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Partitioning of pyrimidine single stranded oligonucleotide using polyethylene glycol – sodium sulfate aqueous two-phase systems; experimental and modeling

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ABSTRACT

In this work partitioning of TAMRA (Tetramethyl-6-Carboxyrhodamine) - oligonucleotide in systems containing 18% (w/w) polyethylene glycol (PEG 600, PEG 1000, PEG 1500 and PEG 4000) and 15% (w/w) sodium sulfate (designated as 18/15) was investigated. To find the optimum condition, effective parameters on biomolecule partitioning including polymer molecular weight, pH, temperature and addition of a second salt were studied. The results showed that as pH increases and molecular weight of polymer decreases, the affinity of TAMRA oligonucleotide to accumulate in the top PEG-rich phase increases. In order to find the temperature effects, partitioning of TAMRA oligonucleotide was studied at two different temperatures 20 and 30 °C). At this range, no significant change was observed. Meanwhile, it is found that addition of 0.5% (w/w) of KH_2PO_4 as the second salt reversed the partitioning behavior of the oligonucleotide. A similar trend with smaller gradients was observed when KH_2PO_4 was substituted by KCl; whereas, substitution by $MgSO_4$ increased the affinity of the oligonucleotide to partition to top phase. The best results achieved using PEG 600 at pH = 6. In this case, almost 100% of TAMRA oligonucleotide was recovered in the upper phase. Finally, the partitioning of the biomolecule was correlated using a model based on UNIFAC-FV group contribution. The results showed that the model can correlate the partitioning of the oligonucleotide with an acceptable accuracy.

1. Introduction

Nowadays demands for purified single and double stranded oligonucleotides are growing up. For many years chromatography was known as the most common way for purification of both single and double-stranded nucleic acids in industrial scale [1]. But because of high costs and operational difficulties especially in scale-up, attempts to find alternative purification methods have been in progress during last decades. Aqueous two-phase systems (ATPSs) as a proper alternative have several preferences in comparison with chromatography technics, especially easiness in scale up and lower costs. Moreover, The combination of several purification steps has made this method more attractive in industrial applications [2–5]. Furthermore, the high water content in both phases of an ATPS, provides an appropriate medium for extraction of a wide range of biomolecules like proteins, enzymes, nucleic acids [6,7]. Aqueous two-phase systems were firstly applied by Albertsson as a systematic method for the partitioning of nucleic acids in a polyethylene glycol – dextran system in 1962 [8]. Such systems are commonly made up of two different hydrophilic polymers or one polymer and one salt. In general, polymer-salt systems are paid more attention than polymer-polymer types due to lower viscosity, higher interfacial tension, and lower prices.

Among different types of biomolecules, oligonucleotides have received lots of attention in biological fields as short nucleic acids strands. They can be applied as sequence-specific binding agents either for revealing the presence of a specific sequence of a DNA or knocking out a specific gene through forming triplex structures [9]. There are numerous applications for triplex-forming oligonucleotides (TFOs) such as DNA cleavage, induction of homologous recombination, generation of site-specific mutations and inhibition of DNA transcription or replication [10]. Such capabilities enable

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oligonucleotides to be used in a new generation of drugs that interact with a particular gene in vivo to modulate its activity [9].

From the experimental point of view, detection and quantitation of oligonucleotide concentration are essential. Spectrophotometric measurement at 260 nm is the common method for quantitation of nucleic acids, but in the presence of PEG, the strategy is not effective because of interference caused by PEG at the same wavelength. To overcome this problem, labeling was employed as a useful technique for both tracing and measuring the oligonucleotide content in the aqueous two-phase systems. A wide range of dyes with fluorescence properties can be used as labeling agents such as TAMRA (Tetramethyl-6-Carboxyrhodamine), FAM (Carboxyfluorescein), Cy 3.5 (Cyanine 3.5), Cy 5 (Cyanine5), etc. In this study, TAMRA was used as the labeling agent because of its potential capability to act as an intercalator in the case of triplex-forming oligonucleotides. Islam [11] reported that Rhodamine B interacts with DNA through binding to its minor grooves. Considering mentioned facts, it was supposed that TAMRA as one of Rhodamine derivatives might also interact with pDNA in a similar way; leading to an increase in the stability of the triplex structure. It should be pointed that, although modification of the oligonucleotide using TAMRA might affect the partitioning behavior, but it was inevitable to avoid such modification in order to guarantee the formation and stability of the triplex structure.

In the previous works, partitioning of long-stranded oligonucleotides was investigated at different operational conditions such as pH, temperature, polymer molecular weight, lysate load and the addition of a second salt [12–19]. Rahimpour et al. [13] investigated the effect of pH and polymer molecular weight in PEG – sodium citrate systems using polyethylene glycols with different molecular weights from 200 to 1500 Da and pH from 5.9 to 7.9 [12]. Johansson, et al. worked on the partitioning of a 2686 bp pDNA in both PEG 4000 and PEH 8000 – Polyacrylate 240000 - Na_2SO_4 systems in a pH range from 5.4 up to 7.3. Luechau et al. [20] investigated the partition behavior of a pDNA in the presence of NaCl as the second salt.

In addition to experimental studies, modeling of the partitioning of biomolecules in ATPSs can be quite helpful from industrial points of view in order to reduce the costs. There is a wide range of models for correlation of partitioning behavior of biomolecules in two-polymer systems [21–23]. Furuya used the modified Flory – Huggins for correlating the partitioning of hydrolytic enzymes in a PEG-dextran system [21]. Lin et al. proposed a correlation based on modified Pitzer to predict the partitioning of proteins [22]. Pazuki et al. applied the artificial neural network model to correlate the partitioning of lysosome and glycylalanine [23]. Correlation of biomolecules in polymer – salt systems were also paid attention in the last decade [24–27]. Partitioning of Bovine Serum Albumin (BSA) was correlated in PEG – K_2HPO_4 systems using both Electrolyte – SAFT [24] and modified Wilson [25]. Dehghani used the artificial neural network model to obtain the activity coefficient of amino acids and peptides [27]. The UNIFAC-FV model was also applied in the prediction of partition coefficient of β-amylase and amyloglucosidase in KH_2PO_4 and Na_2SO_4 systems [26].

To our knowledge, the partitioning of short stranded oligonucleotides in aqueous two-phase systems has not been studied. In this work, the partitioning behavior of a 20bp modified oligonucleotide at pH range 3–6 in systems containing sodium sulfate and polyethylene glycols (with various molecular weights) is studied. Moreover, the temperature effect and presence of the second salts including KH_2PO_4, KCl, and $MgSO_4$ on the partitioning of the TAMRA oligonucleotide is also investigated. Finally, the partitioning of the oligonucleotide in PEG 4000 – sodium sulfate systems is correlated using the UNIFAC free volume group contribution model. The UNIFAC-FV model was chosen to obtain the activity coefficient of the components due to its higher accuracy in comparison with other models such as NRTL, UNIQUAC, and ASOG [28].

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

The chemicals used in this study are shown in Table 1. All other reagents were of analytical grade.

2.1.2. Polypyrimidine oligonucleotide

The modified polypyrimidine 20bp oligonucleotide was synthesized from Bioneer Corporation (South Korea) which had been purified by HPLC method; a GC content of 45% and $OD_{260} = 25$ was reported by the provider. A modification was also performed at the 5′ terminus with TAMRA label. The polypyrimidine oligonucleotide sequence was as follow:

2.1.2.1. 5′-TAMRA – CTTTCTTCCCTTCCTTTCTC -3′. To determine oligonucleotide content in each phase maximum excitation and emission wavelengths was obtained using a Cary Eclipse Fluorescence Spectrophotometer. The results revealed that the maximum excitation and emission happen at a wavelength of 558 and 582 nm, respectively (Fig. S1 in the supplementary materials).

2.2. Methods

2.2.1. Aqueous two-phase preparation

Each system contained a total mass of 5 g comprised of 18% (w/w) polyethylene glycol and 15% (w/w) Na_2SO_4 were prepared in a conical 15 ml centrifuge tube. PEG 600 was added to the system in its pure liquid form while polyethylene glycols with higher molecular weights were added from pre-prepared 50% stock solutions. Meanwhile, the salt was added in the solid form. In continue the components dissolved in water by vortex mixing. The process followed by addition of 40 μl of the oligonucleotide from a stock solution with a concentration of 500 pmol per ml. After adjusting pH, using hydrochloric acid and sodium hydroxide solutions, the tube was shaken up and down several times to distribute the oligonucleotide throughout the system. To accelerate the phase separation, each system was centrifuged at 1000*g* for 5 min with an MSE Mistral 3000i centrifuge. Deionized water was used in the whole experiments.

2.2.2. TAMRA-oligonucleotide quantitation

To quantify the TAMRA-oligonucleotide concentration in the top and bottom phases, separated calibration curves were prepared for each phase in each system. Standards were prepared using the same concentration of PEG and salt to avoid possible interference due to the high concentration of the phase components in the systems. All samples were diluted 10 fold in deionized water. The

Table 1
Table of components.

Component	Supplier	Molar Mass (g/mol)	Purity
KH_2PO_4	Sigma-Aldrich	136.1	≥99%
KCl	AnalaR	74.5	≥99.8%
NaOH	Merck	40.0	≥99%
HCl	Merck	36.5	37%
PEG 600	Merck	570–630	
PEG 1000	Merck	950–1050	
PEG 1500	Merck	1400–1600	
PEG 4000	Merck	3500–4500	