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Purification of plasmid DNA vectors by aqueous two-phase extraction and hydrophobic interaction chromatography

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Abstract

The current study explores the possibility of using a polyethyleneglycol(PEG)–ammonium sulphate aqueous two-phase system (ATPS) as an early step in a process for the purification of a model 6.1 kbp plasmid DNA (pDNA) vector. Neutralised alkaline lysates were fed directly to ATPS. Conditions were selected to direct pDNA towards the salt-rich bottom phase, so that this stream could be subsequently processed by hydrophobic interaction chromatography (HIC). Screening of the best conditions for ATPS extraction was performed using three PEG molecular weights (300, 400 and 600) and varying the tie-line length, phase volume ratio and lysate load. For a 20% (w/w) lysate load, the best results were obtained with PEG 600 using the shortest tie-line (38.16%, w/w). By further manipulating the system composition along this tie-line in order to obtain a top/bottom phase volume ratio of 9.3 (35%, w/w PEG 600, 6%, w/w $(NH_4)_2SO_4$), it was possible to recover 100% of pDNA in the bottom phase with a three-fold increase in concentration. Further increase in the lysate load up to 40% (w/w) with this system resulted in a eight-fold increase in pDNA concentration, but with a yield loss of 15%. The ATPS extraction was integrated with HIC and the overall process compared with a previously defined process that uses sequential precipitations with *iso*-propanol and ammonium sulphate prior to HIC. Although the final yield is lower in the ATPS-based process the purity grade of the final pDNA product is higher. This shows that it is possible to substitute the time-consuming two-step precipitation procedure by a simple ATPS extraction.

Keywords: Aqueous two-phase systems; Liquid–liquid extraction; Plasmid; DNA; Purification; Gene therapy; Polyethylene glycol; Ammonium sulphate

1. Introduction

The development of molecular therapies such as non-viral gene therapy and DNA vaccination have increased the need for high quantities of highly purified plasmid DNA (pDNA) [1,2]. One of the bottlenecks of pDNA manufacturing lies in the purification. Although standard molecular biology protocols are available [3], these are not suitable at large-scale [4]. In addition they frequently use toxic reagents that should not be used in the purification of therapeutic products. These constraints have led to an increase in research directed towards the development of alternative methods for the downstream processing of pDNA.

Aqueous two-phase systems (ATPS) constitute an interesting alternative since several features of early processing steps can be combined in only one operation and phase environment is non-toxic for biomolecules. A number of recent references describe the use of ATPS for the extraction of pDNA from cell lysates [5–8]. A thermoseparating ATPS made of (50% ethylene oxide–50% propylene oxide)/dextran has been developed for the purification of a 6.1 kbp pDNA from a desalted alkaline lysate [8]. The promising results obtained in this study (100% pDNA recovery with 80 and 58% RNA and protein removal respectively) prompted the authors to develop an integrated purification process which combined the thermoseparating ATPS with membrane filtration and chromatography [7]. The more conventional polyethylene glycol (PEG)/salt (K_2HPO_4) [5,6] ATPS was used to study the partial purification of an 8.5 kbp pDNA vector from a

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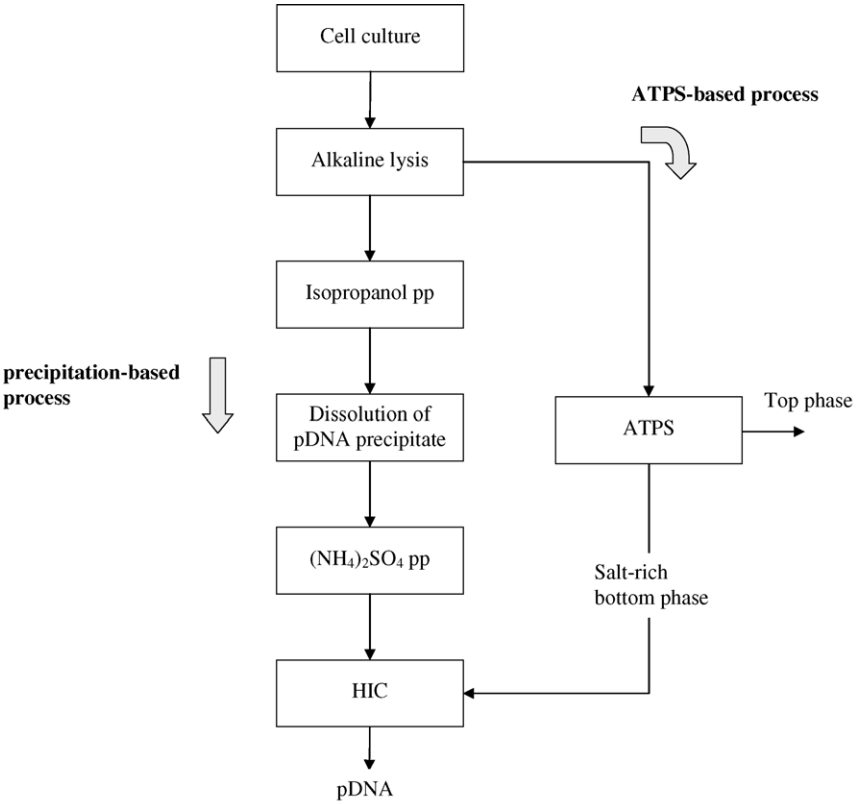

Fig. 1. Process flow sheet describing two alternative routes for the intermediate purification of plasmid DNA prior to preparative hydrophobic interaction chromatography (HIC). The precipitation-based process concentrates and pre-purifies pDNA by precipitation with *iso*-propanol and ammonium sulphate respectively, while the ATPS-based process uses a single extraction step (abbreviations: ATPS – aqueous two-phase system, pp – precipitation).

neutralised alkaline lysate [5]. Results showed that by varying PEG molecular weight, pDNA could be directed towards the top ($M_W < 400$) or bottom phase ($M_W > 400$).

A typical process for the production of pDNA includes cultivation of an *Escherichia coli* host, followed by alkaline lysis and a number of purification steps [4]. Since the complexity of alkaline lysates can severely compromise fixed-bed chromatographic operations, pre-purification steps are usually necessary. For instances, in a process (Fig. 1) based on hydrophobic interaction chromatography (HIC) which has been described for the large scale purification of a pDNA [9], three steps are needed after alkaline lysis and before feeding the HIC column: (i) precipitation of pDNA with *iso*-propanol, (ii) re-dissolution of pDNA precipitate in an appropriate buffer and (iii) precipitation of proteins, RNA and endotoxins with ammonium sulphate ($(NH_4)_2SO_4$). This last step further acts as a suitable conditioning step since the salt type ($(NH_4)_2SO_4$) and concentration (2–2.5 M) in the final pDNA-containing supernatant match the optimal requirements for a HIC feed. In the current study we explore the possibility of using a PEG–salt ATPS to replace the three early processing steps (Fig. 1). In this PEG–salt system, $(NH_4)_2SO_4$ is used instead of the more classical K_2HPO_4. Thus, if conditions are selected such that pDNA is directed towards the salt-rich bottom phase, this stream can be injected directly in the HIC column. A 6.1 kbp pDNA was used as a model molecule and screening of the best conditions for ATPS extraction was performed using three PEG molecular weights (300, 400 and 600) and varying the tie-line length, phase volume ratio and lysate load. An adequate system and extraction conditions were then selected and tested in order to assess its feasibility as a replacement of the two pre-purification steps. Further purification by HIC was tested in order to check the compatibility of the ATPS bottom phase with the chromatographic operation. Yield and purity in terms of contaminants RNA, protein and endotoxin were evaluated in the final preparation.

2. Experimental

2.1. Chemicals

PEG 300, 400 and 600 were obtained from Sigma (St. Louis, MO, USA). Ammonium sulphate and potassium acetate were from Merck (Darmstadt, Germany). All the other reagents used were of analytical grade. The 6050 bp (base pairs) ColE1-type plasmid pVAX1/*lacZ*, designed by Invitrogen (Carlsbad, CA, USA) for the development of DNA vaccines, was used as a model plasmid. This vector