Synthesis And Characterization of Magnetic Nano Particles Embedded in Hydrogels For Drug Delivery Applications

CHAPTER-IV
Chapter 4. Synthesis And Characterization of Magnetic Nano Particles Embedded in Hydrogels For Drug Delivery Applications

4.1 Introduction

The study of Magnetic nano particles (MNPs) has been the subject of intensive research in recent few years because of their prospective applications of as magnetic and biomedical materials [1-6]. Hydrogels were developed in the last couple of decades for various biomedical applications because of their natural unique properties. Hydrogels because of their water withholding capacity network porosity, elasticity properties enables them for very important applications such as adsorbents for various metals [7], scaffolds [8], tissue engineering [9], bioseparation [10], drug delivery [11], and three-dimensional composites [12]. Because of their porous nature, hydrogels can offer suitable environment for the synthesis of various nano particles with enhanced stability. In general hydrogels are three dimensional network polymeric materials containing a large number of hydrophilic groups capable of holding large amount of water in their network structure. They swell by absorbing water and shrink on drying. Hydrogels are utilized in a wide variety of fields. In addition to this the application of the hydrogels are extended to immobilization of enzymes and drug delivery systems. Since hydrogels can absorb water and repond to external stimuli they are being used to develop a number of hydrogel silver nanocomposites for superior antimicrobial applications [13, 14]. Design of hydrogel magnetic nano particle composite materials (ferrogels or magnetic hydrogels) will offer combined magnetic properties in addition to conventional hydrogel characteristics [15]. Variation of cross-linking density, chemical entity and synthetic approach decides the integration of the nano particles with polymer hydrogels that can promote controlled, triggered and hyperthermia applications.
Hydrogels magnetic nanocomposites are synthesized by employing natural polymers and magnetic materials such as iron oxide (Fe$_3$O$_4$) or maghemite (γ-Fe$_2$O$_3$) and are been widely used in many applications, due to their proven biocompatibility, quick response and sensitivity to an external stimulus such as applied magnetic field [16,17].

Because of the importance of magnetic nanocomposites the present work involves incorporation of magnetic nano particles in hydrogels developed by using acrylamide, Poly (Vinyl alcohol) and a natural polymer such as gum acacia. The developed magnetic nanocomposites are characterized, by spectral X-ray, thermal, electron microscopic as well as by VSM analysis. Finally they are used for the drug release of doxorubicin an anticancer drug.

4.2. Experimental

4.2.1. Materials

Acrylamide (AM), PolyVinyl alchol (PVA), Gum acacia (MW 200000) (GA), $N,N$-methylenebisacrylamide (MBA), $N,N,N',N'-$tetramethylthlenediamine (TMEDA), ammonium persulfate (APS), doxorubicin drug were purchased from Aldrich Chemical Company Inc. (Milwaukee, WI, USA), Iron (II) chloride tetrahydrate (99+%) (FeCl$_2$.4H$_2$O) and Iron(III) chloride hexahydrate (99+%) (FeCl$_3$.6H$_2$O) were purchased from Merck (Mumbai, India). Ammonium hydroxide (28% ammonia in water) (99.99%) was purchased from S.D. Fine (Mumbai, India). All the chemicals were used without further purification. Double-distilled water was used for the preparation of all the solutions in this study.
4.2.2. Preparation of Gels

Poly(acrylamide)/Polyvinyl alcohol/Gum acacia (PAM-PVA-GA) hydrogels were synthesized by employing free radical polymerization using \(N,N\)-methylenebisacrylamide (MBA) as a cross-linker and ammonium persulfate/\(N,N,N'\text{,}N'\text{-tetramethylene}diamine (APS/TMEDA) as redox-initiating pair following the usual procedure [22]. In detail, PAM-PVA-GA hydrogels were prepared by first mixing AM (1 g) PVA (0.5 g) with different amounts (0.1, 0.2, 0.3 and 0.4 g) of Gum acacia in 3 mL of water separately. In order to finalize an optimized hydrogel for drug delivery studies, a series of PAM-PVA-GA hydrogels were synthesized by using AM = 1 g PVA = 0.5 g and GA = 0.1 to 0.4 g and varying the cross-linker (MBA), (1.62, 3.24, 4.86, 6.48), with fixed ammonium persulfate (APS), and \(N,N,N'\text{,}N'\text{-tetramethylene}diamine (TMEDA) concentrations (Table 1). The transparent hydrogels were removed, washed with double distilled water, and dried in an oven (GUNA, Chennai, India) at 60°C.

For farther studies except PAM-PVA-GA4 was used since it has the maximum swelling capacity due to higher amount of GA in the sample.
Table I: The feed compositions of the hydrogels

<table>
<thead>
<tr>
<th>Hydrogel code</th>
<th>Concentration in the feed mixture of the hydrogel network (g)</th>
<th>MBA mM</th>
<th>APS mM</th>
<th>TMEDA mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAM-PVA-GA1</td>
<td>1 0.5 0.1 4.86 2.191 0.172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM-PVA-GA2</td>
<td>1 0.5 0.2 4.86 2.191 0.172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM-PVA-GA3</td>
<td>1 0.5 0.3 4.86 2.191 0.172</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PAM-PVA-GA4</td>
<td>1 0.5 0.4 4.86 2.191 0.172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM-PVA-GA5</td>
<td>1 0.5 0.5 1.62 2.191 0.172</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAM-PVA-GA6</td>
<td>1 0.5 0.5 3.24 2.191 0.172</td>
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<tr>
<td>PAM-PVA-GA7</td>
<td>1 0.5 0.5 4.86 2.191 0.172</td>
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</tr>
<tr>
<td>PAM-PVA-GA8</td>
<td>1 0.5 0.5 6.48 2.191 0.172</td>
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4.2.3. Preparation of PAM-PVA-GA4 hydrogel magnetite nanocomposites.

To prepare the desired hydrogel magnetite nanocomposite, PAM-PVA-GA4 hydrogel sample was placed in 60 mL of double distilled water and allowed to swell completely over a period of 24 hours. The swollen hydrogel was taken and transferred to another beaker containing 200 mL of aqueous solution consisting of 2.1 g of iron (II) chloride tetrahydrate and 5.8 g of iron (III) chloride hexahydrate and allowed for 24 hrs to entrap the iron salts throughout the hydrogel networks. Then the hydrogel loaded with iron (II) and iron (III) ions was removed from the iron salt solutions, washed with double distilled water and placed in 60 mL of 0.5 M ammonium hydroxide (28% NH$_3$ in water) and left overnight. The resultant brown or black coloured hydrogel magnetic nanocomposite was removed.
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washed with double distilled water, and allowed to dry in an oven (GUNA, Chennai, India) at 60°C. The overall schematic representation of the formation of magnetic nano particles in hydrogel network i.e., hydrogel magnetic nanocomposite is shown in Figure 1.

![Figure 1](image)

**Figure 1.** (a) PAM-PVA-GA4 Blank hydrogel, (b) PAM-PVA-GA4 iron salt loaded hydrogel (c)PAM-PVA-GA4- magnetic nanoparticles formed in hydrogel.

4.2.4. Swelling Studies

Completely dried PAM-PVA-GA hydrogels and PAM-PVA-GA hydrogel nanocomposites (~50 mg) were equilibrated in distilled water at 30°C for 3 days. The equilibrium swelling ability or swelling ratios ($Q$) of PAM-PVA-GA1 to PAM-PVA-GA4 hydrogels their ion salts and nanocomposites were calculated using the following the equation: $Q = \frac{W_e}{W_d}$, where $W_e$ is the weight of water in the swollen gel at equilibrium and $W_d$ is the dry weight of the dried gel. The swelling ratio values are presented in the form of graph in Fig. 2 in Results & Discussion section.

4.2.5. Characterization

4.2.5.1. FTIR Spectroscopy: FTIR Spectroscopy was performed for hydrogel, iron salts loaded hydrogel, and hydrogel magnetic nanocomposite and were recorded on a Thermo Nicolet Nexus 670 spectrophotometer (Washington, USA). By KBr disc method.
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4.2.5.2. X-Ray Diffraction: The X-ray diffraction studies of the hydrogel, iron salts loaded hydrogel, and hydrogel magnetic nanocomposites were carried out using a Rikagu diffractometer (Rikagu, Tokyo, Japan) employing rotting anode mode Ru-H3R (Cu radiation, k = 0.1546 nm) running at 40 kV and 40 mA.

4.2.5.3. Thermogravimetric Analysis (TGA): The thermal analysis of hydrogel, iron salts loaded hydrogel, and hydrogel magnetic nanocomposites were evaluated on a SDT Q 600 TGA instrument (T.A. Instruments-water LLC, Newcastle, DE 19720, USA), at a heating rate of 10°C/min under a constant nitrogen flow (100 ml/min). The samples were run from 40°C to 800°C.

4.2.5.4. UV-Vis spectrophotometer

UV-Vis absorption spectra of the hydrogels drug delivery were recorded on a Elico SL 160A Model UV-Vis spectrophotometer at λmax 266.5nm.

4.2.5.5 Scanning Electron Microscopy (SEM): The surface morphology of hydrogel, iron salts loaded hydrogel, and hydrogel magnetic nanocomposite were studied using a JEOL JSM 840A (Tokyo, Japan) scanning electron microscope (SEM) at an accelerating voltage of 15 kV. All the samples were dried in vacuum at room temperature and coated with gold before scanning.

4.2.5.6. Transmission Electron Microscopy (TEM): The size of the magnetic nano particles in gel network was determined using a Technai F12 TEM (Philips Electron Optics, Holland). TEM samples were prepared by dropping 2–3 drops of aqueous solutions of magnetic nano particles on a 200 mesh formvar-coated copper TEM grid (grid size: 97 μm) (Ted Pella, Inc., Redding, CA, USA) followed by removing excess solution using a piece of fine filter paper and the samples were allowed to dry in air overnight prior to image the
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particles. The particle size of magnetic nano particles in TEM images were measured using NIH Image software (http://rsbweb.nih.gov/ij/).

4.2.5.7. Magnetization studies using VSM: The magnetization and hysteresis loop were measured at room temperature using a Vibrating Sample Magnetometer (VSM) (Model 7300 VSM system, Lake Shore Cryotronic, Inc. Westerville OH, USA)

4.2.5.8 Drug Loading

Doxorubicin is a drug used in cancer chemotherapy. It is an anthracycline antibiotic, closely related to the natural product daunomycin, and like all anthracyclines it works by intercalating DNA. Doxorubicin is commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma, and soft tissue sarcomas. Therefore, this compound is chosen to load into hydrogels for drug delivery applications. The drug was loaded in to the hydrogel samples by immersing the hydrogel in the drug solution. Experimentally, 50 mg of hydrogel sample was taken and immersed in 20 mL of drug solution (5 mg/ 20 mL distilled water). The amount of drug included in the hydrogel sample was determined by UV-Vis spectroscopy. To determine the maximum absorbance of doxorubicin, the UV-Vis spectra for pure drug solution was taken and the maximum absorbance is found at 492.2 nm. The % of drug loading was calculated using the following equation.

\[
\% \text{ Encapsulation efficiency} = \left[ \frac{\% \text{ actual loading}}{\% \text{ theoretical loading}} \right] \times 100 \quad (3)
\]

4.2.5.9. Release of Doxorubicin

The in vitro release studies of the doxorubicin drug were carried out by placing the dried and doxorubicin loaded hydrogel in definite volume (50 mL) of releasing medium (7.4 pH phosphate buffer (PBS) and the dissolution medium was placed on a rotary shaker (Remi
Instruments Limited, Model No CIS-24BL, Vasai, India) at 100 rpm at 37°C. The amount of drug release was measured spectrophotometrically in pH 7.4 buffer solution. The absorptions of the solutions of doxorubicin drug was measured at λ max 492.2 nm. The results are shown in Fig.9.

4.3 Result Discussion.

4.3.1. Hydrogels Preparation:
Initially a number of hydrogel formulations were prepared following the free-radical solution redox-polymerization of AM (1 g) in the presence of PVA(0.5) by varying different amounts of GA,(0.1,0.2,0.3, and 0.4 g) as constant keeping the concentration of cross-linker(MBA), initiator and activator as constant (Step 1). The corresponding iron salts incorporation into hydrogels (Step 2) and conversion of iron salts into Fe₃O₄ nano particles by reducing with of ammonia solution results in hydrogel magnetic nanocomposites (Step 3). The different hydrogels are shown in Fig.1 of experimental section.

4.3.2. Structure and uses of Gum acacia.
Gum acacia used in Food industry confectionery, beverages, dairy products bakery products, diabetic and dietetic products flavor fixative pharmaceutical industry cosmetic industry and other industries.

4.3.3 Swelling Studies:
The increase of gum acacia (GA) content (0.1 to 0.4 g) in the synthesis of hydrogels had lead to an increased swelling capacity. GA polymer chains impart more hydrophilicity to the hydrogel networks resulting in improved swelling capacity. Additional increase in the swelling capacity was also noticed when these PAM-PVA-GA hydrogels were loaded either with iron salts or after formation magnetic nanoparticles in the hydrogels. The overall order of the swelling capability of these hydrogels was found to be hydrogel magnetic nanocomposite > hydrogel loaded with iron salts > blank hydrogel. When the hydrogels were treated with iron ions, the ions may actually entrap or chemically interact with carboxylate anions of GA polymer or amide groups of PAM polymer chains, alcoholic groups of PVA polymer chains and disperses throughout the gel network.

The increase in the swelling capacity was still more in the case of magnetic nanocomposites. This pattern of swelling is reasonable for magnetic nanocomposites because once the magnetic nanoparticles are formed inside the gel networks, the overall porosity of the system increases allowing more number of water molecules inside the gel [18]. One more reason for this behaviour is that the formed nanoparticles have different sizes with different surface charges in the gel networks causing absolute expansion of the networks. Increase of crosslinker concentration in the hydrogel synthesis leads to decreased swelling capacities of hydrogels. The swelling studies are presented in the form of graph in Fig.2. Therefore, all further studies were limited to only hydrogel, PAM-PVA-
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GA4-Fe salt loded hydrogel and PAM-PVA-GA4-Magnetic Nano Composite hydrogel.

Figure 2. a) Swelling capacity of various hydrogels synthesized with different composition of PAM-PVA-GA4 hydrogel, PAM-PVA-GA4 iron salt loded and PAM-PVA-GA4- Nano hydrogel, b) Effect of MBA concentration on swelling ratio.

4.4 Characterization

4.4.1 FTIR spectroscopy

FTIR spectra of hydrogel, iron ion loaded hydrogel and HMNC are shown in Figure 3. All the hydrogels exhibited a common broad peak at 3438 cm⁻¹ which is due to the NH overlapping of stretching OH stretching and COOH stretching vibrations of the PAM-PVA-GA hydrogel. Peaks at 2911 cm⁻¹ are due to the CH stretching vibration of CH and CH₂ present in the hydrogel network. The peaks at 1636 cm⁻¹, 1425 cm⁻¹ and 1426 cm⁻¹ correspond to the carboxylic groups of GA unit, -NH coupled C-N vibration of -NH₂ group of Polyacrylamide unit respectively [19]. Disappearance of a peak at 1416 cm⁻¹ in iron salts loaded hydrogels suggests that iron salts block the amide bending bands and reappearance of the same peak in magnetic nano particles formed hydrogels confirms the conversion of iron ions into magnetic nano particles. A sharp peak appearing at 581 cm⁻¹ is due to the stretching
vibrations of the Fe-O bond confirming the presence of ion oxide nano particles in the hydrogel networks.

Fig:3 FTIR spectra PAM-PVA-GA4 hydrogel, PAM-PVA-GA4 Fe salt loded hydrogel, and PAM-PVA-GA4 magnetic nano hydrogel.

4.4.2. X-ray Diffraction (XRD):

X-ray diffraction patterns of pure hydrogel and hydrogel loaded with iron salts did not exhibit any sharp peaks (Figure 4). Whereas the hydrogel magnetic nanocomposite has exhibited characteristic $2\theta$ reflections at 20, 32, 35, 43, 55 and 62° arising due to the formation of a crystalline spinal phase of $\text{Fe}_3\text{O}_4$ nano particles encapsulated in the hydrogel network structure.
4.4.3. Thermogravimetric (TGA) analysis:

Thermogravimetric (TGA) analysis not only provides the thermal stability of the hydrogels and hydrogel magnetic nanocomposite but also supports the formation of magnetic nanoparticles. TGA analysis of hydrogels and hydrogel magnetic nanocomposites demonstrates all these samples were degrading in multiple stages (Figure 5. The order of stability of the samples at 800°C as noticed is Hydrogel magnetic nanocomposite > iron salts loaded hydrogel > hydrogel). Hydrogel, hydrogel loaded with iron salts, and hydrogel magnetic nanocomposite exhibited maximum residue of 43.5% 45% and 57.5%, respectively at 800°C. As expected the residue is increased with the content of Fe$_3$O$_4$ in the ferrogel.
Figure 5. Thermograms of PAM-PVA GA4 hydrogel, PAM-PVA-GA4 hydrogel loaded with Fe salts, and PAM-PVA-GA4 Magnetic nanocomposite.

4.4.4. Scanning electron microscopy (SEM).

Morphology of the samples can be exactly determined by scanning electron microscopic studies (SEM). The surface morphology cross-section of the iron ions loaded hydrogel (PAM-PVA-GA4-Fe salts loded) and hydrogel magnetic nanocomposite(PAM-PVA-GA4-Nano) were shown in Figure 6. The iron ions loaded hydrogel showed a randomly aggregated structures throughout the gel (Figure 6 (a)). However, there is a pinpoint variation in the case of magnetic nano particles formed in the hydrogel networks (Fig.6(b)). This clearly indicates the formation of magnetic nano particles along with the PAM-PVA-GA chains rather than just entrapment in the gel networks. The use of GA in the networks is to stabilize the formed nano particles in the gel networks.
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4.4.5 Transmission Electron Microscopy (TEM)

TEM provides very useful information about the particle size and polydispersity profile of the nano particles. Figure 7 shows the magnetic nano particles that were formed inside the hydrogel networks after extraction into aqueous media for about 2 weeks exhibited spherical in shape with an average size of ~ 9 nm. The polydispersity is not much in the system because the size of all the particles formed in the hydrogels is in between 5 and 12 nm. This study indicates that PAM-PVAGA4 sample has provided good platform for not only the formation of uniform magnetic nano particles of size of ~ 9 nm but also has stabilized them without forming any aggregations.
4.4.6 Magnetic properties (VSM Analysis)

Magnetic properties of a magnetic nanocomposite material are of great significance as their magnitude eventually determines the nature of application where the composite material has to be used in. Thus, realizing the need to explore the nature of magnetic behavior of the prepared nanocomposite, variation in their magnetic moments of the prepared hydrogel magnetic nanocomposite was investigated as a function of varying the magnetic field in the range of -14906 to 14906 Oe. The results are shown in Figure 8 which represents the M-H plots for the PAM-PVA-GA4 iron salts loded hydrogel and PAM-PVA-GA4 magnetic nanocomposite. The saturation magnetization (Ms) and coercivity (Hc) of the iron salts loded hydrogel found to be 5.5 emu/g, 1580 Oe and hydrogel magnetite nanocomposite were found to be 7.8 emu/g and 15845 Oe respectively for the above samples. This Ms values is far below compared to iron loded hydrogel (58.6 emu/g), pure magnetic nano particles (Fe₃O₄) (64.8 emu/g). But this values are expected because the presence of only ~9.8% of iron loded hydrogel , (theoretical Ms 4.601 emu/g, experimental 4.723 emu/g) and ~ 15.5% of magnetic nano particles in the hydrogel (theoretical Ms 9.144 emu/g, experimental 9.269 emu/g). Therefore, a single domain of magnetic nano particles formed within the hydrogel networks exhibit a unique phenomenon of superparamagnetism.
Figure 8. Magnetization saturation curves a) PAM-PVA-GA4 Fe salts loded hydrogel 
b) PAM-PVA-GA4 Magnetic nano composite.

4.4.7. Drug Loading and releasing studies

The loading efficiency of doxorubicin in hydrogels varies by the types of hydrogels. The order of efficiency as noticed was hydrogel magnetic nanocomposite > hydrogel loded with iron salt > blank hydrogel. The higher loading in magnetic nanocomposite is due to the presence of more amount of free space between the hydrogel networks because of nano particles formation as well as the doxorubicin molecules can also binds onto the surface of the nanoparticles.

It is well known that the delivery of drugs from the hydrogels system can be controlled by external stimuli. The doxorubicin release from magnetic nanocomposite was found to be in a sustained manner (Figure 9). Since doxorubicin is a hydrophobic molecule low amounts are entrapped in hydrogels. Therefore, these gels show a sustained drug release profiles. But, by employing an external applied magnetic field such as a magnet has improved the delivery of doxorubicin from magnetic nanocomposite into the medium (Figure 9). An improved release is caused by the alignment of magnetic nanoparticles by the external magnetic field which in turn expands the hydrogels networks that allows more number of doxorubicin molecules to release into the medium. This study clearly indicates that the developed hydrogel magnetic nanocomposite can be used as site drug release system for hyperthermia applications.

4.4.8. Doxorubicin structure.

Molecular formula, C_{27}H_{29}NO_{11}.HCl; MW, 580.0 and CAS number 25316-40-9. It is soluble in water and slightly soluble in methanol. Doxorubicin hydrochloride solution for
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injection is red in colour.

Doxorubicin hydrochloride is a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius varians caesius*. The chemical structure of doxorubicin consists of a tetracyclic ring with the sugar daunosamine attached by a glycosidic linkage. Structurally, doxorubicin is related to daunomycin (daunorubicin) and differs only in hydroxyl group substitution (instead of hydrogen) at the alkyl side chain at position '9' of the 'A' ring. It is supplied in solution form containing sodium chloride.
4.5. Conclusions:

An in-situ synthetic method was employed to prepare hydrogel magnetic nanocomposites (HMNCs) in which magnetic nano particles (Fe₃O₄) are scattered throughout the hydrogel networks of (PAM-PVA-GA). The formation of magnetic nano particles was confirmed by FTIR, XRD and thermal properties. The morphology, the size and size distribution of magnetic nano particles was verified by SEM and TEM analysis. The result has shown the formation of uniform magnetic nano particles of the size ~ 9 nm with less polydispersity in the hydrogel networks. The resulted magnetic nanocomposite was used as site drug delivery system for the release of doxorubicin a cancer treatment drug by applying external magnetic field. This is a gooa contribution in the field of site drug delivery systems for hyperthermia applications.
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References:

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