penetration of water or mobile phase solution into the xerogel was recorded automatically for the weight–time curve using the FACE immersion wetting measurement apparatus. The rate of penetration [l^2/t(cm^2/s)] was obtained using the following equation, which is same in principle as Washburn's equation[8]:

$$l^2/t(\mathrm{cm}^2/\mathrm{s}) = \{[L/(SL - W_s/\varphi_2)\varphi_1](Wt_2 - W_0)\}^2/t_2$$
$$W_0 = (\sqrt{t_2}Wt_1 - \sqrt{t_2}Wt_2)/(\sqrt{t_2} - \sqrt{t_1})$$

TABLE I

OXYETHYLENE RESIDUE CONTENT (mg/g DRY GEL) IN PEG–C_{10}–SEPHAROSE 6B COLUMN PACKING GELS

The values are averages of 3–15 determinations.

Bonded phase	*Absorbance photometry*	*^1H NMR*
PEG 400	13.3	13.5
PEG 4000	27.3	26.8
PEG 6000	46.1	46.3
PEG 20M	158.7	157.4

where L is the height of xerogel packed in the column, W_s is the cross-sectional area of the column [$(0.3 \times 0.3 \times \pi)$ cm^2], φ_1 is the specific gravity of water or mobile phase solution, φ_2 is the specific gravity of the xerogel, t_1 and t_2 (s) are the times of the start and end of measurement and w_1 and w_2 are the weight of liquid penetrated into the xerogel at t_1 and t_2 s, respectively.

Collection and isolation of blood cells

Human blood was drawn from normal male adult donors by venous puncture and heparin was added (0.05 ml of a 1000 U/ml solution per 10 ml of blood). A 1-ml volume of 3.8% (w/v) sodium citrate solution was added to 10 ml of blood for collection of platelets. Siliconized glassware was used in all procedures. Centrifugal isolation was used for erythrocytes. The isolation technique based on that of Leeksma and Cohén[10] was employed. For granulocytes, the sodium metrizoate–dextran sedimentation technique of Bøyum[11] was used. The sodium metrizoate–Ficoll sedimentation technique of Thorsby and Bratlie[12] was employed for the collection of lymphocytes. These isolation procedures for blood cells have been described in detail in previous papers[1,2].

Chromatography of blood cells

Sodium phosphate-buffered solution (0.09 *M*) containing 2% (w/w) dextran T40 at the isoelectric points ($pH_{c.p.}$) of the respective blood cells, which were determined previously[3] by the cross-partition method[13], was used as the mobile phase. These $pH_{c.p.}$ values for lymphocytes, erythrocytes and platelets are 5.2, 5.5 and 6.8, respectively. The $pH_{c.p.}$ value for granulocytes is the same as that of platelets. A 0.2-ml volume of the respective cell suspensions containing *ca.* $3 \cdot 10^4$ erythrocytes, *ca.* 10^6 platelets, *ca.* $2 \cdot 10^5$ granulocytes or *ca.* 10^5 lymphocytes was loaded separately onto the PEG–C_{10}–Sepharose 6B (45–165 μm wet particle diameter) column (25 × 0.9 cm I.D.) and eluted at a flow-rate of 10.0–12.0 ml/h by use of a peristaltic pump. The eluate was monitored at 254 and 405 nm. The fractions were collected in volumes of about 1.0 ml. Each fraction was diluted with Isoton and the number of blood cells was counted on a Coulter counter.

RESULTS

Correlations between oxyethylene residue content or number of these units and the rate of penetration

The rates of penetration of water or 0.09 *M* sodium phosphate-buffered solution containing 2% (w/w) dextran T40 (pH 6.8) into eleven kinds of PEG–C_{10}–Sepharose 6B xerogels are given in Table II.

There are four groups of column packing gels, PEG 400–, 4000–, 6000–and 20M–C_{10}–Sepharose 6B, corresponding to Nos. 1, 2–3, 4–5 and 6–11, respectively. Each group has the same number of oxyethylene units but a different oxyethylene residue content. It was observed that the rate of penetration of the liquids into these xerogels having nearly the same oxyethylene residue contents (Nos. 1, 2, 4 and 6) was slower as the number of these units increased from 9 (No. 1) to 470 (No. 6). It can be seen in Table II that the rate of penetration of water or the mobile phase is slower with increasing oxyethylene residue content in each group.

TABLE II

RATES OF PENETRATION OF DIFFERENT PEG-C_{10}–SEPHAROSE 6B XEROGELS

Bonded phase		*Oxyethylene residue content*	*Rate of penetration* (10^{-2} cm^2/s)	
No.	*Component*	*(mg/g dry gel)*	*Water*	*Mobile phase*[a]
1	PEG 400	13.5	34.0	15.0
2	PEG 4000	12.5	24.0	8.8
3	PEG 4000	26.8	18.0	1.2
4	PEG 6000	12.2	15.0	6.9
5	PEG 6000	46.3	3.9	5.1
6	PEG 20M	13.0	8.3	6.3
7	PEG 20M	27.4	5.9	5.9
8	PEG 20M	45.5	5.3	4.8
9	PEG 20M	63.0	4.8	3.4
10	PEG 20M	80.0	3.7	3.4
11	PEG 20M	157.4	3.0	1.4

[a] Contained 2% (w/w) of dextran T40–0.09 *M* NaH_2PO_4–Na_2HPO_4 (pH 6.8).

Plots of the rate of penetration of the liquids into xerogels *versus* oxyethylene residue content for eleven kinds of packings are shown in Fig. 1. In spite of the number of oxyethylene units, the rate increased with decrease in the oxyethylene residue content of the xerogels. The rate of penetration of water into the xerogels having less than 50 mg oxyethylene residues/g dry gel increased considerably (Fig. 1A). The rate of penetration of the mobile phase into xerogels increased linearly with decreasing oxyethylene residue content (Fig. 1B). This suggests that the influence of the oxyethylene residue content on the rate of penetration is greater than that of the number of oxyethylene units. The rate of penetration obtained by use of the mobile phase at pH 5.2 and 5.5, the $pH_{c,p.}$ values of erythrocytes and lymphocytes respectively, was the same as that obtained with the mobile phase at pH 6.8 (data not shown).

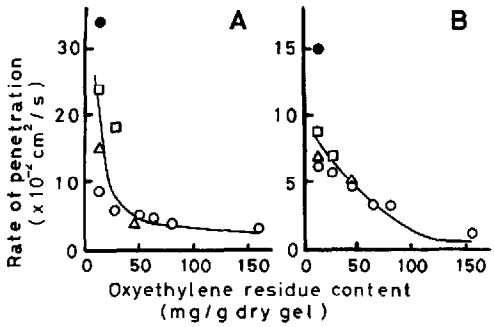

Fig. 1. Relationship between oxyethylene residue content and the rate of penetration of (A) water or (B) the mobile phase into PEG–C_{10}–Sepharose 6B xerogels. ● = PEG 400; □ = PEG 4000; △ = PEG 6000; ○ = PEG 20M.

Capacity factors of blood cells on PEG–C_{10}–Sepharose 6B columns

Table III shows the capacity factors (k') of human blood cells determined by elution from PEG–C_{10}–Sepharose 6B columns (25 × 0.9 cm I.D.) with 0.09 *M* sodium phosphate buffer ($pH_{c.p.}$) containing 2% (w/w) dextran T40 together with the oxyethylene residue contents of the packing gels. These k' values of four kinds of blood cells were calculated by combining the retention volumes of these cells with the interstitial volumes (V_m) of the column determined by the elution volume of native dextran as described previously[6]. The k' values of erythrocytes and platelets were less than 1.0 but not zero, and it was clear that these cells were retained slightly on the PEG–C_{10}–Sepharose 6B columns. The maximum k' values of 0.49 for erythrocytes and 0.71 for platelets were obtained on the column with 80.0 and 157.4 mg/g dry gel oxyethylene residue contents (Nos. 10 and 11), respectively.

On the other hand, the k' values of granulocytes and lymphocytes were higher than 1.0 for every column used. The increase in the oxyethylene residue contents in the groups which bonded the same number of oxyethylene units (Nos. 2–3, 4–5 and 6–11) resulted in an increase in the k' values of granulocytes and lymphocytes, except in a few instances. It found that the k' values of granulocytes were above 2.0 by use of PEG–C_{10}–Sepharose 6B columns having oxyethylene residue contents of more than 63.0 mg/g dry gel. The k' values of lymphocytes were greater than those of granulocytes on every PEG–C_{10}–Sepharose column. The column packings with oxyethylene residues bonded more than 26.5 mg/g dry gel gave the k' values above *ca.* 2.5 for lymphocytes. As the oxyethylene residue content increased above 45.5 mg/g dry gel, the k' values became greater than *ca.* 3.0 (Nos. 8–11).

TABLE III

CAPACITY FACTORS OF HUMAN ERYTHROCYTES (E), PLATELETS (P), GRANULOCYTES (G) AND LYMPHOCYTES (L)

Columns: PEG–C_{10}–Sepharose 6B (25 × 0.9 cm I.D.). The mobile phase contained 2% (w/w) of dextran T40–0.09 *M* NaH_2PO_4–Na_2HPO_4 ($pH_{c.p.}$).

Bonded phase[a]	V_m *(ml)*[b]	*Capacity factors (k')*			
		E	*P*	*G*	*L*
1	3.6	0.19	0.17	1.14	1.56
2	3.6	0.36	0.44	1.47	1.89
3	3.6	0.31	0.53	1.83	2.75
4	3.6	0.39	0.47	1.52	1.89
5	3.9	0.38	0.41	1.90	2.97
6	3.6	0.16	0.44	1.78	1.92
7	4.1	0.17	0.42	1.71	2.49
8	3.8	0.39	0.45	1.97	3.08
9	3.8	0.42	0.50	2.00	3.08
10	3.7	0.49	0.57	2.08	3.22
11	3.8	0.47	0.71	2.00	3.21

[a] See Table II.

[b] V_m: elution volume of native dextran.

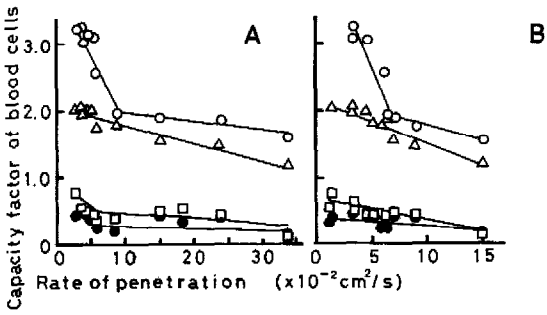

Fig. 2. Relationship between capacity factors of human (●) erythrocytes, (□) platelets, (△) granulocytes and (○) lymphocytes and the rate of penetration of (A) water or (B) the mobile phase into PEG–C_{10}–Sepharose 6B xerogels.

Correlation between the capacity factors of blood cells and the rate of penetration

The rate of penetration of both water and the mobile phase into eleven kinds of PEG–C_{10}–Sepharose 6B xerogels is plotted in Fig. 2 as a function of the k' values of erythrocytes, platelets, granulocytes and lymphocytes obtained on these PEG–C_{10}–Sepharose 6B columns by use of phosphate-buffered mobile phase at the respective $pH_{c.p.}$ values. The packing gel with a rate of penetration of water of less than $10 \cdot 10^{-2}$ cm^2/s and of the mobile phase of less than $7.0 \cdot 10^{-2}$ cm^2/s gave a substantial increase in the k' values of lymphocytes. For granulocytes, linear relationships were found between k' and the rate of penetration of both water and the mobile phase into these xerogels. The k' values of platelets increased slightly when using packing gels with a slower rate of penetration of both water and the mobile phase. The k' values of erythrocytes were almost unchanged even when the PEG–C_{10}–Sepharose 6B packing beads having the slowest rate of penetration were used.

DISCUSSION

It has been shown that the hydrophobic interactions between peripheral blood cells and the bonded PEG or PPG phase of column packing gels play an important role in retaining the cells on these columns in our chromatographic system[4–6]. It has also been shown that the retention of the blood cells depends on the difference in the hydrophobicity of the packing beads based on the oxyethylene or oxypropylene residue content and on the number of these units[4,5].

In previous papers[5,6], the hydrophobicity ($\Delta\log K$ values) of PEG C_{10}–Sepharose, PPG–C_3–Chromagel beads and blood cells were determined by hydrophobic affinity partition[7] using PEG 6000 monopalmitate or Pluronic P84, a block polymer of PEG and PPG, as the hydrophobic ligand. A linear relationship was found between the capacity factors (k') of granulocytes on PPG–C_3–Chromagel columns and the $\Delta\log K$ values of the gel beads. The retention of lymphocytes increased gradually on the columns of packing gel beads with $\Delta\log K$ values <0.5, but increased considerably on the gel beads with values >0.5.

In general, the hydrophobic affinity partition method has been applied to the measurement of the hydrophobicities of soluble proteins, insoluble suspended materials such as cell particles, subcellular organelles and cell membranes[14]. Partition

of the particles such as the blood cells and the packing gel beads was characterized by the partition coefficient (K) calculated as the ratio of the particle concentration in the upper PEG-rich phase to that in the bottom dextran-rich phase. In this method, the number of particles attached to the interface is calculated as the difference between the total number added and the number in the upper or the bottom phase. Half of the number in the interface fraction add to each number in the upper and bottom phases[4]. The partition experiments for the particles are tedious because the separation of the two immiscible aqueous phases is particularly slow and many particles such as blood cells and gel beads become attached to the interface of the two PEG and dextran phases. In addition, the $\Delta\log K$ values determined by the partition method are dominated by chemical species of the hydrophobic ligand, such as PEG palmitate or Pluronic.

It is well known that the wetting of powder particles is of great importance for the measurement of the affinity between particles and a liquid. In general, the wettablity is determined as the contact angle of a liquid droplet which forms on the compressed powder particles. The other method for determining the wetting of powder particles by a liquid is the immersion wetting method[8], in which the wettability is determined as the rate of penetration of the liquid which rises in a column packed with the particles.

In this paper, we determined the rate of penetration of water or the mobile phase used in our chromatographic systems for the separation of blood cells into eleven kinds of PEG–C_{10}–Sepharose 6B xerogels. The rate was considered as a measure of hydrophobicity of these column packing gels. It was found that the hydrophobicity of the packing gels increases with decrease in the rate of penetration of water or the mobile phase (Fig. 1). The rate of penetration of these liquids into PEG–C_{10}–Sepharose xerogels was slow as the oxyethylene residue content increased. It was also found that the rate decreased with increase in the number of oxyethylene units. This suggests that the oxyethylene residue content rather than the number of oxyethylene units affects the hydrophobicity of PEG–C_{10}–Sepharose beads (Table II).

The capacity factors (k') of four kinds of human blood cells were calculated by combining the retention volumes of the cells with the elution volume of the native dextran by using a PEG–C_{10}–Sepharose 6B column and a 0.09 M phosphate-buffered solution containing 2% (w/w) dextran T40 at the respective isoelectric points ($pH_{c.p.}$) of the cells. The relationship between the rate of penetration of water or the mobile phase into PEG–C_{10}–Sepharose xerogels and the k' values of the blood cells was examined (Fig. 2). It is clear that the k' values of lymphocytes on a PEG–C_{10}–Sepharose column increase considerably when the rate of penetration of water is $> 10 \cdot 10^{-2}$ cm^2/s and that of the mobile phase is $> 7 \cdot 10^{-2}$ cm^2/s. A linear correlation was found between the k' values of granulocytes and the rate of penetration of both water and the mobile phase. The k' values of granulocytes and lymphocytes increase with decrease in the rate of penetration. These results are in fair agreement with the preceding study[6] in which the k' values of the granulocytes increased linearly with increasing the hydrophobicity ($\Delta\log K$) of PPG–C_3–Chromagel beads and the retention of lymphocytes increased considerably on PPG-bonded gels with $\Delta\log K > 0.5$. Platelets were retained on the PEG–C_{10}–Sepharose column having the slowest rate of penetration. On the other hand, no change was found for the k' values of erythrocytes even if we used the PEG–C_{10}–Sepharose column with the highest oxyethylene residue content (Fig. 2).

In conclusion, it is clear that hydrophobic interactions play an important role in the retention and separation of granulocytes and lymphocytes on PEG–C_{10}–Sepharose 6B columns. The use of PEG-bonded gels with higher oxyethylene residue contents with a slower rate of penetration of the liquids is necessary for separating erythrocytes and platelets on the chromatographic columns.

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