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Research Article

SYNTHESIS OF PH RESPONSIVE HYDROGEL–SILVER NANOCOMPOSITE FOR USE AS BIOMATERIALS

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ABSTRACT

Hydrogels are the intelligent materials that can absorb and release biological fluids in response to specific external stimuli. Such unique properties of hydrogels prompted us to synthesize a pH responsive semi-interpenetrating polymer network based on cross-linked poly(acrylamide) prepared through an optimized redox initiating free radical polymerization utilizing almond gum as a grafting backbone, N,N'-methylenebisacrylamide (MBA) as the cross-linker and ammonium persulphate (APS) – N,N,N',N'-tetramethylethylenediamine (TEMED) as the redox initiation pair. Silver ions are introduced and silver nanoparticles are generated within the hydrogel networks via insitu reduction of silver ions using Azadirachta Indica (neem) plant extract under atmospheric conditions. The synthesized hydrogel–silver nanocomposite (HSN) was characterized by UV-Visible diffused reflectance spectroscopy (DRS), fourier transform infrared spectroscopy (FT-IR), high resolution scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX) and thermogravimetric analysis (TGA). Swelling properties are studied in solutions of different pH and found that the varying response of HSN in acidic and basic media make it suitable as body water retainers and stomach bulking agents.

Keywords: Hydrogel, nanosilver, green synthesis, swelling, pH responsive polymer.

INTRODUCTION

Hydrogels are the semi-interpenetrating polymeric networks which possess good hydrophilic character. They can swell and imbibe large amount of water without dissolution[1]. The ability of hydrogels to absorb water arises from hydrophilic functional groups that are attached to the polymeric backbone while their resistance to dissolution is attributed to cross-linking that exists within the polymeric networks. These networks swell until the thermodynamic force of swelling is completely counterbalanced by the elastic, retroactive force exerted by the cross-links. The degree of swelling depends on both structural and environmental factors. Structural factors include degree of ionisation, density of cross-linking, extent of hydrophilicity, charge, concentration and pKa of the ionisable group[2,3]. Environmental factors like temperature, pH and ionic strength of the aqueous solution in contact with the polymer also affect the swelling characteristics[4,5].

In addition to the swelling characteristics, the soft, flexible nature, alikeness to natural living tissue, hypotoxicity and biodegradability have made hydrogels suitable for the development of a variety of smart devices such as artificial heart valves, synthetic muscles, fabricated contact lenses and substrates for site specific drug delivery[6-11]. Novel hydrogels based on synthetic, natural or hybrid polymers with excellent swelling ability, biocompatibility, biodegradability, processability and functionalization[12,13] have been synthesised. Innovative products are developed either as water absorbents for specific applications (e.g., personal hygiene products, underwater devices, water reservoirs for dry soils)[14-17] or as biomedical devices, including lubricating surface coatings, phantoms for ultrasound-based imaging, controlled drug release devices[18-20], wound dressings[21], cell immobilization islets, three-dimensional cell culture substrates, tissue engineering[22] and bioactive scaffolds for regenerative medicine[23-28].

It is well established that hydrogels based on polysaccharides possess better biocompatibility, biodegradability, non-toxicity and easily modified ability than various synthetic polymers. Among the polysaccharides, almond gum is used as preservative, binder, antioxidant, antibacterial agent[29], excipient, stabilizer and emulsifier in the food industry. Almond gum is a water soluble polymer which exudates from the trunk of the almond tree (P. dulcis) and consists of arabinose, xylitol, galactose and uronic acid with traces of rhamnose, mannose and glucose, thus suggesting an arabinogalactan structure of the gum[30]. Recently, silver nanoparticles are incorporated in to the hydrogel matrix due to extraordinary enhancement in some properties of hydrogels such as mechanical toughness, large deformability, high swelling/deswelling rates, excellent electrical conductivity, high transparency[31-33], cytotoxicity and remarkably strong antimicrobial activity with a fairly low toxicity against human tissues[34].

Based on the above findings, we report the synthesis of biodegradable semi-interpenetrating hydrogel (SIH) based on cross-linked poly(acrylamide) through a free radical polymerization utilizing almond gum as a grafting backbone, N,N'-methylenebisacrylamide (MBA) as the cross-linker and ammonium persulphate (APS) – N,N,N',N'-tetramethylethylenediamine (TEMED) as the redox initiation pair. Silver ions were embedded into the hydrogel networks and silver nanoparticles have been obtained as nanoreactors via insitu reduction of silver nitrate ($AgNO_3$) using Azadirachta Indica (Neem) plant extract which acts both as reducing and capping agent[35]. The structure of HSN, its swelling behavior, effect of pH and pH reversibility are investigated.

MATERIALS AND METHODS

MATERIALS

Acrylamide (AM), ammonium persulphate (APS), N,N'-methylenebisacrylamide (MBA), N,N,N',N'-tetramethylethylenediamine (TEMED), silver nitrate ($AgNO_3$) of reagent grade were supplied by S.D. Fine Chemicals (Mumbai, India) and used without further purification. Almond gum (AG) was also of reagent grade and purified before use. Double distilled water (DDW) used for the synthesis was procured locally.

Purification of Almond Gum

The gum was dried well, powdered and riddled through sieve No.100. It was solubilised in double distilled water, heated and the concentrated gum was precipitated in an ice cold ethanol[36]. The precipitated gum was separated and dried at 60ºC. The purified gum was finely grained and stored in air tight container.

Preparation of hydrogel silver nanocomposite (HSN)

AM (1.0 g) was dissolved in 5 ml of DDW followed by initiator APS (0.005 g in 1 ml of DDW) and cross-linker MBA (0.01 g in 1 ml of DDW). To this solution, AG (0.1 g in 5 mL of DDW) and TEMED (0.02 mL in 1 mL of DDW) were added. The reaction mixture was stirred and heated at 60° C for 5 minutes. The polymerization reaction results in the formation of SIH (**Fig. 1(a)**) within 10 minutes of reaction time. The formed hydrogel was equilibrated with water for 72 h and with $AgNO_3$ (5mM) solution for 24 h respectively. These silver salt loaded hydrogel was transferred into a beaker containing 50 ml of neem plant extract and allowed to stand for 12 h to reduce the silver ions into silver nanoparticles (**Fig. 1(b)**).

UV-Visible diffused reflectance spectra (DRS)

UV-Visible DRS of SIH and HSN were recorded on the Shimadzu 2550 spectrophotometer using $BaSO_4$ as the reference material.

Fourier transform infrared spectra (FT-IR)

FT-IR spectra of completely dried SIH and HSN were recorded with a Perkin Elmer FT-IR spectrometer – Spectrum.RX1 (USA) of scan range 500–4000 cm^{-1}. The instrument was purged with dry air before data collection and during measurements to eliminate error due to moisture absorption.

High resolution scanning electron microscopy (HR-SEM)

HR-SEM analysis was performed using Tescan Vega3 SBU variable pressure scanning electron microscope with 0.2 ml of finely grinded SIH and HSN dispersions on a copper grid dried at room temperature after removing excess solution using filter paper.

Thermogravimetric analysis

The thermal stability of SIH and HSN was evaluated using SII EXSTAR 6000 thermal system (Japan) at a heating rate of 10 ºC per minute and a flow rate of 10 ml per minute under nitrogen atmosphere.

Swelling studies

Dried sample (Hydrogel, Hydrogel-Ag^+, HSN) of known weights were put in separate tea bags (i.e., a 100 mesh nylon screen) and immersed in 100 mL of DDW at 37°C. After the specific time interval the tea bags were taken out and hung up for 5 min, in order to remove the excess solution, and weighed. Similarly, the swelling ability of HSN in various buffer solutions was studied. The swelling ratio (Q) of the gels was calculated from the equation

$Q = W_e/W_d$, where W_e is the weight of swollen hydrogel and W_d is the weight of the dry hydrogel.

RESULTS AND DISCUSSIONS

In the present work, we have designed a novel pathway for the synthesis of HSN exploiting polysaccharide (AG) which usually couple there biodegradability with stimuli sensitive response. In this method, the reduction potential/anchoring ability of the hydrogel is increased along with the stabilization of embedded nanoparticles[17-20]. In any conventional hydrogel networks, the functional groups present and its cross-linking density decides the stability of nanoparticles. Hence, we have synthesized nanometer sized homogeneous dispersion of silver nanoparticles in SIH networks employing poly(acrylamide) with AG matrices. A prominent feature of this methodology is that the nanoparticles were simply obtained at room temperature in presence of environmental friendly stabilizers. In general, the PAM – AG cross-linked networks acts as reservoir for metal ions uptake and anchor it through carboxylic, amide and hydroxyl groups. The polymeric network also facilitates the reduction of silver ions into nanosilver and stabilizes it by preventing the aggregation of silver nanoparticles. It is quite interesting to indicate that the silver nanoparticles were formed solely inside the hydrogel networks and not in the surrounding medium. This confirms the binding of silver nanoparticles to the hydrogel networks as well as its storage without releasing into the media. The scheme of formation of silver nanoparticles inside the SIH networks is given below:

$$\text{SIH} + AgNO_3 \longrightarrow \text{SIH} - Ag^+ \xrightarrow{\text{Neem leaf extract}} \text{SIH – Silver nanocomposite}$$

UV-Visible diffused reflectance spectra (DRS)

The perseverance of silver nanoparticles in the hydrogel networks was confirmed by a distinct peak around 380 – 400 nm in the diffused reflectance UV-Visible spectra (**Fig. 2(a)**). The peak arises because of surface plasmon resonance (SPR) effect caused by the quantum size of the silver nanoparticles[37] whereas the placebo hydrogel have shown no such peak in the diffused reflectance UV-Visible spectra due to the absence of nanosilver (**Fig. 2(b)**).

Fourier transform infrared spectra (FT-IR)

IR spectra of the poly(acrylamide) based hydrogel (**Fig. 3**(**a**)) shows the presence of absorption bands characteristic to cross-linking bridges. Absorption band attribution was made in agreement with the values given in literature[38]. The absorption bands at 3335 and 1647 cm^{-1} corresponds to –OH stretching vibrations from the cross-linking bridges and –C=O extension vibrations of amide groups respectively. A band at 3202 cm^{-1} arising from the stretching vibration of the amide group, which is obvious in the spectrum of polyacrylamide, proved the presence of acrylamide in the structure of synthesized hydrogel[39]. The symmetric valence vibration at 2916 cm^{-1} is assigned to the $–CH_2$ groups between the macromolecular chains and cross-linking bridges. The asymmetric stretching vibration of ester groups of AG is observed at 1600 cm^{-1}. In the IR spectra of HSN **(Fig. 3(b))**, the peaks were shifted to lower wave numbers (3326, 3192, 1642, 1591 cm^{-1}) due to the coordination between the nanosilver and electron rich groups (relating to –OH, –NH, –C=O, $–COO^-$)[40,41] present in the hydrogel networks. Therefore, we can confirm the presence of silver nanoparticles in the hydrogel networks.

High resolution scanning electron microscopy (HR-SEM)

The SEM micrographs of SIH showed a smooth surface feature (**Fig. 4(a)**) whereas HSN showed shrunken surface due to the presence of nanosilver. The formed silver nanoparticles are rod shaped, well distributed without aggregation and with an average size of about 75 nm (**Fig. 4(b))**.

Energy dispersive X-ray spectrum (EDX) analysis

The EDX spectrum of SIH (**Fig. 5(a)**) shows the presence of C and O signals only whereas the EDX spectrum of HSN **(Fig. 5(b))** shows the presence of Ag in addition to C and O elements. This reveals the presence of silver nanoparticles in the hydrogel and that the weight percentage of silver is about 6.10 % as depicted in **Fig. 5(c)**.

Thermogravimetric analysis

The thermogram of HSN (**Fig. 6(a))** mainly showed two stage decomposition behavior[42]. The first major stage of decomposition is characterized by the initial decomposition temperature (IDT) in the range 230-260 ºC and final decomposition temperature (FDT) in the range 310-380 ºC. This stage resulted in 38 % weight loss. For the second major decomposition stage, the IDT range was 420-450 ºC and FDT range was 500-580 ºC. The weight loss was around 31 % which was attributed to the complete degradation of the hydrogel. However, the TGA thermogram of SIH **(Fig. 6(b))** showed two decomposition curves[43] and degradation of the hydrogel starts around 230 ºC and continues up to 500 ºC. It is important to note that the thermal stability of HSN is higher than SIH. The high thermal stability of the HSN can be attributed to the presence of large quantity of nanosilver inside the hydrogel network.

Swelling capacity of Hydrogel, Hydrogel-Ag$^+$, HSN

The swelling capacity of a hydrogel is because of hydrophilic groups which were entrapped inside the networks. It was known that the polar head groups of polymeric chains such as –OH, –COOH, $–NH_2$, $–CONH_2$, $–SO_3H$ have high affinity for salts[44-46]. The loading of silver ions throughout the gel networks causes repulsion among these networks, which ultimately leads to an improved swelling behavior of hydrogel systems. Further increase in swelling capacity was observed after the addition of reducing agent (neem extract) to silver ions loaded SIH. This is due to the formation of silver nanoparticles throughout the gel networks which increases the overall porosity of system and makes entry for more number of water molecules inside the gel. The order of swelling was**:** **HSN > Ag$^+$ loaded SIH > SIH**

Effect of pH on swelling capacity of HSN

The swelling behavior of HSN in various pH was observed by placing 0.1 g of HSN in pH 2, 4, 6, 8 and 10 solutions respectively and examining them after one hour. It was noticed that the amount of swelling increases with increase in pH from 2 to 6, reaches maximum at pH 8 and then decreases as shown in **Fig. 7**. The repulsion between $–COO^-$ groups in the hydrogel is the main reason for the maximum swelling at pH 8. Below pH 8, the H^+ ions in the external medium effectively suppress the ionisation of the carboxyl and hydroxyl groups of AG. This decreases the number of mobile ions inside the HSN which causes decrease in osmotic pressure and hence the swelling capacity of HSN. Above pH 8, the –OH groups can ionise to –OR which reacts with $–COO^-$ to form esters. This increases the number of networks inside the hydrogel and also decreases the hydrophilicity which is responsible for the decrease in swelling.

Effect of pH reversibility on swelling capacity of HSN

A known weight of HSN was put in pH 2 and pH 8 solutions alternatively at every 30 minutes interval and swelling ratio was recorded. **Fig. 8** shows the reversible swelling-deswelling behavior of HSN in solutions with pH 2 and 8. At pH 8, the hydrogel swells due to anion-anion repulsive electrostatic forces, while at pH 2, it shrinks within a few minutes due to protonation of the carboxylate anions. This swelling-deswelling behavior of the hydrogel makes HSN a suitable option for stomach bulking agent and body water retainers.

APPLICATIONS

Body Water Retainers

In this work, a biocompatible hydrogel has been developed with the aim of removing excess water from the body after ingestion via the faecal route. Water removal is extremely important in the treatment of some pathological conditions such as renal failure and diuretic-resistant oedemas. The HSN in powder form can be administered orally to absorb water in its passage through the intestine, where the pH is about 6-7, without previously swelling in the acidic environment of the stomach. The HSN is then expelled through the faecal way, thus performing its function without interfering with body functions.

Stomach Bulking Agents

Obesity and overweight represent the second cause of death after smoking in the developed countries. These are also the major risk factors for several chronic diseases, such as type 2 diabetes, cardiovascular disease, sleep apnoea, hypertension, stroke and certain forms of cancer. The treatment of overweight and obesity usually consists of a supervised diet, often combined with adequate physical exercise. In the extreme cases, surgical procedures, that involve essentially gastric restriction or particular drug treatments, may be required. However, in the recent years, a number of dietary supplements and meal replacements have been developed and sold as over-the-counter slimming aids. Dietary supplements are claimed to act either by binding fats and so reducing fat absorption, as reported for cellulose-based and chitosan-based products, or by directly reducing the appetite, as for different natural fibers and herbal products, that seem to absorb liquids and swell inside the stomach, thus giving a sense of fullness[47,48]. The basic idea of our work is that HSN-based pill can be administered orally before each meal and that the HSN powder swells once it reaches the stomach. In such a way, the space available for food intake is reduced giving a feeling of fullness. The swollen hydrogel is then eliminated from the body by faecal way. In this perspective, the hydrogel is envisaged to pass through the gastrointestinal tract, thus it is supposed to encounter the different pH environments of the stomach and the intestine. Thus pH-responsive water retention capacity of HSN renders its use as stomach bulking agent.

CONCLUSION

Novel almond gum / poly(acrylamide) semi-interpenetrating hydrogel – silver nanocomposite was successfully prepared via free radical polymerization followed by insitu reduction of silver ions to nanosilver using neem leaf extract as a clean and green reducing agent. The methodology developed for the synthesis of SIH is very simple, rapid and cost effective which makes it easy to implement in the industries. A number of HSN were formulated with high dispersion rates by varying the concentrations of AG. The formed HSN was characterized by different techniques. In the UV-Visible spectra, nanosilver have shown good surface plasmon resonance behavior. In FT-IR spectra, shifting of peaks to lower wave number occurred. SEM images confirm the presence of well defined silver nanoparticles and its stability was further confirmed by thermal analysis. Incorporation of silver nanoparticles increased the swelling capacity of hydrogel. The swelling response of the prepared HSN against pH made it suitable as body water retainers and stomach bulking agents for which specific tests will be performed subsequently.

TABLES AND FIGURES

Fig.1: (a) SIH and (b) HSN

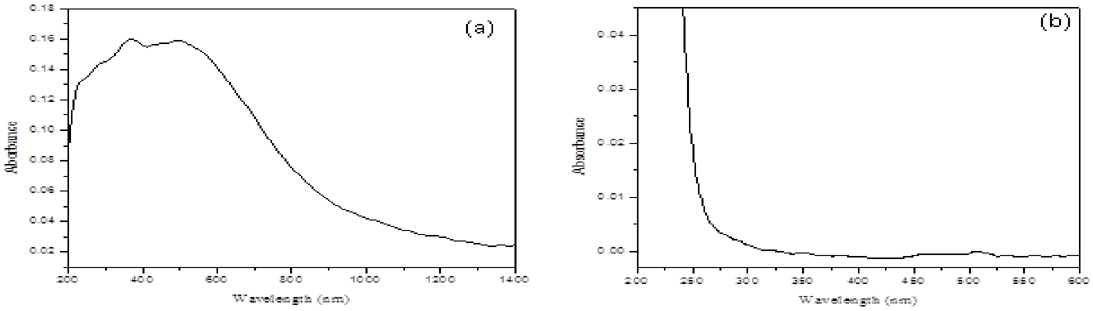


Fig. 2: Diffused Reflectance spectra of (a) HSN (b) SIH

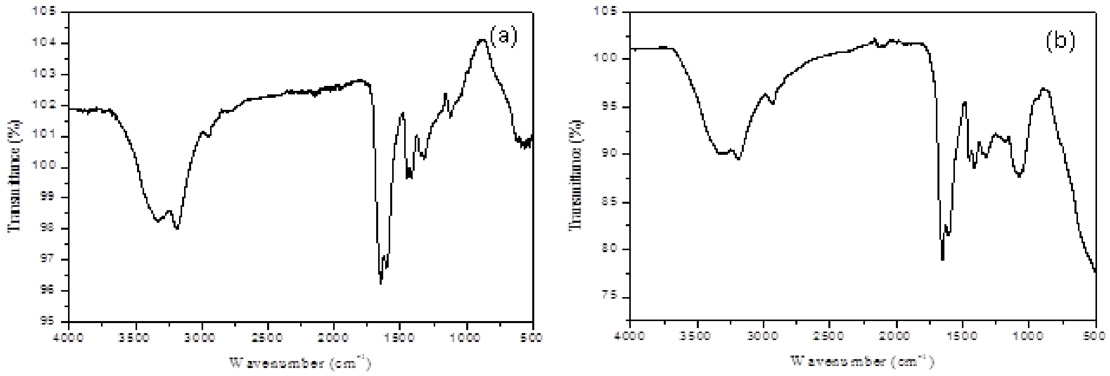


Fig. 3: FT-IR Spectra of (a) SIH (b) HSN

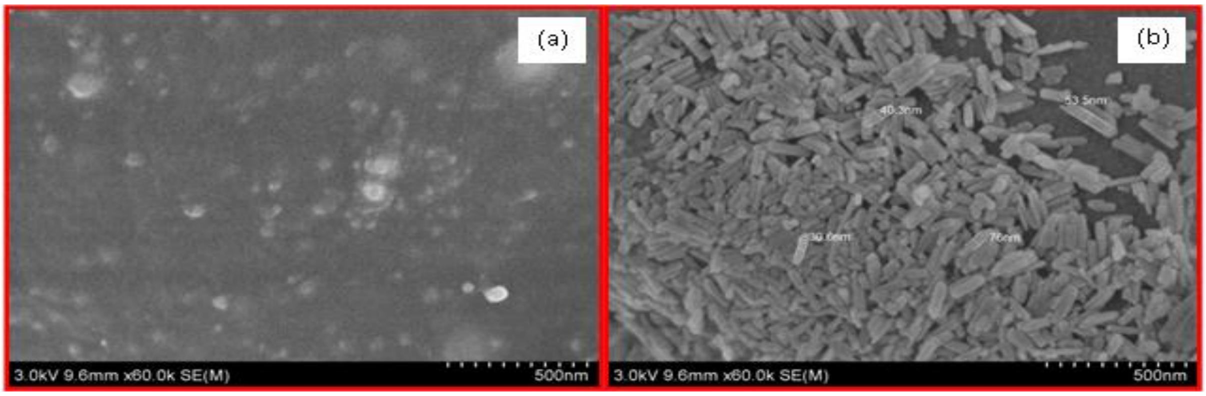
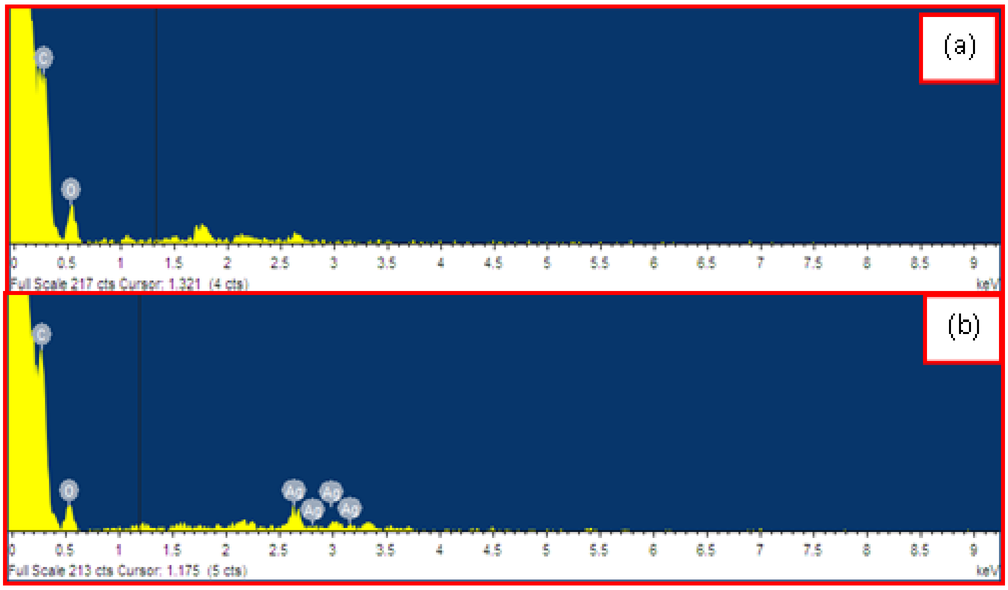


Fig. 4: SEM image of (a) SIH (b) HSN



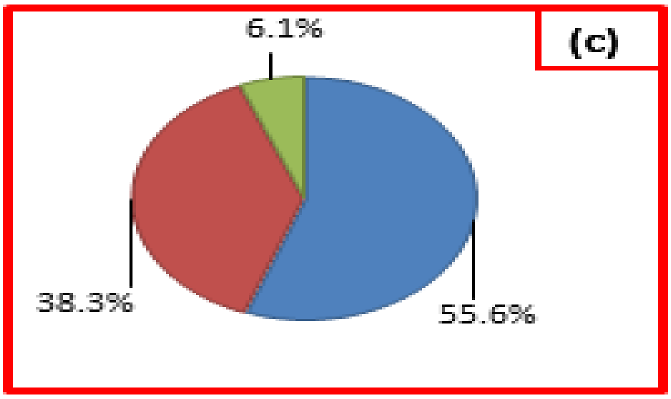


Fig. 5: EDX spectrum of (a) SIH (b) HSN and
(c) Quantitative results of EDX spectrum of HSN

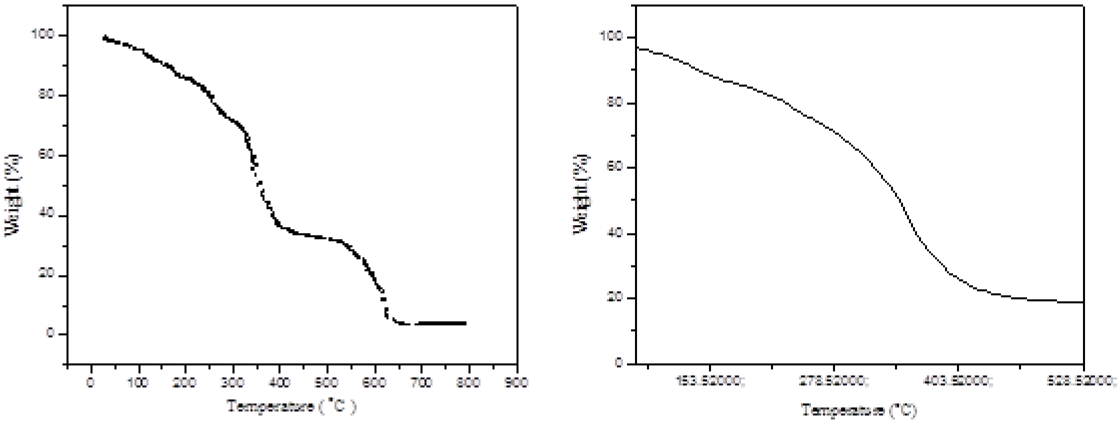


Fig. 6: Thermogram of (a) HSN and (b) SIH

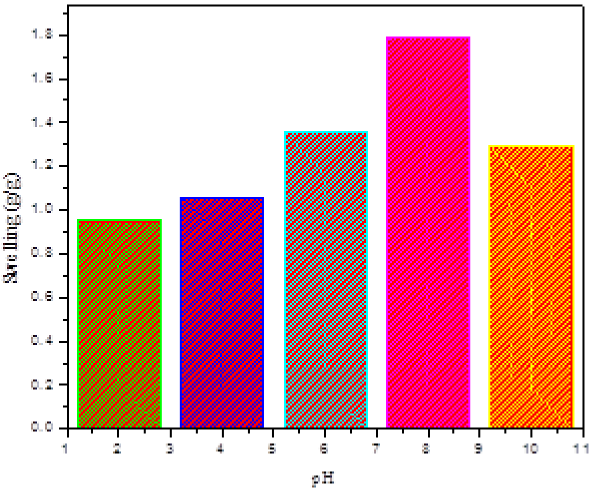


Fig. 7: Effect of pH on swelling
capacity of HSN at 37°C

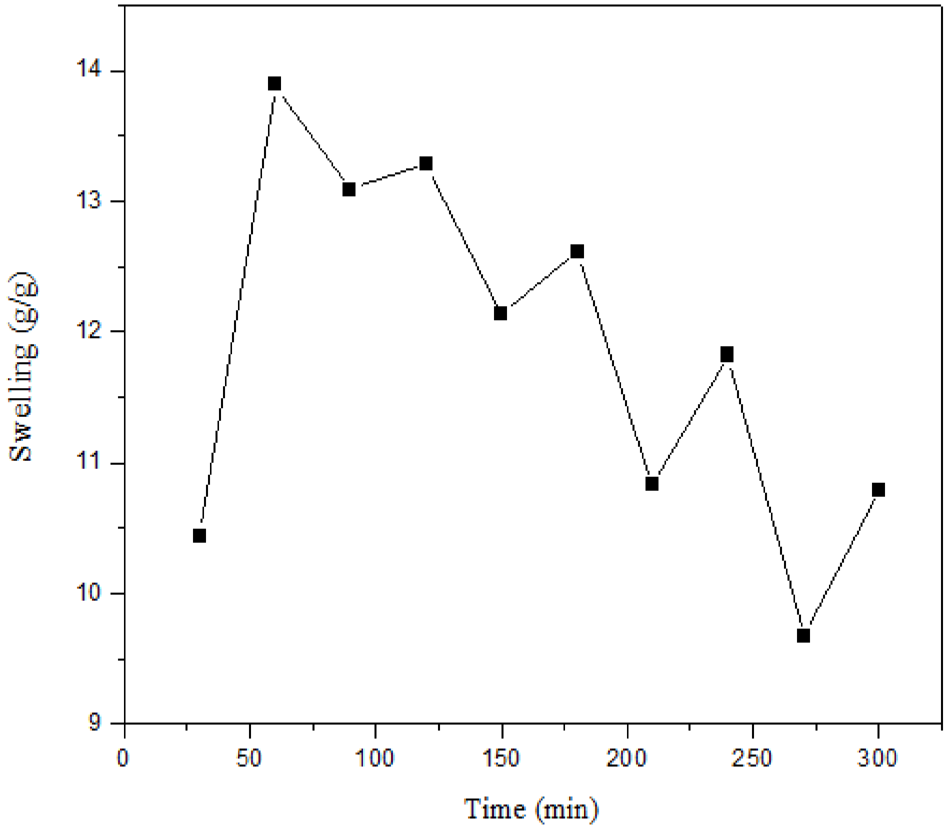


Fig. 8: pH reversibility of HSN with 30 min time interval at 37 °C

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