Medbiotech Journal

MBTJ, 4(4): 157-160, 2020

Research Paper

Study of DNA Separation in Aqueous-Salt Two-Phase System

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Received: 23 September 2020 Accepted: 06 November 2020 Published: 01 December 2020

Abstract

Plasmid DNA (pDNA) based gene therapy belongs to the category of non-viral systems. Generally, their production is considered more scalable, the process is easier to control, monitor and quality control testing is considered easier than that for viral vectors. Since downstream processing techniques for the recovery of nanobioparticles face to problems other than those encountered during the recovery of biomolecules such as peptides and proteins, thus downstream process routes that are currently in use for bioparticles have to be re-designed for such nanoparticles. Aqueous two phase system (ATPS) is one of the processes that are used for this purpose. Partitioning of plasmid DNA in ATPS has been shown to provide a powerful method for separation and purification mixture of nanobioparticles. In this paper, Polymer-salt system have been studying and the partition behavior of pDNA in aqueous two phase systems of polyethylene glycol 300 – potassium phosphate at different temperatures and lysate concentrations were investigated. The volume ratio and recovery of pDNA in top, bottom and interface were measured. The results showed that lysate concentration from 10 to 50 mg/ml as well as temperature from 5 to 45 C have a significant effect on pDNA recovery.

Keywords: Aqueous two phase systems; Nanobioparticle; Plasmid DNA; Polyethylene glycol; Potassium phosphate

How to cite the article:
J. Ghanbarov, Study of DNA Separation in Aqueous-Salt Two-Phase System, Medbiotech J. 2020; 4(4): 157-160, DOI: 10.22034/mbt.2020.122171.

1. Introduction

Gene therapy can be applied to the treatment or prevention of genetic or acquired diseases that categorized in two viral and non-viral systems. The development of molecular therapies such as nonviral gene therapy and DNA vaccination has increased the need for high quantities of highly purified plasmid DNA (pDNA) [1, 2].

A problem that is associated with the large size of nanobioparticles is their greatly reduced rate of diffusion in comparison with smaller biomolecules. Such reduced diffusion rates can cause mass transfer limitation during conventional chromatographic adsorption process [3,4]. Most matrices applied for bioparticle adsorption process were originally designed for protein products, thus the relatively high cost of adsorbents and the apparent high amounts required to meet the supply of vectors still challenges the process economy. Nanobioparticles can be expected to be within the size range of cell debris, so convectional centrifugation methods to remove the cell debris may co- precipitate nanobioparticles and hence are

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inapplicable. Nanobioparticles also have sensitivity to shear. It may be concluded that the above problems led to an increase in research directed towards the development of alternative methods for the downstream processing of pDNA.

Aqueous two-phase systems (ATPS) is one of the non-conventional methods which have recently received attention, since several features of early processing steps can be combined in only one operation and phase environment is non-toxic for biomolecules [5]. A number of recent references describe the use of ATPS for the extraction of pDNA from cell lysates [5-9].

In the present work, the polyethylene glycol (PEG)-salt (K_2HPO_4) ATPS are employed as they have several advantages over polymer-polymer systems [10].Firstly, their lower phase viscosities which makes them easier to handle on a large scale [11].Secondly, the cost of phase-forming chemicals is much lower for polymer-salt systems when compared with polymer-polymer systems. Plasmid DNA, 7 kb in size, was used as a nanobioparticle model and the investigation was performed using different temperatures and lysate load. The volume ratio and recovery of pDNA in top, bottom and interface also were measured to determine the partition behavior of pDNA.

2. Materials and Method

2.1. Materials

PEG 300 was obtained from Sigma (St. Louis, MO, USA). Potassium acetate was from Merck. All other reagents used were of analytical grade. The host-plasmid system used for pDNA extraction by ATPS carried on pGP1-2 plasmid [12], with *E. coli* DH5-a as the host. The pGP1-2 is a 7200bp in size.

2.2. Method

2.2.1. Plasmid and lysate production

Escherichia coli cells harboring plasmid pGP1-2 were cultivated overnight in 1000 ml shake flasks containing 250 ml of Luria Bertani medium supplemented with 30 g/ml of kanamycin (Sigma), at 37ºC and 180 rpm. *E. coli* cells were resuspended in one volume of resuspension buffer (50 mM, Tris-HCL, 10 mM EDTA, pH 8). Two volume of lysis buffer (155 mM NaOH, 1% w/v SDS) were added and mixed for 10 minutes at ambient temperature. One volume of neutralization buffer (3M potassium acetate, 10 mM EDTA, pH 5.5) was then added to the suspension, mixed and incubated for up to one hour at ambient temperature. Finally, this prepared lysate is used for further processing with ATPS as described below.

2.2.2. Preparation of aqueous two-phase system

The ATPS composed of 15% w/w polyethylene glycol(PEG) 300 and 22% w/w potassium hydrogen orthophosphate was designated 15/22. Clean (blank) systems contained only PEG, salt and distilled water. Crude systems contained lysate. The pH of ATPS containing cell lysate was adjusted to 8 with concentrated HCl. After mixing in the experiment temperature until equilibrium and phase separation with centrifugation (1000 g, 3 minutes), the volume ratio was measured and top, bottom and interface phases were analyzed for pDNA with spectrophotometer in 260 nm.

3. Results and Discussion

The binodial curve is a curve, which separated the mono-phase from the bi-phase area, i.e. any mixture of PEG, salt and water to the right of the curve results in two phases. In Figure 1 the phase diagram for PEG300 – salt for crude and clean ATPS is shown. Volume ratio is the ratio of top phase to bottom phase that measured in all the experiments.

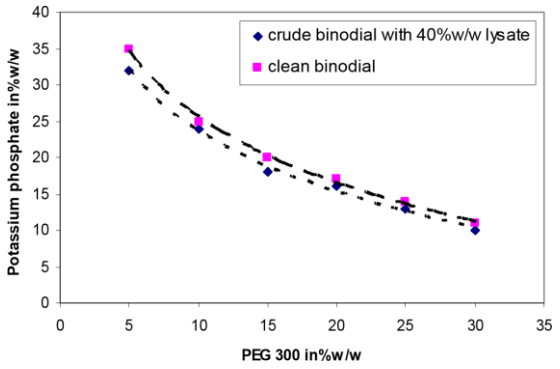

Figure 1. Phase diagram for 15% w/w PEG 300 and 22% w/w potassium phosphate

3.1. The effect of temperature on pDNA partition behavior

An ATPS composed of 15% w/w PEG 300, 22% w/w phosphate and 40% w/w lysate was chosen to study the influence of temperature on the partitioning behavior of pDNA. The distribution profile for 5ºC to 45ºC is shown in Figure 2. The results indicated that the experimental temperature greatly influenced on the partition in a 15/22 ATPS. For temperatures between 5 and 35ºC the partition to the top phase decreased whereas partition to the interface increased. At 35ºC, over 80% of pDNA was recovered in the interface. The volume ratio shifted gradually from 0.78 down to 0.43 with increasing temperature from 5ºC to 45ºC, respectively. The effect of temperature on the partitioning of biomolecules in polymer-salt ATPS

has hardly been reported in the literature, even though some studies exist describing temperature effects in polymer-polymer systems. It has been suggested that the temperature influences the partition behavior of solutes indirectly by changing the chemical compositions of the two bulk phases and that ATPS located close to the critical point are particulary sensitive to temperature changes.

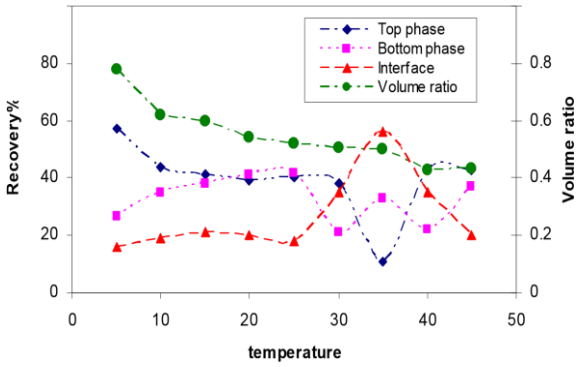

Figure 2. Partition result for plasmid DNA in 15/22 ATPS with respect to the system temperature

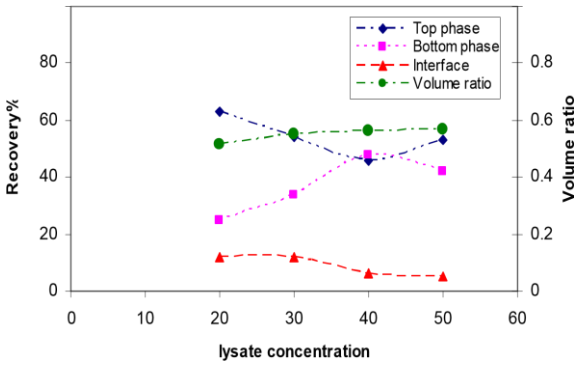

Figure 3. Partition result for plasmid DNA in 15/22 ATPS with respect to the lysate concentration at 20ºC

3.2. The effect of lysate load on pDNA partition behavior

The influence of the amount of crude cell lysate added to a 15/22 ATPS was investigated at three different temperatures , 20ºC, 25ºC and 30ºC.At 20ºC and 25ºC plasmid DNA recoveries as well as the resulting volume ratios for lysate concentration between 20% to 50% w/w are shown in Figure 3 and 4. At 20ºC and 25ºC, with increasing lysate concentration from 20% to 40% , pDNA partitioned to the top phase decreased and in 40% lysate concentration the partition in the top and bottom phase is almost the same. At 30ºC, as is shown in Figure 5 for lysate concentration between 10% and 30% the partition to the bottom phase decreased whereas partition to the interface increased. For all the experiments and increasing lysate concentrations, volume ratio increased.

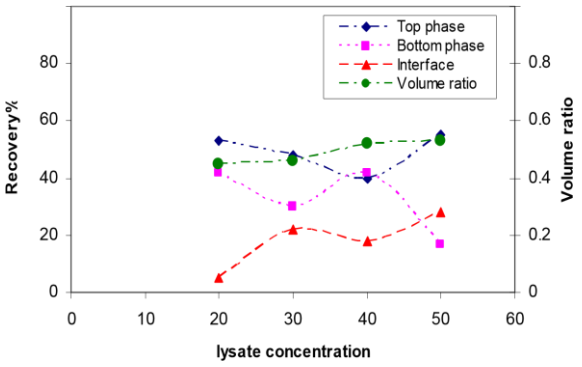

Figure 4. Partition result for plasmid DNA in 15/22 ATPS with respect to the lysate concentration at 25ºC

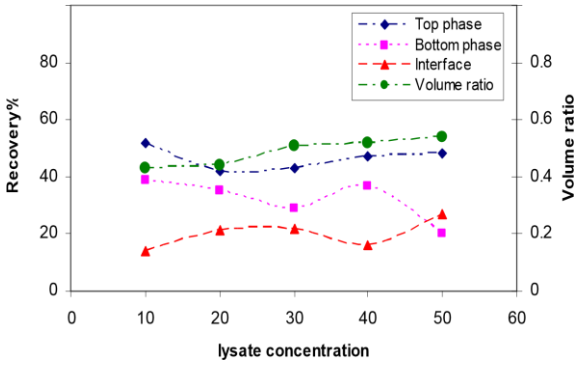

Figure 5. Partition result for plasmid DNA in 15/22 ATPS with respect to the lysate concentration at 30ºC

4. Conclusion

Aqueous two-phase systems (ATPS) shows an interesting alternative and an integration process since several features of early processing steps can be combined in only one operation. The experimental results presented here demonstrate that the plasmid PGP1-2 displayes a varied partition behavior in PEG –phosphate ATPS. It seems that temperature and lysate concentration have great influence on plasmid DNA partitioning. At low lysate concentrations, pDNA partitioned to the top phase, and for three different temperatures, with increasing lysate concentrations, volume ratio increased. It is also obtained that by increasing temperature with 40% lysate concentration, volume ratio gradually decreased.

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