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Supports for liquid–liquid partition chromatography in aqueous two-phase systems: a comparison of LiChrospher and LiParGel

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Abstract

Polyacrylated particles of silica (LiChrospher Diol 4000) or hydrophilic polyvinyl polymers (LiParGel 650) were used as supports for liquid–liquid partition chromatography in an aqueous polyethylene glycol–dextran two-phase system. High packing pressures gave high and reproducible values of the stationary mobile phase volume ratio ($V_S/V_M = 1.5$) on both supports. The volume of one theoretical plate was only 6 μl on LiChrospher and 20 μl on LiParGel. The performance of a 3-ml LiChrospher column was comparable to that of 15-ml LiParGel columns. In addition, the resolution of immunoglobulins was higher on LiChrospher. The influence of the supports on the retention of proteins is discussed.

1. Introduction

Partitioning in aqueous two-phase systems has been used to separate and isolate cells, organelles and macromolecules. As the distribution of a molecule in these systems depends on its conformation and general surface properties [1–4], the method may also be used to obtain information about conformational changes occuring on interaction between molecules [1,5]. By optimizing the composition of the phases, separation may be achieved in only a few steps. However, finding such optimum systems may pose difficulties in order to obtain adequate separation, and various forms of automated counter-current extraction have therefore been developed [6–8]. The time required for the analyses may be further shortened by employing column chromatography. A column chromatographic approach would also increase the plate number and, hence, the sensitivity of the method.

Finding materials suitable as supports for liquid–liquid partition chromatography in aqueous two-phase systems (LLPC) was a problem, however. Several attempts have been made to adsorb the dextran-rich bottom phase of the most thoroughly studied two-phase system, formed by polyethylene glycol (PEG) and dextran, on supports made of, for example, agarose beads [9], polyethers immobilized on Sepharose [10], silicates [11,12] or cellulose [13,14]. Eventually, the problem was solved by combining the affinity of polyacrylamide for the dextran-rich phase with the mechanical strength of hydrophilic vinyl (LiParGel) or silica (LiChrospher Diol) polymers [15]. LLPC on LiParGel has been shown to be a powerful tool for the

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separation and qualitative structural analysis of proteins and nucleic acids [15–18]. Despite their low solubility in the presence of the phase-forming polymers, immunoglobulins and antigen–antibody complexes can also be analysed by LLPC [19–21].

In an attempt to improve the performance of the LLPC columns, we examined the possibility of using dextran-grafted agarose beads as a support [19]. This matrix, Superdex 200, was found to give a larger plate number and a better resolution than LiParGel 650. However, immunoglobulins and other large molecules were strongly retarded, making LLPC on Superdex 200 unsuitable for the analysis of large molecules.

As mentioned above, LiChrospher Diol 4000 consists of silica particles which are polyacrylated [15]. As the LiChrospher particles are smaller than the LiParGel particles, larger theoretical plate numbers may be obtained [15]. In addition, the influence of LiChrospher on the retention of proteins is less pronounced than that of LiParGel [18].

In this work, we compared the properties of LiChrospher Diol 4000 with those of LiParGel 650 with respect to their ability to adsorb the dextran-rich bottom phase of an aqueous PEG–dextran two-phase system. The influence of the supports on the retention of proteins was also studied.

2. Experimental

2.1. Materials

Dextran T 500 (M_r = 500 000) was obtained from Pharmacia–LKB Biotechnology (Uppsala, Sweden) and PEG 8000 (M_r = 6000–7500) from Union Carbide (New York, USA). LiParGel 650 and LiChrospher Diol 4000 were a gift from Merck (Darmstadt, Germany). Protein G-Sepharose 4 Fast Flow, Ampholine PAGplate (3.5–9.5) and Ultropac TSK G3000SW columns (600 × 7.5 mm I.D.) were supplied by Pharmacia–LKB Biotechnology. Horseradish peroxidase was obtained from Merck, human albumin and bovine thyroglobulin from Pharmacia–LKB Biotechnology, human immunoglobulin G (IgG) from Sandox (Basle, Switzerland), Ig fractions of rabbit anti-human albumin and anti-human IgG from Dakopatts (Glostrup, Denmark), human apo-transferrin and whale skeletal myoglobin from Sigma (St. Louis, MO, USA) and bovine serum albumin (BSA) from Wilfried Smith (Edgware, UK). Sera from four patients with multiple myeloma were a gift from Dr. F. Lindström (Department of Medicine, Linköping, Sweden).

2.2. Isolation of myeloma IgG1

IgG was isolated from myeloma sera by affinity chromatography on Protein G-Sepharose in 20 m*M* sodium phosphate (pH 7.0). Bound immunoglobulins were eluted with 0.1 *M* glycine–HCl (pH 2.7). The purity of the preparations (95%) was checked with agarose gel electrophoresis [0.8% agarose in 75 m*M* barbital buffer–2m*M* calcium lacate (pH 8.6)], double immunodiffusion and high-performance size-exclusion chromatography HPSEC on the Ultropac TSK G3000SW column in 10 m*M* sodium phosphate–0.14 *M* NaCl (pH 7.4).

2.3. Characterization of the proteins

Molecular masses were determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) on a 5% or 7.5% polyacrylamide gel [22] and by HPSEC on an Ultropac TSK G3000SW column in 10 m*M* sodium phosphate–0.14 *M* NaCl (pH 7.4). Isolectric points were determined by isoelectric focusing on an Ampholine PAGplate (limits 3.5–9.5).

2.4. Two-phase system

All experiments were performed in a 4.4% (w/w) PEG 8000–6.2% (w/w) dextran T 500 two-phase system containing 50 m*M* sodium phosphate–0.1 *M* NaCl–0.1 M glycine (pH 7.0). The two-phase system was prepared by thoroughly mixing PEG, dextran and water. In experiments where LiParGel 650 was used as a support, sodium chloride, glycine and sodium

phosphate were added to the phase system and the pH was adjusted with HCl. The system was then equilibrated at 20°C for 72 h and the clear phases were separated. In experiments where LiChrospher Diol 4000 was used as a support, the two-phase system was equilibrated at 20°C for 72 h and the clear phases were separated. Salts were added to the top phase whereas the bottom phase was adsorbed on LiChrospher without any additional salts or buffer substances.

2.5. Determination of partition coefficients in batch

A 4-g amount of the two-phase system was thoroughly mixed with 0.5–4 mg of protein and the phases were allowed to separate at 20°C overnight. No precipitates were observed. The partition coefficient, K_{batch}, which describes the ideal partitioning of the protein in the phase system, was defined in accordance with the notation commonly used in aqueous two-phase partitioning, *i.e.*,

$$K_{batch} = C_{top\ phase}/C_{bottom\ phase} \quad (1)$$

where $C_{top\ phase}$ and $C_{bottom\ phase}$ are the concentrations of the protein in the top phase and bottom phase, respectively, determined spectrophotometrically at 280 nm.

2.6. Preparation of the LLPC columns

The matrix, LiParGel 650 or LiChrospher Diol 4000, was equilibrated with the dextran-rich bottom phase (stationary phase) overnight. The coated matrix was rinsed with the PEG-rich top phase (mobile phase) to remove any excess of stationary phase, suspended in mobile phase and poured into a thermostated (20°C) column with a filling reservoir. LiParGel was packed in steel columns (200 × 4.5 mm I.D. or 300 × 8.0 mm I.D.) under gravitational sedimentation for 5–30 h. The columns were further packed at a flow-rate of ≤0.2 ml/min (≤0.7 MPa), giving a volume ratio of the stationary phase to the mobile phase (V_S/V_M) of ≤1.0, or at ≤1.0 ml/min (≤25 MPa), giving a V_S/V_M of 1.5. Amounts of 20–150 μg of protein were applied to the columns in 0.1 ml of mobile phase at a flow-rate of 0.01–0.30 ml/min. LiChrospher (200 × 4.5 mm I.D. steel columns) was packed at a flow-rate of ≤0.025 ml/min (≤2.6 MPa), giving a V_S/V_M of ≤1.5 Large LiChrospher columns (300 × 8.0 mm I.D.) were not used as the dense packing considerably reduced the flow-rate. The columns were equilibrated with at least three volumes of mobile phase until the eluates were clear. Amounts of 100–200 μg of protein were applied to the columns in 0.1 ml of mobile phase at a flow-rate of 0.025 ml/min. The eluates were continuously monitored at 280 nm.

2.7. Calculations

The parameters of the LLPC columns were determined as described previously [17]. Two reference proteins, peroxidase and myoglobin (0.02–0.2 mg), were applied to the columns. Ideal partitioning was assumed for the references, *i.e.*, K_C was taken as $1/K_{batch}$ (Eq. 1). K_C is the ratio of the concentration of a molecule in the stationary phase to that in the mobile phase:

$$K_C = C_{stationary\ phase}/C_{mobile\ phase} \quad (2)$$

The volumes of the stationary and mobile phases, V_S and V_M, were calculated form the retention volumes of the references, V_R, using the relationship

$$V_R = V_M + K_C V_S \quad (3)$$

The plate number, N, was calculated from the peak width at half-height (w_h) of the myoglobin peak according to

$$N = 5.54\,(V_R/w_h)^2 \quad (4)$$

The resolution of the peroxidase and myoglobin peaks, R_s, was calculated as

$$R_s = (\sqrt{N}/4)[k/(1+k)](\alpha - 1) \quad (5)$$

where k is the capacity factor [$k = (V_S/V_M)K_C$] and α is the ratio of the partition coefficients of the references ($\alpha = K_{batch,\ peroxidase}/K_{batch,\ myoglobin}$).

3. Results

The influence of packing pressure on the prestanda of LiParGel 650 columns was examined. The parameters of columns packed at low pressure (in accordance with the packing procedure commonly used) or at higher pressure (see Experimental) are compared in Table 1. While the content of the stationary phase (V_S) was not affected by the higher packing pressure, the amount of mobile phase (V_M) decreased, indicating that the elevated pressure did not affect the volume of the LiParGel particles. The denser packing increased not only the ratio of V_S to V_M but also the plate number and the resolution of the reference proteins. The column performance in terms of plate numbers increased with decreasing flow-rate in the range 0.01–0.30 ml/min. It should be noted that the flow-rates used in this study were those required to elute the reference proteins within 2 h (Table 1).

In an attempt to reduce the consumption of material, small, densely packed LiParGel columns were prepared. The parameters of the 3.2-ml columns were compared with those of the 15.1 ml columns (Table 1). It is interesting that V_S and V_M per unit column volume and the volume of one theoretical plate (about 20 μl) were independent of the column volume. Running two small, densely packed columns in tandem did not improve the performance as the flow-rates had to be doubled. Thus, by employing a higher packing pressure, reproducible column packings were obtained and smaller columns with adequate performance could be used.

The influence of the properties of the support on the chromatographic performance was also examined. A polyacrylate support with a smaller particle size than LiParGel, LiChrospher Diol 4000, was used as a support in small, densely packed LLPC columns (Table 1). Although the volume of the LiChrospher matrix was smaller than that of LiParGel (10% of the column volume compared with 30% for LiParGel), the volume of stationary phase adsorbed on LiChrospher was larger per unit column volume. Further, the plate numbers were larger for the LiChrospher columns (the volume of one theoretical plate was only 6 μl), which would imply that the surface area of the stationary phase that is available for partitioning is larger on the LiChrospher particles. The resolution between the references was also higher on the LiChrospher columns than on LiParGel. Hence, using LiCrospher as a support improved the chromatographic performance of LLPC columns.

The variation in column parameters with repeated use is exemplified by the ratio of V_S to V_M in Fig. 1. V_S/V_M was found to be constant on LiParGel columns. The columns could even be stored at 20°C for several weeks without any change in the performance. In contrast, V_S/V_M

Table 1
Influence of packing pressure, size of the column and choice of support (LiParGel 650 and LiChrospher Diol 4000) on the parameters of the LLPC columns used

Matrix	Pressure (MPa)	I.D. (cm)	L (cm)	V_S/V_M	V_S/V_C	V_M/V_C	N	R_s	Flow-rate[a] (ml/min)
LiParGel	0.7	0.80	30	1.0 ± 0.1	0.36 ± 0.01	0.38 ± 0.01	595 ± 50	2.63 ± 0.07	0.12
LiParGel	25	0.80	30	1.5 ± 0.1	0.41 ± 0.01	0.27 ± 0.01	725 ± 50	3.64 ± 0.07	0.12
LiParGel	25	0.45	20	1.5 ± 0.1	0.41 ± 0.01	0.27 ± 0.01	200 ± 10	1.92 ± 0.05	0.025
LiParGel	25	0.45	2 × 20	1.5 ± 0.1	0.41 ± 0.01	0.28 ± 0.01	250 ± 25	2.10 ± 0.07	0.05
LiChosper	2.6	0.45	20	1.5	0.53	0.35	500	3.00	0.025
LiChrosper	2.6	0.45	20	1.0	0.45	0.44	200	1.64	0.025

I.D. = Column internal diameter; L = column length; V_S = volume of the stationary phase; V_M = volume of the mobile phase; V_C = column volume; N = plate number for myoglobin; R_s = resolution of peroxidase and myoglobin.
[a] Flow-rate required to elute the reference proteins within 2 h.

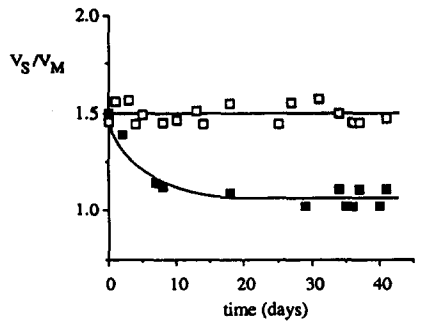

Fig. 1. Variations in the column parameters, exemplified by the volume ratio of the stationary phase (V_S) to the mobile phase (V_M), for (□) LiParGel 650 and (■) LiChrospher Diol 4000 columns run 8 h per day, 1–5 days per week, during a period of 6 weeks.

on LiChrospher columns varied until a value around 1.0 was achieved. The performance was considerably reduced with decreasing V_S/V_M, but still comparable to that of LiParGel (Table 1). After several weeks' of storage at 20°C, the plate numbers had returned to the original value whereas the ratio of V_S to V_M was still about 1.0.

Fig. 2 shows the chromatogram for rabbit IgG (anti-albumin), human IgG and albumin obtained by LLPC on LiParGel and LiChrospher. In order to facilitate the comparison of results obtained on columns with different parameters, retention volumes were expressed as K_C according to Eq. 3. The relative standard deviation of

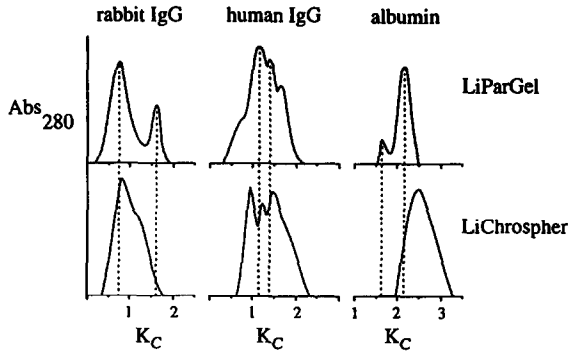

Fig. 2. Chromatograms for rabbit IgG (anti-albumin), human IgG and albumin obtained on LiParGel 650 (polyvinyl–polyacrylamide) ($V_S/V_M = 1.5 \pm 0.1$; $N = 725 \pm 50$; $R_s = 3.64 \pm 0.07$) and LiChrospher Diol (silica–polyacrylamide) 4000 ($V_S/V_M = 1.1 \pm 0.1$; $N = 470 \pm 100$; $R_s = 2.6 \pm 0.3$).

K_C for a given component was <5% on LiParGel columns and <15% on LiChrospher columns. Rabbit IgG was eluted as two peaks on LiParGel whereas LLPC on LiChrospher gave two poorly resolved components. Human IgG was fractionated into three components on both matrices. Human albumin was eluted as two peaks on LiParGel but as a single, broad component on LiChrospher. The differences between the elution patterns obtained for a given protein on the two matrices indicated a profound influence of the properties of the supports on the partitioning of proteins.

In order to evaluate the influence of the supports on the retention of proteins in LLPC columns, seven proteins with different physicochemical properties was analysed. The experimentally obtained retention volumes were compared with those calculated according to Eq. 3, using $1/K_{batch}$, where K_{batch} is determined in the absence of any matrix, as K_C (Fig. 3). The calculated volumes are assumed to describe ideal partitioning. It should be noted that for proteins that were fractioned into more than one component, all calculations refer to the average

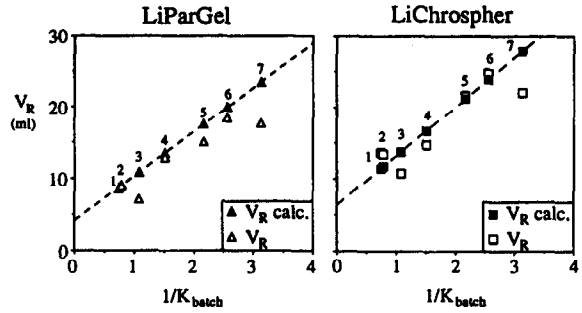

Fig. 3. Retention volumes calculated from Eq. 3 using $1/K_{batch}$ as K_C ($V_{R,calc}$) as a function of $1/K_{batch}$, plotted together with the experimentally obtained retention volumes (V_R), for LiParGel 650 ($V_S/V_M = 1.5 \pm 0.1$; $N = 725 \pm 50$; $R_s = 3.64 \pm 0.07$) and LiChrospher Diol 4000 ($V_S/V_M = 1.1 \pm 0.1$; $N = 425 \pm 100$; $R_s = 2.4 \pm 0.2$). All retention volumes given refer to a column volume of 15.1 ml. Molecular masses (M_r) were determined by SDS-PAGE and isoelectric points (pI) were determined by isoelectric focusing. 1 = Rabbit anti-IgG (M_r 160 000, pI 5.8); 2 = rabbit anti-albumin (M_r 160 000, pI 5.8); 3 = thyro-globulin (M_r 670 000, pI 4.5); 4 = human IgG (M_r 160 000, pI 6.8); 5 = transferrin (-Fe) (M_r 80 000, pI 6.2); 6 = BSA (M_r 65 000, pI 4.9); 7 = albumin (M_r 70 000, pI 4.9).

retention volume for the entire population. Whereas ideal partitioning was observed for bovine serum albumin (BSA), human serum albumin (albumin) was eluted earlier than expected on both matrices. As the molecular mass and the isoelectric point are similar for these proteins, this difference in retention would indicate that the conformation of the molecules is of importance to their ability to gain access to the stationary phase. Thyroglobulin, which has an isoelectric point similar to that of BSA and albumin but is considerably larger, was eluted earlier than expected on both matrices, indicating that molecules might be partially excluded owing to their size. The partitioning of polyclonal antibodies did not seem to be disturbed by either LiParGel or LiChrospher to any great extent, although the elution patterns on LiParGel differed from those on LiChrospher (*cf.*, Figs. 2 and 3).

In order to investigate further the influence of the matrices on LLPC of immunoglobulins, four monoclonal IgG1 antibodies (mIgG) were analysed. The chromatograms are shown in Fig. 4. The proteins were eluted mainly as single peaks with different partition coefficients. Although the resolution between the references was lower on LiChrospher than on LiParGel (2.7 compared with 3.6), the separation of the monoclonal antibodies was better on LiChrospher than on LiParGel. As the K_C values obtained on LiChrospher differed from those obtained on LiParGel, the two matrices appeared to affect the retention of immunoglobulins in different

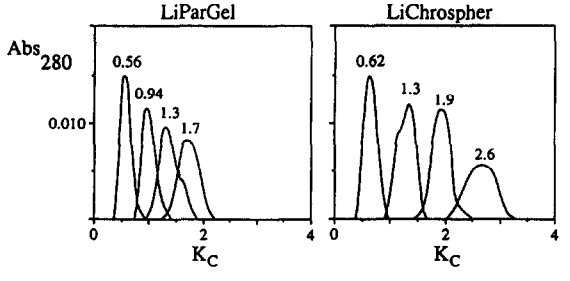

Fig. 4. Chromatograms for four monoclonal IgG1 antibodies obtained on LiParGel 650 ($V_S/V_M = 1.5 \pm 0.1$; $N = 725 \pm 50$; $R_s = 3.64 \pm 0.07$) and LiChrospher Diol 4000 ($V_S/V_M = 1.1 \pm 0.1$; $N = 530 \pm 100$; $R_s = 2.7 \pm 0.4$).

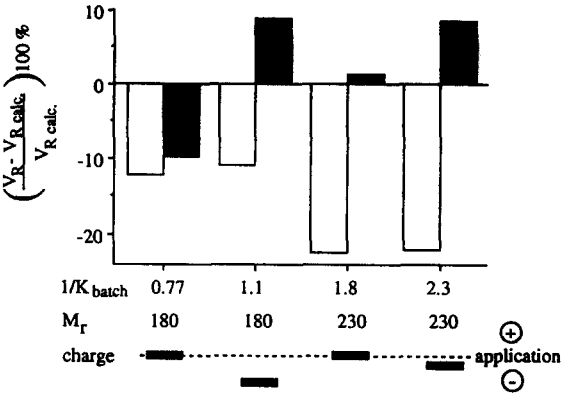

Fig. 5. Differences between the retention volumes calculated as described in Fig. 3 ($V_{R,calc}$) and those obtained experimentally (V_R) for four monoclonal myeloma IgG1 on (□) LiParGel 650 ($V_S/V_M = 1.5 \pm 0.1$; $N = 725 \pm 50$; $R_s = 3.64 \pm 0.07$) and (■) LiChrospher Diol 4000 ($V_S/V_M = 1.1 \pm 0.1$; $N = 530 \pm 100$; $R_s = 2.7 \pm 0.4$). Molecular masses (M_r) were determined by HPSEC and electrophoretic mobilities were determined by agarose gel electrophoresis at pH 8.6.

ways (Fig. 5). In addition, all monoclonal antibodies were eluted earlier than expected on LiParGel whereas antibodies favouring the stationary phase tended to elute later than expected on LiChrospher. The non-ideal behaviour could not be ascribed solely to either the size or the net charge of the otherwise homogeneous mIgGs (Fig. 5). Further, plate numbers calculated from the mIgG peaks were found to be independent of K_C (Table 2). Hence, the non-ideal retention of proteins in LLPC columns may not be ascribed to variations in column-bed packing structure or to poor diffusion in the stationary phase.

Table 2
Plate numbers calculated for four monoclonal IgG1 antibodies on LiParGel 650 and LiChrospher Diol 4000

$1/K_{batch}$	Plate number	
	LiParGel	LiChrospher
0.77	145	250
1.1	155	270
1.8	145	310
2.3	155	295

4. Discussion

LiParGel 650 and LiChrospher Diol 4000 have previously been used as supports for LLPC in aqueous PEG–dextran two-phase systems [15,18,23,24]. In this paper we have shown that LiParGel and LiChrospher columns could be packed at considerably higher pressures than recommended (25 and 0.7 MPa compared with 2.6 and 0.15 MPa [15,16], respectively) without damaging the supports.

The amount of stationary phase in the LLPC columns was larger than that reported by others ($\leq$30% of the column volume on LiParGel [16] and $\leq$20% on LiChrospher [15]; *cf.*, Table 1), indicating a strong adsorption of the dextran-rich bottom phase to both LiParGel and LiChrospher. The coating of LiParGel and LiChrospher is considered to be due to incompatibility of the polyacrylamide chains of the supports with PEG in the mobile phase rather than to attraction of the dextran-rich phase to the matrix [16]. Both supports have been shown to carry negative charges [18], which may be neutralized by high concentrations of salts (see below). In addition, a high ionic strength would increase the relative hydrophobicity of the PEG-rich top phase and decrease the repulsion of the dextran-rich bottom phase from the support, in this way facilitating adsorption.

A normal procedure to improve resolution is to increase the separation selectivity, determined as α in Eq. 5. This may be achieved by altering the composition of the two-phase system, *e.g.*, by reducing the size of the dextran molecules [1]. However, the concentration of PEG in the top phase would then increase, reducing the solubility of many proteins. We have previously shown that also immunoglobulins, which are easily precipitated by PEG, are soluble in appropriate concentrations (1 mg/ml) at a biological pH in two-phase systems containing dextran 500 but not dextran 40 [21]. Hence, we had to find other approaches to increase the resolution in our two-phase system.

Another common procedure to improve the resolution is to increase the capacity factor, k, in Eq. 5. This is accomplished by increasing the volume ratio of the stationary phase (V_S) to mobile phase (V_M). As the mechanical strength of both supports allows high packing pressures [16], we were able to achieve a ratio of V_S to V_M of 1.5 by packing the columns densely. This values ensures a high resolution for a wide range of K_C values. We were also able to achieve large theoretical plate numbers, in this way improving the resolution considerably (Table 1). A comparison with LLPC performance obtained by others may be misleading owing to differences in phase composition, temperature and flow-rates. However, it might still be of interest that our improved packing procedure reduces the volume of one theoretical plate from 45 to 20 μl for LiParGel and from 11 to 6 μl for LiChrospher [16]. In this context it should also be noted that the flow-rates used here were those required to elute the reference proteins within 2 h. Lower flow-rates would improve the column performance even further.

The partitioning properties of a biomolecule in a given aqueous two-phase system reflects a combination of its conformation and general surface properties, *e.g.*, net charge, hydrophobicity, shape and size. The distribution of the molecule between the phases depends mainly on the balance between its interactions with the phase-forming polymers, as interactions with the solvent, water, may be neglected [25]. The presence of salts partitioning towards one of the phases creates an electrical potential between the phases which may obstruct the free movement of proteins across the interphase. Considering our system, the concentrations of phosphate and sodium chloride are not likely to affect the distribution of proteins [2,26]. As glycine is distributed evenly between the phases [20], each protein should be able to partition freely towards the most energetically favourable phase. Thus, the partition coefficients determined in batch experiments are likely to describe the ideal partitioning properties of the molecules.

However, in LLPC, partitioning may be disturbed by the presence of the support. Although non-ideal retention of several proteins was indeed observed (Fig. 3), deviations from ideal partitioning could not be related to their charge.

LLPC of four monoclonal antibodies with different partition coefficients and physico-chemical properties strengthened this indication (Fig. 5). Our results confirm that the presence of negatively charged groups on the supports may be neglected at the ionic strength used in our two-phase system [16].

As the stationary phase is considered to be adsorbed inside the pores of the particles of both matrices [15,24], size-exclusion phenomena might be a problem in LLPC columns. However, the retention of IgM and antigen–antibody complexes ($M_r \leq 1\,000\,000$) on LiParGel agrees well with their partitioning properties determined in batch [20,21]. Ideal partitioning has been observed for large proteins ($M_r < 500\,000$) on LiChrospher 1000 [24] with a pore size that is four times smaller than that of LiChrospher 4000 [15]. Thus, the non-ideal behaviour of thyroglobulin (M_r 670 000) that was observed on both matrices may not be ascribed to exclusion phenomena.

Whereas polyclonal immunoglobulins were eluted in accordance with their partitioning properties (Fig. 3), four monoclonal IgG1 antibodies were found to deviate from ideal retention (Fig. 5). This apparent contradiction may be explained by the heterogeneity of polyclonal immunoglobulins of which a monoclonal preparation may be considered to represent a homogeneous subfraction. In polyclonal populations, positive and negative deviations from ideal partitioning by such subfractions may cancel each other out. This should be taken into account when ideal partitioning of heterogenous proteins in LLPC is discussed.

As the size of antibodies of a particular subclass is similar [27], the differences in M_r estimated by size-exclusion chromatography would indicate a difference in conformation. The degree of deviation from ideal partitioning in LLPC columns may then be determined by the conformation of the molecule together with the properties of the support with respect to pore size and polyacrylamide coating.

Although the influence of the LLPC supports on immunoglobulins and other proteins is obvious, the general applicability of the matrices is not invalidated as the interactions may be exploited as an additional parameter for separation.

5. Acknowledgements

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