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# Preparation of polyacrylamide based microgels with different charges for drug encapsulation

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ABSTRACT

The unique physicochemical properties of hydrogels have attracted considerable attention in their drug storage and delivery applications. Herein, a series of acrylamide based hydrogels with different charges (cationic, neutral, and anionic) under different pH (1, 7, and 13) were prepared via an inverse emulsion polymerization process. The effects of pH and aqueous to organic phase (w/o) ratio on the size of the produced hydrogels were studied. Fluorescein was used as the model drug to assess and optimize the encapsulation efficiency of the hydrogels. Optical microscopy and confocal fluorescence microscopy were carried out to examine the existence and distribution of fluorescein inside the hydrogels. It was found that the hydrogels were swollen to the greatest extent at pH = 13 and their size increased with decreasing w/o ratio. Fluorescein was molecularly dispersed within the cationic and anionic hydrogels at pH = 13 with an encapsulation efficiency of ~45 wt%. This study provides a convenient method to prepare hydrogels with the required properties and demonstrates the potential of using hydrogels to store and stabilize therapeutics.



## 1. Introduction

Hydrogels are three-dimensional polymeric networks with the ability to respond to their environment [1,2]. Their ability to respond to an external stimulus as well as their biocompatibility are attributed to the high content of water, which makes hydrogels unique materials for applications in drug delivery, pharmacology, and cosmetics [3–5]. The network forming polymers in hydrogels can be both ionic and non-ionic. Ionic polyelectrolyte hydrogels offer additional tunable properties arising from the presence of ions/charges in the network. Polyelectrolyte hydrogels carrying positive or negative charges on their backbones show extreme sensitivity toward the ionic strength and/or pH of the medium. These hydrogels are often referred to as stimuli responsive materials (smart materials), and may exhibit significant volume changes in response to small changes in pH, temperature, electric field, solvent, ionic strength of the medium, and light [6–9]. Besides their responsiveness to external stimuli, the resemblance of hydrogels to living tissues is another attractive feature for biological applications such as tissue regeneration, cell growing media, and artificial organs [10–12]. Hydrogels have been widely explored for their applications in the preparation of DNA-based biosensors [13], biological laboratory devices [14,15], separation and purification processes [16], recognition of biomolecules and proteins [17,18], muscle actuation applications [19–21], and controlled drug delivery vehicles [22–31].

As a proof of concept, Liu and co-workers recently described a process to prepare a polyacrylamide hydrogel based biosensor by immobilizing a modified mercury binding DNA [2]. This biosensor can be also used to detect and remove highly toxic mercury from water. Based on the mercury sensing hydrogel described above, they continued to prepare the cationic gels and anionic gels to explore the influence of the properties of the hydrogel on the interaction between DNA and target molecules [13]. We are

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particularly interested in microgels since they are potentially useful for drug delivery applications. The objective of the current research is thereby to extend the functions of the hydrogels based on previous studies [2,13], and incorporate charged monomers. In this work, a series of hydrogel microparticles having different charges were prepared via a convenient inverse emulsion polymerization method and a drug with an encapsulation efficiency of ~45 wt% can be stored in the prepared hydrogels through controlling the charges of the hydrogel and pH value of the solution environment. Different methods to control the size of the hydrogel beads during or after the polymerization were also examined.

## 2. Experimental

### 2.1. Materials

The monomers used for the hydrogel synthesis were acrylamide (99%), 2-acrylamido-2-methylpropane sulfonic acid (AMPS, 99%) (used for preparation of negatively charged hydrogels) and allylamine (99+%) (used for preparation of positively charged hydrogels). N,N′-methylenebisacrylamide (bis-acrylamide, 99+%) was used as the crosslinking agent. Span 80 was used as the surfactant to form the inverse emulsion. Ammonium persulphate (APS, 98%) and N,N,N′,N′-tetramethylethylenediamine (TEMED, 99%) redox system was used to initiate the polymerization. APS was purified by recrystallization from ethanol and dried under vacuum at room temperature before use. Cyclohexane (99%) was used as the organic solvent in the w/o inverse emulsion and 3′,3″,5′,5″-tetrabromophenolsulfonphthalein (bromophenol blue, 95%) was a color indicator used to test the charge of the hydrogels synthesized. Fluorescein (98%) was used as the fluorescent model drug. Phosphate buffer saline (PBS) solutions with pH = 1, 7, and 13 were used to soak the prepared hydrogels (MP Biomedicals Inc. USA). All of the chemicals used were purchased from Aldrich. Distilled water was obtained from the Department of Chemical Engineering, University of Waterloo, Canada.

### 2.2. Synthesis of hydrogels with different types of charge

Three types of hydrogels with different charges characterized by cationic, neutral and anionic hydrogels were prepared according to the formulation recipe presented in Table 1.

Cationic, neutral, and anionic polyacrylamide hydrogel beads were prepared using an inverse emulsion polymerization [32]. The aqueous phase (~9 mL total) contained water, acrylamide, bis-acrylamide, allylamine (only for cationic hydrogels) and AMPS (only for anionic) hydrogels, and APS. The organic phase consisted of 9 mL cyclohexane with dissolution of 900 μL of Span 80 as surfactant to stabilize the emulsion droplets. The aqueous phase was dispersed into the oil phase in a 20 mL glass vial. The solution was stirred at 800 rpm using a 1 in. magnetic stirrer to form an inverse emulsion in an ice bath. Then the solution was purged with nitrogen for 2 min to remove oxygen in the reaction system. Afterwards the solution was stirred for 4 h. While the solution was being stirred (after 30 min) a certain amount of TEMED was then added. When the polymerization starts there should be a measurable increase in temperature, and also a change in color from white to white–orange should be visible. After stirring the solution for 4 h, the solution was allowed to separate into an aqueous and an organic phase. The top cyclohexane layer was then removed. 200 μL of the aqueous phase was then pipetted into a microcentrifuge tube containing 1 mL ethanol. After 1 h of soaking and mixing with ethanol, the microcentrifuge tube was centrifuged at 15,000 rpm for 15 min. The ethanol was removed and the hydrogels were soaked in 1 mL of water for 1 h. The tube was centrifuged again at 15,000 rpm and the water was removed. This washing cycle was repeated five times in order to remove unwanted reagents including unreacted monomers, TEMED, and Span 80. Finally the hydrogels were dispersed in a volume of 1 mL water.

### 2.3. Preparation of hydrogels with different pH

After cationic, neutral, and anionic hydrogels were synthesized (Section 2.2.), these three types of hydrogel were first centrifuged at 15,000 rpm for 10 min and then placed into 1 mL of buffers with different pH = 1, 7, and 13 respectively to soak the hydrogels. Then the hydrogels were vortexed inside the buffer for 5 min so as to replace the water with the buffer solution. Subsequently the microcentrifuge tube was centrifuged again and 1 mL of buffer was then replaced with the same buffer as used previously. After soaking inside the buffer for 2 h, the hydrogels with different pH values were obtained.

### 2.4. Determination of average particle size and distributions

The particle size distributions of the hydrogel particles were determined by counting around 500 particles under an optical microscopy using a Matlab (Mathworks Inc. Natick, MA) image processing system. The average particle size was reported as the number average based diameter.

**Table 1**
Formulation recipe of three types of polyacrylamide hydrogels represented by cationic, neutral and anionic hydrogels.

| Group no. | Charge | Water (mL) | Monomer (mmol) | Bis-acrylamide (mmol) | Allylamine (mmol) | AMPS (mmol) | Span 80 (μL) | Cyclohexane (mL) | APS (mmol) | TEMED (mmol) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Cationic | 9.0 | 16 | 0.8 | 11 | – | 900 | 9.0 | 0.88 | 2.64 |
| 2 | Neutral | 9.0 | 22 | 1.1 | – | – | 900 | 9.0 | 0.088 | 0.264 |
| 3 | Anionic | 9.0 | 16 | 0.8 | – | 3 | 900 | 9.0 | 0.88 | 2.64 |