



## Synthesis of Nanoparticles Based on pH-sensitive Alginate-g-polyacrylonitrile Copolymer and Its Application in Drug Loading



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**D**IFFERENT samples of sodium alginate grafted by polyacrylonitrile copolymer were synthesized via the grafting process through a free radical polymerization mechanism. Graft structures were characterized and confirmed using Fourier Transform Infrared (FT-IR) spectroscopy and X-Ray diffraction (XRD). Thermogravimetric analysis (TGA) showed that the graft copolymers acquired higher thermal stability compared to native alginate. Moreover, its solubility in several solvents was examined as compared to that of native alginate. The main outcome of this paper was that a grafted copolymer with hydrophobic enrichment (Wt% = 150 %) of polyacrylonitrile as a core was sufficient to form stabilize colloidal system within nanoparticles have Z-average diameter equal 55 nm and sodium alginate as a shell. The SA-g-PAN graft copolymer showed a higher degree of swellability in the basic medium than in acidic medium. The consequence of that was the SA-g-PAN graft copolymer exhibited pH-responsive properties. Moreover, the formed nanoparticles were loaded by a hydrophobic model and proofed by a transmission electron microscope (TEM).

**Keywords:** Sodium alginate, Polyacrylonitrile, Graft, Nanoprecipitation, pH-sensitive, Encapsulation.

### Introduction

Natural polysaccharides and their derivatives constitute a group of polymers broadly used in biomedical and pharmaceutical fields for the controlled delivery of drugs. Conventional drug delivery systems targeting specific sites are restricted by the non-targeting properties of drug carriers, breakdown of active drugs during circulation, solubility, and stability of drugs and the nature of drug carriers [1-3]. The superiority of controlled drug delivery systems is due to their enhancement to drug activity as they protect them against the environment and minimize the drug side effects [4,5]. Natural polysaccharides have many advantages as they are available, less expensive, nontoxic and biodegradable [4,6,7]. Sodium alginate (SA) is one of the most efficient biopolymers used in pharmaceutical and medical industries due to its biocompatibility and biodegradability [8,9].

Sodium alginate (SA) is mainly extracted from the cell walls of brown algae. It is a linear copolymer with homo-polymeric blocks of (1-4)-linked-D-mannuronate and -L-guluronate, covalently bonded together [10]. Nanoparticles formation from SA could offer a synergistic therapeutic effect [11-13]. Graft copolymerization with vinyl monomers using radical initiators is the most suitable method for adding new properties to SA for broadening its medical use. Acrylonitrile (AN) is one of the most frequently used vinyl monomers due to its high grafting efficiency [14,15]. Recently, poly(acrylonitrile) (PAN) has also been considered as a good drug carrier [16].

Several techniques were used to encapsulate drugs within SA-g-PAN such as ethanol injection, emulsion solvent evaporation, emulsion solvent diffusion and ionic gelation. But nanoprecipitation-solvent displacement or interfacial deposition of a polymer following the

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displacement of a semi-polar solvent miscible with water from a lipophilic solution [17-19] seems to be the simplest technique and the most reproducible in nanoparticles preparation. In this study, SA-g-PAN copolymers have been prepared and characterized using FTIR, XRD and TGA. Also, SA-g-PAN nanoparticles were prepared using nanoprecipitation technique, and then they were characterized by TEM and applied in drug loading using *p*-phenylenediamine, as a model drug.

## Experimental

### Materials:

Sodium alginate (SA) was supplied from Nice Chemicals Pvt. Ltd., Kerala, India. Acrylonitrile (AN) was purchased from Merck (Schuchardt OHG, Hohenbrunn, Germany); it was purified by simple distillation before use. Potassium persulfate (KPS) was supplied from Hopkin & Williams, Ltd., London. *p*-Phenylenediamine was purchased from Sigma-Aldrich. Solvents were of analytical grades and used without further purification.

### Synthesis of grafted copolymer (SA-g-PAN)

The grafting process was performed as follow; 0.5 g of SA with predetermined amount of KPS was dissolved in 25 ml distilled water under N<sub>2</sub> gas flow for 15 min in definite temperature. Then, definite amount of AN was added dropwisely and the grafting process were continued for the required time and temperature. PAN (homopolymer) was removed by soxhlet extraction using dimethylformamide (DMF) till constant weight of dried samples.

The grafting parameters were calculated according to the following equations[20,21];

$$\text{Graft yield (\%G)} = ((W_1 - W_0) / W_0) \times 100 \quad (1)$$

$$\text{Homopolymer (\%H)} = ((W_2 - W_1) / W_3) \times 100 \quad (2)$$

$$\text{Grafting efficiency (\%GE)} = ((W_1 - W_0) / (W_2 - W_0)) \times 100 \quad (3)$$

Where;

$W_0$ ,  $W_1$  = the weights of the initial alginate and grafted copolymer, respectively.

$W_2$  = Total weight of grafted copolymer and that of the homopolymer before soxhlet extraction.

$W_3$  = weight of acrylonitrile.

### Nano-precipitation technique for nanoparticles formation

50 mg of the prepared grafted copolymer was dissolved in a solvent mixture of THF:H<sub>2</sub>O (95:5) v/v. Then, using a micropipette, 10 ml of distilled water was added with a rate of 0.1 ml.min<sup>-1</sup>. After complete addition, certain amount of distilled water was added one time to freeze the nanoparticles dispersion. Finally, the solution is centrifuged at 10,000 rpm at 20°C for 30 min to remove THF[22].

### Encapsulation of *p*-phenylenediamine:

Encapsulation of *p*-phenylenediamine took place during the formation of nanoparticles. *p*-phenylenediamine was chosen because it is colored so efficiency of loading onto nanoparticles can be easily measured (In section 2.4.1. Loading efficiency onto nanoparticles). Firstly, 1 mg of *p*-phenylenediamine was dissolved in 1 ml of THF. Then, 0.1 ml of this solution was added before the nanoprecipitation step. The successful encapsulation was verified by TEM.

### Loading efficiency onto nanoparticles

Firstly, colorimetric absorbance of 1 mg ml<sup>-1</sup> solution of PPD in THF was measured and found to be equal to 0.218.

Second, this amount was added to the solution of copolymer in THF and nanoparticles were collected by centrifugation and the residual was obtained by evaporation of water and the solid was dissolved in THF. The absorbance of PPD in THF was equal to 0.01

From the two absorbance values, the loading efficiency was calculated to be equal to 92 %.

### Instruments used in the study:

FT-IR spectra were recorded on Testcan Shimadzu Infra-Red-Spectrophotometer (model 8000), the range of wave number from 400-4000 cm<sup>-1</sup> at 25°C. The thermal behavior of the grafts was analyzed using Thermogravimetric analysis, TGA-50H Shimadzu analyzer. The temperature range was 0-700°C with heating rate 10°C.min<sup>-1</sup> under N<sub>2</sub> atmosphere. XRD patterns were recorded using equipment model (X'Pert PRO PANalytical, Netherland), which operated at 45 kV and 30 mA using filtered Cu K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) in

the  $2\theta$  range from  $10^\circ$  to  $50^\circ$  and high score plus software. TEM images were taken by transmission electron microscope (JEOL, TEM 1400, Japan) at acceleration voltage of 80-100 kV.

## **Results and Discussion**

### *Effect of various reaction parameters onto grafting process*

In this section, various copolymerization parameters affecting the grafting process have been studied as follows;

#### *Effect of KPS concentration*

The effect of KPS concentration onto the grafting of AN onto SA was studied keeping the other reaction conditions constant (Fig.1). The KPS concentration was changed from 0.02 to 0.10 mol.L<sup>-1</sup> while all the other parameters were kept constant being AN concentration 4 mol.L<sup>-1</sup>, reaction temperature 60° C and the copolymerization reaction was conducted for 2 h. The obtained data revealed that both graft yield (G%) and graft efficiency (GE%) increased with increasing the initiator concentration reaching their maximum values (224% and 92%, respectively) at 0.08 mol.L<sup>-1</sup> of KPS. At this concentration of KPS, the homopolymer percent (H%) acquired the lowest value (12%). A further increase in KPS concentration led to a decrease in both G% and GE%. This may be due to the competition between initiation and termination reactions through chain transfer to initiator.

#### *Effect of AN concentration*

The effect of monomer concentration onto the grafting process was studied keeping other reaction conditions constant (Fig.2). The AN concentration was changed from 1 to 5 mol L<sup>-1</sup> while the KPS concentration was kept constant at 0.08 mol.L<sup>-1</sup>, the temperature at 60°C and the reaction time for 2 h. The results indicated that both graft yield (G%) and graft efficiency (GE%) increased with increasing the AN concentration reaching their maximum values (224% and 95%, respectively) at 4 mol.L<sup>-1</sup> of AN. At this concentration of AN, the homopolymer percent (H%) acquired the lowest value (12%).

A further increase in AN concentration led to a decrease in both G% and GE%. This is because of the degradative chain-transfer which form unreactive species with low ability for propagation[21].

#### *Effect of reaction temperature*

The effect of reaction temperature onto the grafting process was studied keeping other reaction conditions constant (Fig.3). The temperature of the polymerization process was changed from 55 to 70°C, while the KPS concentration was kept constant at 0.08 mol.L<sup>-1</sup>, the AN concentration at 4 mol.L<sup>-1</sup> and the reaction time for 2h. It was found that the optimum temperature at which maximum G% and GE% (224% and 97%, respectively) and lowest H% (12%) were obtained is 60°C. Further increase in the reaction temperature led to a decrease in G% and GE% which was probably due to possible chain-transfer reactions which came on the expense of the grafting process.

#### *Effect of reaction time*

The effect of reaction time onto the graft copolymerization process was studied keeping the other reaction conditions constant (Fig.4). The reaction time range was changed from 1 to 4 h, while the KPS concentration was kept constant at 0.08 mol.L<sup>-1</sup>, the monomer concentration at 4 mol.L<sup>-1</sup> and the reaction temperature at 60 °C. It was found that the maximum G% and GE% (224% and 87%, respectively), with the lowest H% (12%), were obtained at a reaction time of 2h and then their values decreased. The decrease in graft yield % at higher reaction time (after 2h) was probably due to the consumption of the majority of the monomer and the partial depolymerization of the growing chains due to the difficulty of termination by recombination as a result of increase in the viscosity of the medium with the time of copolymerization.

### *Characterization of sodium alginate-g-polyacrylonitrile*

#### *Fourier-transform infrared spectroscopy (FTIR)*

FTIR spectra of SA and SA-g-PAN are shown in Fig.5. FTIR of SA (Fig. 5a) confirmed the presence of four strong absorption bands at 1155, 1073, 1030 and 895 cm<sup>-1</sup> which are characterizing the polysaccharide structure. The strong broad absorption band at 3400 cm<sup>-1</sup> is attributed to -OH stretching vibration of sodium alginate with -OH bending at 1030 cm<sup>-1</sup>.

Two bands were observed around 1620 and 1420 cm<sup>-1</sup> due to stretching vibrations of carboxylate group -COO<sup>-</sup> (asymmetric and

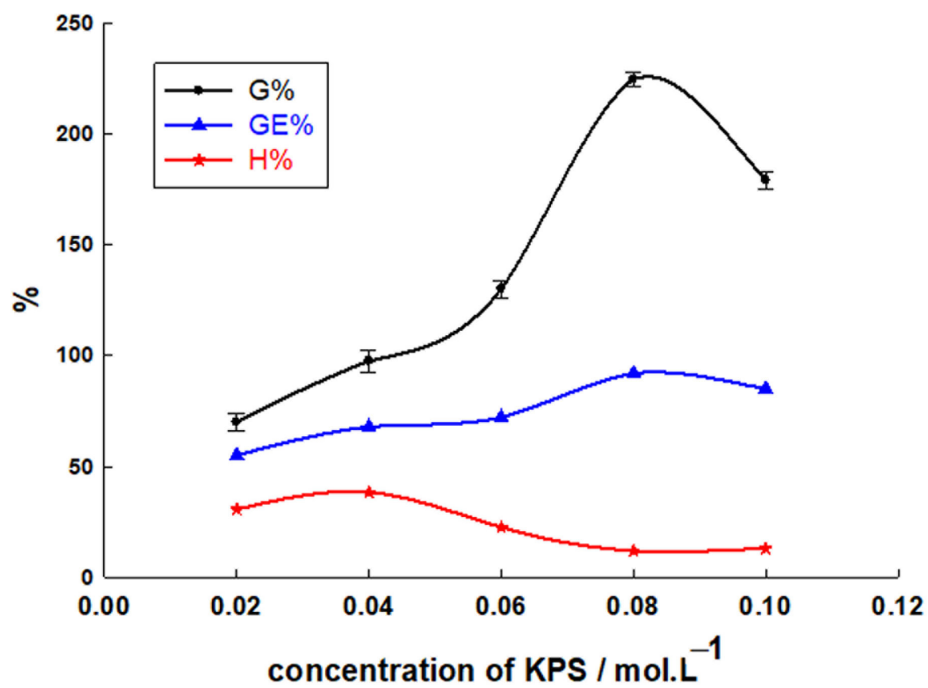


Figure 1. Effect of KPS concentration on graft yield (G%), graft efficiency (GE%) and homopolymer percent (H%).([AN]= 4 mol.L<sup>-1</sup>, reaction time = 2 h and reaction temperature = 60 °C)

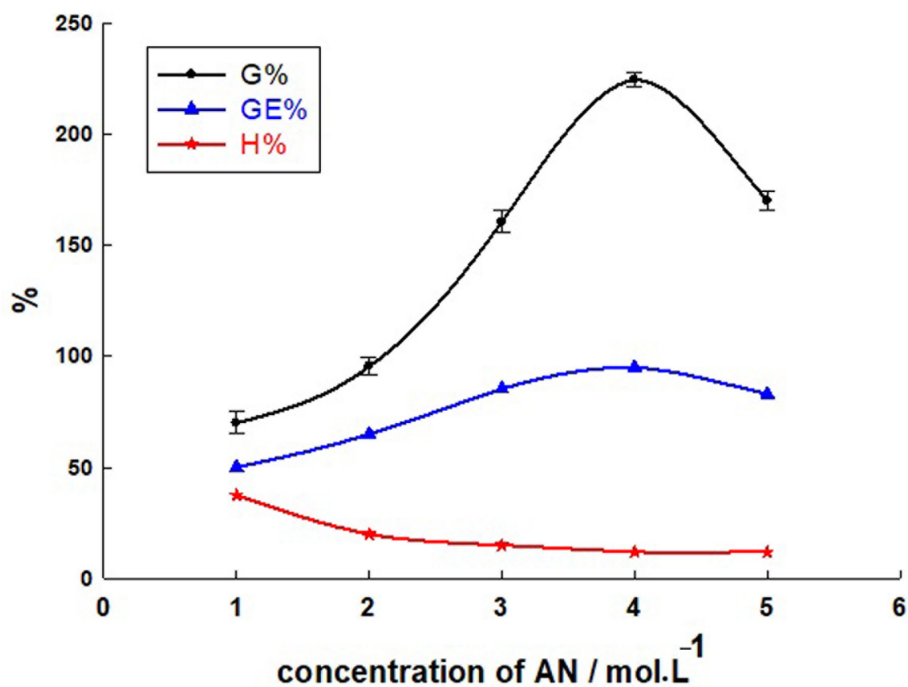


Figure 2. Effect of AN concentration on graft yield (G%), graft efficiency (GE%) and homopolymer percent (H%) ([KPS]= 0.08 mol L<sup>-1</sup>, reaction time = 2 h and reaction temperature = 60 °C)

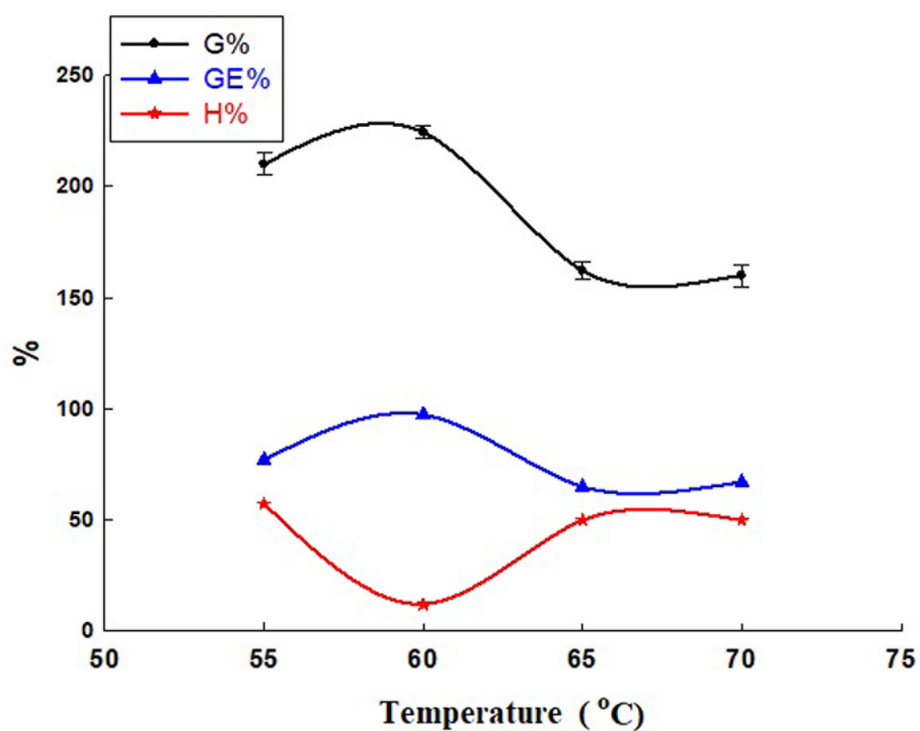


Figure 3. Effect of reaction temperature on graft yield (G%), graft efficiency (GE%) and homopolymer percent (H%) ([KPS]=0.08 mol.L<sup>-1</sup>, [AN] = 4 mol.L<sup>-1</sup> and reaction time =2h)

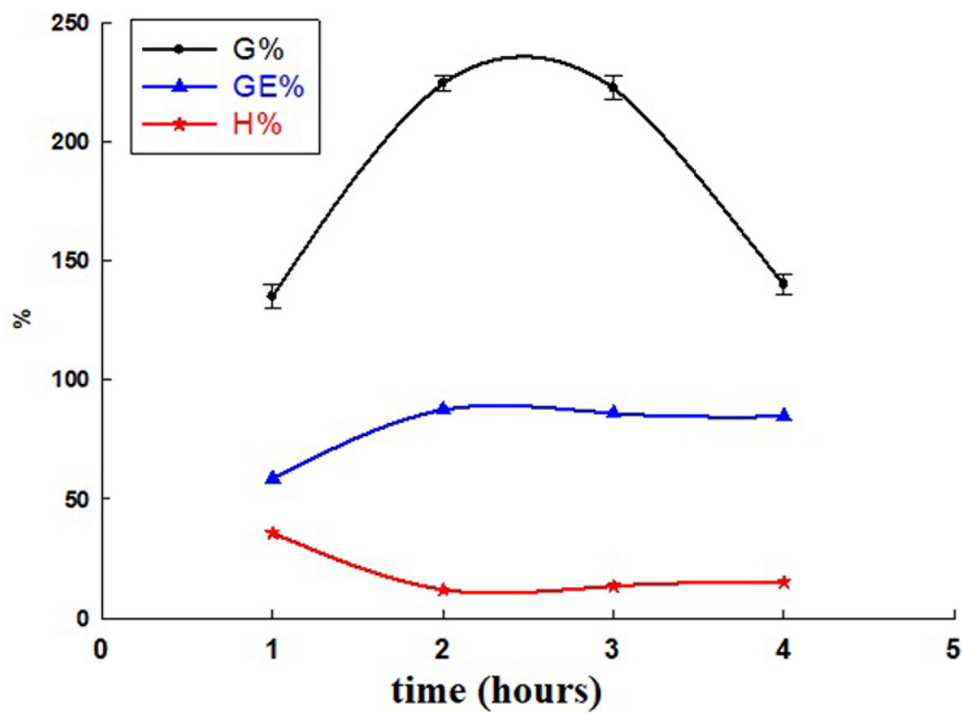


Figure 4. Effect of reaction time on graft yield (G%), graft efficiency (GE%) and homopolymer percent (H%) ([KPS]=0.08 mol.L<sup>-1</sup>, [AN] = 4 mol.L<sup>-1</sup> and the reaction temperature = 60 °C)



symmetric), respectively. These observations are in a good agreement with those reported in several literature [23-25]. Upon grafting SA with PAN (SA-g-PAN, Fig. 5b), additional new absorption bands appeared at 2244 and 2934  $\text{cm}^{-1}$  specific for  $-\text{CN}$  and  $-\text{CH}_2-$ , respectively of PAN backbone which confirm the grafting of PAN onto SA [26].

#### *Thermal Analysis:*

TGA of sodium alginate and its grafted copolymer (SA-g-PAN) are shown in Fig. 6. TGA of SA (Fig. 6a) showed two degradation steps. The first step occurred over temperature range 225-275°C which was due to the decarboxylation and liberation of  $\text{CO}_2$  gas. The second degradation step, which occurred over temperature range 625-700°C, was due to the degradation of SA to carbonaceous residue and  $\text{Na}_2\text{CO}_3$ . 10% and 20% weight losses of SA were observed at 240, 253, and 300°C, respectively. This is a quite well matching with other previous data in literature [23-25]. However, in case of SA-g-PAN (Fig. 6b), the initial decomposition temperature was observed at 300°C. Weight losses 8%, 9% and 13% of the copolymer were observed at 240, 253, and 300°C, respectively. In other words, the temperature at which the graft loses 20% of its weight (344°C) proved that PAN has improved the thermal stability compared to SA alone (253°C). Table 1 summarizes some readings obtained from Fig. 6.

#### SA-g-PAN

##### *X-Ray diffraction (XRD)*

X-Ray diffraction pattern of SA showed three peaks at 27.4°, 31.7° and 45.2° (Fig. 7a) indicating its crystalline nature [23]. At SA-g-PAN (Fig. 7b), a new diffraction peak appeared at 16.9° corresponding to the presence of PAN [27] indicating successful grafting of PAN onto SA.

##### *Solubility of SA and SA-g-PAN*

The solubility of SA and SA-g-PAN was tested in different solvents. Table 2 summarizes these

data. SA was freely soluble in water and insoluble in organic solvents at room temperature. The grafted copolymer, SA-g-PAN, was insoluble in DMF and DMSO but it was soluble in a mixture of THF:H<sub>2</sub>O (95:5 v/v).

##### *Swelling in different pH values*

Swelling of SA-g-PAN was studied in different pH values (pH = 4 and 10). The grafted copolymer showed a higher degree of swellability in basic medium (DS% = 166) than in acidic medium (DS% = 114). That could be explained that PAN has its highest degree of water uptake in basic medium [22,28].

##### *Transmission Electron Microscopy (TEM)*

Transmission electron microscope (TEM) showed spherical nanoparticles of SA-g-PAN. As shown in Fig. 8a, nanoparticles are clearly observed with average diameter about 55 nm, indicating the successful synthesis of nanoparticles using nanoprecipitation process. On the other hand, in diluted suspension of loaded nanoparticles with *p*-phenylenediamine as a hydrophobic model drug (Fig. 8b), the diameter of encapsulated nanoparticles was increased to 156 nm.

#### **Conclusions**

Grafted copolymers based on SA-g-PAN were prepared and characterized by FTIR, TGA, and XRD. FTIR and XRD confirmed the synthesis of the grafted copolymer. TGA showed that PAN has improved SA thermal stability in SA-g-PAN compared to SA alone. Nanoparticles based on SA-g-PAN were fabricated by nanoprecipitation technique with an average diameter of about 55 nm. The diameter of these nanoparticles was increased to 156 nm after loading with *p*-phenylenediamine as a hydrophobic drug model indicating the successful encapsulation process. Based on these obtained results, the prepared nanoparticles could be used in drug loading therapeutic applications.

#### **Declarations of interest**

none

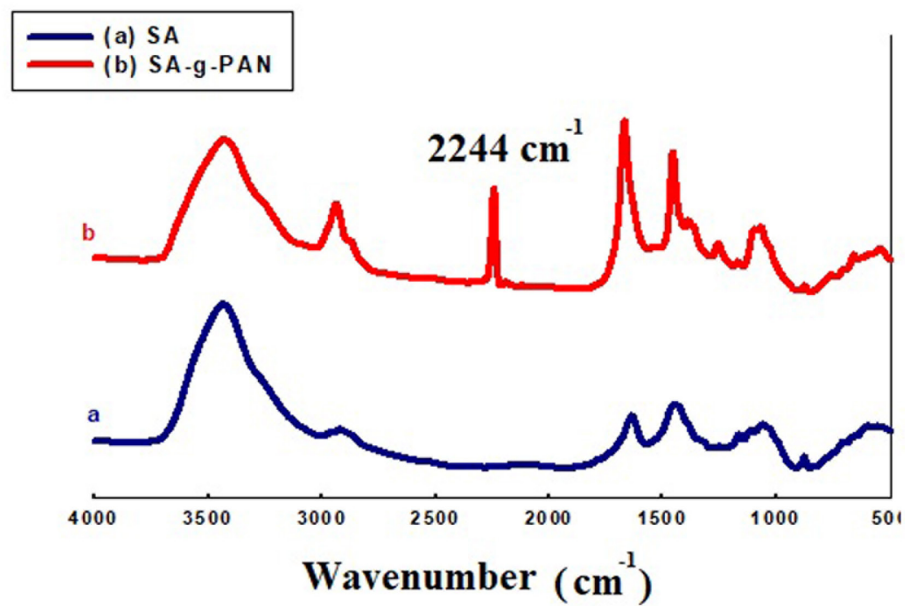


Figure 5. Infrared spectra of (a) sodium alginate SA and (b) grafted copolymer SA-g-PAN

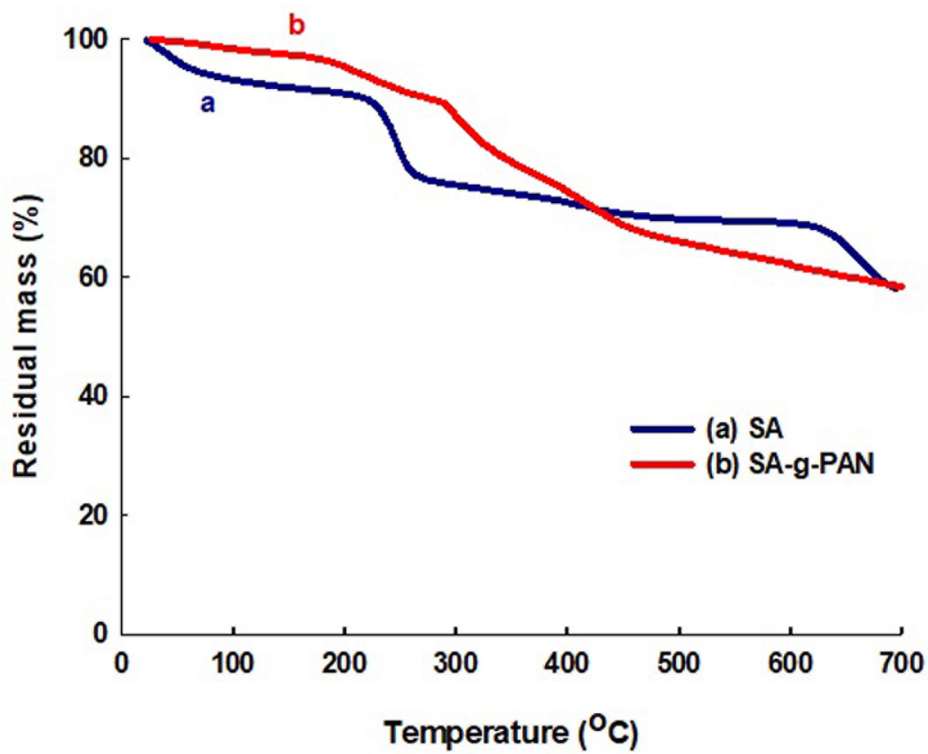


Figure 6. Thermal gravimetric analysis of (a) sodium alginate and (b) grafted copolymer

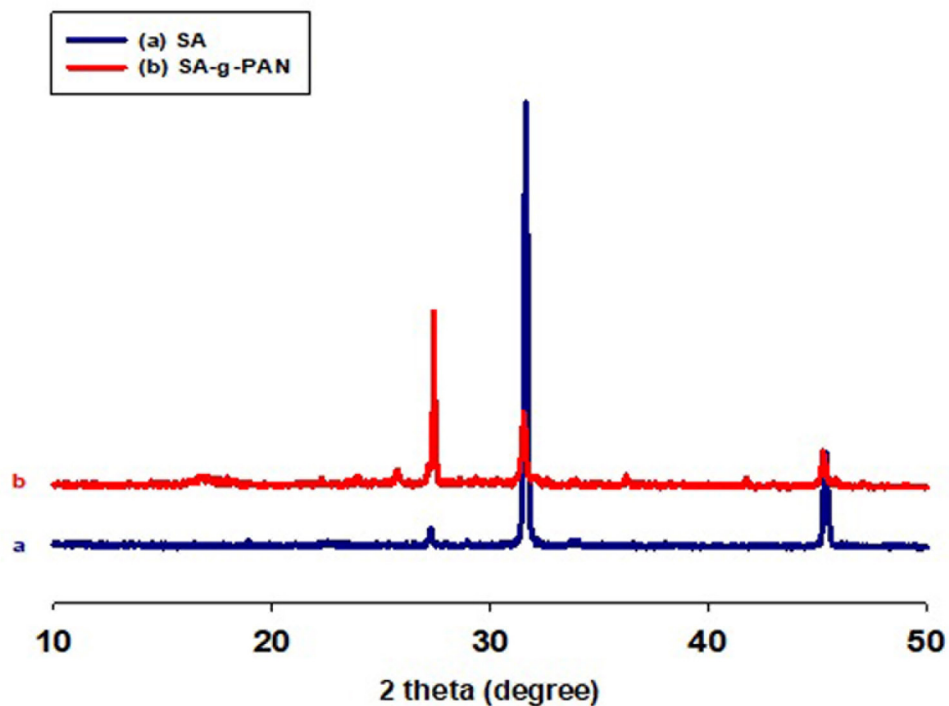


Figure 7: X-Ray diffraction of (a) sodium alginate (SA) and (b) grafted copolymer SA-g-PAN

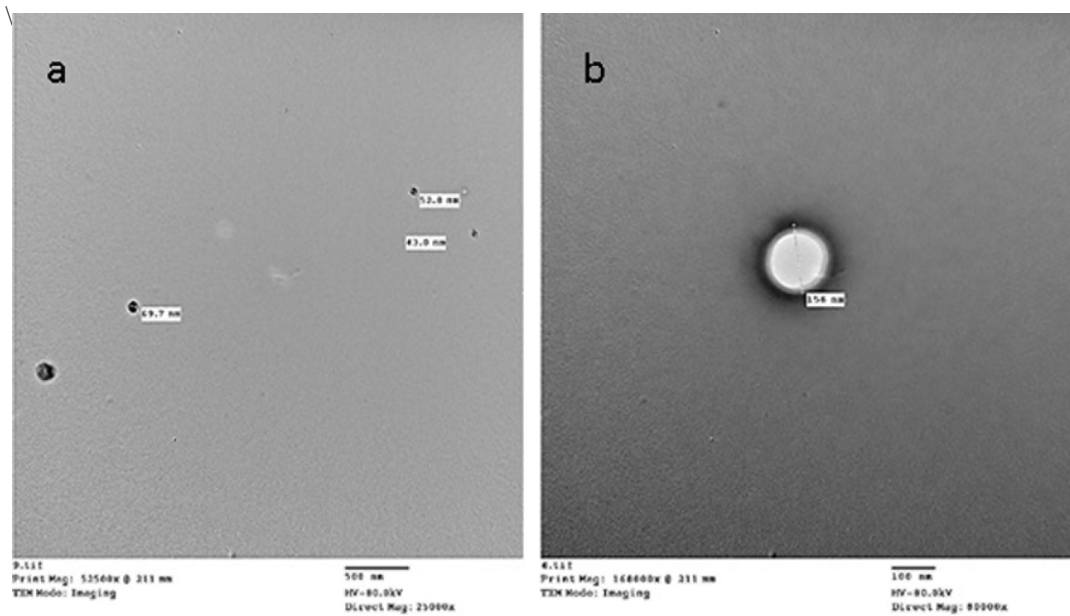


Figure 8. TEM images of prepared nanoparticles based on grafted copolymer SA-g-PAN a) unloaded and b) loaded with p-phenylenediamine.



**TABLE 1. TGA of Sodium alginate and Grafted copolymer SA-g-PAN**

| Polymers | Initial Decomposition Temp. (IDT) (°C) | Temperature (°C) |              | Weight loss (%) at 300 °C |
|----------|--|------------------|--------------|---------------------------|
|          |  | 10 % wt. loss    | 20% wt. loss |                           |
| SA       | 225                                    | 240              | 253          | 25                        |
| SA-g-PAN | 360                                    | 277              | 344          | 13                        |

**TABLE 2. Solubility behavior of Sodium alginate (SA) and Grafted copolymer SA-g-PAN in different solvents**

| Solvent                       | SA        | SA-g-PAN                                     |
|-------------------------------|-----------|--|
| Water                         | Soluble   | Insoluble                                    |
| Methanol                      | Insoluble | Insoluble                                    |
| Ethanol                       | Insoluble | Insoluble                                    |
| Dimethylsulphoxide (DMSO)     | Insoluble | Insoluble                                    |
| N,N'- Dimethylformamide (DMF) | Insoluble | Insoluble                                    |
| Tetrahydrofuran (THF)         | Insoluble | Soluble in THF:H <sub>2</sub> O 95:5 % (v/v) |

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## تحضير جسيمات نانوية مبنية علي بوليمر مشترك من الجينات والبولي اكليرونيتريل الحساسة لدرجة الحمضية وتطبيقه في تحميل الدواء

مجدي سبع وديد فرج، ريهام رشاد محمد علي، ياسمين عبد السلام السيد محمد، سليمان مهاود عبد اللطيف سليمان  
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تم تحضير ثلاث مختلفة من صوديوم ألجينات المطعم بالبولي أكريلونيتريل عن طريق تقنية التطعيم خلال البلمرة بالالكترن الحر. تم تأكيد و توصيف ألجينات المطعم باستخدام التحليل الطيفي للأشعة تحت الحمراء و انحراف أشعة-اكس. التحليل الحراري أظهر انبوليمر المشترك اعلي ثبات حراري عن ألجينات الأصلي. بالعلوة علي ذلك تم فحص قابليته علي الذوبانية في مذيبات مختلفة بالمقارنة مع ألجينات الاصلي. النتيجة الرئيسية من هذا البحث هي ان البوليمر المشترك غني بالبولي اكريلونيتريل الكاره للماء وهذا كان كافيا للمحافظة علي ثبات النظام المعلق مكون من جسيمات نانوية بقطر ٥٥ نانوميتر. أظهر البوليمر المطعم المشترك صوديوم ألجينات مطعم بالبولي اكريلونيتريل درجة انتفاش في الوسط القوي اكبر من الوسط الحمضي. نتيجة ذلك البوليمر صوديوم ألجينات مطعم بالبولي اكريلونيتريل اصبح له حساسية اتجاه درجة الحموضة الوسط. علوة علي ذلك تم تكوين جسيمات النانوية محملة بنموذج كاره للماء وتم اثباته الميكروسكوب الالكتروني النافذ.