Radiation synthesis and anticancer drug delivery of poly(acrylic acid/acrylamide) magnetite hydrogel

Nasser Mohammed Hosny¹ · Mohammed Abbass¹ · Faten Ismail² · Horia M. Nizam El-Din²

Received: 7 February 2022 / Revised: 3 May 2022 / Accepted: 11 May 2022 / Published online: 29 May 2022 © The Author(s) 2022

Abstract
Hydrogels had gained considerable importance in drug delivery systems. Polyacrylic acid/acrylamide (AAC/AAm) and polyacrylic acid/acrylamide doped with magnetite (AAC/AAm)Fe₃O₄ hydrogels have been prepared by γ-radiations and characterized by Fourier transform infrared spectra (IR), X-ray diffractions (XRD), scanning electron microscope (SEM) and transmittance electron microscope (TEM). The morphology and the particle size were determined from TEM images. The average particle size of Fe₃O₄ was found to be 12 nm. The degree of cross-linking of Poly (AAC/AAm) hydrogel was determined from the gel fraction at different radiation doses and compositions. Swelling of the hydrogel was studied at different conditions. The magnetism of (AAC/AAm) Fe₃O₄ was studied by vibrating sample magnetometer and the results indicated super-paramagnetic character of (AAC/AAm)Fe₃O₄. The band gap ($E_g$) was determined and the values suggested semi-conductivity of the hydrogels. Doxorubicin loading and release by (AAC/AAm)Fe₃O₄ hydrogel was studied and indicated that the hydrogel can load 78% of doxorubicin.

Keywords Polyacrylic acid/acrylamide · Radiation synthesis · Swelling · Doxorubicin · Drug delivery

Nasser Mohammed Hosny
Nasserh56@yahoo.com

1 Chemistry Department, Faculty of Science, Port Said University, P.O. BOX 42522, Port Said, Egypt
2 Polymer Chemistry Department, National Center for Radiation Research and Technology, Cairo, Egypt
Introduction

Hydrophilic gels are an important category of cross-linked polymers that absorb water without dissolution. Hydrogels have interesting properties as softness, smart and ability to store water [1].

The polymers that can change their properties by changing the chemical or physical stimuli, are known as “smart” polymers. Hydrogels are sensitive to the change in the surrounding environmental conditions due to the presence of various functional groups along the polymer chains. Poly acrylic acid is affected by the change in the pH of the medium, so it has wide applications in the drug delivery to the gastrointestinal tract [2]. Hydrogels are soft materials that have wide biological applications for half a century. They have good biocompatible advantages which come from their higher swelling ability. On the contrary, they exhibit some limitations to mechanical properties and consequently the applications. The effects of starting materials, fabrication factors as mechanical stress and swelling have been studied [3].

The unique properties of metal oxide nanoparticles are associated with its surface area [4–7]. The application of hydrogel in drug delivery, biosensors and tissue engineering was recently reviewed [8]. The role of hydrogel in transdermal drug delivery was also reviewed [9]. In addition to that, the rheological behavior was extensively studied by Hissiou et al. [10–14].

Delivery of water soluble or insoluble pharmaceutics is a key challenge in nanomedicine. Polymer hydrogels had gained considerable importance in drug delivery systems. Hydrogels have several advantages as they are easily synthesized, elastic, soft and environmentally safe materials which make them suitable for nanomedical applications [15].

In this manuscript, the synthesis, spectral, swelling, magnetic and drug release properties of polyacrylic acid/acrylamide, magnetite hydrogel have been studied to shed some light on the potential medical applications of these hydrogels.

Previously, acrylic acid/ acrylamide was prepared by potassium persulfate as an initiator and N,N’-methylene bisacryl-amide as a cross-linking agent [16]. The present work has some advantages as it used radiation in synthesis and as a cross-linking agent. In addition to that, the obtained composites have higher swelling ability and could be applied as a carrier of the well-known anticancer drug Doxorubicin.

Experimental

Acrylic acid, 99%, Acrylamide, 99%, FeSO₄.7H₂O, 99% and FeCl₃.6H₂O, 99% were purchased from Sigma-Aldrich.

Preparation of acrylic acid (AAC)/acrylamide (AAM) by gamma radiations

Stock solutions of AAc and AAm were prepared as follows: 20 mL of acrylic acid in 100 ml distilled water; 5 mL of acrylamide was dissolved in 100 ml distilled water.
From the stocks the following ratios of AAc/AAm (10/90, 30/70, 50/5, 70/30 and 90/10 were prepared. Nitrogen gas was passed in the mixture for 5 min and then subjected to gamma radiation.

The source of radiation is $^{60}$Co gamma cell and the dose rate was 5 KGY/h.

**Preparation of acrylic acid, acrylamide, magnetite (AAc/AAm)Fe$_3$O$_4$**

FeSO$_4$.7H$_2$O and FeCl$_3$.6H$_2$O were mixed in 1:2 ratio in 100 mL distilled water. Let the mixture overnight. To the above mixture AAc/AAM in different ratios were added. Then, 1 M ammonium hydroxide was added drop by drop until black color appears then irradiated with different doses of gamma radiations with dose rate 5 KGY/hr.

**Swelling study**

Swelling of AAc/AAM was carried out as follows: A dry weight of the hydrogel ($W_1$) was immersed in distilled H$_2$O at room temperature for various time intervals. Then remove excess of water by a filter paper and reweigh ($W_2$). The % of swelling ($S$) was found from the relation $S = (W_2 - W_1 / W_1) \times 100$.

**Gel fraction determination**

A weighed sample ($W_1$) of the polymer was boiled in H$_2$O (distilled) for 4 h and dry in a vacuum oven at 80 °C, till constant weight ($W_2$). The gel fraction% ($F$) was calculated: $F = (W_2 / W_1) \times 100$.

**Doxorubicin loading**

0.1 g of AAM/AAc/Fe$_3$O$_4$ nanocomposite was immersed in 2 mL of 0.5 mM of Doxorubicin in distilled water for 24 h. Finally, the polymer was washed by distilled water then, dried at 50 °C. The drug loaded was studied at pH 5.0, 7.4 and 8.0 for 24 h. The loaded amount of the drug was determined using UV–vis spectrophotometer at 470 nm.

**Drug release measurements**

Doxorubicin release were followed at 37 °C for approximately 24 h. Release experiments were done by placing various nanocomposite samples loaded with drug (conc. 0.5 mM) into 30 mL buffer solutions of pH 5, 7.4, 8. A sample was taken and measured at 470 nm at different time intervals.
The amount of drug released from nanocomposite hydrogel was calculated using the equation: Drug release (\%) = \left[ \frac{\text{amount of drug released}}{\text{amount of drug loaded}} \right] \times 100.

**Working procedures**

Infrared spectra (FT-IR): The prepared hydrogels were measured using (FT-IR-4100 type A) at Damietta University. Electronic spectra (UV–Vis) of the isolated compounds were recorded on (Hach Model DR-6000) at Egyptian propylene and poly propylene company. X-ray powder diffraction patterns were taken on Philips X’PERT-PRO diffract meter, with wavelength is 1.5406 Å. Transmission electron microscope images of hydrogels were taken on transmission electron microscope (JEOL JEM-2100) at Egyptian petroleum research institute (EPRI), Cairo.

**Results and discussion**

Polyacrylic acid/acrylamide (AAc/AAm) hydrogel was synthesized by gamma irradiation in aqueous solution. The cross-linking of poly (AAc/AAm) in aqueous media was mediated by radiation. The suggested mechanism of cross-linking is as follow: The solvent (HOH) and the monomers (AAc and AAm) absorb gamma radiation forming activated compounds (AAc*, AAm* HOH*). Then, the activated compounds can undergo fragmentations forming free radicals of AAc., AAm., H. and .OH. The radicals of the solvents can transfer to AAc and AAm forming more of these radicals and increasing the rate of cross-linking [17, 18].

\[
\text{AAc} + \text{H}^- \text{and} \cdot \text{OH} \rightarrow \text{AAc}^-
\]

\[
\text{AAm} + \text{H}^- \text{and} \cdot \text{OH} \rightarrow \text{AAm}^-
\]

In the termination, step (n) or (m) repeating units combine to form cross-linked polymers

\[
(\text{AAc}^-)_n + (\text{AAm}^-)_m \rightarrow (\text{AAc})_n - (\text{AAm})_m
\]

**Encapsulation of magnetite in P(AAc/AAm) hydrogel matrix**

XRD pattern of magnetite doped (Fig. 1) shows the peaks at 2θ= 30.2, 35.7, 43.0, 57.0 and 63.0° attributed to the planes (220), (311), (400), (422) and (440), respectively. These planes confirm that the sample is Fe₃O₄ with face centered cubic structure (JCPDS) 19-0629. The broadening of the peaks is attributed to the presence of the particles in the nanoscale.
Debye–Scherer equation $D = 0.94b \lambda/\beta \cos \theta$ \cite{19} ($D$ is the crystallite size, $\lambda = 1.5406$ Å, $\beta$ is the width at half maximum) was applied to determine the crystallite size from the peak at 35.7° and it was 12 nm.

One of the most characteristic features of the hydrogel is its high ability to uptake water. So, magnetite was doped from aqueous media in the hydrogel matrix by in situ procedures.

TEM image of Poly (AAc-co-AAm) Fe$_3$O$_4$ hydrogel is indicated in Fig. 2A. The high contrast in TEM images was used to confirm encapsulation of Fe$_3$O$_4$ nanoparticles in P(AAc/AAm) hydrogel matrix. As indicated from TEM analysis, the black spheres point to magnetite nanoparticles that encapsulated inside the hydrogel core and they are not outside the hydrogel. It is clear (Fig. 2B) that some magnetite nanoparticles aggregated to form rods. The particle size distribution curve of magnetite encapsulated in P(AAc/AAm) (Fig. 2C) indicates the distribution of the particle in small range from 6 to 13 nm with two maxima at 7.5 and 11.5 nm.

**FT-IR spectra**

IR of Poly(AAc/AAm) Fe$_3$O$_4$ spectrum (Fig. 3) reveals a band at 568 cm$^{-1}$ characteristic to $\nu$ (Fe–O) in octahedral site of Fe$_3$O$_4$. The bands at 637 and 609 cm$^{-1}$ are characteristic also to Fe–O bonds in magnetite \cite{3}.

The stretching vibrations of $\nu$ (OH) of acrylic acid appear at 3412 cm$^{-1}$ \cite{3}. The bands at 3337 cm$^{-1}$ and 3189 cm$^{-1}$ are due to $\nu_{as}$ and $\nu_{s}$ of NH$_2$ of acrylamide \cite{3}. The absence of the bands assigned to (CH$_2$=CH–) in both AAc and AAm confirms the polymerization of AAc and AAm through this group.

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**Fig. 1** XRD pattern of Poly (AAc/AAm)Fe$_3$O$_4$
Fig. 2  TEM images of (AAc/AAm)Fe₃O₄. C Particle size distribution of Fe₃O₄ encapsulated in Poly(AAc/AAm)
spectrum of Poly(AAc/AAm) Fe₃O₄, the positions of the bands at 1617 and 1650 cm⁻¹, which were attributed to protonated (COOH) group [20] and ν₅ (CO) of acrylamide did not change compared with their positions in the free polymer, while there are shifts to lower wavenumber in the bands 1607 and 1402 cm⁻¹ that were attributed to νₐs and νₛ (COOH). These shifts suggest the possibility of electrostatic attraction between Fe₃O₄ and this group.

**Gel fraction**

To determine the degree of cross-linking of Poly (AAc/AAm) hydrogel, the gel fraction was calculated at different radiation doses and compositions (Fig. 4A). The gel fraction was calculated by applying the relation.

\[
\text{Gel} \% = \frac{W_d}{W_i} \times 100 \quad \text{(where } W_d \text{ is the weight of extracted hydrogel and } W_i \text{ is the weight of dry hydrogel)}.
\]

From the curves, it is clear that the degree of cross-linking increases with increase of the radiation dose. At composition ratio AAc:AAm (30:70), the hydrogels have approximately the same gel fraction % regardless of the applied radiation doses. The hydrogel synthesized at radiation dose 50 KGY exhibits the maximum cross-linking at composition ratio 50:50 (AAc:AAm); then, the cross-linking decreases with increasing composition ratios. The cross-linking of the hydrogels synthesized by radiation doses 10 and 30 KGY begins to decrease after composition ratios (30:70) AAc:AAm. In the beginning when aqueous solution of AAc/AAm was subjected to radiation, free radicals of AAc, AAm and water will generate and they can combine randomly forming cross-link of co and homo-polymer AAc. When two radicals of neighboring polymeric chains combine a cross-linked macromolecules will be formed. As the concentration and radiation dose increase the presence of higher number of the rigid cross-linked polymer with
low mobility will increase. In the same time, the possibility of formation of more cross-linked polymer will decrease [21].

From Fig. 4B, it is clear that the gel fraction percentage increases as the concentration of magnetite increases as Fe$^{2+}$ and Fe$^{3+}$ in magnetite can bind neighbor polymeric chains together through coordination with the active donor sites in the polymers.

**Swelling**

Hydrogels exist in three-dimensional (3D) networks with hydrophilic character. They have excellent capacity of water swelling through bonding the water molecules
Fig. 5 Effect of Ac composition, radiation dose and pH on swelling of (AAc/AAm) hydrogel
by the polar hydrophilic groups or by occlusion the water molecules in the gaps between the chains [22]. The effect of radiation doses from (10 to 50 KGY) on the swelling of (AAc/AAm) showed that the highest swelling value of the hydrogel is observed at the lower radiation dose 10 KGY (Fig. 5A). There is a decrease in swelling at higher dose (50 KGY) (Fig. 5B). This behavior can be interpreted on the basis of increasing the cross-linking associated with decreasing the swelling [23]. It is noticeable that, with increasing cross-linking density, there is a decrease in the separating space between the chains leading to rigidity of the formed polymer. The rigid copolymer structure will resist expansion and holding large quantities of water [24, 25].

The swelling increases also, as the concentration of AAc increases. The increment in swelling values at lower concentration is attributed to the higher hydrophilicity of the hydrogel. Then, the decrease in swelling with concentration could be resulted from the increased chance of terminating AAc radicals. In addition, the viscosity of the reaction increases at higher AAc concentration, which restricts the mobility of the monomeric radicals and deactivates grow of the polymeric chains. It can be suggested also that there will be an enhancement in homo-polymerization reaction over copolymerization [26–28].

With respect to the increase in the swelling, the pH increases (Fig. 5C). It is noticeable that at higher pH the alkaline medium will affect on the hydrophilic groups as hydroxyl, carboxyle and amide of the reactants leading to increase the possibility of hydrogen bonding and consequently, increasing the swelling.

The swelling of hydrogels was studied in the temperature range (30–70 °C). The results indicated that, by raising temperature, the swelling % increases until 50 °C, then the swelling % decreases by raising temperature. In the first stage raising temperature may increase the mobility of the polymers and activate them to absorb water molecules, at higher temperature there will be breaking in hydrogen bond and liberation of water molecules.

**Magnetic properties**

The super-paramagnetism of Fe₃O₄ nanoparticles is important for their application in therapeutic applications. Super-paramagnetic nanoparticles have magnetism in the presence of external magnetic field and lose it in the absence of the field. If there is no hysteresis, both the retentivity (Mr) and coercivity (Hc) are zero or very close to zero, the material is characterized as super-paramagnetic.

The critical size of super-paramagnetic material is ca. 20 nm for soft magnets and 4 nm for hard ones [29]. Also, there is a direct relation between saturation magnetization of the nanoparticles and the size. By decreasing the particle size, the magnetization will be lowered. When the size of magnetite is reduced to the nanoscale, they exhibit super-paramagnetism [30, 31].

The magnetism of (AAc/AAm) Fe₃O₄ was studied by vibrating sample magnetometer at 25 °C (Fig. 6). The curve shows no hysteresis and the saturation magnetization (Ms) is 2.2 emu/g due to the presence of Fe₃O₄ in nanoscale and
the effect of diamagnetic polymers. Both retentivity \((M_r)\) and coercivity \((H_c)\) are \(48 \times 10^{-3}\) emu/g and 7.8 G, respectively, which are close to zero. From these findings it could be concluded that \(\text{Fe}_3\text{O}_4\) (AAC/AAm) is a super-paramagnetic material.

**Optical band gap \((E_g)\)**

Electronic absorption spectrum of poly (AAC/AAm) shows an absorption band centered at 280 nm due to \(n-\pi^*\) transitions. This band was shifted to 295 nm in case of (AAC/AAm) \(\text{Fe}_3\text{O}_4\) as a result of interaction between the metal orbital with that of the polymer, forming charge transfer from organic polymer to the metal.

The spectra of hydrogels were used to determine \(E_g\) of these compounds by applying Tauc’s equation: \[ (\alpha h\nu)^2 = B (h\nu-E_g)^m \]

- \(m\) is equal to 1/2 for indirect transition and 2 for direct transition. The absorption coefficient \(\beta\) is given from the relation \(A/d\) where \(A\) = absorbance, \(d\) is the path length through the sample. The parameter \(B\) represents transition probability. The graphical representation of \((\alpha h\nu)^2\) against \(h\nu\) gives direct transitions when extrapolating the linear portion of the curve to \((\alpha h\nu)^2 = 0\) (Fig. 7). \(E_g\) values are 4.47 and 4.36 eV for poly (AAC/AAm) and (AAC/AAm) \(\text{Fe}_3\text{O}_4\), respectively. The reduction in \(E_g\) value after encapsulation \(\text{Fe}_3\text{O}_4\), comes from the interaction between the polymers orbitals with that of the metal orbital forming new larger molecular orbitals. The increment in the orbital leads to reduction of \(E_g\). The values of \(E_g\) indicate that theses hydrogels are semiconductors [33, 34].

**Release of doxorubicin**

Doxorubicin is a well-known anticancer chemotherapy drug. Doxorubicin belongs to anthracycline antibiotic.
Fig. 7 Optical band gap of A (AAc/AAm) and B (AAc/AAm) Fe$_3$O$_4$

- **A**: Optical band gap of A (AAc/AAm) Fe$_3$O$_4$
  - $E_g = 4.47$ eV ($\alpha h\nu$)
  - $250 - 650$ nm

- **B**: Optical band gap of B (AAc/AAm) Fe$_3$O$_4$
  - $E_g = 4.36$ eV ($\alpha h\nu$)
  - $250 - 650$ nm

- **C**: Optical band gap of (AAc/AAm) Mag Co$^{2+}$
  - $E_g = 4.27$ eV ($\alpha h\nu$)
  - $250 - 650$ nm

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*Figures and data points are not transcribed into text due to the nature of the content.*
The drug-loading % of hydrogel was calculated using the following relation:

\[
\text{Loading of drug \%} = \frac{\text{Weight of drug in hydrogel}}{\text{Weight of hydrogel loaded with drug}} \times 100
\]

The drug loading percent was found to be 78.0%.

The release profile of doxorubicin from (AAc/AAm)Fe₃O₄ at pH’s 5, 7.4 and 8 has been studied (Fig. 8). The release is high in the beginning at the three pH, then it becomes slow at equilibrium. The release rate increases as pH increases, the slowest release is observed in the acid medium, while the release is high in the basic medium. This can be explained based on adsorption of Doxorubicin onto AAc/AAm magnetite hydrogel either through coordination of doxorubicin hydroxyl groups with magnetite or through formation of hydrogen bonds with COOH and amide group of AAc/AAm.

At high pH, there is a competition between the hydroxyl group of medium and that of Doxorubicin, so, the rate of release increases [35, 36].

**Conclusion**

Polyacrylic acid/acrylamide (AAc/AAm) and polyacrylic acid/acrylamide magnetite (AAc/AAm)Fe₃O₄ hydrogels have been synthesized and tested for loading and release of the anticancer drug Doxorubicin. Swelling study was carried out to determine the suitable conditions for swelling the hydrogel. The optical band gap was determined for the two polymers and indicated semi-conducting nature of the two polymers. (AAc/AAm)Fe₃O₄ hydrogel can load 78% of Doxorubicin and release
it in the alkaline medium. (AAc/AAm)Fe₃O₄ hydrogel is a potential candidate for chemotherapy anticancer drugs delivery.

**Funding** Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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