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# A Study on Microgel System as a Template for Dispersion and Separation of Chiral Tartaric Acid

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**Abstract**

Separation of tartaric acid enantiomers in organic compounds using conventional techniques requires high energy demand, high chemicals consumption, lengthy, complicated and expensive processes. This research demonstrates utilization of PNIPAM microgel system as a template for incorporation of chiral D- and L- tartaric acids (TA). The poly(n-isopropylacrylamide) (PNIPAM) microgel was synthesized using surfactant free emulsion polymerization (SFEP) and was characterized using standard procedures. The combination of 1.4×10-3 g/ml microgel with 3.02×10-4 g/ml TA gave the most stable system. Subsequently, a two-phase system was obtained after centrifugation. Both phases were subjected to dynamic light scattering (DLS) analysis and the corresponding separated systems gave particle sizes ranging from 80-90 nm for the upper phase, and 1200-1500 nm for the lower phase. Scanning electron micrographs showed the presence of agglomeration in the lower phase, supporting the DLS results. Nuclear magnetic resonance (1H NMR) analysis confirmed the presence of TA indicated by a peak at δ = 13.1 ppm. Based on these findings, the system can be a potential alternative template for TA racemix acid dispersion and separation.

**Keywords:** microgels; tartaric acid; dispersion; aggregation; smart polymers

## 1. INTRODUCTION

Chiral compounds (CC) are an important category of molecules in pharmaceuticals [1]. However these compounds are usually exist in racemix mixtures, in which one of the enantiomer has good biological activity and the other will not have the same ability. Therefore the separation of pure enantiomer into distinguish compounds has been the subject of increasing attention.

Up to now, the techniques employed for chiral separation mainly involved chromatography such as HPLC [2], GC [3], micellar MEKC and capillary gel electrophoresis (CGE) [4]. Although these techniques offer promising approaches, there are still drawbacks such as the separation process which is challenging, resulting hard to predict elution order of enantiomers and poor selectivity for enantiomers. Obviously, these techniques are time consuming and expend solvent. Therefore, it is desirable to analyse alternative, of a more efficient methods.

Of relevance to this work are salt-induced separation [2], surfactant- protein complexes separation [3] and membrane separation [4] which are prepared in microemulsion systems. Although microemulsions offer good supporting systems, however these systems are complicated and difficult to maintain their stability [5]. Attempts were also made using molecularly imprinted polymers (MIPs) where it has been developed and used for the separation of racemates due to their high selectivity [6]. Yet, interactions between MIPs functional monomers and template would lead to aggregation. This instability becomes the limitation factor for the applicability of MIPs. Recently, by controlling microgel properties, simple phase separation has elegantly been demonstrated by Lazimet. al [7,8]. The authors have shown microgel systems could be manipulated as a template for supporting material, phase separation and recovery

By virtue of the polymer chemistry aqueous microgels can possess different charges (anionic or cationic) in one medium which leads to important properties. Interestingly, these responsive polymers can be formulated from single components, or synthesized as polyampholytes. There are no literature found describing microgels with potential to incorporate with CC or used these smart polymers for separation and recovery purposes.

Therefore in this work a cationic microgels poly(N-isopropylacrylamide) (PNIPAM) will be designed to interact with CC (e.g L-tartaric acid, D-tartaric acid, D-alanine and L-alanine). The microgel-CC complexes will then characterized morphologically, chemically and physically. Based on their unique properties, further investigation for separation ability will be carried out using real samples (which contains D and L chiral). This research offers alternative for low cost, efficient and utilize less chemicals.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

All reagents unless otherwise stated, were purchased from Aldrich Ltd.

### 2.2 Analysis Technique

For all samples, pH was measured by using a dip-cell pH meter (HI98127, pHep Hanna).

### 2.3 Microgel Preparation

Microgel was synthesized using the following reagents and quantities [9,10]. In a reaction flask, 800 mL of purified water (PureLab, Elga) was added to 0.51 g of potassium persulfate initiator. Separately, 5.0 g of NIPAM and 0.51 g of cross-linking agent N,N-methylenebisacrylamide (BA) were dissolved in 200 mL of purified water (mili-Q). The dissolved reagents were added to the reaction flask and the polymerization reaction was set at 70 oC under an inert atmosphere. The polymerization reaction was left to proceed for 6 hours with continuous stirring (~150 rpm). The outcome of the dispersion was filtered through glass wool

### 2.4 Dynamic Light Scattering

Hydrodynamic diameters were determined by dynamic light scattering (DLS) using a Malvern Instruments Zeta Nano Series, Nano S90 apparatus, equipped with a 15 mW laser ($\lambda$ = 678 nm). Electrophoretic mobility measurements on the microgel particles were performed by phase analysis light scattering (PALS) using a Malvern Instruments Nano Z mobility apparatus.Sample for analysis contained a microgel concentration with dilution factor of 10.

### 2.6 Transmission Electron Microscopy (TEM)

TEM was used to determine the size and shape of synthesised microgel particles. In order to prepare the TEM samples, each microgel concentration was diluted (10x) with water before a small volume of sample was pipetted onto a carbon-coated copper grid. The sample was then left overnight for drying. In this research all images of transmission electron microscopy were obtained using a Philips CM12 microscope operating at 120 kV with a digital camera. Particle size distributions were generated using Soft Imaging analysis software.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Microgel Stability

Aggregation behavior for each complex sample is possibly influenced by the increasing of ionic strength due the pH adjustments, as described in detail by Snowden et. al. [11]. Based on that approach TA-microgels samples were prepared with varying concentrations, dispersion compositions, and mixed microgel ratios. Hence a large matrix of samples was screened, varying all composition parameters over certain appropriate ranges. Results obtain from the screening test are very important in order to build a model for further applications whereby the L/D acid tartaric will be used.

In this section five most significant samples from that array were discussed (Table 1) in comparison between results obtained from the samples prepared by author (A-E) and previously reported [11, 12] All samples were examined based on their aggregation as a function of pH at different mass concentration microgels used. The physical state of all samples was determined visually at room temperature.

**Table 1:** Stability observations of TA-microgel mixtures at room temperature

| Sample | Microgel used/gml-1 | TA used/gml-1 | Observation at room temperature |
|---|---|---|---|
| A | 1.4 x 10 -3 | 7.55 x 10 -5 | Dispersed / clear |
| B | 1.4 x 10 -3 | 1.51 x 10 -4 | Dispersed / clear |
| C | 1.4 x 10 -3 | 3.02 x 10 -4 | Well dispersed |
| D | 1.4 x 10 -3 | 4.53 x 10 -4 | Aggregated |
| E | 1.4 x 10 -3 | 6.04 x 10 -4 | Aggregated |

Based on the stability observation shown in Table 1, aggregation occurred for last two samples except for sample A, B and C. Although the first two samples showed stable dispersion, however due to a very low concentration, it was very difficult to characterize them respectively. On the other hand at a mixture of (1.4 x 10 -3 : 1.51 x 10 -4 gml-1) sample C was steadily dispersed almost for 6 months. Since this sample showed its homogeneity without any separation, hence sample C mixtures component was chosen for further optimization and applications.

### 3.2 Particle Size

Figure 1 shows the dependence of effective diameter of microgels at different pH as a reference. Due to its insensitivity to pH, the size of the microgel is consistent over the pH range of 3 to 10. This result is significant as reported previously [12]. On contrary, the TA- microgels complexes at pH 3-10 shows opposite trends where their effective diameter rose with pH increment. As a result of adding NaOH for pH adjustment, consequently leads to the TA protonation [13]. Under these conditions the carboxylate groups within the microgel particles are dissociated [12-14]. Therefore the increment of carboxylate (COO-) charged groups raise the hydrophobic interactions which caused the microgel swelling through electrostatic repulsion between these negatively charged [15].

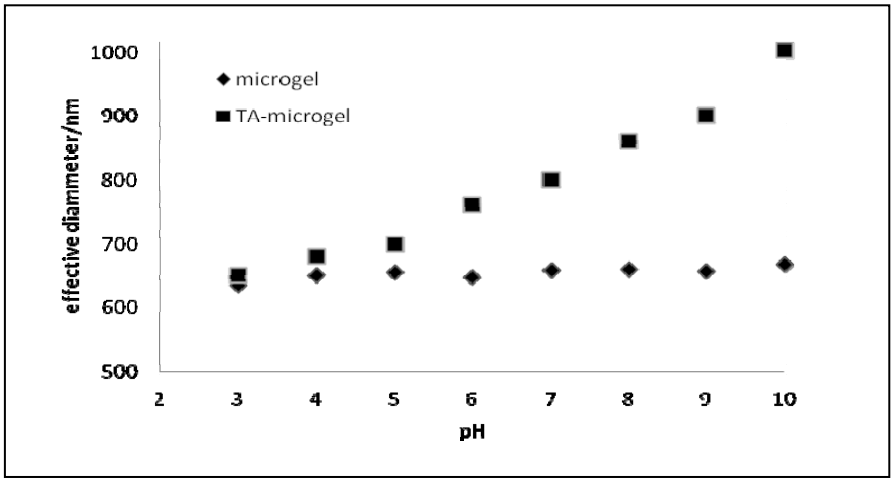


**Figure 1:** Effective diameter of microgel systems and TA-microgels in a function of pH

### 3.3 Separation

A biphasic system was obtained with a lower turbid concentrate separating from a clear upper portion after the centrifugation made to the sample (Figure 2). The same results were obtained at different pH. Therefore for further optimization, samples at pH 3 and pH 10 were selected and characterized by using the DLS in order to determine their particle sizes.

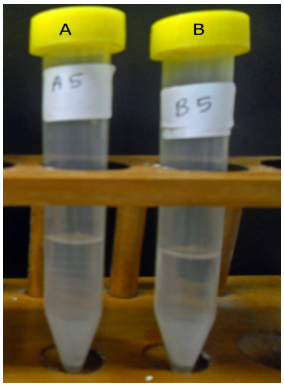


**Figure 2:** Biphasic systems of TA-microgel mixtures where A is (D- tartaric acid) and B is (L-tartaric acid) after the centrifuge

**Table 2:** Particle sizes for upper phase and lower phase for racemic TA-microgels (D and L) at pH 3 and pH 10

| Sample | pH 3 | pH 10 |
|---|---|---|
| Upper phase<br>D-tartaric acid microgel<br>L-tartaric acid microgel | <br>80 nm<br>85 nm | <br>90 nm<br>93 nm |
| Lower phase<br>D-tartaric acid microgel<br>L-tartaric acid microgel | <br>1350 nm<br>1200 nm | <br>1500 nm<br>1420 nm |

The hydrodynamic particle size shown in Table 2 has been divided into two categories: i) upper phase and ii) the lower phase of the biphasic systems. In the range of pH 3-10, it clearly showed an absence of polymer in the upper phase, whereas the apparent TA-microgel complex mostly separated into the lower phase. The increasing of hydrodynamic diameters can be seen in every sample, in range of 1200 nm to 1500 nm. The effect of adding TA into microgel dispersions causes repulsion in microgel surface charge which influencing so greatly the hydrodynamic diameter, since only the surface of microgel is charged due to the polymerization initiator group [16]. Hence TA adsorbs preferentially to the surface of microgel causing some charge will repel each other [17].On the other hand this is also confirms that essentially of the TA-microgel complex has been separated into the lower phases.

### 3.4 SEM Image

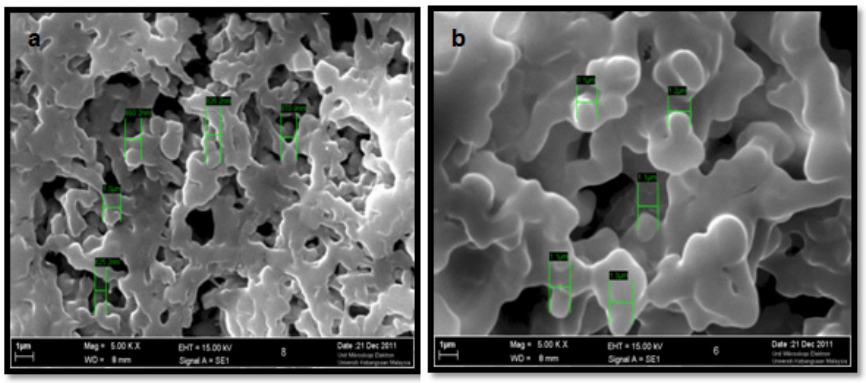


**Figure 3:** SEM image for lower phase of TA-microgel after the centrifuge process where a) D-tartaric acid microgels and b) L- tartaric acid microgels

The high resolution image of the TA-microgel complex shown in Figure 3 verifies the aggregation occurred between the tartaric acid and microgels. The average diameter is 900-100 nm, which is in agreement with DLS data reported in the previous section. The existence of large agglomeration was also observed, possibly an artefact from the interaction of Na+/COO- with the microgel systems [18]. The Na+ as co-ion increased due to the pH adjustments. Therefore their existence is considered as one of the inducing factor for interparticle aggregation whereby the COO- group acts as a 'sticker' in the complexation [19]. Moreover, it becomes more pronounced with increasing of ionic strength. A similar qualitative study was reported by Daly and Saunders [19] the particles were collapsed and flocculated at a higher ionic strength due to absence of electrostatic stabilization.

### 3.5 NMR Spectroscopy Analysis

The preliminary result from NMR spectroscopy confirmed the cooperation between the TA and microgel systems, hence the separation was occurred. 1H NMR of TA-microgel showed signals at δ 13.1 ppm corresponding to the acidic proton of TA and 13.1 ppm corresponding to the NH of microgel PNIPAM. However, due to the present of unreacted colloid/microgel in the mixture, the chemical shift of other protons was difficult to interpret.

## 4. CONCLUSION

These microgel systems have successfully incorporated with both chiral D- and L-tartaric acid. A combination of microgels 1.4 x 10 -3 g/ml and tartaric acid 3.02 x 10-4 g/ml exhibited as the most stable system. Further optimization and characterizations were made, where two phase systems were obtained after the centrifugation. The dynamic light scattering (DLS) showed the lower phase has bigger sizes due to the settlement of microgels colloid. In comparison, the range of particle sizes of the upper phase was very small (80-90 nm) confirmed that most of TA-microgels settled to the bottom phase. These results were supported by the SEM micrographs which clearly shown agglomeration occurred. Based on these promising results, these systems are potentially useful as a template for complex racemix dispersion and separation.

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