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SURFACE AFFINITY CHROMATOGRAPHIC SEPARATION OF BLOOD CELLS

V*. RETENTION BEHAVIOUR OF HUMAN PERIPHERAL BLOOD CELLS ON POLY(PROPYLENE GLYCOL) BONDED AGAROSE COLUMNS AND ITS RELATIONSHIP TO SURFACE HYDROPHOBICITIES OF THE GEL BEADS AND THE CELLS

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SUMMARY

Glycidyl ethers of poly(propylene glycol) (PPG) 200, 400 or 950 were oxirane-coupled with several kinds of agarose bead as the bonded phase of packing materials. An eluent was composed of 0.09 *M* phosphate-buffered 2% (w/w) dextran T40 solution at pH of respective isoelectric points of human blood cells. A linear relationship was found between the retention volumes of platelets, granulocytes and lymphocytes on the PPG–agarose columns and a measure of surface hydrophobicities of the cells, $\Delta \log K$ values, which were determined by using hydrophobic affinity partition. Furthermore, the retention volumes of granulocytes and lymphocytes increased according to the increase of $\Delta \log K$ of PPG–agarose beads. The retention of these two cells must be due to the hydrophobic interaction with the bonded PPG phase.

INTRODUCTION

In previous papers[1,2], the chromatographic behaviour of human peripheral blood cells has been studied by use of an oxirane-coupled poly(ethylene glycol) (PEG) 20M–Sepharose 6B (PEG 20M–C_{10}-Sepharose) column and phosphate-buffered mobile phases containing dextran T40 or T500. Except in a few instances, erythrocytes and platelets were eluted first from the column, followed by granulocytes and lymphocytes in that order. This elution order seems to be independent of cell characteristics such as size, adhesiveness and surface negative charge.

The hydrophobic affinity partition proposed by Shanbhag and Axelsson[3] has been used for the measurement of the degree of hydrophobicity of soluble proteins

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and suspended substances such as cell particles, subcellular organelles and cell membranes[4]. In our preceding paper[5], $\Delta\log K$ values, which are the difference between the logarithm of the partition coefficients in aqueous polymeric two-phase systems with and without hydrophobic ligand, of four kinds of blood cell have been determined by the partition method using PEG 6000 monopalmitate as the hydrophobic ligand. The $\Delta\log K$ value as a measure of surface hydrophobicities of the cells increases in the order platelets, granulocytes, lymphocytes and erythrocytes in a PEG 6000–dextran T40 two-phase system at the isoelectric point pH for the respective cells[5]. A relationship between the retention volumes of the cells, except erythrocytes, on PEG 20M–C_{10}-Sepharose column and their $\Delta\log K$ values has been found[5]. On the basis of the linear relationship, it is suggested that the hydrophobic interaction between the PEG 20M stationary phase and the blood cells contributes to the retention on the column.

The PEG 20M content of 15.7–17.4 μmol per gram of dry PEG 20M–C_{10}-Sepharose[1,2] is limited because commercial epoxy-activated Sepharose 6B contains a rather small amount of epoxy groups, 45–60 μmol per gram of dry powder. In this work, in order to increase the hydrophobicity of column packing gels, glycidyl ethers of poly(propylene glycols) (PPGs) were coupled to several kinds of bead-formed agarose gel by a method similar to that of Hjertén *et al.*[6]. PPGs with number-average molecular weights of 200, 400 and 950 are used as the bonded phase of column packings, because PPGs with molecular weights over 1000 are known to be insoluble in aqueous media. We applied the hydrophobic affinity partition method to specify the surface hydrophobicity of the PPG-bonded agarose beads. The relationship between the retention behaviour of blood cells and the $\Delta\log K$ values of the packing gel beads and those of the blood cells is also discussed.

EXPERIMENTAL

Materials

Dextran T40 (weight-average molecular weight, M_w = 40 000), epoxy-activated Sepharose 6B and Sepharose 6B (wet diameters 45–165 μm) were obtained from Pharmacia (Uppsala, Sweden). Poly(ethylene glycol) 6000 (number-average molecular weight, M_n = 6000–7500; extra-pure grade) was purchased from Wako Junyaku (Osaka, Japan). Poly(propylene glycol) 200 diglycidyl ether (DER 732) and poly(propylene glycol) 400 diglycidyl ether (DER 736) were from Dow Chemical Japan (Tokyo, Japan). Poly(propylene glycol) 950 (M_n = 950; chemical-pure grade) was from Sanyo Kasei (Kyoto, Japan). Chromagel A4 and A6 (wet diameters 50–150 mesh) were from Dojin Yakkagaku (Kumamoto, Japan). Bio-Gel A5-m (150–300 μm, 50–100 mesh in wet diameters) was from Bio-Rad Labs. (Richmond, CA, U.S.A.). Other reagents were of analytical reagent grade.

Poly(ethylene glycol) 6000 monopalmitate (PEG 6000 palmitate) was synthesized from poly(ethylene glycol) 6000 and palmitoyl chloride in the presence of triethylamine in toluene solution[7]; *ca.* 60% of the total hydroxyl groups in the glycol were substituted.

Instruments

A Coulter Counter Model D (Coulter Electronics, Harpenden, U.K.) was used

for counting the number of blood cells and column-packing gel beads. 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 580 nm simultaneously. An LKB 2112 Varioperpex II or 2132 Microperpex peristaltic pump and a RediRac fraction collector (LKB, Bromma, Sweden) were employed for elution of blood cells and for fractionation of the eluates.

Collection and isolation of blood cells

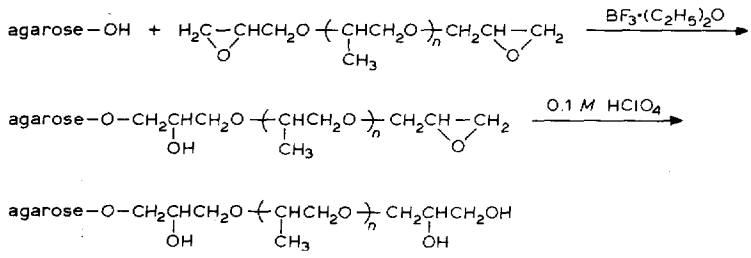
Human peripheral blood was drawn from normal male adult donors by venous puncture, and heparin, 0.05 ml of a 1000 U/ml solution per 10 ml of blood, was added. For collection of platelets, 1 ml of 3.8% (w/v) sodium citrate was added to 10 ml of blood. Siliconized glassware was used in all procedures. The isolation procedures for erythrocytes and platelets by centrifugation or the metrizoate-dextran T500 sedimentation technique for granulocytes and the metrizoate-Ficoll (Lymphoprep) sedimentation for lymphocytes have been described in detail in previous papers[1,2].

Preparation of PPG-bonded agarose gels

PPG 400–C_{10}-Sepharose 6B. Epoxy-activated Sepharose 6B (1 g) was coupled with 10 g of PPG 400 for 16 h at 40°C in an aqueous solution of pH 12. The method was similar to that previously used for the oxirane coupling of activated Sepharose with PEG 20M[1]. The coupling product was shaken with 0.1 *M* perchloric acid for 1 h at room temperature to hydrolyse the residual free epoxy groups[8].

PPG 950 glycidyl ether. The glycidyl ether was prepared by a method similar in principle to that for preparing alkyl and aryl glycidyl ethers[9]. Epichlorohydrin (4.6 g, 0.05 mol) was added dropwise to a mixture of PPG 950 (47.5 g, 0.05 mol) in dioxane (50 ml) and 2 ml of 48% boron trifluoride etherate in diethyl ether at 55°C under vigorous stirring. After stirring overnight at room temperature, 5 g of 50% sodium hydroxide solution was added dropwise at 20–25°C. The reaction mixture was washed with water and diethyl ether was added. The ether extract was washed with water until the washings were neutral. After the extract had been dried over anhydrous sodium sulphate, the solvent was removed by distillation.

PPG 200–C_3-Chromagel A6, PPG 400–C_3-Chromagel A4 and PPG 950–C_3-Bio-Gel A5. Oxirane coupling of diglycidyl ethers of PPG 200 (DER 732) and PPG 400 (DER 736) and glycidyl ether of PPG 950 to agarose beads was performed by a method similar in principle to that of Hjertén *et al.*[6]. The following scheme shows the oxirane coupling with agarose and hydrolysis of the residual epoxy groups:



A 100-ml volume of wet agarose beads was transferred to a glass filter funnel and washed with 100-ml portions of each solvent, water–dioxane (4:1), water–dioxane (3:2), water–dioxane (2:3), water–dioxane (1:4), and seven times with dioxane. After exchanging water in the gel beads with dioxane, the beads were transferred to a reaction vessel equipped with a stirrer, and 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 the following amounts of PPG glycidyl ethers in 10 ml of dioxane were added dropwise: PPG 200 diglycidyl ether, 1.1 g ($3.5 \cdot 10^{-3}$ mol); PPG 400 diglycidyl ether, 7.1 g ($1.4 \cdot 10^{-2}$ mol); PPG 950 glycidyl ether, 20.8 g ($2.0 \cdot 10^{-2}$ mol). The reaction mixture was stirred for a further 40 min at 20–25°C. After the reaction, the product was separated by filtration, and was shaken with 0.1 *M* perchloric acid for 1 h at room temperature to hydrolyse the residual epoxy groups. PPG-bonded agarose, thus obtained, was transferred to a glass filter funnel and washed with dioxane, followed by dioxane–water according to the same procedure as described above but in the reverse order, and finally washed with distilled water.

Hydrophobic affinity partition of blood cells

Human peripheral erythrocytes, platelets, granulocytes and lymphocytes were partitioned in two-phase systems containing 8% (w/w) of dextran T40, 8% (w/w) of PEG 6000 with and without 10% (w/w) of PEG palmitate in the total PEG 6000 (0.8% of the final concentration in the system), 0.15 *M* sodium chloride and 0.01 *M* sodium phosphate buffer, $pH_{c.p.}$ (= pH values at the cross-point, which were determined by a cross-partition method for the four kinds of blood cell[8]. These pH values may be regarded as the isoelectric points of the cells) of the cells, as have been previously described in detail[5].

Hydrophobic affinity partition of column-packing gel beads

The two-phase system contained 8% (w/w) of dextran T40, 8% (w/w) of PEG 6000 (including PEG palmitate), 0.12 *M* potassium chloride and 1.2 m*M* potassium phosphate buffer (pH 5.2, 5.5 and 6.8); these $pH_{c.p.}$ values were determined previously by the cross-partition method as the isoelectric points of four blood cells[8]. All the solutions were filtered through a Millipore filter (pore size 0.47 μm) prior to use to remove particles that might be mistakenly counted in the Coulter Counter.

The phase systems of total weight 8.0 g were prepared by mixing 3.2 g of a 20% (w/w) dextran T40 solution with 3.2 g of a 20% (w/w) PEG 6000 solution including 20% (w/w) of PEG 6000 monopalmitate (2.0% palmitate in the final phase systems), and contained potassium phosphate buffer with 0.07 g of potassium chloride. A 0.1-ml volume of gel bead suspensions in the buffer solution was added in the 8.0 g of the two-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 the lower phases were carefully removed with a pipette. An aliquot of each phase was diluted with 30 ml of 0.9% saline, and the number of packing gel beads was counted on a Coulter Counter. The bead number was measured with an orifice tube of 280-μm aperture.

Partition of the gel beads was characterized by the partition coefficient, *K*, calculated as the ratio of the number of the beads per millilitre in the upper phase to that in the lower phase. The number of the gel beads attached to the interface was

calculated as the difference between the total number added and the number in the upper or the lower phase. One-half the number at interface were added to each number of the upper or the lower phase.

The partition experiments were repeated more than five times for each sample, and the gel bead concentration determined in the phases deviated from the mean value by less than 5%.

Chromatography of blood cells

Sodium phosphate buffer solutions (0.09 *M*, $pH_{c.p.}$) containing 2% (w/w) dextran T40 were used as a mobile phase. The $pH_{c.p.}$ values, which are the pH values for the isoelectric points of blood cells determined by a cross-partition method, were found to be 5.5 for erythrocytes, 6.8 for platelets and granulocytes and 5.2 for lymphocytes, respectively[8].

The packing gels were passed through a set of standard sieves. The 48–65 mesh (210–300 μm) wet fraction was suspended in the mobile phase and packed into the column (25 × 0.9 cm I.D.) by the slurry-packing method. The columns were equilibrated with the mobile phase. The total amount of erythrocytes, platelets, granulocytes or lymphocytes isolated as above was suspended independently in 0.3 ml of the mobile phase. A 0.2-ml volume of 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 column. Then the column was eluted at a flow-rate of 6–12 ml/h with a peristaltic pump. The eluate was monitored at 254 and 580 nm and 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.

RESULTS

Chromatography of blood cells on PPG-bonded agarose columns

PPG 400 was coupled with epoxy-activated Sepharose 6B through a ten-carbon and two-oxygen spacer derived from 1,4-bis(2,3-epoxypropoxy)butane of the activated Sepharose (PPG 400–C_{10}-Sepharose) in a similar manner for the coupling of PEG 20M as previously described[1,8]. Another preparation was produced by the oxirane coupling of PPG 400 diglycidyl ether (DER 736) to Chromagel A4, a bead-formed agarose gel produced in Japan, through the mediation of a three-carbon linkage as described in the Experimental section (PPG 400–C_3-Chromagel). PPG 200 was coupled as its diglycidyl ether (DER 732) with Chromagel A6 (PPG 200–C_3-Chromagel), and PPG 950 glycidyl ether was linked similarly with Bio-Gel A5 (PPG 950–C_3-Bio-Gel).

Table I shows the retention volumes of four kinds of human peripheral blood cell on three PPG–C_3-agarose columns and on a PPG 400–C_{10}-Sepharose column. Included in Table I for comparison are the retention volumes of the cells obtained with Sepharose 6B, which lacks any PPG bonded phase. These retention volumes were obtained by separate elution of respective cell fractions at their isoelectric points. Table I shows that erythrocytes and platelets are eluted first, followed by granulocytes and lymphocytes from the four different PPG-bonded agarose columns. The retention volumes of erythrocytes, granulocytes and lymphocytes increase with the de-

TABLE I

RETENTION VOLUMES OF HUMAN PERIPHERAL ERYTHROCYTES (e), PLATELETS (p), GRANULOCYTES (g) AND LYMPHOCYTES (l) ON PPG–AGAROSE COLUMNS AT THEIR ISOELECTRIC POINTS AND ΔLOG K VALUES OF THE PACKING GEL BEADS

Mobile phases, 2% (w/w) dextran T40 buffered with 0.09 *M* sodium phosphates. Isoelectric points: pH 5.5, erythrocytes; 6.8, platelets and granulocytes; 5.2, lymphocytes. Column, 25 × 0.9 cm I.D. The retention volumes on the PEG 20M–C_{10}-Sepharose column are quoted from our previous paper[8]. All the retention volumes are averages of several column runs. The constituents of the two-phase partition systems are 8% (w/w) of dextran T40, 8% (w/w) of PEG 6000 with or without 20% (w/w) of PEG palmitate, 0.12 *M* potassium chloride and 1.2 m*M* potassium phosphate buffer, at the pH of the isoelectric points of the cells.

No.	*Column*	*Retention volume (ml)*				*Δlog K*		
		e	*p*	*g*	*l*	*pH 5.2*	*pH 5.5*	*pH 6.8*
1	PPG 200–C_3-Chromagel A6	4.8	4.6	13.5	21.8	0.68	0.48	0.67
2	PPG 400–C_3-Chromagel A4	4.6	4.5	12.7	16.5	0.50	0.33	0.65
3	PPG 950–C_3-Bio-Gel A5	5.2	6.5	8.5	14.1	0.41	0.21	0.57
4	PPG 400–C_{10}-Sepharose 6B	5.3	5.2	8.9	11.4	0.06	0.27	0.16
5	PEG 20M–C_{10}-Sepharose 6B	5.6	6.5	11.4	16.0	0.33	0.28	0.37
6	Sepharose 6B	4.7	4.9	9.9	10.9	0.11	0.08	0.18

crease in the number of oxypropylene units from 16 to 4. Granulocytes and lymphocytes show appreciable retention on all the columns examined. In spite of rather limited retention on the PPG–C_3-Bio-Gel and PPG 400–C_{10}-Sepharose columns, granulocytes and lymphocytes increase their retention volumes on PPG 200–C_3- and PPG 400–C_3-Chromagel columns.

The retention volumes of erythrocytes and platelets are changed only to a small extent according to the number of oxypropylene units of the bonded phase. These values, except those on the columns of Nos. 3, 4 and 5, show only slight differences from those obtained with Sepharose 6B as a reference. Incidentally, it seems to be difficult to separate the two kinds of blood cell with the PPG-bonded agarose columns and the mobile phase.

Relationship between retention volumes of blood cells and their Δlog K values

The retention volumes of platelets, granulocytes and lymphocytes on the four PPG-bonded agarose columns (Nos. 1, 2, 3 and 4) are plotted in Fig. 1 against their Δlog K values. These Δlog K values, determined at the respective isoelectric points of the cells are quoted from our preceding paper[5]; the values are found to be -0.04 for platelets, 0.36 for granulocytes, 0.67 for lymphocytes and 1.17 for erythrocytes, respectively[5]. Erythrocytes should be excluded from the plots because of their much higher Δlog K values.

The retention volumes of platelets, granulocytes and lymphocytes increase linearly with their Δlog K values, on all the columns examined.

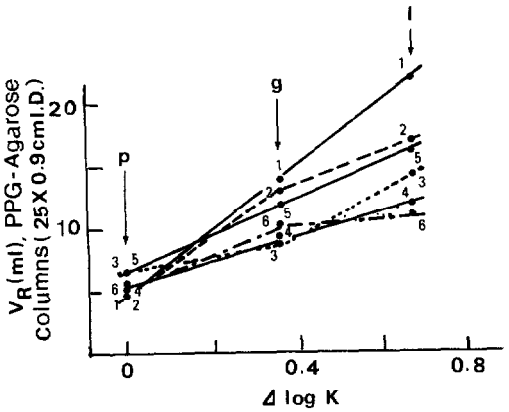


Fig. 1. Relationship between retention volumes of human platelets (p), granulocytes (g), and lymphocytes (l) and their $\Delta\log K$ values at isoelectric points of the cells. Packing gels: 1 = PPG 200–C_3-Chromagel A6; 2 = PPG 400–C_3-Chromagel A4; 3 = PPG 950–C_3-Bio-Gel A5; 4 = PPG 400–C_{10}-Sepharose 6B; 5 = PEG 20M–C_{10}-Sepharose 6B; 6 = Sepharose 6B. The mobile phase for the chromatography and the isoelectric point values of the cells are listed in Table I. Hydrophobic affinity partition systems for the cells are described in the Experimental section.

Hydrophobic affinity partition of column-packing gel beads

A measure of the surface hydrophobicity of packing gel beads is given in terms of $\Delta\log K$, defined as $\Delta\log K = \log K_p - \log K_0$, where K_p is the partition coefficient of gel beads in dextran T40–PEG 6000 two-phase system containing PEG palmitate as a hydrophobic ligand, and K_0 is the partition coefficient in the two-phase system in the absence of the palmitate[3,5].

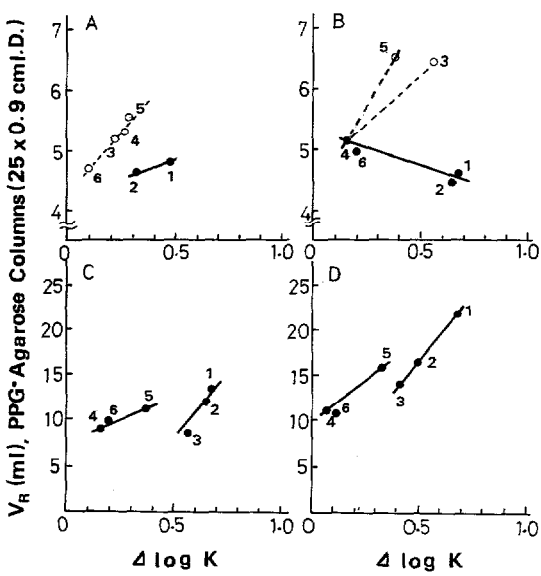


Fig. 2. Relationship between retention volumes of (A) human erythrocytes, (B) platelets, (C) granulocytes, and (D) lymphocytes and $\Delta\log K$ values of packing gel beads at isoelectric points of the cells. Packing gels 1–6 as in Fig. 1. The mobile phase and the affinity partition systems for the packing gel beads were as in Table I.

Table I shows the $\Delta\log K$ values of four PPG-bonded agarose gels, together with those of PEG 20M–C_{10}-Sepharose and Sepharose 6B for comparison. These values were determined at the isoelectric point pHs of the four different blood cells. The $\Delta\log K$ values of the PPG-bonded gel beads increase in the following order: PPG 400–C_{10}-Sepharose, PPG 950–C_3-Bio-Gel, PPG 400–C_3-Chromagel, and PPG 200–C_3-Chromagel. The $\Delta\log K$ value of PEG 20M–C_{10}-Sepharose beads is comparable with that of PPG 950–C_3-Bio-Gel, perhaps because of a low PEG content and despite a large number of bonded oxyethylene units. Similarly, the PPG content of PPG 400–C_{10}-Sepharose may be limited because commercial epoxy-activated Sepharose 6B has only a lower epoxy content of 45–60 μmol per gram of dry weight.

Relationship between retention volumes of blood cells and $\Delta\log K$ of column-packing gel beads

Figs. 2 A–D show plots of the retention volumes of four blood cells on four PPG-bonded agarose columns, together with those on PEG 20M–C_{10}-Sepharose and Sepharose 6B columns, *versus* the $\Delta\log K$ values of these gel beads. An approximately linear relationship is found between the retention volumes of granulocytes and lymphocytes and $\Delta\log K$ values of the three PPG–C_3-agarose beads (Nos. 1, 2 and 3) (Figs. 2C and D). 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, do not correlate with the plots for the PPG–C_3-agarose groups.

The retention volumes of erythrocytes slightly increase in proportion to the $\Delta\log K$ values of the two PPG–C_3-Chromagels and Sepharose 6B (Nos. 1, 2 and 6, Fig. 2A). However, no increase is observed between the retention volumes of platelets and the $\Delta\log K$ of PPG-bonded agarose beads (Fig. 2B).

DISCUSSION

It has been shown that the substances partitioned between dextran–PEG two-phase systems can significantly be influenced by the introduction of aliphatic hydrocarbons bound covalently to PEG; the affinity of the substances for the PEG-rich upper phase increases several-fold[3]. The difference in the partition of the substances in phase systems with and without PEG-bound hydrocarbons provides a measure of the interaction of the hydrocarbon with the substances. The difference, $\Delta\log K$, between the logarithms of the partition coefficients is taken as a measure of the hydrophobicity of the substances[3].

In our preceding paper[5], $\Delta\log K$ values of four kinds of human peripheral blood cell were determined by hydrophobic affinity partition using PEG 6000 monopalmitate as a hydrophobic ligand. In the absence of the palmitate, blood cells partition between the upper phase and the interface of dextran T40–PEG 6000 systems. The partition experiments were carried out at the isoelectric points ($pH_{c.p.}$) for the four different blood cells[8] to quench electrostatic effects and to manifest the hydrophobic interactions between the cell surface and the phase components or the ligand[5]. We have used the partition method extensively to determine the surface hydrophobicities of several PPG-bonded agarose beads.

In the hydrophobic affinity partition of the packing gels, 20% (w/w) of PEG 6000 palmitate in the total PEG 6000 (2.0% concentration of the palmitate in the

final partition systems) was adopted as the concentration of the ligand. Potassium phosphate buffer and potassium chloride were used in place of respective sodium salts that were used in blood cell partition[5]. This composition is chosen to distribute most gel beads at the interface of the two phases in the absence of PEG palmitate, in order to increase the K_p values and to increase the $\Delta\log K$ values of the gel beads.

On the chromatography of blood cells, sodium phosphate buffer (0.09 *M*) containing 2% (w/w) of dextran T40 was used as a mobile phase, because it is the best concentration of dextran T40 for the separation of four blood cells on the PEG 20M–C_{10}-Sepharose column[2]. The pH value of the mobile phase was adjusted to one of the $pH_{c.p.}$ values, which was determined for four blood cells by a cross-partition method[8]. The $pH_{c.p.}$ values are found to be 5.5 for erythrocytes, 6.8 for platelets and granulocytes and 5.2 for lymphocytes, respectively[8].

Excellent linear plots are obtained between the retention volumes of platelets, granulocytes and lymphocytes on all the three PPG–C_3-agarose columns (Nos. 1, 2 and 3) and the $\Delta\log K$ values of these cells (Fig. 1). The dependence of the retention volumes on their $\Delta\log K$ values suggests that the retention behaviour of these cells is predominantly based on the hydrophobic interaction between the cell surface and the PPG stationary phase, and is probably independent of electrostatic interactions between them. These results seem to be in line with our previous observations concerning the retention behaviour of these cells on PEG-C_{10}-Sepharose columns[5,8].

The relationship between the retention volumes and $\Delta\log K$ values of packing gel beads suggests, furthermore, that hydrophobic interactions influence the retention of granulocytes and lymphocytes on PPG-bonded agarose columns (Fig. 2C and D). Longer retentions for granulocytes and lymphocytes occur with PPG 200–C_3- and PPG 400–C_3-Chromagel columns, because either these cells or the two packing gels have higher $\Delta\log K$ values. However, the lowest retention of platelets is observed on these PPG–C_3-Chromagel columns (Fig. 2B).

The retention behaviour of granulocytes and lymphocytes on PPG 200–C_3-, PPG 400–C_3- and PPG 950–C_3-agarose columns does not depend on the increase in the number-average molecular weight of bonded PPG; the molecular weights of 200, 400 and 950 correspond to number of oxypropylene units of *ca.* 4, 7 and 16, respectively. The difference in hydrophobicities of the gel bead surface is probably the main factor affecting the retention of these cells on the columns. It is suggested that the $\Delta\log K$ values are probably dependent on the amount of bonded PPG, but are independent of their molecular weights (Table I).

In conclusion, the surface hydrophobicities of granulocytes, lymphocytes and PPG-bonded phase of column packing gels play a dominant role in determining the retention behaviour in this chromatographic system. The hydrophobicity of the packing gels should depend on the amount of bonded PPG, and it can be surmised that in PPG 200–C_3-Chromagel the PPG 200 content is the highest in the prepared packings. A study on the relationship between the amount of bonded PPG and retention volumes of blood cells will be reported in a subsequent paper.

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