

Enhancing the Detection of Urinary Tract Infections Using Two-Phase Aqueous Micellar Systems

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Urinary tract infections (UTIs) are a leading cause of health expenditures, in part due to the expensive and lengthy diagnostic method that involves culturing bacteria. A new technique has been developed allowing for faster UTI diagnosis through an electrochemical chip to measure unique ribosomal RNA (rRNA) found in bacteria associated with UTIs. Although this technique has had success, concentrating the bacterial rRNA through the use of two-phase aqueous micellar systems in the urine sample prior to utilizing the chip may provide increased sensitivity. This approach is appealing because these systems are relatively inexpensive, easily scalable, and simple to use. In this study, we examined the partitioning behavior of bacterial RNA fragments, purified from *Enterococcus faecalis*, in a two-phase micellar system comprised of the nonionic surfactant Triton X-114 and phosphate-buffered saline. Experimentally measured results were compared to theoretical values to determine the governing factors involved in RNA partitioning. We demonstrated that RNA fragments partition primarily due to steric, excluded-volume interactions that exist between the RNA and micelles. However, the presence of entrained micelle-poor, RNA-rich domains in the macroscopic micelle-rich, RNA-poor phase limits the extent of RNA partitioning in this system. Additionally, by manipulating the volume ratio, or the volume of the top, micelle-poor phase divided by that of the bottom, micelle-rich phase, we demonstrated that RNA fragments can be concentrated up to four-fold in a predictive manner. Concentrating the bacterial RNA with these two-phase micellar systems prior to detection with the UTI chip may facilitate the earlier detection of UTIs.

INTRODUCTION

Bacterial infections of the bladder, kidneys, and other components of the urinary tract are some of the most common infections in the United States (Liao et al., 2006; Griebeling, 2007). A study in 2000 reported that over \$3.5 billion was spent on medical expenses related to UTI patient care, making UTIs one of the leading causes of health expenditures in the United States (Litwin and Saigal, 2007). These high costs are in part due to the expensive and lengthy diagnostic method, requiring urine sample retrieval, cell culture to increase the amount of bacteria present in the sample, and finally, uropathogen identification. Because the process can take several days, patients with a UTI may receive treatment, primarily antibiotics, without a conclusive diagnosis (Liao et al., 2006). This early treatment could result in inappropriate prescriptions and a rise in uropathogen resistance to common antibiotics. Faster and more sensitive diagnostic tools, which could decrease the amount of time necessary to detect infections, could circumvent such problems (Ivancic et al., 2008).

Thus, a novel device has been developed which allows for a more rapid approach to UTI diagnostics. The device is an electrochemical chip containing an array of fixed probes for capturing different ribosomal RNAs (rRNAs), which are unique to bacteria commonly found in UTIs (Sun et al., 2005; Liao et al., 2006). Upon lysis of the uropathogens in the urine sample of a patient, the rRNA hybridizes to the capture probes on the chip, producing a measurable current unique to the type of bacteria present. The UTI chip has the potential to diagnose UTIs in less than one hour, allowing for individualized treatments (Liao et al., 2006) and, thereby, potentially slowing the emergence of antibiotic-resistant uropathogens. While the chip yields faster and more sensitive detection of UTIs than standard UTI screening methods, concentrating the RNA fragments in the urine sample prior to the detection step could further improve its potential as a diagnostic tool.

One possible method to concentrate the uropathogens' RNA is through the use of two-phase aqueous micellar systems. Two-phase micellar systems exploit the chemical properties of surfactants and their interactions with hydrophilic and hydrophobic molecules. Surfactants are amphiphilic molecules that have a hydrophilic, or polar, "head" and a hydrophobic, or nonpolar, "tail." In an their critical micelle concentration (CMC), surfactants self-assemble into aggregates called micelles. In these micelles, the surfactants' hydrophobic tails face inward to minimize their contact with water, while their hydrophilic heads face outward to maximize their contact with water (Quina and

Hinze, 1999). Since these two-phase systems would ideally be used in a device at the site of patient care, they should be scalable to small volumes, as is possible with liquid-liquid extraction. Furthermore, unlike other liquid-liquid extractions involving organic solvents, the two phases of micellar systems are primarily composed of water, thus providing a mild environment for biomolecules (Liu et al., 1998; Quina and Hinze, 1999). Finally, two-phase micellar systems are also advantageous due to the relative simplicity in forming the two phases.

In this investigation, we theoretically and experimentally examined the use of two-phase aqueous micellar systems for partitioning and concentrating bacterial RNA. Bacterial RNA was purified from *Enterococcus faecalis* (*E. faecalis*) and used in the experiments, since it is one of the bacteria commonly found in UTIs (Sun et al., 2005). The micellar system selected was comprised of the nonionic surfactant Triton X-114 and phosphate-buffered saline. The Triton X-114 micellar solution exhibits a homogenous, isotropic phase at room temperature. Upon increasing the temperature, the solution separates into a bottom, micelle-rich phase and a top, micelle-poor phase. Hydrophilic molecules, such as water-soluble proteins and viruses, have been previously found to be driven into the micelle-poor phase due to repulsive, steric, excluded-volume interactions with the micelles (Kamei et al., 2002a). The extent of this partitioning behavior can be quantified through the partition coefficient, which is the ratio of the concentrations in the two phases. RNA was experimentally partitioned at various temperatures and, as in the case of the water-soluble proteins and viruses, was found to partition unevenly between the two phases. By treating the micelles as cylinders and the RNA as spheres, a theoretical expression was employed to predict the partition coefficient of RNA based on excluded-volume interactions with the micelles in solution. Previous studies have shown that entrainment may be a limiting factor in the extent of the partitioning of biomolecules in two-phase systems (Kamei et al., 2002b; Mashayekhi et al., 2009). Therefore, we hypothesized and confirmed that RNA will be partitioned primarily due to excluded-volume interactions, but will be limited due to the effects of entrainment. We also derived a theoretical expression for the concentration factor of RNA which can be used to predict the fold-concentration of RNA in these systems.

MATERIALS AND METHODS

GENERATING TRITON X-114 PHASE DIAGRAM The phase diagram of Triton X-114 in Dulbecco's phosphate-buffered

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saline (PBS; Invitrogen, Carlsbad, CA) was generated by determining the surfactant concentrations in both the micelle-rich and micelle-poor phases at temperatures ranging from 22 - 36°C. For a given temperature, three solutions with identical concentrations of Triton X-114 in PBS were made and incubated for 18 hours at the desired temperature. The two phases were then carefully extracted using syringe and needle sets. The bottom, micelle-rich phase was withdrawn by piercing the side of the tube with the needle to avoid disrupting the liquid-liquid interface. The sampled phases were then diluted below the Triton X-114's CMC with PBS, and the Triton X-114 concentration was determined using UV absorbance at 274 nm with an extinction coefficient of 2.51 (mg Triton X-114/g of solution)-1cm-1. All reagents and materials were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

CELL CULTURE *E. faecalis* (American Type Culture Collection) was grown in brain heart infusion media (Fisher Scientific, Pittsburgh, PA). The bacteria were incubated overnight at 37°C in a shaker incubator to an approximate density of 10⁹ bacteria/mL before being used in subsequent RNA purification steps.

HeLa cells (American Type Culture Collection) were grown in MEM (Invitrogen, Carlsbad, CA) supplemented with 2.2 g/L sodium bicarbonate, 10% FBS (Hyclone, Logan, UT), 1% sodium pyruvate (Invitrogen), 100 µg/mL streptomycin (Invitrogen), and 100 units/mL penicillin (Invitrogen), at a pH of 7.4. The cells were incubated at 37°C in a humidified atmosphere with 5% CO₂ to an approximate density of 105 cells/cm² before being used in DNA purification steps.

NUCLEIC ACID PURIFICATION AND QUANTIFICATION Bacterial RNA fragments were purified from *E. faecalis* using the RNeasy Mini Kit and RNAProtect Bacteria Reagent (QIAGEN, Valencia, CA). Briefly, a stock solution of *E. faecalis* was incubated with RNAProtect Bacteria Reagent, followed by centrifugation to form a pellet. The cells were then resuspended in a lysis buffer comprised of 20 mM Tris•Cl, 2 mM sodium EDTA, 1.2% Triton X-100, and 20 mg/ml lysozyme at a pH of 8.0, and incubated at room temperature for ten minutes. The buffer conditions of the lysate were adjusted with solutions provided in the kit. The lysate was then loaded onto spin columns, which were equipped with membranes that bind specifically to RNA. After the spin column membranes were washed with buffers provided in the kit to remove other biomolecules, the RNA fragments were eluted from the columns with RNase free water. Purified bacterial RNA fragments were

stored at -20°C in RNase free water until used.

RNA was quantified using the Quant-iT RNA Assay Kit (Invitrogen, Carlsbad, CA) following the manufacturers' protocol. The kit provided a buffer solution containing a fluorescent dye that non-covalently binds to RNA in a sample. Twenty microliters of samples with an unknown concentration of RNA was added to a 96-well plate containing 200 µL of the provided buffer solution. When the dye binds to RNA, it has excitation and emission peaks at approximately 620 to 670 nm, respectively. The fluorescence intensity was directly proportional to the mass of RNA present in solution in the range of 5 to 100 ng. To find this proportionality constant, a standard curve of fluorescence intensity versus mass of RNA was generated using triplicates for each data point. Since the presence of Triton X-114 affects the fluorescence intensity, the standard curves were generated in the presence of the appropriate concentration of surfactant. Specifically, in addition to known amounts of RNA, 20 µL of either the micelle-rich or micelle-poor phase of the control solution, which contained Triton X-114 but no RNA, were added to wells used for generating the standard curves.

Double-stranded DNA fragments were purified from HeLa cells using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA). HeLa cells were centrifuged to form a pellet, resuspended in PBS, and then lysed enzymatically with proteinase K. To obtain RNA-free DNA fragments, 6 µL of 100 mg/mL RNase A was added to the lysate, and the solution was incubated at room temperature for two minutes. Solutions provided in the kit were added to the lysate to adjust the buffer conditions, followed by centrifugation of the lysate in a spin column which was equipped with a membrane that selectively binds to double-stranded DNA fragments. Other biomolecules present in the lysate were washed away using solutions provided in the kit, followed by DNA elution with Buffer AE, which was also provided in the kit. Purified DNA was stored in Buffer AE at -20°C until it was used.

DNA was quantified using the Quant-iT dsDNA High Sensitivity Assay Kit (Invitrogen, Carlsbad, CA). The buffer provided in this kit contained a fluorescent dye which non-covalently and selectively binds to double-stranded DNA fragments. When the dye binds to double-stranded DNA, it has approximate excitation and emission peaks at approximately 502 and 523 nm, respectively. Similar to the RNA quantification procedure, 96-well plates were used to quantify the amount of DNA in a solution. The fluorescence intensity from each well was directly proportional to the amount of DNA in the range of 0.2 ng to 100 ng of DNA. To find this proportionality constant, similar to RNA

quantification procedure described above, standard curves were also generated for DNA quantification.

DETERMINING OPERATING CONDITIONS The operating conditions, namely incubation temperatures and initial Triton X-114 concentrations, were chosen to obtain a volume ratio of 1.0 and 0.35 for RNA partitioning and concentration experiments, respectively. Solutions of varying concentrations of Triton X-114 were incubated for 1 hour (or 1.5 hours for RNA concentration experiments) at a given temperature, centrifuged for 1 minute at 162 g using the Allegra 6 benchtop centrifuge (Beckman Coulter, Fullerton, CA), and the volume ratio determined. Based on these values, the Triton X-114 concentration was adjusted accordingly until the desired volume ratio was obtained.

PARTITIONING RNA IN TWO-PHASE AQUEOUS MICELLAR SYSTEMS The PBS used in RNA experiments was treated with diethylpyrocarbonate (DEPC) to inhibit RNase activity. 0.1 mL of DEPC was added to 100 mL of PBS and incubated at 37°C for 12 hours, followed by autoclaving for 15 minutes to remove traces of DEPC. For each RNA partitioning experiment, four identical Triton X-114 solutions in DEPC-treated PBS with a volume of 3.5 mL were made and stored at 4°C to ensure the solutions were one homogenous phase. 20 µg of bacterial RNA was added to three of the Triton X-114 solutions, while an equivalent volume of RNase free water was added to the fourth solution, which served as the control. These solutions were incubated at the desired temperature for 1 hour in a water bath. The solutions were then centrifuged at 162 g for 1 minute using the Allegra 6 bench top centrifuge to minimize the entrainment of micelle-poor domains in the macroscopic micelle-rich phase and that of micelle-rich domains in the macroscopic micelle-poor phase. After centrifugation, the two phases were carefully extracted using syringe and needle sets, and the concentration of RNA in the two phases was determined by the method described above. Photographs of the two phases were taken before and after the centrifugation step

PARTITIONING DNA IN TWO-PHASE AQUEOUS MICELLAR SYSTEMS To estimate the degree of entrainment of micelle-poor domains in the macroscopic micelle-rich phase in the Triton X-114/PBS micellar system, mammalian DNA fragments were partitioned. Four identical Triton X-114 solutions were prepared in PBS, which was not treated with DEPC. Ten micrograms of DNA were added to three of the solutions, while an equal volume of DNA storage buffer (buffer AE, provided in the DNeasy Blood and Tissue Kit) was added to the fourth solution, which served

as the control. The solutions were then incubated at the desired temperature for 1 hour, and centrifuged at 162 g for 1 minute before extraction of each phase using syringe and needle sets. DNA was quantified in each phase using the procedure described above.

CONCENTRATING RNA IN TWO-PHASE AQUEOUS MICELLAR SYSTEMS The RNA concentration experiment was conducted following the procedure described above for RNA partitioning, except that the operating conditions (initial Triton X-114 concentration and incubation temperature) were adjusted to obtain a smaller volume ratio. Furthermore, the incubation time was increased to 1.5 hours instead of 1 hour since phase separation occurred at a slower rate for solutions with lower volume ratios.

RESULTS

EXCLUDED-VOLUME THEORY With an increase in temperature, a homogeneous solution with Triton X-114 will separate into two macroscopic phases. During phase separation, the interaction between biomolecules and micelles present in the solution will drive the biomolecules preferentially into one of these macroscopic phases. To quantify the degree of separation, the partition coefficient is evaluated and defined as:

$$K_{bm} \equiv \frac{C_{bm,t}}{C_{bm,b}} \quad (\text{Eq. 1})$$

where $C_{bm,t}$ and $C_{bm,b}$ are the concentrations of the biomolecule in the top, micelle-poor phase and the bottom, micelle-rich phase, respectively.

The excluded-volume theory derived by the Blankschtein and Deen research groups states that, if separation occurs only as a result of repulsive, steric, excluded-volume interactions, the partition coefficient of a dilute biomarker of known geometry is given by the following (Lazzara et al., 2000; Nikas et al., 1992):

$$K_{bm} = \exp \left[U_{b-m} (C_{m,b} - C_{m,t}) \right] \quad (\text{Eq. 2})$$

where $C_{m,t}$ and $C_{m,b}$ are the concentrations of micelles in the top and bottom phases in units of number per mL, respectively, and U_{b-m} is the excluded volume between biomarker and micelle. Note that $\exp[y]$ is equivalent to e^y , the exponential function of y . The excluded volume

between a biomolecule and micelle is given by (Jansons and Phillips, 1990):

$$U_{b-m} = V_m + \frac{1}{4\pi} (S_m M_b + S_b M_m) + V_b \quad (\text{Eq. 3})$$

where V_b and V_m are the volumes, S_b and S_m are the surface areas, and M_b and M_m are the integrals of the local mean curvature over the surfaces of biomolecules and micelles, respectively. Previously, the partitioning of globular, hydrophilic proteins in two-phase aqueous nonionic micellar systems was investigated. It was shown that if only repulsive, steric, excluded-volume interactions are considered, the partition coefficient could reasonably be predicted by modeling the micelles as cylinders and proteins as spheres, with the following expression (Nikas, 1992):

$$K_p = \exp \left[-(\phi_t - \phi_b) \left(1 + \frac{R_p}{R_0} \right)^2 \right] \quad (\text{Eq. 4})$$

where ϕ_t and ϕ_b are the surfactant volume fractions in the top and bottom phases, R_p is the hydrodynamic radius of the protein, and R_0 is the cross-sectional radius of the cylindrical micelles, respectively. While the structure of RNA is variable depending on the length and structural folding of these molecules, we approximated the bacterial RNA to be spherical in shape, and the Triton X-114 micelles to be cylindrical in shape. Therefore, Eq. (4) can be used to predict the partition coefficient of RNA in Triton X-114/PBS systems.

ENTRAINMENT One source of observed inconsistency between empirical partitioning data and the predictions of the excluded-volume theory is the effect of entrainment. One of the assumptions of the excluded-volume theory is that macroscopic phase separation equilibrium has been attained. Entrainment corresponds to the situation where, after phase separation, domains of micelle-poor phase remain in the bottom, macroscopic micelle-rich phase, while domains of micelle-rich phase remain in the top, macroscopic micelle-poor phase. If a biomolecule partitions extremely into the micelle-poor phase, even a small number of the micelle-poor, biomolecule-rich domains entrained in the macroscopic micelle-rich, biomolecule-poor phase will cause the measured concentration of biomolecule in the bottom, micelle-rich phase to be much higher (and therefore the partition coefficient to be much lower) than that predicted solely by the excluded-volume theory, in

which no entrainment is assumed to occur. However, the reverse situation, in which micelle-rich, biomolecule-poor domains are entrained in the macroscopic, micelle-poor, biomolecule-rich phase, should not significantly affect the measured biomolecule concentration in the top, micelle-poor phase. The effect of entrainment has been attributed to deviations from theoretical predictions for DNA fragment partitioning in previous investigations of the same system, and has been accounted for using a single fitted parameter (Mashayeki, 2009):

$$K_m = \frac{K_t}{1 + x(K_t - 1)} \quad (\text{Eq. 5})$$

where K_t is the theoretical partition coefficient (based solely on excluded-volume interactions), K_m is the measured partition coefficient (when both excluded-volume interactions and entrainment are considered), and x is the volume fraction of micelle-poor domains entrained in the macroscopic, micelle-rich phase. The fitted parameter x can be determined from a partitioning experiment with the same volume ratio, since x has been shown to be only a function of the volume ratio and not the partitioned biomolecule (Kamei et al., 2002b).

CONCENTRATION FACTORS The total amount of biomolecules present in both phases should equal the initial amount present in solution prior to separation, which is represented by the following equation:

$$C_{bm,0} \cdot (V_t + V_b) = C_{bm,t} \cdot V_t + C_{bm,b} \cdot V_b \quad (\text{Eq. 6})$$

where $C_{bm,0}$ is the initial concentration of the biomolecule in the solution prior to phase separation, $C_{bm,t}$ is the concentration of the biomolecule in the top, micelle-poor phase, $C_{bm,b}$ is the concentration of the biomolecule in the bottom, micelle-rich phase, and V_t and V_b are the volumes of the top and bottom phases, respectively. Algebraic rearrangement of this equation yields the concentration factor:

$$\text{Concentration factor} \equiv \frac{C_{bm,t}}{C_{bm,0}} = \frac{V_r + 1}{V_r + K_{bm}^{-1}} \quad (\text{Eq. 7})$$

where V_r is the volume ratio of the top phase to that of the bottom phase (V_t/V_b), and K_{bm} is the measured partition coefficient of the biomolecule ($C_{bm,t}/C_{bm,b}$). Therefore, based on Eq. 7, assuming the value of the measured partition

coefficient of the biomolecule is significantly larger than 1 and by adjusting the volume ratio to less than 1, a hydrophilic biomolecule such as RNA can be concentrated in the top, micelle-poor phase.

PARTITIONING NUCLEIC ACIDS IN THE TRITON X-114 MICELLAR SYSTEM The phase diagram of the Triton X-114/PBS micellar system, shown in Figure 1, was used to approximate the initial Triton X-114 concentrations and incubating temperatures needed to obtain the desired volume ratios. These approximations were subsequently tested, and if needed, adjusted to determine the operating conditions for the RNA and DNA partitioning experiments (Table 1) as well as the RNA concentration experiments (Table 2).

To qualitatively demonstrate the presence of entrainment, after incubating the Triton X-114 solutions in a water bath for 1 hour, photographs of the solutions were taken before and after the centrifugation step (Figure 2). The presence of entrained domains in a macroscopic phase can scatter light, resulting in the phase appearing noticeably turbid or “cloudy.” The micelle-rich phase of the two-phase solutions before centrifugation (Figure 2A) appeared significantly more turbid than that of the solutions after centrifugation (Figure 2B).

A previous investigation into the partitioning of DNA in the Triton X-114/PBS micellar system has shown that DNA fragments partition extremely into the micelle-poor phase of the micellar system (Mashayekhi et al., 2009). Therefore, Eq. 5 could be simplified to obtain the following

approximation to predict the partition coefficient of DNA fragments in this system:

$$\lim_{K_t \rightarrow \infty} K_m = \frac{K_t}{1 + x(K_t - 1)} \approx \frac{1}{x} \quad (\text{Eq. 8})$$

In a given micellar system, x has been shown to be only a function of volume ratio, rather than the molecule being partitioned (Kamei et al., 2002b). Therefore, for the Triton X-114/PBS micellar system studied in this paper, x was estimated from a single DNA partitioning experiment with the same volume ratio of 1. Using the DNA partition coefficient obtained at 32.3°C, and Eq. 8, the value of x was estimated to be 0.08 for the Triton X-114/PBS micellar system. The theoretically predicted DNA partition coefficients using Eq. 8 are presented in Figure 3.

As RNA fragments are generally smaller than the DNA fragments studied, based on Eqs. 4 and 5, RNA fragments were expected to partition less extremely. The inability to accurately estimate the effective hydrodynamic radii of RNA fragments due to the variable length and structural folding of these fragments precludes an accurate, purely theoretical prediction of RNA partition coefficients in micellar systems due to excluded-volume interactions. However, certain parameters of the system may still be approximated. Since the expression of ribosomal RNA (rRNA) cannot be amplified similar to that of the RNA that codes for proteins, and since ribosomes are required in large amounts, rRNA makes up a large portion of the RNA present in bacterial cells. The method used to purify RNA is known to only retain fragments longer than 200

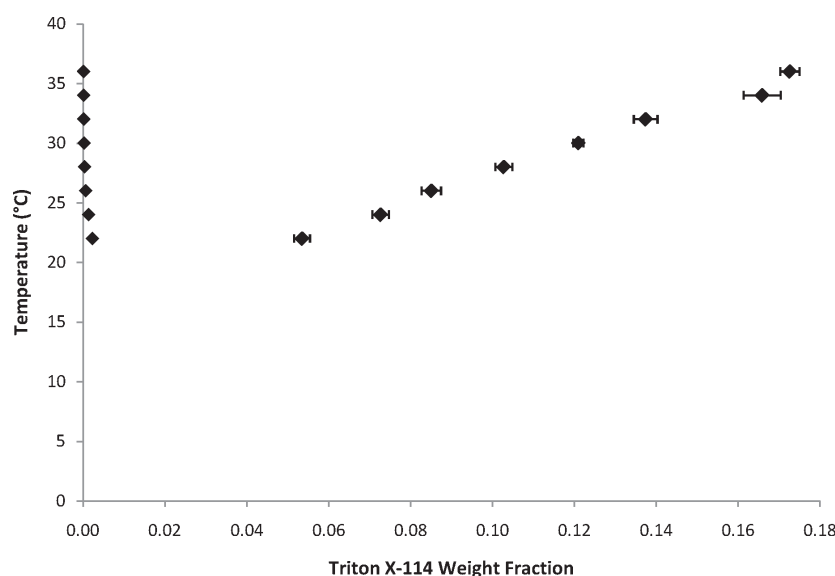


Figure 1. Phase diagram of the Triton X-114 and PBS system. The phase diagram was generated for temperatures ranging from 22°C to 36°C and was utilized to determine operating conditions for the partitioning and concentration experiments. Error bars represent standard deviations from triplicate measurements.

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Temperature (°C)	Initial Concentration (%w/w)	Measured Volume Ratio	Tie Line
29.1	Triton X-114: 6.15%	1.01 ± 0.01	0.131
32.3	Triton X-114: 7.20%	1.00 ± 0.05	0.143
38.6	Triton X-114: 9.89%	1.00 ± 0.05	0.196

Table 1. Operating conditions, volume ratios, and tie-line lengths for the RNA and DNA partitioning experiments. RNA was partitioned at 29.1°C and 38.6°C, while DNA was partitioned at 32.3°C and 38.6°C.

Temperature (°C)	Initial Concentration (%w/w)	Measured Volume Ratio
29.1	Triton X-114: 6.15%	1.01 ± 0.01
27.5	Triton X-114: 8.50%	0.35 ± 0.01

Table 2. Operating conditions and volume ratios for the RNA concentration experiments. These conditions were used for experiments in which RNA was concentrated.

base pairs (Quina and Hinze, 1999). Therefore, the larger ribosomal segments should compose a significant portion of the RNA fragments purified. The 30 S segment, the larger of the two rRNA segments and therefore the segment more likely to be purified, had been previously found to have a hydrodynamic radius of approximately 109 Å around the operating temperatures used in the experiments (Tam et al., 1981). The hydrodynamic radius of the Triton X-114 micelles in the proposed system is also sparingly characterized. However, based on studies of similar systems (Brown et al., 1989), the radius of the Triton X-114 micelles is approximated to be 32 Å. Based on these values obtained from literature and the value of x estimated from the DNA partitioning experiment, Eqs. 4 and 5 were used to predict the partition coefficient of RNA fragments in Triton X-114/PBS micellar systems as a function of the tie-line length, which is the difference between the surfactant volume fractions in the top and bottom phases, namely $(-)$. These theoretical RNA partition coefficients are presented in Figure 3. Furthermore, the experimentally measured partition coefficients of both DNA fragments and RNA fragments obtained in the Triton X-114/PBS two-phase aqueous micellar system are shown in Figure 3. The values of the tie-length corresponding to the partitioning experiments were extrapolated from the phase diagram of the Triton X-114/PBS micellar system (Table 1).

CONCENTRATION FACTORS The measured partition coefficients (Figure 3), the experimental volume ratios (Table 1), and Eq. 7 were used to predict the concentration factor of RNA fragments in Triton X-114/PBS micellar systems. Additionally, experiments were performed by decreasing the volume ratio of the two phases to further concentrate RNA fragments in the top, micelle-poor phase.

The predicted values, along with results obtained in the RNA fragments concentration experiments, are shown in Figure 4. By decreasing the volume ratio from 1.0 to 0.35, the experimental concentration factors were varied from approximately 1.5 to 4 and were in good agreement with the predicted values

DISCUSSION

Concentrating bacterial RNA in patient urine samples prior to detection with the UTI chip may allow for earlier

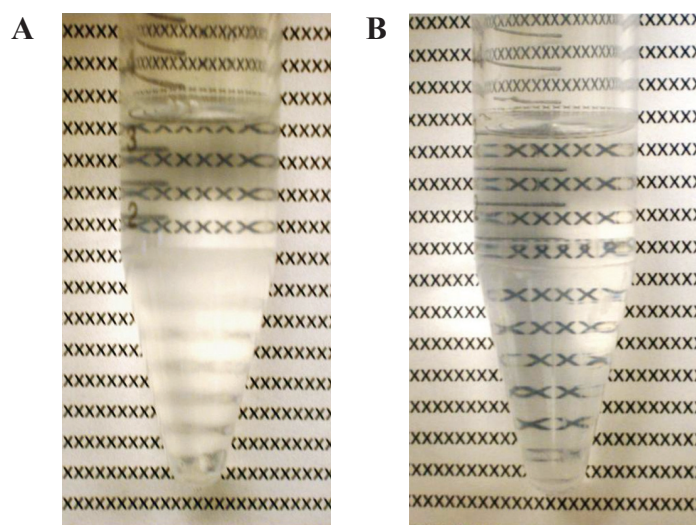


Figure 2. The Triton X-114/PBS system after 1 hour of incubation (A) before centrifugation and (B) after centrifugation. The white piece of paper with the letter “x” in the background was used to help visualize the turbidity of the phases. The turbidity of the micelle-rich phase before centrifugation suggests that entrainment is present in this system which may affect the measured partition coefficient. While the centrifugation step decreased the turbidity, and likely the level of entrainment, it was not expected to completely remove its presence.

diagnosis and therefore additional treatment options. In this study, the ability of Triton X-114/PBS two-phase micellar systems to partition and concentrate bacterial RNA was examined. Additionally, both theory and experiments were used to determine the main driving forces behind RNA partitioning in the Triton X-114/PBS micellar system.

The presence of entrainment was qualitatively demonstrated through photographs taken of the two phases before and after the centrifugation step (Figure 2). The turbidity of the micelle-rich phase suggests that entrainment was indeed present in the Triton X-114/PBS micellar system, since the presence of entrained domains in a macroscopic phase can scatter light and result in the phase appearing turbid and “cloudy.” Additionally, the micelle-rich phase is significantly less turbid in the solution after centrifugation, suggesting that the centrifugation step reduces the entrainment of micelle-poor domains in the macroscopic, micelle-rich phase.

The deviation of the experimentally obtained partition coefficients from those predicted by the excluded-volume theory alone (determined from Eqs. 4 and 5), indicates that the centrifugation step reduced the amount of entrained micelle-poor domains in the macroscopic, micelle-rich phase (Figure 3). However, some level of entrainment was still present. Entrainment in a two-phase aqueous micellar

system is independent of the biomolecule partitioned and is only a function of the volume ratio (Kamei et al., 2002b). Therefore, to determine the extent of the entrainment in the Triton X-114/PBS micellar system, mammalian DNA fragments were partitioned in this system. Using the DNA partition coefficient obtained experimentally at 32.3°C and Eq. 8, the value of x , or the volume fraction of micelle-poor domains entrained in the macroscopic, micelle-rich phase, was estimated to be 0.08 for the Triton X-114/PBS micellar system. Additionally, the partition coefficient for the DNA fragments measured at the 38.6°C operating temperature, which also resulted in a volume ratio of 1.0, was similar to that of the DNA fragments at 32.3°C. This verified that x is a function of the volume ratio, and not the operating temperature.

Equation 4 represents the theoretical RNA partition coefficient based solely on the excluded-volume theory, which considers the repulsive, steric, excluded-volume interactions between RNA fragments and micelles, however it does not account for the effects of entrainment. Equation 5 represents the theoretical partition coefficient when both the excluded-volume theory and entrainment effects are considered. Since RNA partition coefficients obtained experimentally were closer to those predicted theoretically using Eq. 5 rather than Eq. 4 (Figure 3), it is concluded

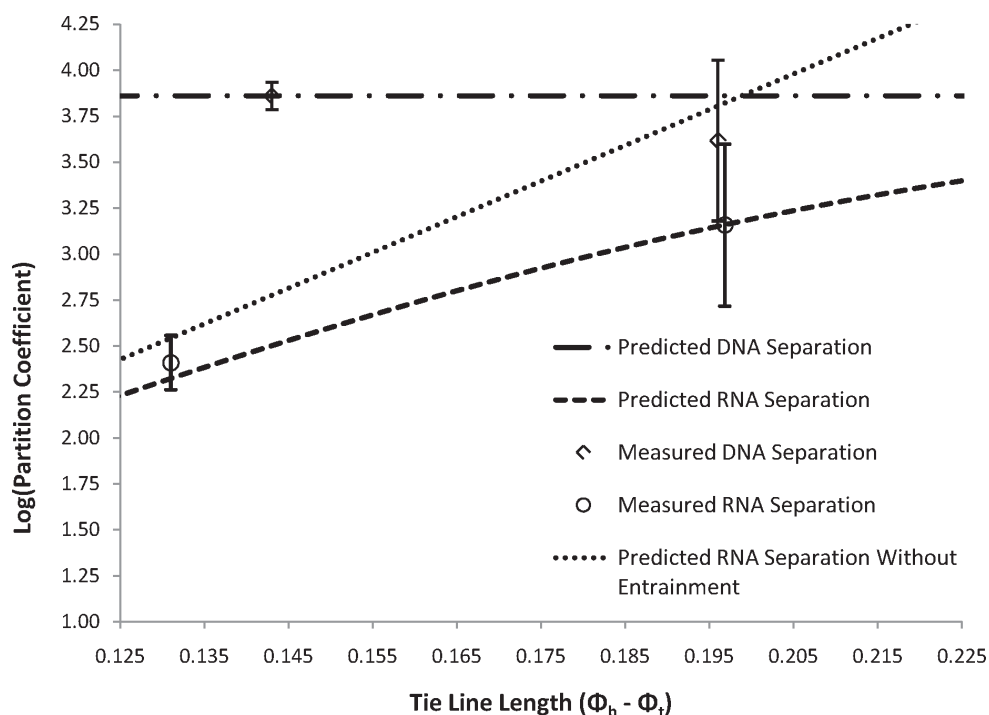


Figure 3. Theoretically predicted and experimentally measured partition coefficients for RNA and DNA at various tie-line measurements. Both DNA and RNA were partitioned at various temperatures in the Triton X-114/PBS micellar system. The measured values for RNA and DNA partitioning experiments showed reasonable agreement with the predicted values based on the excluded-volume theory and entrainment effects. Error bars represent standard deviations from triplicate measurements.

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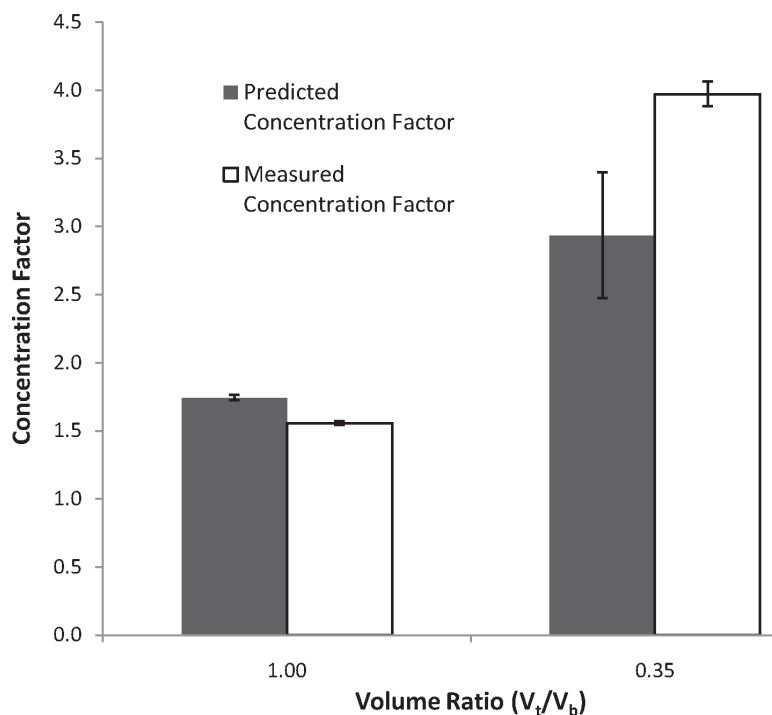


Figure 4. Theoretically predicted and experimentally measured concentration factors for RNA fragments in Triton X-114/PBS micellar systems. RNA was concentrated by decreasing the volume ratio. The experimental values were in good agreement with the predicted values, suggesting that RNA can be concentrated from approximately 1.5- to 4- fold in a predictive manner by decreasing the volume ratio from 1.0 to 0.35. Error bars represent standard deviations from triplicate measurements.

that partitioning of RNA fragments in Triton X-114/PBS micellar system is primarily driven by repulsive, steric, excluded-volume interactions between RNA fragments and micelles, but is limited by entrainment of micelle-poor, RNA-rich domains in the macroscopic micelle-rich, RNA-poor phase. This mechanism is the same as that previously identified for viruses and DNA fragments partitioning in these types of micellar systems.

The effects of entrainment are difficult to remove because of the small density difference and interfacial tension between the micelle-rich and micelle-poor domains (Kamei et al., 2002b). However, because the measured partition coefficients are much larger than 1, RNA fragments can be concentrated by adjusting the volume ratio. We were able to achieve approximately a 4-fold concentration factor by decreasing the volume ratio from 1.0 to 0.35 as shown in Figure 4. Additionally, the predicted concentration factors, determined from Eq. 7, reasonably agreed with the measured experimental values (Figure 4). This suggests that RNA fragments can be concentrated in the Triton X-114/PBS micellar systems in a predictive manner by decreasing the volume ratio. Though the measured concentration factors were the result of a single concentration step, extraction-in-series, a technique in which the top phase from each separation is subsequently phase separated, could be

utilized to further concentrate RNA fragments.

Future work will focus on using these two-phase systems to concentrate bacterial RNA in solutions that mimic bodily fluids, such as a urine like electrolyte and serum. Subsequently, these biomolecules will be concentrated in actual bodily fluids to determine if the other compounds present in these samples effect the separation performance of the two-phase systems. Finally, for this concentration process to have clinical applicability, it will need to be scaled down. Therefore, future work will also focus on miniaturizing these two-phase systems and integrating them upstream of a UTI diagnostic test, such as the UTI chip.

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