Journal of Chromatography, 398 (1987) 153–164
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 552

SURFACE AFFINITY CHROMATOGRAPHIC SEPARATION OF BLOOD CELLS

VI. CAPACITY FACTORS OF HUMAN PERIPHERAL BLOOD CELLS ON POLY(PROPYLENE GLYCOL) BONDED CHROMAGEL COLUMNS AND THEIR CORRELATION WITH SURFACE HYDROPHOBICITIES OF THE GEL BEADS AND THE CELLS

YŌICHI SHIBUSAWA*, USHIHO MATSUMOTO and MASAHIRO TAKATORI
Division of Analytical Chemistry, Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-03 (Japan)
(Received March 11th, 1987)

SUMMARY

The chromatographic behaviour of human peripheral blood cells was investigated on poly(propylene glycol) (PPG)-bonded Chromagel. The mobile phase was 0.09 *M* phosphate-buffered 2% (w/w) dextran T40 solution at the pH of the respective isoelectric points of human blood cells. The elution order of four kinds of blood cells from PPG–C_3–Chromagel columns coincided with that of the $\Delta \log K$ values of these cells determined by the hydrophobic affinity partition method using Pluronic P84 as a hydrophobic ligand. The capacity factors of granulocytes and lymphocytes increased with increase in the $\Delta \log K$ values of PPG–C_3–Chromagel beads. Hydrophobic interactions contributed to the retention of the four kinds of blood cells on PPG-bonded Chromagel columns.

INTRODUCTION

In previous studies[1,2], the chromatographic behaviour of human blood cells was studied using bisoxirane-coupled poly(ethylene glycol) 20000 (PEG 20M)–Sepharose 6B (PEG 20M–C_{10}–Sepharose 6B) as the column packing with sodium phosphate buffer (pH 7.5) containing 2% (w/w) dextran T40 or T500 as the mobile phase. The blood cells were eluted from the column in the order erythrocytes, platelets, granulocytes and lymphocytes, with some exceptions. An increase in the sodium chloride concentration in the mobile phase while maintaining the isotonicity resulted in an increase in the retention volumes of granulocytes and lymphocytes with the PEG 20M–C_{10}–Sepharose 6B system. This is consistent with the well known fact that in hydrophobic interaction chromatography the affinity between the stationary phase and the hydrophobic moiety of soluble proteins increases proportionally to the neutral salt concentration in the mobile phase[3]. It was shown that the retention

0021-9673/87/$03.50

volumes of granulocytes and lymphocytes showed a marked dependence on the number of bonded oxyethylene units in the range from 9 (PEG 400) to 450 (PEG 20M) at the isoelectric points of blood cells ($pH_{c.p.}$), increasing with increasing number of oxyethylene units[4]. It was assumed that the hydrophobic interaction between the surface membrane of the blood cells and the bonded PEG phase contributed to the retention of, at least, granulocytes and lymphocytes.

The hydrophobicity ($\Delta\log K$ values) of four kinds of cells were determined by the hydrophobic affinity partition method proposed by Shanbhag and Axelsson[5] using PEG 6000 monopalmitate as a hydrophobic ligand. A linear correlation between the retention volumes of platelets, granulocytes and lymphocytes on PEG 20M–C_{10}–Sepharose 6B and the $\Delta\log K$ values of these cells was found[6].

In previous work[7], glycidyl ethers of poly(propylene glycols) (PPGs) were coupled to several kinds of agarose beads (PPG–C_3–agarose column packings) by a method similar to that of Hjertén *et al.*[8]. PPG 400–C_{10}– and PEG 20M–C_{10}–Sepharose, which differ from PPG–C_3–agarose in the coupling mode of the bonded stationary phase, were also prepared and the chromatographic behaviour of blood cells was compared with that using PPG–C_3–agarose columns. The retention volumes of platelets, granulocytes and lymphocytes increased linearly with their $\Delta\log K$ values on all the columns examined. However, erythrocytes did not conform to this correlation because of their much higher $\Delta\log K$ values. An approximately linear relationship was found between the retention volumes of both granulocytes and lymphocytes and the $\Delta\log K$ values using PPG–C_3–agarose beads. In studies of the chromatographic behaviour of human peripheral blood cells, the retention volumes of erythrocytes and platelets are smaller than those of granulocytes and lymphocytes on all PEG– and PPG–agarose columns.

In this study, we prepared several PPG–C_3–Chromagel A4 column packings with different numbers of oxypropylene units and with different amounts of oxypropylene residues. The $\Delta\log K$ values of four kinds of blood cells and PPG–C_3–Chromagel beads were measured by using Pluronic P84, a block polymer of PEG and PPG, instead of PEG 6000 monopalmitate as the hydrophobic ligand. Further, in order to measure the interstitial volumes of PPG–C_3–Chromagel columns, five kinds of large hydrophilic polymers were eluted independently from these columns. The relationship between the capacity factors of blood cells and the $\Delta\log K$ values of cells and the values for the packing gel beads is also discussed.

EXPERIMENTAL

Materials

Dextran T40 (weight-average molecular weight, M_w = 40000) was obtained from Pharmacia (Uppsala, Sweden). Poly(ethylene glycol) 6000 (PEG 6000, extra-pure grade; number-average molecular weight, M_n = 6000–7500) was purchased from Wako Junyaku (Osaka, Japan), poly(propylene glycol) 200 and 400 diglycidyl ether (DER 732 and 736) from Dow Chemical Japan (Tokyo, Japan) and poly(propylene glycol) 950 (PPG 950, extra-pure grade; M_n = 950) from Sanyo Kasei (Kyoto, Japan). Chromagel A4 (wet diameter 50–150 mesh) was obtained from Dojin Yakkagaku (Kumamoto, Japan) and Pluronic P84, a block polymer of PEG 1950 and PPG 2250 (PEG 40%, PPG 60%, M_n = 4200) from Asahi Denka (Tokyo, Japan).

Poly(ethylene oxide) (PEO 18, $M_w = 4.3 \cdot 10^6$–$4.8 \cdot 10^6$) was from Seitetsu Chemicals (Osaka, Japan), beef liver glycogen ($M_w = 2.7 \cdot 10^5$–$3.5 \cdot 10^6$) from ICN Nutritional Biochemicals (Cleveland, OH, U.S.A.), pullulan P-2400 ($M_w = 2.46 \cdot 10^6$), polysaccharide for gel permeation chromatography from Showa Denko (Tokyo, Japan) and native dextran ($M_w = 5.0 \cdot 10^6$–$4.0 \cdot 10^7$) from Tokyo Kasei (Tokyo, Japan). Other reagents were of analytical-reagent grade. PPG 950 glycidyl ether was prepared from PPG 950 and epichlorohydrin as described previously[7].

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 the oxypropylene residue content of Chromagel A4. A Coulter Counter Model D (Coulter Electronics, Harpenden, U.K.) was used for counting the number of blood cells and packing gel beads. A Model 5100 C osmometer (Wescor, Logan, UT, U.S.A.) was used for the measurement of the osmotic pressure of the mobile phase or dextran–PEG two-phase systems. 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 404 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 Model R-403 differential refractometer (Waters Assoc., Milford, MA, U.S.A.) was used for measuring the refractive indices of polymers eluted from the PPG–C_3–Chromagel columns.

Preparation of PPG–C_3–Chromagel

Oxirane coupling of PPG 200 or 400 diglycidyl ether or PPG 950 glycidyl ether to Chromagel A4 beads was performed in dioxane as described previously[7]. A 100-ml volume of wet Chromagel A4 beads was washed successively with 100-ml portions of water–dioxane (4:1, 3:2, 2:3 and 1:4) and seven times with dioxane. Gel beads were suspended in 100 ml of dioxane and 2 ml of 48% boron trifluoride etherate in diethyl ether were added. The mixture was stirred for 5 min and various amounts of PPG glycidyl ethers ($3.0 \cdot 10^{-3}$–$5.6 \cdot 10^{-2}$ mol) in 10 ml of dioxane were added dropwise. The reaction mixture was stirred for 40 min at 20–25°C. After the reaction, the products were washed according to the procedure mentioned above but in the reverse order, and finally washed with water. The residual epoxy groups on the PPG–C_3–Chromagel were hydrolysed by shaking with 0.1 *M* perchloric acid for 1 h at room temperature.

Determination of the oxypropylene residue content bonded to PPG–C_3–Chromagel by ^1H NMR spectroscopy

PPG–C_3–Chromagel beads were carefully washed with acetone on a glass filter-funnel and about 5 g of the beads were dried *in vacuo* (P_2O_5) overnight at 70°C. A 40-ml volume of 88% (w/v) formic acid was added to about 200 mg of gel beads and the mixture was heated for about 4 h on a boiling water-bath for complete hydrolysis and then evaporated to dryness under reduced pressure at 70°C. The hydrolysed product was dissolved in ^2H_2O and lyophilized. This deuterium exchange procedure was repeated three times. Finally, 100 mg of lyophilized powder were

weighed and dissolved in 0.5 ml of [$^2H_6$]dimethyl sulphoxide for ^1H NMR measurement.

As an example, a ^1H NMR spectrum of hydrolysed PPG 400–C_3–Chromagel is shown in Fig. 1A. The methyl proton signal from PPG is observed at 1.0 ppm and is completely resolved from the spectral background originating from galactose oligomers in the hydrolysed Chromagel. Thus, the oxypropylene residue content of PPG 400 in the gel (mg/g dry powder) was obtained from the integrated peak intensity of the methyl proton using a calibration graph. Three calibration graphs of concentration of PPG 200 and 400 diglycidyl ethers and PPG 950 glycidyl ether *versus* the integrated peak intensity of the methyl proton of PPG 200, 400 and 950 were constructed (Fig. 1B). Linear relationships occur up to 5 mg/ml (16, 10 and 5 μmol/g) of PPG 200 and 400 diglycidyl ethers and PPG 950 glycidyl ether. Hence the oxypropylene residue content in PPG 200–, 400– and 950–C_3–Chromagel (mg/g dry gel) could be calculated.

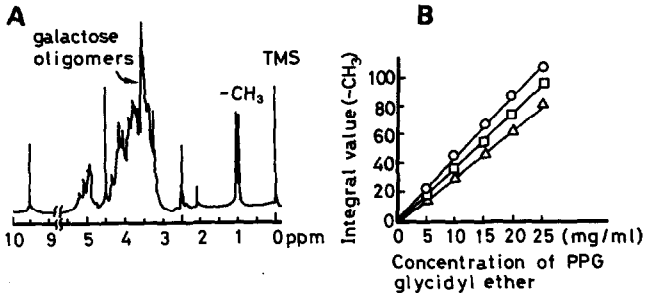

Fig. 1. (A) ^1H NMR spectrum of hydrolysed PPG 400–C_3–Chromagel A4 in [$^2H_6$]dimethyl sulphoxide at 200 MHz and (B) calibration graphs of integral value of methyl proton of PPG 200 (○), 400 (□) and 950 (△) glycidyl ethers. Measurement conditions: spectral width, 2000 Hz; pulse width, 5 μs; pulse recycle time, 20 s; accumulation, 4 times.

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. The isolation procedures for erythrocytes, platelets, granulocytes and lymphocytes have been described in detail in previous papers[1,2].

Hydrophobic affinity partition of blood cells and PPG–C_3–Chromagel beads

The dextran T40–PEG 6000 two-phase system was similar to that used by Shanbhag and Axelsson[5] except that dextran T40 was used instead of T70. Human peripheral erythrocytes, platelets, granulocytes and lymphocytes were partitioned in two-phase systems containing 8% (w/w) of dextran T40 and 8% (w/w) of PEG 6000 with and without 10, 20, 30, 40 and 50% (w/w) of Pluronic P84 in the total PEG 6000 [0.8, 1.6, 2.4, 3.2 and 4.0% (w/w) final concentration in the system]. The systems also contained 71.3 m*M* sodium chloride in order to maintain the isotonicity. The pH of each two-phase system was adjusted to the isoelectric points ($pH_{c.p.}$) of the

blood cells, which was determined previously by the cross-partition method[9]. These $pH_{c.p.}$ values for lymphocytes, erythrocytes and platelets are pH 5.2, 5.5 and 6.8, respectively. The $pH_{c.p.}$ of granulocytes is the same as that of platelets.

A 0.1-ml volume of a suspension of blood cells (erythrocytes, $2.7 \cdot 10^5$; platelets, 10^3–$6 \cdot 10^3$; granulocytes, $7 \cdot 10^4$; lymphocytes, 10^5) or PPG–C_3–Chromagel beads ($7.3 \cdot 10^3$–$10.9 \cdot 10^3$) in 0.9% (w/w) saline was added to 8.0 g of the phase systems. The phase systems were gently mixed by 30 inversions and were allowed to separate for 10–15 min at 20–25°C. After phase separation, 1 ml each of both the upper and lower phases were carefully removed with a pipette. An aliquot of each phase was diluted with 30 ml of saline and the number of blood cells or PPG–C_3–Chromagel beads was counted on a Coulter Counter as described previously[7].

The hydrophobicity ($\Delta\log K$) of blood cells or gel beads is given by $\Delta\log K = \log K_p - \log K_0$, where K_p is the partition coefficient in the two-phase system containing Pluronic P84 as a hydrophobic ligand and K_0 is the partition coefficient in the two-phase system in the absence of Pluronic P84. The partition experiments were repeated more than five times for each sample, and the blood cell or gel bead concentration determined in the phases deviated from the mean value by less than 5%.

Chromatography of blood cells

Sodium phosphate buffer solution (0.09 *M*) containing 2% (w/w) dextran T40 at $pH_{c.p.}$ was used as the mobile phase. PPG–C_3–Chromagel beads (210–300 μm) were suspended in the mobile phase and packed into the columns (25 × 0.9 cm I.D.) by the slurry-packing method. 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 on to the column and eluted at a flow-rate of 6.0–12.0 ml/h by use of a peristaltic pump. The eluate was monitored at 254 and 404 nm. The fractions were collected in volumes of 1.0–2.0 ml. An aliquot of each fraction was diluted with Isoton and the number of blood cells was counted on a Coulter Counter.

Interstitial volume of PPG–C_3–Chromagel columns

Five polymers (Fig. 2) were eluted from PPG–C_3–Chromagel columns (25 × 0.9 cm I.D.) independently with 0.09 *M* sodium phosphate buffer containing 2% (w/w) dextran T40 ($pH_{c.p.}$). A 0.2-ml volume of the respective polymer solution containing 0.25% (w/v) poly(ethylene oxide), 0.2% (w/v) Blue Dextran 2000, 0.5% (w/v) beef liver glycogen, 0.5% (w/v) pullulan P-2400 and 0.1% (w/v) native dextran was loaded separately on to the column. The Blue Dextran 2000 was detected at 254 nm and the other polymers were monitored with a refractometer.

RESULTS

Hydrophobic affinity partition of blood cells

In order to determine the $\Delta\log K$ values of four kinds of human peripheral blood cells, we used Pluronic P84, a surfactant of a block polymer of PEG 1950 and PPG 2250, as a hydrophobic ligand in affinity partition systems. The $\Delta\log K$ values of the blood cells were measured as a function of the ratio of Pluronic P84 to PEG

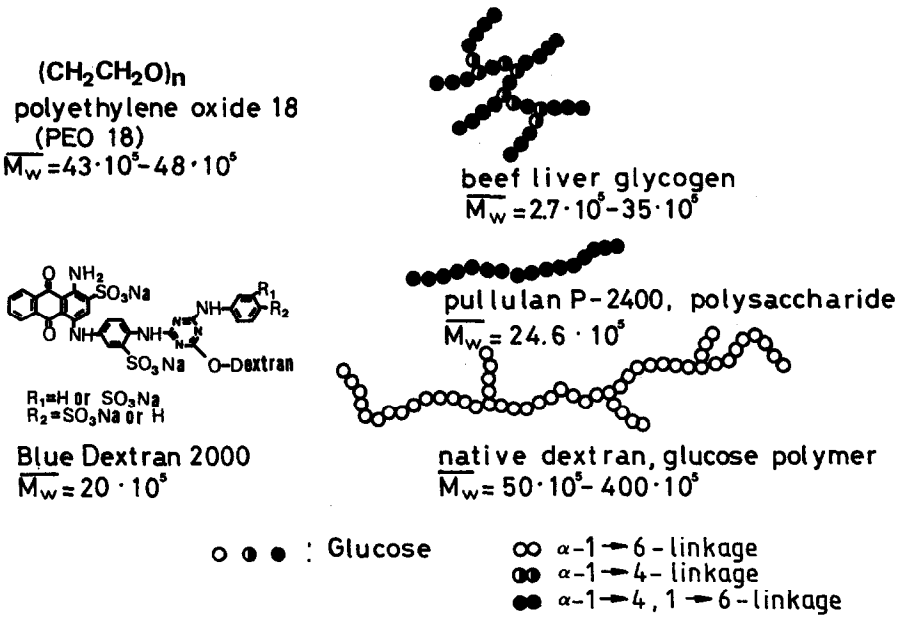

Fig. 2. Five large hydrophilic polymers used for determination of interstitial volume of the PPG–C_3–Chromagel columns.

6000 in the PEG-rich upper layer of the PEG 6000–dextran T40 two-phase system. The $\Delta\log K$ values of the four kinds of blood cells increased monotonously with increase in the ratio of Pluronic P84 in the upper layer (data not shown). As Pluronic P84 is not dissolved in the PEG-rich layer beyond about a 1:1 (w/w) ratio, it is kept at this concentration in the dextran T40–PEG 6000 two-phase system. The final concentration of Pluronic P84 is 4% (w/w) in the two-phase system. The $\Delta\log K$ values determined in this two-phase system were found to be -0.19 for erythrocytes, 0.23 for platelets, 0.42 for granulocytes and 0.47 for lymphocytes.

Hydrophobic affinity partition of PPG–C_3–Chromagel beads

About a 1:1 ratio of Pluronic P84 to PEG 6000 in the PEG-rich upper layer of the dextran T40–PEG 6000 two-phase system gives the largest $\Delta\log K$ values of four kinds of blood cells, as mentioned above. Therefore, the $\Delta\log K$ values of PPG–C_3–Chromagel beads were also determined at this ratio (*i.e.*, Pluronic P84:PEG 6000 = 1:1). The $\Delta\log K$ values of the gel beads are summarized in Table I together with the oxypropylene residue content per gram of dry PPG–C_3–Chromagel.

There are three groups of column packing gels, PPG 200–, 400– and 950–C_3–Chromagel A4, corresponding to Nos. 1–3, 4–6 and 7–9, respectively. Each group has the same number of oxypropylene units but a different oxypropylene residue content. It can be seen in Table I that the hydrophobicity ($\Delta\log K$ values) of the packing beads at any pH increases with increasing oxypropylene residue content in each group.

TABLE I

ΔLOG *K* VALUES OF PPG–C_3–CHROMAGEL A4 BEADS AT THE ISOELECTRIC POINTS OF FOUR KINDS OF BLOOD CELLS

Isoelectric points: erythrocytes, pH 5.5; platelets and granulocytes, pH 6.8; and lymphocytes, pH 5.2. The constituents of the two-phase partition systems are 8% (w/w) of dextran T40, 8% (w/w) of PEG 6000 with or without 50% (w/w) of Pluronic P84, 71.3 m*M* sodium chloride, at the pH of the isoelectric points of the cells.

No.	*Bonded phase*	*Oxypropylene residue content (mg/g dry gel)*	*Δlog K*		
			pH 5.2	*pH 5.5*	*pH 6.8*
1	PPG 200	15.5	0.08	0.03	0.10
2		37.0	0.40	0.28	0.27
3		86.6	0.60	0.56	0.42
4	PPG 400	6.8	0.08	0.05	0.08
5		36.9	0.34	0.24	0.13
6		73.8	0.55	0.42	0.35
7	PPG 950	36.4	0.14	0.19	0.23
8		72.0	0.53	0.31	0.27
9		100.6	0.80	0.71	0.77

Chromatography of blood cells

Table II shows the retention volumes of human blood cells determined by eluting from PPG 200–, 400– and 950–C_3–Chromagel A4 with 0.09 *M* sodium phosphate buffer ($pH_{c.p.}$) containing 2% (w/w) dextran T40. Granulocytes and lymphocytes show a strong affinity for the columns that have the highest oxypropylene

TABLE II

RETENTION VOLUMES OF HUMAN PERIPHERAL ERYTHROCYTES (E), PLATELETS (P), GRANULOCYTES (G) AND LYMPHOCYTES (L)

Mobile phase, 2% (w/w) dextran T40 buffered with 0.09 *M* sodium phosphate. Isoelectric points of blood cells as in Table I. Column, 25 × 0.9 cm I.D. The retention volumes on the PPG–C_3–Chromagel A4 are averages of several column runs.

No.	*Bonded phase*	*Oxypropylene residue content (mg/g dry gel)*	*Retention volume (ml)*			
			E	*P*	*G*	*L*
1	PPG 200	15.5	4.9	5.7	7.3	15.6
2		37.0	5.0	5.1	8.2	16.2
3		86.6	5.0	4.6	11.8	20.2
4	PPG 400	6.8	4.6	4.5	9.5	14.4
5		36.9	4.7	5.0	10.1	16.3
6		73.8	4.6	4.5	12.7	16.5
7	PPG 950	36.4	4.8	5.8	10.4	16.3
8		72.0	3.8	5.4	10.6	18.9
9		100.6	4.8	7.3	15.4	26.2

residue contents (Nos. 3, 6 and 9) in each group (Nos. 1–3, 4–6 and 7–9). Lymphocytes especially show a notable increase in retention on the columns that have about 16 oxypropylene units (PPG 950) (Nos. 7–9). Further, the retention volumes of the four kinds of blood cells were determined on the columns packed with PPG–C_3–Chromagels having nearly the same oxypropylene residue contents with different numbers of oxypropylene units (Nos. 2, 5 and 7). As the number of oxypropylene units increases from about 7 (No. 2) to 9 (No. 5) the retention volumes of granulocytes increase, but those of lymphocytes remain unaltered. However, the retention volumes of erythrocytes and platelets exhibit only small but non-systematic changes with regard to the oxypropylene residue content and the number of oxypropylene units.

Determination of interstitial volumes of PPG–C_3–Chromagel columns and capacity factors of blood cells

Five large hydrophilic polymers shown in Fig. 2 were selected for the determination of interstitial volume of PPG–C_3–Chromagel columns. These polymers were eluted from PPG–C_3–Chromagels (Nos. 3, 6 and 9) that have the highest oxypropylene residue content in each group (Nos. 1–3, 4–6 and 7–9) with 0.09 *M* sodium phosphate buffer solution containing 2% (w/w) dextran T40 ($pH_{c.p.}$). Poly(ethylene oxide) (PEO-18) and Blue Dextran 2000 were retained longer on every PPG–C_3–Chromagel A4 than on the Chromagel A4 without any PPG-bonded phase. It seems that PEO-18 or Cibacron Blue residue of Blue Dextran interacts with the PPG-bonded phase. Beef liver glycogen and pullulan P-2400 permeate into the cross-linked gel matrix of the Chromagel A4 support material of the PPG-bonded phase and these two polymers also did not appear to be suitable for the determination of the interstitial volume of PPG–C_3–Chromagel columns. The elution volume of native dextran is about 3.8–3.9 ml on both PPG–C_3–Chromagel and Chromagel A4 columns. These values are adequately approximated by the calculated interstitial volume of the column. This calculated interstitial volume (V_m) of the column is given by $V_m = V_c - (w/d)$, where V_c is the column volume (15.9 cm^3), w is the weight of the packing in the column (25 × 0.9 cm I.D.) and d is the specific gravity of packing beads. Consequently, the elution volume of native dextran provides an estimate of the interstitial volumes of PPG–C_3–Chromagel columns. The capacity factors of four kinds of blood cells were calculated by combining these retention volumes with the interstitial volumes of the column.

Table III shows the capacity factors of four kinds of blood cells on nine PPG–C_3–Chromagel columns together with interstitial volumes (V_m) of the columns. The capacity factors of erythrocytes and platelets are less than 1.0 but not zero, except for erythrocytes on the PPG 950–C_3–Chromagel column (No. 8). It is clear that these two kinds of cells are retained slightly on these PPG–C_3–Chromagel columns. The capacity factors of granulocytes are higher than 1.0 except on column No.1, which has the lowest oxypropylene residue content of the PPG 200 bonded packing group (Nos. 1–3). Lymphocytes have much higher capacity factors than those of the other three kinds of blood cells on every column.

Correlations between capacity factors of blood cells and their Δlog K values

The capacity factors of four kinds of blood cells on the PPG 200–, 400– and

TABLE III

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

Mobile phase and columns as in Table II.

No.	*Bonded phase*	V_m *(ml)**	*Capacity factor*			
			E	*P*	*G*	*L*
1	PPG 200	3.9	0.26	0.46	0.87	3.00
2		3.9	0.28	0.31	1.08	3.15
3		3.8	0.32	0.21	2.11	4.32
4	PPG 400	3.9	0.18	0.28	1.44	2.69
5		3.9	0.21	0.28	1.59	3.18
6		3.9	0.18	0.15	2.26	3.23
7	PPG 950	3.9	0.23	0.49	1.67	3.18
8		3.8	0.00	0.42	1.79	3.97
9		3.9	0.23	0.87	2.95	5.72

* Elution volumes of native dextran.

950–C_3–Chromagel are plotted against their $\Delta\log K$ values in Fig. 3. The blood cells that have higher $\Delta\log K$ values, such as granulocytes and lymphocytes, are retained longer on PPG–C_3–Chromagel than erythrocytes and platelets with lower $\Delta\log K$ values. It can be seen that the order of elution of blood cells from column is ery-

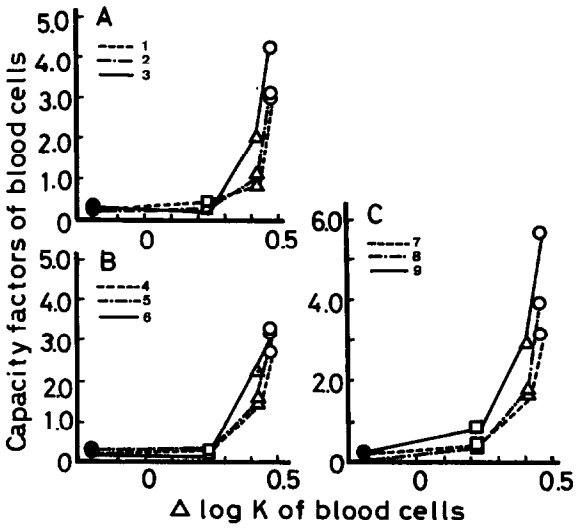

Fig. 3. Relationship between capacity factors of human erythrocytes (●), platelets (□), granulocytes (△) and lymphocytes (○) and their $\Delta\log K$ values at the isoelectric points of the cells on (A) PPG 200–, (B) 400– and (C) 950–C_3–Chromagel columns. Packing gels 1–9 as in Table I. The mobile phase for the chromatography and the isoelectric points of the cells are listed in Table I. The hydrophobic affinity partition systems for the cells are described under Experimental.

throcytes, platelets, granulocytes and lymphocytes, which corresponds to the hydrophobicity (Δlog K values) of the four kinds of blood cells.

The capacity factors of platelets granulocytes and lymphocytes which have Δlog K values higher than 0.20 increase linearly with their Δlog K values on columns with higher oxypropylene residue contents (Nos. 3, 6 and 9) among each group.

Correlations between capacity factors of blood cells and Δlog K values of PPG–C_3–Chromagel beads

The capacity factors of erythrocytes, platelets, granulocytes and lymphocytes obtained on PPG–C_3–Chromagel columns at the respective $pH_{c.p.}$ values are plotted against the Δlog K values of several packing particles in Fig. 4. An approximately linear relationship is found between the capacity factors of granulocytes and the Δlog K values of six gel beads. The capacity factors of lymphocytes increase slightly when using gel beads with Δlog K values smaller than 0.5. On the other hand, the packing beads with Δlog K values of more than 0.5 cause a rapid increase in the retention of lymphocytes. The capacity factors of platelets increase on packing beads with the highest Δlog K values.

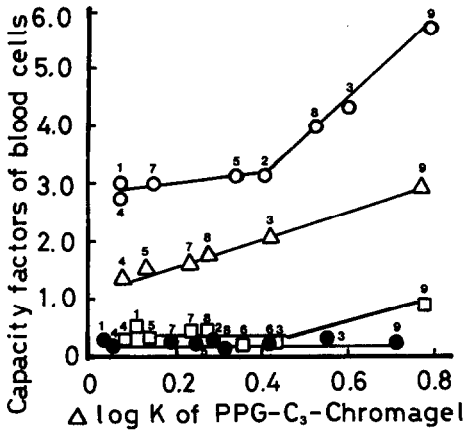

Fig. 4. Relationship between capacity factors of human erythrocytes (●), platelets (□), granulocytes (△) and lymphocytes (○) and Δlog K values of the PPG–C_3–Chromagel beads. Mobile phase and packing gels 1–9 as in Table I. The hydrophobic affinity partition systems for the packing beads are described under Experimental.

DISCUSSION

It has been shown that the surface hydrophobicity of four kinds of blood cells and the column packing beads play an important role in the chromatographic behaviour of blood cells. In previous studies[6,7], the hydrophobicity (Δlog K values) of the four kinds of blood cells and PEG– and PPG–agarose beads were obtained by the hydrophobic affinity partition method using PEG 6000 monopalmitate as a hydrophobic ligand. It was found that as the Δlog K values of the cells, platelets, granulocytes and lymphocytes increased, their retention volumes also increased linearly in this order. However, the Δlog K values of erythrocytes were the highest among

the four kinds of blood cells in the PEG–dextran two-phase system containing sodium chloride and was next to that of lymphocytes in the system without sodium chloride. Despite the large $\Delta\log K$ values of erythrocytes, their retention volumes were the smallest of the four or after platelets on every PEG- and PPG–agarose column. Eriksson *et al.*[10] distinguished ervthrocytes from various species and classified them in two groups based on their affinity for the PEG–palmitate in dextran–PEG two-phase system; it was suggested that the ability of the PEG ester to bind to the erythrocyte membrane may be species-dependent. It was concluded that both hydrophobic interactions and biochemical affinity would be taken place between PEG monopalmitate, a hydrophobic ligand, and the erythrocyte membrane. It was not clear whether erythrocytes and platelets were retained on the column because the retention volumes of these two kinds of cells were smaller than those of granulocytes and lymphocytes.

An approximately linear relationship was found between the retention volumes of both granulocytes and lymphocytes and the $\Delta\log K$ values of the three PPG–C_3–agarose beads. The plots for PPG 400–C_{10}– and PEG 20M–C_{10}–Sepharose, which differ from PPG–C_3–agarose in the mode of coupling of the bonded stationary phase, did not correlate with the plot for the PPG–C_3–agarose groups.

On the basis of this previous work[7], we used PPG–C_3–Chromagel A4 packings which have different numbers of oxypropylene units and have different oxypropylene residue contents. A 0.09 *M* sodium phosphate buffer containing 2% (w/w) of dextran T40 was used as the mobile phase. The pH of the mobile phase was adjusted to the isoelectric points ($pH_{c.p.}$) of four kinds of cells for quenching electrostatic interactions.

It is considered that Pluronic P84, a block polymer of PEG and PPG, gives suitable $\Delta\log K$ values for the four kinds of blood cells because PPG is used as the bonded phase in this chromatographic system. We determined the $\Delta\log K$ values of the four kinds of blood cells in the dextran–PEG two-phase system containing a 1:1 ratio of Pluronic P84 to PEG 6000. The same system that gives the highest $\Delta\log K$ values for the cells is also used for the determination of the $\Delta\log K$ values of the PPG–C_3–Chromagel beads.

The $\Delta\log K$ values of blood cells increased in the order erythrocytes, platelets, granulocytes and lymphocytes, which coincided with the elution order from the PPG–C_3–Chromagel columns, except for columns 3, 4 and 6 (Table II). An approximately linear correlation was found between the capacity factors of platelets, granulocytes and lymphocytes on PPG–C_3–Chromagel columns with the highest oxypropylene residue contents (Nos. 3, 6 and 9) in each group and the $\Delta\log K$ values of these cells. This observation suggested that the blood cells that have high hydrophobicities ($\Delta\log K$ values), such as granulocytes and lymphocytes, would have a strong affinity for the PPG-bonded phase.

The capacity factors of both erythrocytes and platelets are greater than zero, except for the former cells on the PPG 950–C_3–Chromagel column (No. 8) (Table III), and it is found that these two kinds of cells are retained slightly on PPG–C_3–Chromagel.

The $\Delta\log K$ values which were obtained from PPG–C_3–Chromagel A4 beads were plotted against the capacity factors of the four kinds of blood cells shown in Fig. 4. The capacity factors of the granulocytes increased linearly with increasing

$\Delta\log K$ values of the packings. The retention of the lymphocytes increased gradually on PPG–C_3–Chromagel with $\Delta\log K$ values less than 0.5, but increased rapidly on packing beads with values above 0.5. The PPG–C_3–Chromagel having the highest $\Delta\log K$ values retained the platelets.

In conclusion, it is clear that hydrophobic interactions play an important role in retaining the four kinds of blood cells at $pH_{c.p.}$ and the PPG–C_3–Chromagel A4 columns retain both granulocytes and lymphocytes sufficiently even if they contain a small amount of oxypropylene residues. On the other hand, erythrocytes and platelets are retained slightly on these columns but are not separated from each other. It is thought that the hydrophobicity of the PPG–C_3–Chromagel beads is too low to separate these two kinds of cells.

It seems necessary to prepare gel beads having higher hydrophobicity and to determine the optimal $\Delta\log K$ values of the beads for sufficient retention or separation of erythrocytes and platelets.

ACKNOWLEDGEMENT

We express our gratitude to Dr. H. Shindo for valuable comments on the ^1H NMR measurements.

REFERENCES

1 U. Matsumoto and Y. Shibusawa, *J. Chromatogr.*, 187 (1980) 351.
2 U. Matsumoto and Y. Shibusawa, *J. Chromatogr.*, 206 (1981) 17.
3 S. Hjertén, *J. Chromatogr.*, 87 (1973) 325.
4 U. Matsumoto, Y. Shibusawa and Y. Tanaka, *J. Chromatogr.*, 268 (1983) 375.
5 V. P. Shanbhag and C.-G. Axelson, *Eur. J. Biochem.*, 60 (1975) 17.
6 U. Matsumoto, M. Ban and Y. Shibusawa, *J. Chromatogr.*, 285 (1984) 69.
7 U. Matsumoto and Y. Shibusawa, *J. Chromatogr.*, 356 (1986) 27.
8 S. Hjertén, J. Rosengren and S. Påhlman, *J. Chromatogr.*, 101 (1974) 281.
9 P.-Å. Albertsson, S. Sasakawa and H. Water, *Nature (London)*, 228 (1970) 1329.
10 E. Erikson, P.-Å. Albertsson and G. Johansson, *Mol. Cell. Biochem.*, 60 (1975) 17.