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# Fabrication of Hollow poly acrylic acid Nano gels via Emulsion polymerization as a Model for loading of Drugs

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**ABSTRACT:** Nanogels are nano-sized hydrogel networks formed by chemically or physically crosslinked polymer particles. Their colloidal stability affords them as good candidates for drug delivery systems. Like nanoparticles, nanogels are injectable and responsive to environmental factors, such pH, and temperature. This work presents a facile and large-scale fabrication of poly(acrylic acid) (PAA) hollow nanogels via in situ Pickering miniemulsion polymerization method. Cross-linked polyacrylonitrile (PAN) nanoparticles with hollow structure were prepared by using hydrophobic solvent as liquid core. The complete hydrolysis reaction process of PAN shell leads to the successful formation of hollow PAA nanogels. The properties of PAA nanogel were characterized by FT-IR, Scanning electron microscope and Dynamic light scattering. It is found that the nanogels have a hollow core-porous shell structure. Protein, bovine serum albumin (BSA) was used as model drugs to investigate their loading abilities as versatile drug-delivery vehicles. The nanogel exhibits high loading ability to protein. The maximum BSA loading capacity of PAA nanogel can reach at pH=5. This high loading capacity may be related to the hollow core-porous shell structure of PAA nanogels. Considering the high stability of the materials, simple and mild preparation procedure, high loading capacity, and ability to protect biological agents from denaturation, PAA nanogels should be promising drug-delivery carriers for drug-delivery systems.

**Keywords:** *BSA, Drug delivery, Nanogel, Pickeing miniemulsion.*

## INTRODUCTION

Today, studies on the controlled release of drugs and other bioactive agents from drug-delivery systems have attracted considerable attention from researchers worldwide. In these methods, chemical or biological active agents are delivered to the intended target at the appropriate speed and for the desired duration. The main goal of these methods is the maintenance of the desired speed of release and drug concentration level in the body [1,2]. Polymer carriers are of special importance among numerous compounds for obtaining systems in which drug is released at variable speeds. In fact, polymers are the most important materials for preparing novel drug-delivery systems due to their unique properties, including substance permeability control, mixing with other substances, easy manufacture, and biocompatibility [3-5]. Of various types of polymers used for this purpose, hydrogels have attracted consid-

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erable attention for having a membrane with swelling property. Hydrogels are completely permeable to hydrophilic active agents with a high molecular mass. This property in addition to the high biocompatibility of hydrogels has motivated studies on these polymers for use in the slow release of proteins such as insulin, aprotinin, tumor antigens, and luteinizing hormone [6-8]. Small water-soluble drugs often pass hydrogels quickly and in desired amounts. Furthermore, hydrogel membranes have numerous applications as carriers of water-insoluble drugs such as steroids. These hydrogels are utilized for the release of hydrophilic as well as hydrophobic drugs which can both be placed inside hydrogels. For instance, the temperature/pH-sensitive oligo(β-amino ester urethane) hydrogel is used for controlling the release of the hydrophilic drug doxorubicin, photo-crosslinked chitosan is utilized for loading the hydrophobic drug paclitaxel, and the hydrogel based on glycol chitosan and poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) is employed for simultaneously releasing paclitaxel and doxorubicin [6, 9, 10]. Among the various synthetic methods of micro and nanogels, in situ Pickering mini emulsion polymerization is one of the promising approach with different applications in drug delivery, optical and mechanical properties, self-cleaning, biological engineering and so on. Pickering emulsion is one of the common synthesis methods in polymerization by using solid particles as a stabilizer to fabricate polymer beads. Due to the therapeutic potential and cosmetic applications of the polymeric compounds, the use of Pickering emulsion is now emerging and scientists are studying and reinventing Pickering emulsion concept [11].

In this study by using the method of in situ Pickering miniemulsion polymerization, PAA hollow nanogels are synthesized. With regards to extensive studies of PAA abilities in drug loading and drug delivery, the ability of produced nanogels in drug loading are investigated. The BSA protein is used as drug model. The Bradford assay is applied to determine the ability of nanogels in drug loading. The PAN and PAA nanogels were characterized by scan electron microscopy (SEM) and Infrared spectroscopy. UV-Vis spectrophotometer was applied in Bradford method.

## EXPERIMENTAL

### *Materials and methods*

Hexadecane (HD, Fluka), sodium nitrite (Aldrich), 2,2,4-trimethylpentane (TMP, Aldrich), γ-(trimethoxysilyl) propyl methacrylate (MPS, Aldrich), tetraethoxysilane (TEOS, Aldrich), and trimethylamine (TMA, Aldrich) were used without further purification. Acrylonitrile (AN) and divinylbenzene (DVB) were purified by passing through a short basic $Al_2O_3$ column before use. 2,2`-azobis(isobutyronitrile) (AIBN, Fluka) was purified by recrystallization in absolute ethanol and kept refrigerated until use. A Bradford protein assay reagent, Coomassie blue, phosphoric acid and methanol was purchased from Sigma. Surface morphology and distribution of nanoparticles were investigated via a SEM instrument (LEO1430VP). Fourier transforms IR spectrometer in KBr pellets over the range 400-4000 $cm^{-1}$. Bradford assay was investigated by UV-Vis spectrophotometer to monitor the amount of drug adsorbed. The mean diameter and size distribution of the PAA nanogels were measured by dynamic light scattering (DLS) using a Brookhaven BI9000AT system (Brookhaven Instruments Corp., Holtsville, NY). The laser wavelength of 658.0 nm and an incident angle of 90° at 25 °C were used to all DLS measurements. All analyses were triplicated, and the result was the average of six runs.

### *Preparation of PAA hollow nanogels*

Synthesis of PAA nanohalogels with Pickering miniemulsion method was reported previously. Briefly, the monomer mixtures of AN (0.2 g), TEOS (10 g), and TMP (0.095 g) were added HD (0.8 g) and ABVN (0.15 g) to form an oil phase. $NaNO_2$ (0.15 g) in water (100 ml) was added to the oil phase. After stirring the mixture solution to make a pre-emulsified for 5 min, TMA in water (1ml in 70 ml water) the mixture was stirred for 30 min. For preparation mini emulsion, the solution was homogenized by hemogenizer at 19000 rpm for 5 min in an ice bath. For completing the polymerization, the solution homogenized at 1100 rpm in an oil bath at 55°C for 21 h. After centrifuge, silica/PAN microspheres were obtained. To obtain nanogel of PAA, silica/PAN (0.5 g) was added to 30 ml NaOH

solution (5 M) and stirred for 5h at 80°C. After centrifuge, PAA nanogel was dried under freeze drying.

***Preparation of BSA-loaded PAA naanogel***

The Investigation of BSA loading in PAA nanogels was done with incubation method. PAA nanogel loading capacity was investigated in 1mg/ml BSA solution at pH 4, 5 and 6. For drug loading, the suspension solution of PAA nanogel (1mg) in 25 ml of BSA (1 mg/ml) at different pH was prepared and stirred over the night at room temperature.

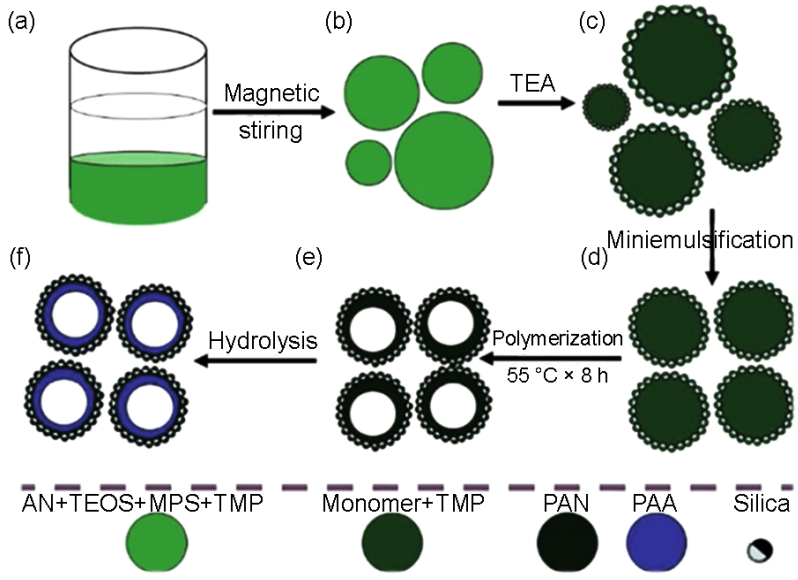
***Evaluation of drug entrapment***

The PAA nanogels were separated from drug solution after 24 h through centrifugation (15000 rpm for 1 h). The amount of free BSA in solution was measured by Bradford assay. In this method, binding of protein to coomassie dye in acidic conditions causes the change in color from brown to blue. For BSA/dye complex the absorbance band appears in 595 nm. By using UV-Vis spectrophotometer, and calibration curve, BSA loading by PAA nanogel was determined. For drawing calibration curves, 5 standard solutions of BSA/dye solutions with different concentrations in different pH were prepared. The linear standard curve of absorbance versus micrograms protein was prepared. The BSA concentration of solution was determined by comparison with standard curves.

## RESULTS AND DISCUSION

In this study poly(acrylic acid) (PAA) hollow nanogels were synthesized via in situ Pickering miniemulsion polymerization by modifying previous procedure [11]. Silica nanoparticles as emulsifier were applied for the stabilization of Pickering miniemulsions. By taking advantages of phase separation between hydrophobic trimethylpentane (TMP) and the growing polymers, TMP phase was compressed and restricted as a liquid core. Hollow polyacrylonitrile (PAN) nanoparticles of below 100 nm were obtained successfully after monomers polymerized. Furthermore PAA hollow nanogels were fabricated after the hydrolysis of PAN shell under base conditions. Scheme 1 shows the fabrication of silica/PAA hollow nanogels in situ by Pickering miniemulsion polymerization. The mixture of acrylonitrile (AN), divinylbenzene (DVB), γ–(trimethoxysilyl) propyl methacrylate (MPS), TMP, and hexadecane (HD) were used as an oil phase and sodium nitrite ($NaNO_2$) in aqueous solution was used as water phase (Scheme 1a).

The coarse emulsion was obtained by mechanical stirring of the solution and dispersing oil phase in liquid phase (Scheme 1b). After the added triethylamine (TEA) diffuses to the oil/water interface of coarse emulsion, nanosilica particles were in situ formed through the hydrolysis condensation of tetraethoxysi-




**Scheme 1.** The fabrication of silica/PAA hollow nanogels in situ by Pickering miniemulsion polymerization [11].

lane (TEOS) under basic conditions. Then the nanosilica particles were partially modified by MPS showing amphiphilic properties, and located on the surfaces of monomers droplets (Scheme 1c). Miniemulsion droplets as nanoreactors were obtained subsequently via miniemulsification process using a Fluko FM200 homogenizer. The miniemulsion nanodroplets were stabilized by amphiphilic nanosilica particles (Scheme 1d). 7,8 Due to a small amount of $NaNO_2$ solution was added to the miniemulsion, the formation of PAN nanoparticle occured primarily via monomer droplet nucleation. When organic monomers polymerized, the great immiscibility between TMP and the growing polymer resulted in phase separation. TMP phase was compressed and accumulated to form a liquid core within the initially formed PAN particles (Scheme 1e). After the complete polymerization of monomers, silica/PAN hollow nanoparticles were obtained (Scheme 1e). When NaOH solution was added, large amounts of hydroxyl ions penetrated into the interior of silica/PAN hollow nanoparticles. Silica/PAA hollow nanogels were fabricated successfully after the hydrolysis reactions of PAN polymer (Scheme 1f). And a high yield synthesis of PAA nanoparticles was achieved. After the hydrolysis reactions of PAN nanoparticles, the hydrophobic-CN groups were converted to highly hydrophilic-COOH groups.

### *Characterization*

Fig. 1 shows the comparison between IR spectra of

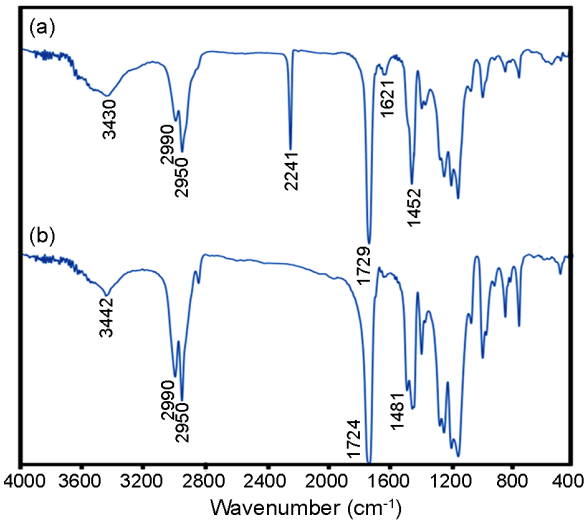



**Fig. 1.** IR spectra of a) polyacrylonitril (PAN), b) polyacrylic acid.

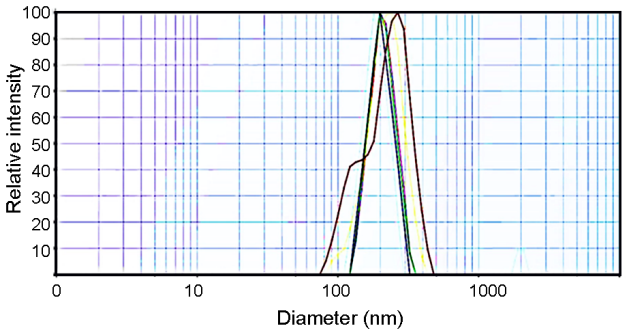



**Fig. 2.** Size distribution of PAA nanogels.

PAA nanogel and PAN. The presence of silica groups in nanogels are proved by observation of asymmetric stretching broad band of Si-O-Si in 1000-1200 $cm^{-1}$. The bands in 1729 and 1170 $cm^{-1}$ were assigned to C=O and C-O in the co-monomers at the body of acrylonitrile polymers. The band was appeared in 2241 $cm^{-1}$ assigned to nitril stretching vibration in the body of acrylonitrile polymer. The bands in 2950-2990 $cm^{-1}$ can be assigned to aliphatic groups ($CH_2$, CH3).

After completing conversion of PAN to PAA by adding NaOH to the solution, the stretching band of -CN in 2240-2260 $cm^{-1}$ disappears and a new band as-

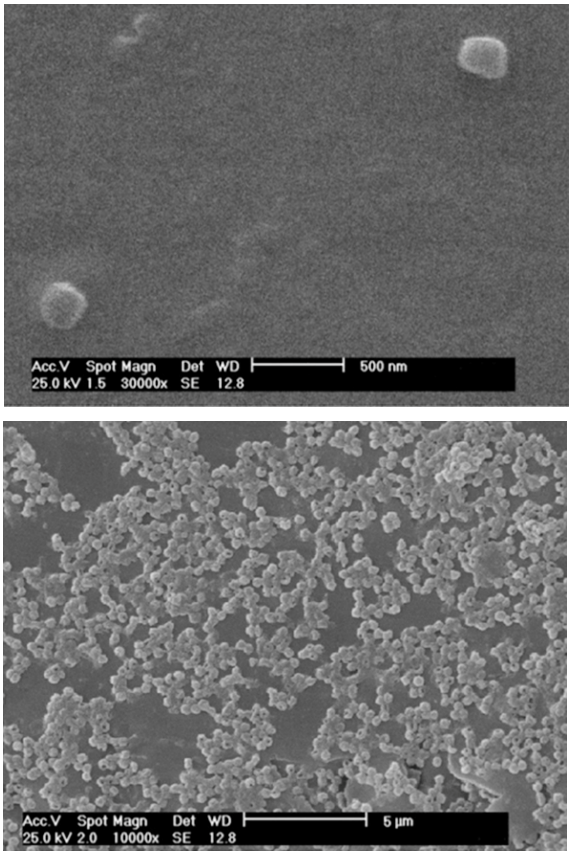



**Fig. 3.** The SEM image of PAA nanoparticles.

signed to stretching band of -COOH appears in 1724 $cm^{-1}$. The disappearance of -CN band and appearance of –COOH band approve PAN nanoparticles hydrolyzed under base condition and PAA nanogel is formed. For investigation of size and distribution of PAA nanogel, DLS measurement was done. The results are the average of 6 runs. With regards to the distribution curves the repeatability of nanogel is high. The mean diameter of a PAA nanogel was about 207 nm, which is an acceptable size for nanogels. As we can see in Fig. 2 a narrow distribution in size of angel is observed.

Fig. 3 shows the SEM image of PAA nanoparticles. The morphology and structure of PAA nanogels are spherical with an average size of 200 nm, and their sizes were almost uniformly. It is found that the nanogels have a hollow core-porous shell structure. With regards to DSL, SEM and IR data, mini emulsion polymerization method is a suitable method for synthesis of PAA nanogels to apply for drug delivery.

### *Drug Loading of the PAA Nanogels*

With regards to SEM data, the halo nanogel size is 200 nm. It has a hollow core-porous shell structure. With this condition, PAA nanogel can be a good candidate to apply as drug carriers. For investigating of this ability, BSA molecule with prolate ellipsoid shape and major axes of 13.8 nm and minor axes of 4.6 nm is a good candidate to use as a drug model [12]. Incubation method was applied for BSA loading in PAA nanogels at room temperature. PAA nanogel loading capacity was investigated in 1mg/ml BSA solution at pH 4, 5 and 6. The Bradford assay was used to determine the protein concentration in solution. With this assay we can find the amount of protein loading ability of nanogel. For this reason, the standard curves with 6 standard concentrations of BSA/dye solutions in different pH were drown. The linear standard curve of absorbance versus micrograms protein was prepared (Fig. 4). With applying these curves, BSA concentration after drug loading was measured.

With regards to the previous reports, the maximum BSA adsorption takes place near the iso electric point (IEP) of BSA. The IEP of BSA is 4.9. The BSA protein has no charge in pH=5 and positive charge in pH=4 and negative charge in pH=6. Because of minimum

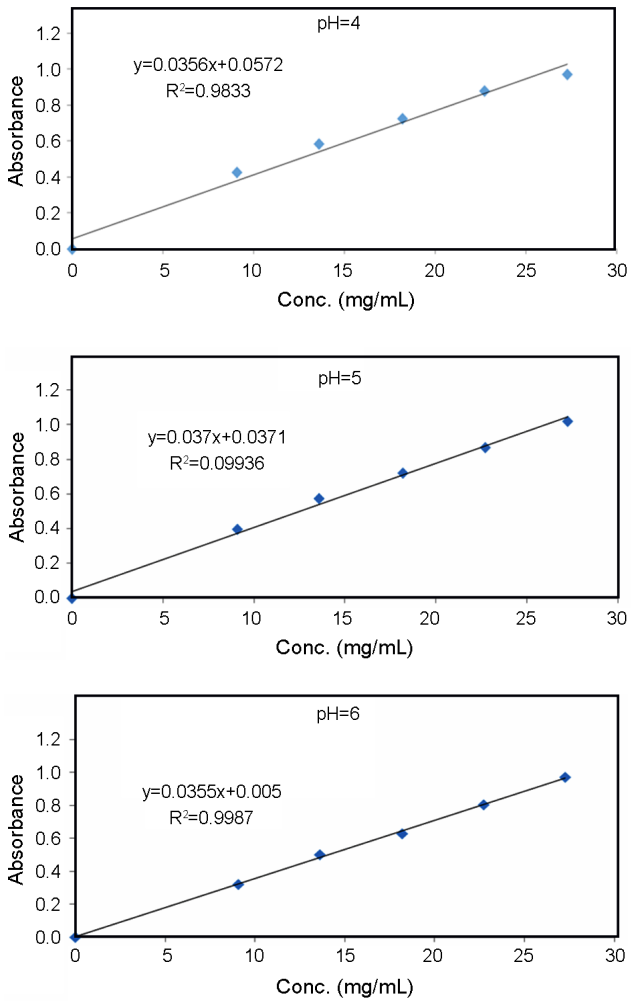



**Fig. 4.** The standard curves in 3 different pH.

solubility of BSA near the IEP in pH=5, we expected to have maximum loading of BSA on the PAA nanogels. Table. 1 shows the comparison between BSA loading in this three pH. The BSA loading is 0.136 mg in pH 5. In pH=4 and 6, BSA adsorption reduce in compare with pH=5. In pH=4, electrostatic attraction and hydrophobic interaction are important parameters and influence amount of drug loading. The electrostatic interaction between BSA and nanogel is the important reason to decrease the amount of drug loading in

**Table 1.** PAA nanogel loading capacity in 1mg/ml BSA solution at pH 4, 5 and 6.

| pH | Abc | Conc(mg/l) Free BSA | Conc(mg/l) Loaded BSA |
|---|---|---|---|
| 4 | 0.0602 | 0.086 | 0.114 |
| 5 | 0.0394 | 0.064 | 0.136 |
| 6 | 0.0053 | 0.138 | 0.062 |

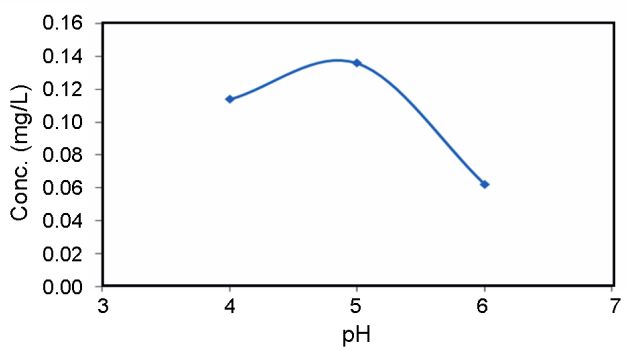



**Fig. 5.** The changes in BSA adsorption in different pH.

pH=6 even this amount is less than amount of loading in pH=4. The changes in BSA adsorption are depicted in Fig. 5 in different pH.

## CONCLUSIONS

Stability of nanohallow gels is good reason to use them as drug carrier. PAA was synthesized by Pickering mini emulsion methods and charectrized with. Surface morphology and distribution of nanoparticles were investigated via a SEM, DLS and Fourier transform IR spectrometer. The morphology and structure of PAA nanogels are spherical with an average size of 200 nm, and their sizes were almost uniformly and the nanogels have a hollow core-porous shell structure. The IR results show that the disappearance of –CN band and appearance of –COOH band approve PAN nanoparticles hydrolyzed under base condition and PAA nanogel is formed. The DLS results show that the mean diameter of a PAA nanogel was about 207 nm, which is an acceptable size for nanogels. With regards to DSL, SEM and IR data, mini emulsion polymerization method is a suitable method for synthesis of PAA nanogels to apply for drug delivery. Bradford assay was investigated by UV-Vis spectrophotometer to monitor the amount of drug adsorbed. BSA was applied as a drug model. Bradford method can help to determined the amount of protein loading. Maximum BSA loading capacity of PAA nanogels is 130% . As a best result, 1mg of nanogels can load 1.3 mg BSA in pH 5. This high loading capacity may be related to the hollow core-porous shell structure of PAA nanogels.

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