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Preparation and characterization of glucose-sensitive hydrogel submicron particles using inverse microemulsions

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A novel inverse microemulsion mediated synthesis of glucose-sensitive hydrogel submicron particles was developed for self-regulated insulin delivery system. Pseudo-ternary phase diagram of inverse microemulsions was prepared and the effects of temperature and monomer concentrations on phase behavior were evaluated. Results showed that the reverse microemulsion existence field expands as temperature increases, but phase behavior does not change with the monomer concentration varying. Then, the glucose integration property of hydrogel beads and hydrogel submicron particles was compared in order to prove the glucose response and concentration dependent property. Results showed that the glucose sensitivity of the submicron is higher than hydrogel beads.

Key words: Hydrogel – Submicron – Insulin – Inverse microemulsion.

The feasibility of administering insulin more precisely and effectively through a self-regulating insulin delivery system capable of controlling glucose concentration has gained increasing attention over the past few years. Three types of system, the glucose oxidase-loaded system [1-6], the lectin-loaded system [7-9] and the phenylboronic acid moieties containing system have been developed. These systems all could be sensitive to changes in glucose concentration. But the first two systems use proteins, such as glucose oxidase and Con A, which may cause undesirable immune response once exposed to the body. In order to develop a super glucose-sensitive polymeric system, it is necessary to circumvent these proteins. Thus hydrogel, a totally synthesized polymer, was developed [10-12].

Several groups earlier reported that smaller particles of hydrogel materials possess the following advantages: i) high drug (insulin) loading as a result of being able to dissolve more water soluble compound than other systems; ii) easy modification of surface characters by attaching appropriate ligands due to the presence of many reactive groups on the particle to encapsulate water soluble drugs [13] ; iii) long circulating.

Microemulsions are transparent, liquid isotropic dispersions composed of water, oil and surfactants and are thermodynamically stable. At precise compositions of ingredients their formation is spontaneous and high shear energies are not required for their preparation. Microemulsions have been reported to facilitate polymerization reaction. There are relatively fewer studies that address the preparation of glucose-sensitive hydrogel nano-particles using microemulsions [14]. In this report we introduce the procedure to prepare water-in-oil microemulsions and polymerize monomers using the microemulsions to obtain nano/submicron hydrogel particles. The physicochemical characterization of these hydrogel submicron particles was described and the results of studies on the glucose integration using select hydrogel submicron particles were compared to hydrogel beads.

I. MATERIALS AND METHODS

1. Materials and chemicals

m-Aminophenylboronic acid hemisulphate (APBA), 1-ethyl-3-(3-methylaminopropyl) carbodiimide hydrochloride (EDC HCl), and 2-(dimethylamino) ethyl methacrylate (DMAEMA) were purchased from Acros Organics (Belgium). 1-Vinyl-2-pyrrolidone (NVP) was obtained from Merck Corp. (Germany). Acrylic acid (AAc), N,N'-methylene-bis-(acrylamide) (BisAAm), Span-60, potassium peroxo disulphate (KPS), 2,2'-azobisisobutyronitrile (AIBN), and glucose were purchased from Shanghai Chemicals (PR China). Labrafac CC (medium chain triglycerides EP), Labrafil M 1944 CS (oleoyl macrogolglycerides EP), Labrasol (caprylocaproyl macrogolglycerides EP), Plurol Oleique CC 497 (polyglyceryl oleate FCC) were kindly supplied by gattefossé Corporation (France). All other chemicals were reagent grade and used without further purification.

2. Synthesis of m-acrylamidophenylboronic acid

m-Acrylamidophenylboronic acid was synthesized according to the procedure published previously [15]. Briefly, 0.055 mol of EDC HCl was added to 0.05 mol of m-aminophenylboronic acid and 0.05 mol of acrylic acid in 100 ml of water, adjusted to pH 4.8 with 3 mol/l NaOH and cooled to 4°C. The mixture was stirred for 1 h after the addition was completed. White solid was precipitated, filtered, and recrystallized from H_2O. The ^{1}H-NMR (Bruker AM-400 NMR, Bruker Company, USA) and mass spectrum (VG AutoSpec 3000 mass spectrometer, VG, Manchester, UK) were used to verify the structure of m-acrylamidophenylboronic acid. The reaction scheme is shown in *Figure 1*.

3. Synthesis of hydrogel beads with phenylboronic acid groups

The hydrogel beads with phenylboronic acid were made using a reverse phase suspension polymerization and its procedure was as follows. Toluene (96 ml), 1,1,2,2-tetrachloroethane (28 ml), Span-60

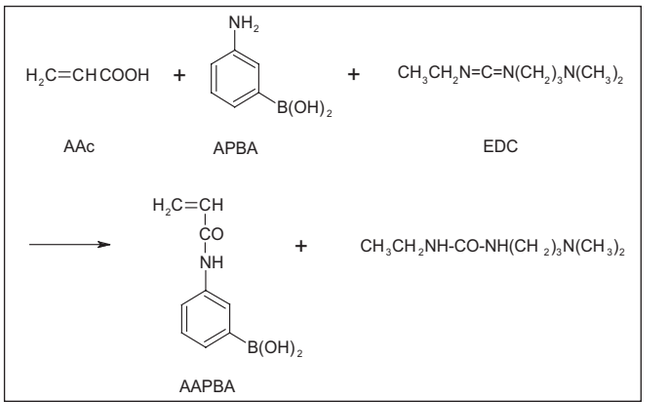

Figure 1 - Scheme route of m-acrylamidophenylboronic acid synthesis.

(0.106 g) were mixed, stirred and bubbled with Ar over 1 h. AAPBA (0.091 g), DMAEMA (0.155 g), NVP (0.117 g), BisAAm (0.077 g), KPS (0.032 g) were dissolved in 20 ml of distilled water. pH of the aqueous solution was adjusted to 8.4 with 1 mol/l HCl to bind the amine to the boron moiety. This aqueous solution was then added to the organic solution with stirring followed by performing the suspension polymerization for 7 h at 50°C. The solvents were removed by rotating evaporation. The residue was washed with ethanol, acetone and purified water in order to remove unreacted monomers and surfactants. After vacuum drying, 0.4 g of hydrogel beads was obtained. The structural formula of polymer beads is shown in *Figure 2*.

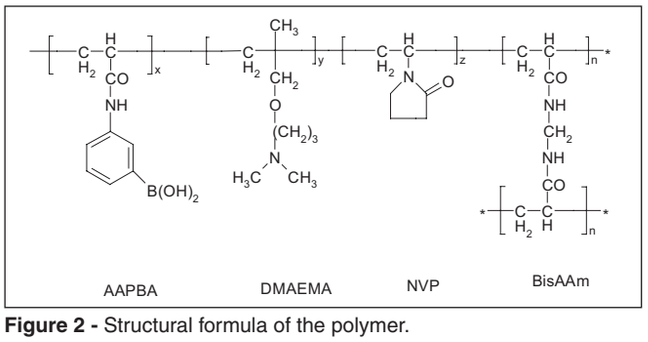

Figure 2 - Structural formula of the polymer.

4. Preparation of pseudo-ternary phase diagram of inverse microemulsions

Phase diagrams were constructed by titration of a series of oil/surfactant mixtures with water at ambient temperature (Labrafac CC/Labrafil M 1944 were used as oil phase, Labrasol/Plurol Oleique CC 497 as surfactant). Typically, the surfactants were mixed at the desired ratio and allowed to equilibrate overnight. A certain amount of the surfactants mixture was placed in a 20-ml vial. The aqueous phase was then added to the surfactant mixture within the vial in small aliquots. Following addition of the aliquot of water, the vial was capped and after vortexing the mixture was visually examined for clarity. Titration was carried out until the mixture became hazy or turbid to establish the region of clear isotropic mixtures along the water-surfactant axis in the pseudo-ternary diagram. At this juncture, small aliquots of oil mixture were added to the surfactant-water mixture to establish isotropic regions along the axis from the surfactant-water baseline towards the oil apex. If the mixtures appeared hazy, small aliquots of the surfactant mixture were added till it became clear. This process was continued to determine the entire domain of clarity from an oil-poor isotropic phase to an oil-rich isotropic one. The above process was also carried out at different temperatures (35 and 50°C) to evaluate the effect of temperature on microemulsion phase behavior. No attempt was made to distinguish between micelles, swollen micelles, oil-in-water microemulsions, water-in-oil microemulsions, bicontinuous microemulsions or liquid crystalline phases.

5. Submicron preparation using microemulsion polymerization

Appropriate amounts of Labrafac CC, Labrasol/Plurol Oleique CC 497 were added to a beaker and mixed with an aqueous solution of monomers (AAPBA, DMAEMA, NVP, BisAAm), and vortexed for 5 min to obtain clear isotropic systems. The ratios of Labrasol to Plurol oleique CC 497 used in the preparation of a variety of microemulsions were almost 1:1 by weight.

To evaluate the effect of dissolved monomer on reverse microemulsion phase behavior, varying concentrations of each monomer were dissolved in the aqueous phase before addition to the surfactant/oil mixture. The aqueous phase consisted of the following:
1. AAPBA (20 or 40 mg/ml) dissolved in distilled water,
2. DMAEMA (40 or 80 mg/ml) dissolved in distilled water,
3. NVP (30 or 60 mg/ml) dissolved in distilled water,
4. BisAAm (20 or 40 mg/ml) dissolved in distilled water,

After formation of microemulsions, the mixture was vortexed continuously and bubbled using argon for 20 min. Polymerization was then performed for 5 h at 50°C following addition of AIBN solution by dropping onto the microemulsions. At pre-set time point, the same volume acetone with the reacting solution was added to the microemulsions and mixed followed by centrifugation of the mixture for 30 min at a speed of 10,000 rpm. The supernatant was discarded and solid residue remaining in the centrifuge tube was mixed with 2 ml of acetone again followed by centrifugation. This step was repeated to remove residual oil, surfactants and unreacted monomers. Finally, the fine white powders were collected and dried in a vacuum dessicator at ambient temperature. All dried powders were stored in a refrigerator at 4°C in tightly capped centrifuge tubes until used in the experiments. Structural formula of the polymer is shown in *Figure 2*.

6. Determination of size and size distribution

Size and size distribution of blank microemulsions and particle-containing microemulsions were analyzed by photon correlation spectroscopy (PCS) using a Zetasizer 3000HS (Malvern Instruments, UK). The samples of hydrogel submicron particles (2 mg) were suspended in 2 ml alcohol [16] and sonicated using a probe sonicator (Model JY-92, Xinzhi Inc., PR China) at 50 W for 2 min to minimize aggregation before the measurement of particle size that was conducted using the same instrument (Zetasizer 3000HS).

7. Microscopic observations

A BVPM-8 Braford Variable Projection Microscopy Systems (American Biologics) was used for observing the size and shape of the submicron product. For the microscopic study, the hydrogel submicrons were mounted on a sample holder, the sample was observed through the projection. Micrographs were taken after the observations.

8. Evaluation of glucose integration

Hydrogel submicron particles (20 mg) were added to 1 ml of 10 mmol/l phosphate buffered (pH 7.4) containing two different amounts of glucose. The test concentrations of glucose in the phosphate buffer were 100 and 400 mg/dl, respectively. The hydrogel submicron particles in such glucose buffer solutions were swollen and incubated for 12 h at room temperature. Aliquots were centrifuged for 30 min to separate the particles from the glucose solution, the amount of glucose solution was assayed using the glucose kit and the amount of glucose integration was calculated. Twenty milligrams of hydrogel beads were used as a reference for comparison study.

II. RESULTS AND DISCUSSION

1. Characterization of monomer compound

The monomer compound obtained was a white solid. The results of mass spectrum were consistent with proposed structure shown in *Figure 1*.

EI-MS m/z (%): 191$[M]^+$.

^{1}H-NMR (CD_3COCD_3) spectrum: 7.31 (1H, dd), 7.58 (1H, d), 7.86 (1H, d), 8.06 (1H, s), belong to phenyl, 5.71 (1H, dd, J = 10.1, 2.01 HZ), 6.35 (1H, dd, J = 16.1, 2.1 HZ), 6.51 (1H, dd, J = 16.1, 2.0H Z), belong to CH_2=CH.

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2. Microemulsion phase behavior

The pseudo-ternary phase diagrams of the systems investigated are shown in *Figure 3*. At a fixed ratio of surfactants, the region along the surfactant/water axis is significantly affected as the temperature is increased from 25 to 50°C. Thus at 25°C it was possible to obtain a mixture containing 42% water and 58% surfactant mix that is optically clear and isotropic. The amount of water that could be included