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Plasmid DNA partitioning and separation using poly(ethylene glycol)/poly(acrylate)/salt aqueous two-phase systems☆

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ABSTRACT

Phase diagrams of poly(ethylene glycol)/polyacrylate/Na_2SO_4 systems have been investigated with respect to polymer size and pH. Plasmid DNA from *Escherichia coli* can depending on pH and polymer molecular weight be directed to a poly(ethylene glycol) or to a polyacrylate-rich phase in an aqueous two-phase system formed by these polymers. Bovine serum albumin (BSA) and *E. coli* homogenate proteins can be directed opposite to the plasmid partitioning in these systems. Two bioseparation processes have been developed where in the final step the pDNA is partitioned to a salt-rich phase giving a total process yield of 60–70%. In one of them the pDNA is partitioned between the polyacrylate and PEG-phases in order to remove proteins. In a more simplified process the plasmid is partitioned to a PEG-phase and back-extracted into a Na_2SO_4-rich phase. The novel polyacrylate/PEG system allows a strong change of the partitioning between the phases with relatively small changes in composition or pH.

1. Introduction

Aqueous two-phase systems (ATPS) have been used extensively as a bioseparation tool, particularly in protein research and studies of larger biomolecular structures such as organelles and virus particles [1–15]. The most common system, discovered by Albertsson [1] is composed of poly(ethylene glycol) (PEG) and dextran. Its separation property is attractive from several points of view, namely the phase diagram is almost independent on pH, salt content and temperature of the system. However, the cost of dextran limits its use in large scale. The PEG/salt systems are less expensive, but rather low molecular weight PEGs (typically less than 600 g/mol) must be used for pDNA partitioning to the PEG-rich phase [9]. Recently, a two-phase system composed of poly(ethylene glycol), sodium polyacrylate (Na-PAA) and salt was developed and used as a separation tool for proteins [16–20]. Contrary to the PEG–dextran system it is dependent on pH and salt content and relatively insensitive to temperature. The driving force for separation seems to be an enthalpic effective repulsion between the charged carboxylate groups of polyacrylate and ethylene oxide units of PEG. The addition of salt is necessary to allow compartmentalization of the polyelectrolyte into one of the phases. Although this system contains more complex driving forces of enthalpic and entropic nature, than the PEG–dextran system, it has the advantage of being less expensive and more practical, for instance by the relatively low polymer content necessary to induce two-phase formation. In spite of the strong electrostatic interactions of the polyelectrolyte, the dominant force for protein partitioning is, under high salt concentration conditions, hydrophobic, as shown for lysozyme [18]. Herein we have studied the partition of plasmid DNA (pDNA), where the electrostatic repulsion to the polyelectrolyte is relatively strong although the salt concentration is relatively high. Since almost all ATPS-partitioning studies are performed with PEG/dextran or PEG/salt systems, this new PEG/polyacrylate system is an important addition to those methods of easily available and well defined systems for pDNA partitioning and separation. The aim of this investigation is to find conditions in which pDNA is partitioned to the polyelectrolyte rich phase or the PEG-rich phase. Recently, the interest in large-scale production of pure plasmids has increased due to the possibility to use plasmids in gene therapy [21]. However, the plasmid purification in large scale is a challenge as reviewed by Prather

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et al. [21]. The removal of genomic DNA (gDNA), RNA, proteins and endotoxins has been successful with different types of chromatography [21,22]. However, since aqueous two-phase extraction systems have several attractive advantages in terms of scalability and high loading capacity [23], we investigated here the potential of PEG–PAA two-phase system for pDNA purification. A semi-pure plasmid has been used for studies of the pH-dependent partitioning. At pH close to 5 relatively many monomer units of polyacrylate become protonated and uncharged. Their electrostatic repulsion to DNA is turned off and may be turned into attractive hydrogen bonding interactions to different groups of DNA (possibly phosphate groups [24,25]). In this work pDNA recovery from the polymer-phases has been studied using a low protein content homogenate as starting material. In order to investigate potential protein contamination in a developed process, partitioning studies were performed using *Escherichia coli* protein rich homogenate. Since these proteins are easily precipitated in the presence of SDS, partitioning studies were also performed with BSA as a highly soluble model protein.

2. Materials and methods

2.1. Materials

Polymers: sodium polyacrylate having a molecular weight of 240,000 g/mol and poly(ethylene glycol) 8000 g/mol were obtained from Sigma–Aldrich. Poly(ethylene glycol) 4000 g/mol was obtained from Merck. All other reagents were of analytical grade.

2.2. Methods

2.2.1. Two-phase systems

The two phase systems were made by mixing stock solutions of sodium polyacrylate (Na-PAA), PEG, Na_2SO_4, and water. Sodium dodecyl sulfate (SDS) was added in some cases. Semi-pure stock solutions or homogenate with pDNA was added lastly and always after the previous solutions had been mixed. The polyacrylate stock solution concentration was 14–15% and the pH was adjusted to the desired pH with NaOH (50%). The final pH of the two-phase system was adjusted by adding small quantities of HCl (37%) or NaOH (50%). The mass of the systems was 5–10 g and mixed thoroughly in a graded glass tube, by gently mixing the system by turning the tube several times up-side down. The homogenate-free systems separated into clear two-phase systems by gravity within 15 min. Systems with homogenate were left standing for 1 h or centrifuged 5 min with a table centrifuge. All separations were performed at 22 °C.

2.2.2. Phase diagram

The binodal curve that separates the single phase from the two-phase region was determined as described by Albertsson [1], by stepwise dilution of a point in the two-phase region (indicated by turbidity upon mixing), with a 4 wt% Na_2SO_4 solution. However, at some points close to the binodal curve, the difference in refractive index is very small and turbidity may not be observed in the two-phase region. Therefore, all non-turbid systems were centrifuged and inspected for two-phase formation.

2.2.3. Tie-lines

Tie-lines were determined by analyzing the PEG concentration in the top and bottom phases and fitting the composition line to the binodal curve. The PEG concentration in the top- and bottom-phases was determined by taking known amounts of the phases, which were dried in an oven at 50 °C, and then the PEG content was extracted with warm (40–50 °C) acetone. The acetone with PEG was placed in beakers and left standing for evaporation and drying. The PEG content was then determined gravimetrically.

2.2.4. Plasmid DNA and protein analysis

The pDNA concentration was determined with the Picogreen fluorescence reagent and was quantified by using a pure lambda phage standard of known concentrations using the Quant-iT PicoGreen kit (Invitrogen Life Technologies, Grand Island (NY), USA). The partition coefficient of the pDNA, K_p is the ratio of the pDNA concentration in the top and bottom phases, respectively. The pDNA yield in the phases are given in percentage of added pDNA to the total system. The pDNA concentration in the (low protein) homogenate was between 60 and 100 μg/g. The concentration of soluble pDNA in the phases was 3–20 μg/g. At least a 20-time dilution of the phase was performed before analyzing with the fluorescence probe. The BSA content was determined by absorbance at 280 nm and the presence of *E. coli* proteins was visualized by coomassie or silver staining in SDS-PAGE gels.

2.2.5. Plasmid DNA preparation and treatment of homogenate

E. coli strain TG1 was used as host in all experiments for production of plasmid pUC18 with 2686 bp (GE Healthcare, Uppsala, Sweden). Cell growth was carried out in a shake-flask at 37 °C using a Terrific Broth medium (20 g/l tryptone, 24 g/l yeast extract, 4 ml/l glycerol, 0.017 M KH_2PO_4, 0.072 K_2HPO_4) supplemented with 30 μg/ml of ampicillin. After fermentation process, cells were centrifuged at 5400 × *g* (20 min, 4 °C) and pellet conserved at −20 °C. Cells were lysed using a modification of the alkaline method. Cells pellets were resuspended in 20 ml of P1 Buffer (50 mM glucose, 25 mM Tris–HCl, 10 mM EDTA, pH 8.0). Alkaline lysis, based on the Birnboim and Doly procedure [26] was performed by adding 20 ml of P2 Buffer (200 mM NaOH and 1% (w/v) SDS) and incubated for 5 min at room temperature. Cellular debris, gDNA and proteins were precipitated by adding 16 ml of P3 (3 M potassium acetate, pH 5.0) and incubated 15 min on ice. The precipitate was removed by centrifugation at 20,000 × *g* (30 min, 4 °C). A second centrifugation step was carried out under same conditions. Briefly, the pDNA in the supernatant was precipitated by adding isopropanol (0.7 times the volume of the supernatant) and incubation on ice for 30 min. The pDNA was recovered by centrifugation at 16,000 × *g* (20 min, 4 °C). The pellets were then re-dissolved in 2 ml of 10 mM Tris–HCl (pH 8.0).

2.2.6. E. coli homogenate protein preparation

The homogenate prepared for pDNA purification had a low protein concentration. In order to observe protein partitioning an additional (protein rich) homogenate from *E. coli* cells was prepared as follows. Wet *E. coli* cells were dispersed in TE-buffer (Tris–HCl, 10 mM, EDTA, 1 mM, pH 8.0). The concentration was 13.5 wt%. The cells were sonicated with a 6 mm wide sonicator tip 600 s on ice. The energy output was a total of 9000 J on a 21 g *E. coli* mixture.

2.2.7. Plasmid DNA partitioning processes

In one process (I) pDNA was partitioned to the bottom phase in a PEG 8000/polyacrylate two-phase system. In step A pDNA was partitioned in a PEG 8000 (3.6%)/polyacrylate (1.8%)/Na_2SO_4 (4%) aqueous two-phase system. In the initial step (A) the pDNA concentration in the system was 5.7 μg/g and the SDS concentration was 0.18%. The pH was set to 5.2–5.3 by adding HCl (37%), ca. 90 mg/10 g system. The top-phase was discarded and the bottom phase was used for step B. In step B a new two-phase system was made, where the content of PEG was changed into PEG 4000. Thus the system in step B was composed by a bottom phase a solution (37.45% of the mass of system B) and a solution (62.55% of mass of system B) containing PEG 4000 and polyacrylate to compensate the discharged

top-phase in step A. The added PEG solution had the following composition: PEG 4000 (4.8 wt%), polyacrylate (1.13 wt%, Na_2SO_4 (4.0 wt%), glycine–NaOH buffer (0.33 mol/kg, pH 9.0), NaOH (0.8%). The pH in step B was set between 10 and 11 to assure all polyacrylate was completely deprotonated. The pDNA was now partitioned strongly to the PEG-rich top-phase and this phase was collected and used to form the system in step C. This phase was formed by adding 0.86 g of a 15.0 wt% solution of Na_2SO_4 per 1 g top-phase of system B. This induced a PEG/Na_2SO_4 two-phase system where practically all pDNA was partitioned to the salt-rich bottom-phase. In a more simplified process (II) the pDNA is first partitioned to a PEG 4000 rich top-phase and then extracted to a salt phase as above, corresponding to steps A and B (process II), however without addition of SDS. In this process II, the system in step A was composed of 3.0 wt% PEG 4000, 3.0 wt% polyacrylate 240,000, 4.0 wt% Na_2SO_4, and pH 5.6 ± 0.4 adjusted with HCl (37%). The pDNA concentration was 8 μg/g system. In step B of process II, the top-phase was transferred into a separate tube and solution of 15.0 wt% Na_2SO_4 was added to the system. The added salt was 0.82 g salt solution/g top-phase, which induced a PEG/Na_2SO_4 aqueous two-phase system at room temperature.

2.2.8. Removal of polymers before agarose gel electrophoresis analysis

The polyacrylate present in the bottom-phases of the samples was removed as follows: 0.4 g of bottom phase was mixed with 0.4 g of a solution containing PEG 8000 5 wt%, Glycine–SO_4 buffer 0.8 mol/kg, pH 2.0. Upon mixing polyacrylate and acidic PEG a complex was formed and precipitated from the solution (pH 2.3). The mixture was centrifuged immediately in an Eppendorf centrifuge (14,000 rpm, 2 min). The supernatant, which was practically polymer-free was transferred to another Eppendorf tube and 0.060 g of a buffer (Gly–NaOH, 2.0 mol/kg, pH 9.5) was added to adjust the pH to ca. 8.5, in order to avoid acid catalyzed degradation of DNA.

3. Results

3.1. Phase diagram

The phase behavior of solutions containing PEG 8000 and polyacrylate were tested at different pH values. In Fig. 1a and b the phase diagrams of various PEG–polyacrylate systems are shown. The diagrams are displayed as a (pseudo-)ternary system since the systems are quaternary (two polymer, water and salt). The binodal shape of the curve is typical of similar two-polymer segregating systems. Two tie lines based on determination of PEG-concentration in the phases are also shown. As expected there is a substantial decrease in size of the two-phase region when the PEG molecular weight is halved. The position of the binodal curves shows strong pH dependence between pH 4.8 and 5.5, while between 5.5 and 7.3 there is a relatively small change. The positions of the binodal curve and tie-lines indicate that there is relatively much polyacrylate in the PEG-rich phase.

3.2. Plasmid DNA partitioning in PEG–polyacrylate system

The pDNA (low protein content) was partitioned in PEG–polyacrylate at different pH values. In Fig. 2 the effect of pH is shown for pDNA partitioning in the system PEG 8000-sodium polyacrylate. The partition is expressed as percentage pDNA in the different phases. At pH 7.3 pDNA is preferentially partitioned into the top PEG-rich phase whereas at pHs 5.4 and 5.8 a more even distribution between the phases is observed. At pH 6.2 partitioning was not reproducible, but a trend can be seen where pDNA is more partitioned to the top-phase at higher pH. Interfacial partition occurs in the whole pH interval. The mass balance does not add to 100% and that could be due to inhibition in the fluorescence analysis used in pDNA detection, or to undissolved precipitated pDNA.

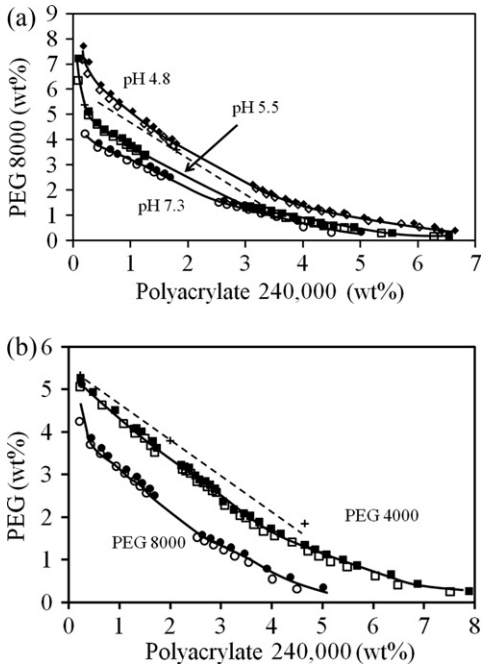

Fig. 1. (a) Phase diagram of the PEG 8000/polyacrylate 240,000/Na_2SO_4 system. Effect of pH. Concentration of Na_2SO_4 is 4 wt%. Systems with pH 5.5 and 4.8 contained sodium acetate buffer 2–4 mmol/kg. The two-phase region is above the corresponding binodal curve. The dashed line is tie-line for the system at pH 5.5. Temperature 22 °C. (b) Phase diagram of the PEG/polyacrylate 240,000/Na_2SO_4 system. Effect of molecular weight. Concentration of Na_2SO_4 is 4 wt% and pH is 7.3. The two-phase region is above the corresponding binodal curve. The dashed line is tie-line for the system with PEG 4000. Temperature 22 °C.

3.3. Partitioning of a model protein (BSA) in PEG–polyacrylate two-phase system: effect of SDS and pH

In Fig. 3 comparisons between systems with or without SDS, and systems with low or high pH are shown. It can be seen that the presence of SDS in a system at low pH (5.25) causes a strong partitioning of BSA to the PEG-rich phase, and without SDS the protein is partitioned to the bottom phase. Changing pH from 6.0 to 12, causes a dramatic increase in the BSA partition coefficient *K* (defined as the concentration ratio of target molecule in the phases).

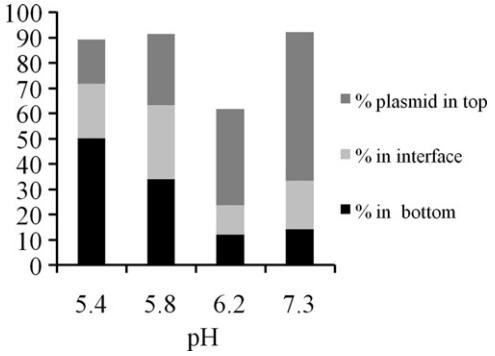

Fig. 2. Plasmid DNA partitioning in PEG 8000 (3.6%)/polyacrylate 240,000 (1.8%)/Na_2SO_4 (4%) two-phase system. pDNA concentration: 16–20 μg/g system. The percentage of pDNA in the different phases versus pH is shown.

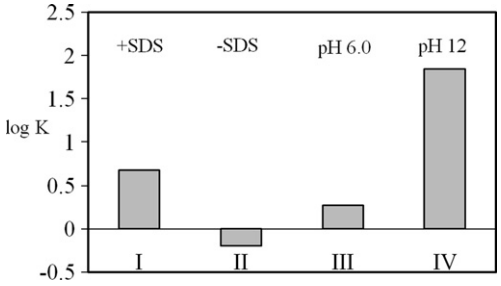

Fig. 3. Partitioning of BSA in PEG–polyacrylate two-phase systems. Effect of SDS and pH. All systems contained Na_2SO_4 (4 wt%) and BSA (0.2 wt%). Polymer composition as in Table 1 (systems I–IV): pH 5.25 (I, II), pH 6.0 (III), pH 12 (IV). The difference between systems I and II is 0.18 (wt%) SDS in system I.

3.4. Partitioning of E. coli homogenate proteins in PEG–polyacrylate systems: effect of SDS and pH

A protein-rich *E. coli* crude homogenate, free from cell-debris was mixed in four different systems (I–IV) in order to study effects of SDS or pH on the protein partitioning. The composition of the systems and protein concentration in the phases are shown in Table 1. All systems contained 40% *E. coli* sonicated homogenate. The cell debris was removed by centrifugation before mixing the homogenate with the system. The systems were mixed and left standing 10 min before centrifuging for 5 min using a table centrifuge. Samples taken from the phases were analyzed using a SDS-PAGE gel (not shown) and protein concentration determined in clear centrifuged phases. Precipitation of proteins occurred in all systems particularly in system I (with PEG 8000) which contained SDS. This explains the low value on mass-balance (assuming the rest is the precipitated protein, ca. 60%). Partial precipitation on systems II–IV was relatively low, but clearly visible.

3.5. Plasmid DNA partitioning processes

Based on the results above two cross-partitioning processes were developed as shown schematically in Fig. 4. In these experiments a homogenate containing 60–100 μg/g pDNA with low protein content was used and no extra protein-rich homogenate was added to the systems. In process I the pDNA preference for polyacrylate at low pH is utilized in the step A. In step B the pH is raised and the PEG 8000 is partially replaced with PEG 4000, which causes the pDNA to partition to the PEG phase in step B. In process II pDNA is directly partitioned to a PEG phase in step A. In both processes the pDNA is separated from PEG by inducing a PEG/salt system where pDNA is partitioned almost exclusively to the salt-rich phase. Table 2 contains the partitioning data for

Table 1
Systems for *E. coli* homogenate protein partition. Systems I and II contained PEG 8000, and systems III and IV contained PEG 4000. All systems contained 4.0 wt% Na_2SO_4 and 40% homogenate and Na-PAA 240,000. Polymer and SDS concentrations below are given in wt%. The polyacrylate concentration is given as polyacrylate excluding the sodium counterion. Protein concentrations in mg/g. Mass balance in %. T- and B-phase mean top (PEG-rich) and bottom (PAA-rich) phase, respectively. Average total protein concentration in all systems: 3.4 mg/g (0.34 wt%).

Component	System I	System II	System III	System IV
PEG	3.6	3.6	3.0	3.0
Polyacrylate	1.8	1.8	3.0	3.0
SDS	0.18	–	–	–
pH	5.4	5.5	5.8	9.6
Protein partitioning				
Conc. in T-phase	1.2 ± 0.2	2.6 ± 0.6	5.1 ± 0.8	7.0 ± 0.3
Conc. in B-phase	1.1 ± 0.2	3.6 ± 0.2	2.7 ± 0.5	1.1 ± 0.1
Mass balance	34± 5	91± 8	100± 10	110± 10
K-value	1.2 ± 0.1	0.7 ± 0.1	1.9 ± 0.1	6.4 ± 0.9

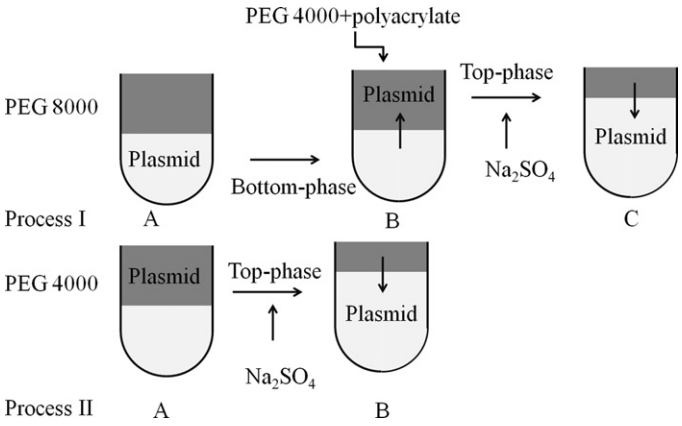

Fig. 4. Partitioning of plasmid DNA in PEG/polyacrylate aqueous two-phase systems. In process I pDNA is cross-partitioned between the PAA and PEG phase and then finally back-extracted to a salt phase. In process II the pDNA is back-extracted directly after the primary extraction to the PEG-rich phase. See Section 2.2.7 for composition details.

the processes. The total yields as pDNA obtained in the final salt rich phases compared to the added pDNA in steps A were 60% in process I and 70% in process II. As the result of protein precipitation after obtaining the pDNA homogenate, no protein bands were observed in the final phases of the two processes upon analysis through SDS-PAGE (coomassie and silver staining).

3.6. Plasmid DNA conformation

The analysis of pDNA conformations (supercoiled and open circle) was determined using agarose gel as shown in Fig. 5. Samples from different conditions were analyzed: the bottom phases at pH 5.4 and 5.8 from the partitioning studies in Fig. 2 above, lanes 1 and 2, respectively. Furthermore, the final Na_2SO_4-containing phases in the separation processes I and II (shown in Fig. 4 above), lanes 4 and 5, respectively. The concentrated pDNA fraction in lane 6 is a reference. The three bands furthest down in lane 6 are (starting from the furthest down) RNA, supercoiled pDNA and open circle pDNA. It can be seen in Fig. 5 that there is no significant change in pDNA conformation for all the different cases.

Table 2
Plasmid DNA partitioning in phases of bioseparation processes with PEG/polyacrylate/salt two-phase systems. Top/bottom volume ratios are: in process I, step A (1.1), B (1.55), C (0.08), in process II: step A (0.5 ± 0.1), B (0.1). Values are averages of 4 experiments (process I) or 2–5 experiments (process II). Standard deviations are given. Process I: initial pDNA concentration in step A: 5.7 μg/g. Process II: initial pDNA concentration in step A: 8 μg/g.

		Yields in the phases (%)			
		Top-phase	Bottom phase	Interface	pH
Process I					
Step	*K*-value				
A	0.33 ± 0.19	16 ± 6.5	49 ± 14	6.2 ± 4.6	5.2
B	12 ± 5.0	110 ± 6.8	7.5 ± 3.4	15 ± 14	9.5
C	0.06 ± 0.03	0.8 ± 0.8	110 ± 10	0.2 ± 0.3	9.5
Total Process[a] I yield: 60%					
Process II					
Step	*K*-value				
A	6.1 ± 2.2	57 ± 31	16 ± 11	3.3 ± 2.7	5.7
B	<0.1	0 ± 0	130 ± 0	0 ± 0.0	5.5 ± 0.5
Total Process[a] II yield: 70%					

[a] The total process yield refers to the yield when starting with step A and consider the final quantity in the salt-rich bottom phase in steps C and B, for processes I and II, respectively.

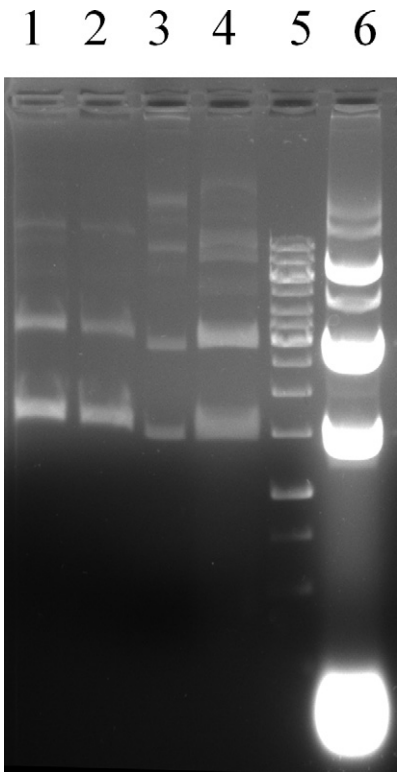

Fig. 5. Plasmid DNA analysis in different phases. Lane 1: polyacrylate rich bottom phase of a system with pH 5.4; lane 2: polyacrylate rich bottom phase of a system at pH 5.8; lane 3: salt rich bottom phase from process I; lane 4: salt rich bottom phase from process II; lane 5: Mw-ladder; lane 6: pDNA extracted from *E. coli* by alkaline lysis.

4. Discussion

4.1. Phase diagram of PEG–polyacrylate–salt two-phase systems

The shape of the binodals in the PEG–polyacrylate phase diagrams are similar to the corresponding systems of PEG/dextran, with exception that the content of polyacrylate in the PEG-rich phase is relatively high for its molecular weight. This can be understood from the stronger mixing entropy of the polyelectrolyte relatively to the corresponding non-charged hydrophilic polymer. An extensive experimental and theoretical study on similar PEG–PAA systems is found in reference [20]. The pK_a of polyacrylate is reported to be between 4 and 4.5 [27]. The increased compatibility of polyacrylate with PEG at lower pH is counter-intuitive according to the entropic driving forces, since, the lower the charged is on the PAA, the lower is the entropic cost of compartmentalization, and thus the easier is the phase separation. However, the PEG–PAA compatibility can be understood by an increased hydrophobic attraction between parts of uncharged polyacrylate with the PEG molecules or as frequently reported in the literature, increased hydrogen bonding between uncharged carboxylic groups with the ethylene oxide groups [28]. The tie-lines are not closing into the binodals which may be due to the fact that the system is quarternary, an indication that the phase boundary determined by turbidity titration, may be different from the binodals determined by composition points given by tie-lines.

4.2. Plasmid DNA partition

The strong influence of pH on pDNA partitioning is at first surprising. Polyacrylate and DNA repel each other electrostatically and DNA is expected to partition to the PEG-phase. However, since the hydrophobic groups of double stranded super coiled DNA are mostly non-exposed to the solution, the chemical character of DNA is strongly hydrophilic, which in the high salt concentration of the system may facilitate partition to the polyacrylate phase.

Generally, the driving force of entropic repulsion favours biomolecule partitioning to the polymeric phase with the smallest polymer size [29,30]. At lower pH there is an increased polyacrylate content in the PEG-phase, making the PEG-phase less entropically attractive. An alternative explanation could be hydrogen bonding between the non-charged carboxylic groups of polyacrylate at pH 5.2 with the hydrogen bond acceptor groups of the diester-phosphate groups as has been suggested in the literature [24,25]. It should be noted that the presence of the Na_2SO_4 favours partitioning of anions to the PEG phase by the well known electro-chemical driving force acting in aqueous two-phase systems containing a dominant salt [31]. Since, there are both entropic and enthalpic driving forces for DNA partitioning to the PEG-rich phase, another stronger enthalpic force favours partitioning to the polyacrylate phase. The partitioning of pDNA is complicated by the possibility of pDNA to adopt different structures, for instance a more compact form as discussed by Frerix et al. [8]. Polymers may induce compaction of DNA, which may also explain the occurrence of precipitation of a fraction of the pDNA [32].

4.3. Protein partitioning

The strong difference in BSA partitioning upon adding SDS to the system at pH 5.3 can be explained as follows: SDS associates with and denature BSA, creating a complex that is highly charged, and therefore repelling polyacrylate, and at the same time increasing the hydrophobicity of BSA, all this favouring a strong partitioning to the PEG-phase. In systems without SDS, the hydrophilic nature of BSA drives it to the polyacrylate phase. At pH 5.3 the charge of BSA is close to zero and no significant repulsion to the polyelectrolyte is expected. When BSA is completely charged at pH 12 it turns into a strong polyelectrolyte with a strong repulsion to the polyacrylate phase. Since BSA unfolds at pH between 9.5 and 10.9 [33,34] the exposed hydrophobic residues may have a strong influence on the increase of the partitioning to the PEG-rich phase. Using these findings, SDS was included in a system with *E. coli* proteins (Table 1). The presence of moderately high SDS concentration in a system with *E. coli* homogenate causes extensive aggregation and precipitation of most of the proteins, which in a bioseparation process for pDNAs is advantageous. Furthermore, hydrophobic endotoxins may be removed by the SDS addition and by partitioning to the PEG-rich phase. Increasing pH close to 10 in systems with *E. coli* homogenate proteins leads to the same effect explained above for BSA. However, since pDNA also partitions to the PEG-phase at high pH the process with SDS at pH 5.3 is expected to be more efficient in removing proteins from pDNA.

4.4. Bioseparation process

The partitioning driving forces (enthalpic and entropic) of flexible polymeric macromolecules are very large since they scale with molar volume [30]. Insolubility or precipitation into a separate interfacial phase is very common [6]. One way to overcome this is to choose aqueous two-phase system composition close to the critical point at the binodal curve. At this point phase differences are relatively small and partitioning becomes less extreme and precipitation can be avoided. However, if the starting material is a homogenate, whose composition may vary substantially due to changes in protein composition, the risk of strong changes in phase volume ratios and even prevention of two-phase formation is high, if the system composition is close to the critical point. If the composition point is far from the critical point the system becomes more

stable, separates faster, but more proteins and nucleic acids precipitate. The systems chosen here have been a compromise between these effects. In process II the pDNA is directly partitioned to the PEG-phase and then back-extracted to a salt rich phase. Proteins seems to partition also to the top-phase and it is therefore advantageous to add SDS to the homogenate or to the system as in process I, to remove most of the contaminating proteins by precipitation. The determined yield values and their total sum deviate strongly from 100% (complete mass balance). A trend can be seen, in the systems of process I step A, that they are lower than 100% and in the other steps they are higher. This may be due to an underestimation of pDNA content in step A, due to the presence of SDS, which may interfere with the fluorescence measurements. The total recovery in the presented system is in the same range as reported for other aqueous two-phase systems [13]. The novel polyacrylate/PEG system allows a strong change of the partitioning between the phases with relatively small changes in composition or pH.

4.5. Plasmid DNA conformation

As presented above (Fig. 5) there is no dramatic change in fraction of pDNA that is supercoiled. Since a supercoiled pDNA is relatively hydrophilic due to the high fraction of buried nucleotides, the partition force to the bottom-phase is probably of hydrophilic nature as discussed above. The high salt concentration and the presence of hydrophilic polymer may favor the more compact supercoiled structure of pDNA. The treatment of pDNA in a polyacrylate-rich bottom phase with a low pH buffer and PEG did not significantly change its conformation. This process of removing the polyacrylate polymer by co-precipitation with PEG at pH 2, could in principle be used in a recovery process for pDNA. However, since the pDNA is exposed to acidic conditions, the possibility of degradation of purines [35,36] should be evaluated. The pDNA can more safely be recovered from the polymer by cross-partitioning to the PEG and then back-extracted into a salt-phase as shown in process I.

5. Conclusion

A bioseparation process in which pDNA can be directed to PEG or to polyacrylate in an aqueous two-phase system has been found. In one process the pDNA is partitioned first to a polyacrylate-rich phase and then back extracted to a PEG-phase by replacing the PEG with one having a lower molecular weight. In another process the pDNA is directly partitioned to a PEG-phase. In both processes the pDNA is separated from the PEG by addition of salt which induces the formation of a PEG/salt system. The yield of pDNA obtained in the final salt phases is 60–70%. pDNA seems to have an attractive interaction with polyacrylic polymer at pH 5–6, and a repulsive interaction above pH 6. It is possible to enhance partitioning by lowering the molecular size of PEG thus favoring partitioning of pDNA to the PEG-rich phase. The recovery of pDNA from the PEG–polymer is performed by addition of salt (e.g. Na_2SO_4) to the PEG-rich phase which thereby induces a PEG/salt two-phase system, where pDNA is obtained in the salt phase. The supercoiled conformation of pDNA is retained in the separation processes.

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