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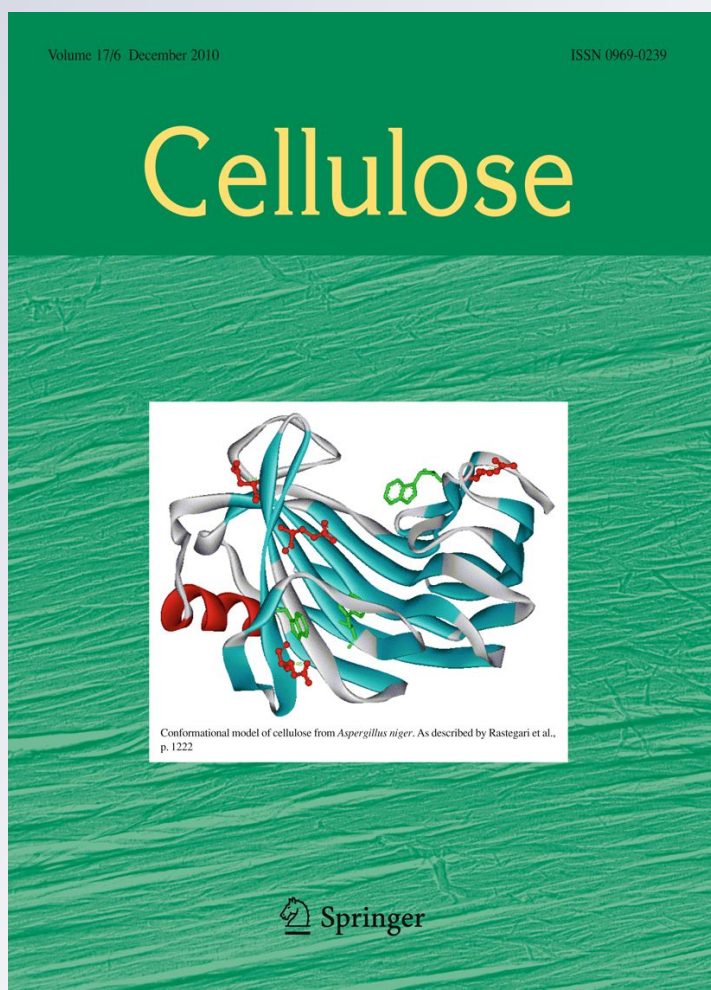
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Synthesis and characterization of antibacterial semi-interpenetrating carboxymethyl chitosan/poly (acrylonitrile) hydrogels

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Abstract Blend hydrogels composed of carboxymethyl chitosan (CMCh) and poly (acrylonitrile) (PAN) were synthesized via crosslinking method. Several analyses were made to investigate both physical and thermal properties of CMCh/PAN hydrogels like; FTIR, scanning electron microscope, XRD and thermogravimetric analysis (TGA). TGA results showed that CMCh/PAN hydrogels are thermally more stable than CMCh and their thermal stability increases as PAN content increases in the hydrogel. Moreover, the swelling behavior of CMCh/PAN hydrogels was studied in different buffer solutions. It was found that CMCh/PAN hydrogels swell much more than PAN especially at pH 9. The hydrogels sorption for different dyestuff and various metal ions like; Cu^{2+} , Cd^{2+} and Co^{2+} were also studied. In this work, antibacterial characteristic of hydrogels was mainly investigated towards *Escherichia coli* (*E. coli*) as a serious disease-leading bacterium. All tested hydrogels have clearly presented good antibacterial activity as CMCh content increases in the hydrogels.

Keywords Hydrogels · Swell ability · Metal uptake · Dyes · Antibacterial activity · Thermal stability

Introduction

Hydrogels are important class of polymeric materials that have been utilized in a wide variety of biomedical and pharmaceutical applications. Hydrogels are three-dimensional crosslinked polymeric networks that can swell dramatically in an aqueous medium and can imbibe water or biological fluids, while maintaining their structures. Highly hydrated hydrogels are biocompatible as their structures are very similar to natural tissues which can be partially attributed to their soft, flexible nature and high water content. During the past several decades, hydrogels thereby found various biomedical and pharmaceutical interests and applications (Simonida et al. 2010; Langer and Peppas 2003). Hydrogels consist of polymeric chains with either physical or chemical cross-links that prevent their dissolution and instead result in swelling of the material upon interaction with aqueous solutions. Currently, a variety of novel crosslinking methods have been employed to prepare different kinds of hydrogels (Hennink and Van Nostrum 2002). From the main polymers used in hydrogels preparation are Chitosan and its derivatives. Chitosan is the *N*-deacetylated product of chitin, a biocompatible and biodegradable polysaccharide, which has been known long for its gel forming ability and has been extensively studied as physical gels (Guoming et al. 2007; Kim et al. 2000; Yan et al. 2001; Cheng et al. 2003). Chitosan has many useful properties such as mucoadhesive, antimicrobial, anticoagulant, and immune

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stimulant. Because of its unusual biological activity, the use of chitosan as drug carrier would have dual therapeutic effects, which made chitosan a novel drug carrier candidate. Thereby chitosan has been intensively investigated in the biomedical (Long et al. 2003) and pharmaceutical fields (Singh and Ray 2000). To improve the solubility of chitosan, generally, chemical modification is required. Partially acetylated chitosans, which have a degree of deacetylation around 50%, are able to dissolve in pure water. Recently, attention of various researchers was directed towards carboxymethyl chitosan as a chitosan derivative having carboxymethyl substituents on some of either amino or primary hydroxyl sites of the glucosamine units of the chitosan structure (Liu et al. 2001). This property gives carboxymethyl chitosan more solubility and chelating properties. It was reported that carboxymethyl chitosan is non-toxic, either in vitro in fibroblast culture assays or in vivo in, testing with intraperitoneal, oral, or subcutaneous treatments (Kennedy et al. 1996). Additionally, it is suitable as an excipient in ophthalmic formations to improve the retention and bioavailability of drugs (Muzzarelli et al. 1999).

As carboxymethyl chitosan is a hydrophilic polymer, it imparts hydrophilicity to the prepared hydrogels by the formation of some composites with another mechanically stronger and hydrophobic materials such as poly (acrylonitrile). Polyacrylonitrile (PAN) is a semicrystalline vinylic homopolymer with the repeating unit—(CH₂—CHCN)—, usually in atactic form. Several studies have shown that the use of PAN in electrolyte systems can have several advantages (Dissanayake et al. 2002). PAN systems show good mechanical strength as fibres and are thermally stable. PAN is mainly used in soft cloth furnishings, but it is also an important carbon fiber precursor. Grafting copolymers of CMCh with PAN (CMCh-g-PAN) were found to adsorb more heavy metals ions than CMCh alone and that the adsorption is more pronounced in case of their amidoxime derivatives (Saba et al. 2010a, b). Moreover, it was shown that as the graft % increases, the ability of CMCh to attract more dye molecules increases and that this ability increases also for the amidoxime derivatives. Seon et al. studied the temperature- and pH-responsive of the semi-interpenetrating polymer network (semi-IPN) hydrogels constructed from chitosan and polyacrylonitrile (PAN) (Seon et al. 2003a, b). The characterizations of the prepared semi-

IPN hydrogels were investigated using FTIR and DSC. Semi-IPN hydrogels exhibited a relatively high swelling ratio at room temperature. The swelling ratio of hydrogels depends on pH and temperature. DSC was used to determine the amount of free water in semi-IPN hydrogels as the amount of free water increased with increasing chitosan content in the hydrogels. Synthesis and controlled release of a model drug, gentamicin from a superabsorbent hydrogel based chitosan and polyacrylonitrile (PAN) was studied by Hossein (2011). Swelling capacity was conducted in solutions with pH ranged from 1 to 13. The hydrogels exhibited a pH-responsiveness character so that a swelling-deswelling behavior was recorded at pHs 2 and 8. This on-off switching behavior makes the hydrogel as a good candidate for controlled delivery of bioactive agents. Therefore, loading and the in vitro controlled drug-release behaviors of these hydrogels were investigated in detail.

The present work aimed to prepare hydrogels from CMCh and PAN in presence of agar as a chemical cross-linking agent. The prepared semi-IPN hydrogels have been examined for their swellability and their potential applications in many environmental areas for removal of heavy metals ions and other toxic materials, as well as for the sorption of a number of dyes in solution as an alternative to conventional sorbents. This could be a “green” solution for environment treatment form hazardous materials. This study has been extended to the examination of the prepared hydrogels for their thermal stability and their potential as antibacterial agent for *E. Coli*.

Experimental

Materials

Chitosan (code KB-002) was purchased from Funakoshi Co. LTD, Japan. Deacetylation content = 88.2%. Acrylonitrile (AN) was purchased from Kanto Chemical (Tokyo, Japan) and used without further purification. Nutrient Agar was purchased from EDM Company, Egypt. Monochloroacetic acid was purchased from LOBA chemie PVT. Ltd.—Bombay, India. Congo red dye (acidic dye), Maxilon Blue dye (cationic dye) and Methylene Blue (basic dye) were purchased from G.T. Gurr, London, s.w.6. Cu²⁺, Co²⁺ and Cd²⁺ chloride salts were purchased from Aldrich.

Preparation of carboxymethyl chitosan

Carboxymethylation was carried out by stirring 5 g chitosan in 100 mL 20% NaOH (w/v) for 15 min. 15 g monochloroacetic acid was added dropwisely to the reaction medium and the reaction was continued for 2 h at 40 °C with continuous stirring. The reaction mixture was then neutralized with 10% acetic acid, poured into an excess of 70% methanol, filtered by using a G₂ sintered funnel and washed with methanol. The produced carboxymethyl chitosan was dried in a vacuum oven at 55 °C for 8 h to give 6.5 g dried carboxymethyl chitosan, CMCh (Cheng et al. 2003; Sabaa et al. 2010a, b). The degree of substitution of CMCh was determined to be 0.75 according to the method described in literature (Wu et al. 2003).

Preparation of poly (acrylonitrile) (PAN)

Solution polymerization of acrylonitrile (AN) was done by adding 1.5 mol/L of the monomer in 25 mL distilled water in a round- bottomed flask and the reaction temperature was adjusted at 60 °C (using a thermo-stated water bath). Freshly prepared potassium persulphate (6.75 g)/sodium bisulfite (2.6 g) solution initiator was added portion wise and the reaction was conducted for 3 h with continuous stirring. PAN was precipitated in water, collected with filtration and dried in an air-oven (%) yield = 88%. M_w was found to be 11.58×10^5 g/mol and M_n 7.26×10^5 g/mol, [PD] = 1.59.

Preparation of CMCh/PAN hydrogels

CMCh (4 g) was dissolved in 25 mL degassed distilled water and PAN (4 g) was dissolved in 25 mL DMF. Some turbidity was noticed upon mixing. CMCh/PAN in the final solution was made up of weight ratio 1:1, 1:3, 3:1. Agar (2 g) was added to the above mixture and stirred by mechanical stirring for 2 h. The mixed solution was poured into a Petri-dish and dried in an oven at 60 °C for 48 h. The hydrogel was then removed and washed with deionized water to remove the unreacted materials; the hydrogel was dipped into deionized water for 48 h and dried in a 40 °C vacuum oven for 2 days, as reported in literature for preparation of chitosan/PAN hydrogels (Seon et al. 2003a, b).

Gel fraction

Hydrogel samples were soxhlet extracted first in dimethyl formamide for 72 h to get rid of uncross-linked PAN then soxhlet extracted with boiling water for 4 h to remove any uncrosslinked CMCh. The remained crosslinked gel was separated by centrifugation, dried to constant weight at 70 °C. Gel fraction was measured gravimetrically:

$$\text{Gel fraction (\%)} = W_d/W_o \times 100$$

where W_d is the weight of dry gel after extraction and W_o is the initial weight of dry gel.

Instrumentation

FTIR spectra were recorded in KBr discs on (FTIR model 8000) Testcan Shimadzu IR-Spectrometer under dry air at room temperature within the wave number range of 4,000–600 cm^{-1} .

SEM analysis was done in a JEOL-JXA-840A Electron probe microanalyzer at 20 kV. The dry sample, spread on a double sided conducting adhesive tape, pasted on a metallic stub, was coated (100 μ) with gold in an ion sputter coating unit (JEOL S150A) for 2 min.

Atomic absorption was done on AAnalyst 100 winlab- Perkin Elmer to determine the amount of metal ions remaining in the hydrogel liquor.

Colorimetric Spectrophotometry was done on Unico 1200 Spectrophotometer at λ_{max} 480 nm for Congo red dye, λ_{max} 580 nm for Maxilon blue dye and λ_{max} 660 nm for Methylene blue dye, respectively.

Thermal analysis was done on TGA-50H Shimadzu thermogravimetric analyzer. Samples were heated from 0 to 500 °C in a platinum pan with a heating rate 10 °C/min in N₂ atmosphere at a flow rate of 25 mL/min.

Applications done on CMCh/PAN hydrogels

Swelling studies

Water uptake of hydrogels was studied at 30 °C in doubly distilled water and in buffered solutions of different pH values; 4 and 9. A known weight of pre-dried sample was placed into a flask with 25 mL water or buffer solution of the given pH and kept undisturbed

in a thermostated water bath (at 30 °C) until equilibrium swelling was reached (~24 h). After wiping off the excessive water on the sample surface with filter paper, the weight of the swollen samples was measured. The swelling percentage was determined according to the following equation (Yingshan et al. 2009):

$$\text{Water uptake \%} = [(W_s - W_o)/W_o] \times 100$$

W_s = weight of wet sample, W_o = weight of dry sample.

The results obtained represent the average of three comparable experiments for each sample.

Dye uptake

Three types of dyes were used; Congo red (acidic dye), Maxilon Blue (cationic dye) and Methylene blue (basic dye). 25 mL of the dye solution of a known concentration was added to 100 mg of the hydrogel in 100 mL flat bottomed flask and stirred continuously at room temperature for 24 h to reach equilibrium. After filtration, the concentration of the dye in the filtrate was determined colorimetrically at 480, 580 and 660 nm for the three investigated dyes, respectively. The quantity of the adsorbed dye was calculated according to the following equation (Crinia et al. 2008):

$$Q = (N_a - N_s)/W$$

Q = fixed quantity of dye (mg)/hydrogel (g), N_a = quantity of original dye (mg), N_s = quantity of remaining dye after adsorption (mg), W = mass of hydrogel (g).

Adsorption of heavy metals ions

Chloride salts of the heavy metals ions (Co^{2+} , Cd^{2+} , Cu^{2+}) solutions of known concentrations were prepared (0.05 mol/L), then 0.1 g of the hydrogel was soaked into 25 mL salt solution, stirred for 24 h at room temperature till equilibrium is attained. After filtration, the remaining metals ions in the solution was estimated using atomic absorption technique—after soaking the hydrogel—to determine the amount of metal ions remaining in the liquor and consequently,

the amount of adsorbed metal ions can be calculated by difference according to the following relation:

$$q(\text{mg/g wet weight}) = V(C_o - C_A)/1000 W$$

where $V(L)$ is the volume of the salt solutions, $W(g)$ is the weight of the hydrogel, $C_o(\text{mg/L})$ is the initial metal concentrations and $C_A(\text{mg/L})$ is the metal concentration at definite time (Qunwei et al. 2009).

Antibacterial activity tests for CMCh/PAN hydrogels

All tests were performed under aseptic conditions. The method of (Jiang et al. 2005) was adopted. One loopful of fresh bacteria (*E. coli*) was suspended in an appropriate amount of sterilized saline solution, forming a bacterial cell suspension. The viable cell number in the suspension was controlled via the turbidity comparison method. This suspension was diluted to a prescribed cell concentration with sterilized distilled saline solution, thus preparing a bacterial cell suspension that was directly used for the antibacterial tests for the hydrogels.

In each test, 20 mL of the bacterial suspension and 0.2 g (dry weight) of the tested hydrogels were placed in a sterilized glass container with a cotton stopper. The container was shaken for 2 h (150 rpm), 1 mL of this suspension system was pipetted out from the container and quickly mixed with sterilized saline and then decimal serial dilutions were prepared. The viable cell number in each of the hydrogel/bacterial suspension systems at the contact time was determined by conventional spread-plate method. Replicates were made and colonies were counted after 24 h of incubation on Nutrient Agar medium at 37 °C. The percentage of inhibition was counted as follows:

$$\% \text{ Inhibition} = \frac{\text{Untreated} - \text{Tested hydrogel}}{\text{Untreated}} \times 100$$

Results and discussion

IR analyses of gel portion

To elucidate the formation mechanism of the CMCh/PAN hydrogels, the FTIR spectra of the formed gels were studied. The results are shown in Fig. 1. FTIR spectra of PAN and CMCh were also shown for comparison.

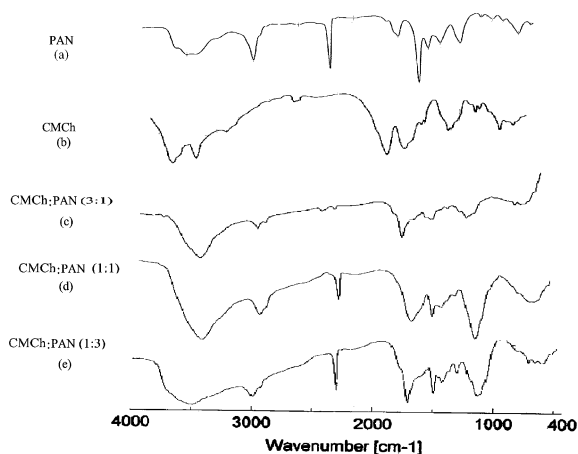


Fig. 1 IR Spectrum for PAN, CMCh and CMCh/PAN hydrogels with different ratios

The FTIR spectrum of CMCh shows that the main characteristic peaks: at $3,429\text{ cm}^{-1}$ strong broad band ascribed to both the hydrogen-bonded $-\text{OH}$ and $-\text{NH}$ groups, $2,879\text{ cm}^{-1}$ ($\text{C}-\text{H}$ stretching), $1,657\text{ cm}^{-1}$ ($\text{N}-\text{H}$ bending), $1,320\text{ cm}^{-1}$ ($\text{C}-\text{N}$ stretching), $1,156\text{ cm}^{-1}$ (bridge O stretching), and $1,075\text{ cm}^{-1}$ ($\text{C}-\text{O}$ stretching). $1,765\text{ cm}^{-1}$ ascribed to the amino group ($-\text{NH}_2$ deformation), the FTIR spectrum of CMCh also showed a characteristic peak (at $1,602\text{ cm}^{-1}$) for associated carboxylic acid salt ($-\text{COO}$ asymmetric stretching) suggesting the presence of carboxymethyl groups. Furthermore, the peaks observed at $1,069\text{ cm}^{-1}$ specific for the secondary hydroxyl groups (characteristic peak of $-\text{CH}$, $-\text{OH}$ in cyclic alcohols, $\text{C}-\text{O}$ stretch).

PAN characteristic functional groups of nitrile ($-\text{CN}$) stretching band appears at $2,240\text{ cm}^{-1}$. In the FTIR spectra of the CMCh/PAN hydrogels, the doublet peak of $-\text{NH}_2$ at both $3,429$ and $3,600\text{ cm}^{-1}$ disappeared and a single peak appeared instead, indicating its conversion to $-\text{NH}-$ group due to the interpenetration with PAN. A peak appears at $2,246\text{ cm}^{-1}$ characterizing $\text{C}\equiv\text{N}$ groups, which increases in intensity as PAN content increases in hydrogel. This indicates that the $\text{C}\equiv\text{N}$ groups are free, which means that the PAN doesn't contribute into the crosslinking process of CMCh by using Agar. Also a sharp band appears at $1,091\text{ cm}^{-1}$ characteristic for $\text{C}-\text{N}$ stretching vibration (Seon et al. 2003a, b; Bumsuk et al. 2004). Both bands of CMCh and PAN appear in the FTIR chart of the

prepared hydrogel showing the formation of semi-IPN hydrogels.

Scanning electron microscopy: (magnification $\times 1,000$)

The scanning electron micrographs of Chitosan, Carboxymethyl chitosan (CMCh), CMCh/PAN hydrogels are shown in Fig. 2. It is known that carboxymethylation process to chitosan, followed by hydrogel formation, completely modified the surface morphology of chitosan. The flaky nature of chitosan was totally modified due to the formation of carboxymethyl chitosan (CMCh) as there appear more lumps on the smooth surface of chitosan due to the formation of the bulky $-\text{COOH}$ groups on the surface. Study of the scanning electron microscopy (SEM) verified that the addition of PAN indeed made the hydrogel surface become quite fibrous in nature, thus may provide maximum surface area for metal ions sorption (Fig. 2), similar behavior has been shown for chitosan/poly (vinyl alcohol) chemically cross-linked blends (Ezequiel et al. 2009).

CMCh/PAN hydrogels showed a fibrous morphology indicating that blend homogeneity between PAN and CMCh took place. The results of SEM demonstrate that the good compatibility was sustained by the hydrogen bonds and the intermolecular interaction between PAN and CMCh. As the content of PAN increases in the hydrogels, the fibrous nature increases (Ezequiel et al. 2009).

X-ray diffraction

X-ray patterns of CMCh and CMCh/PAN blends are shown in Fig. 3. The diffraction peaks of Carboxymethyl chitosan are located at around 16.5° , 20° and 36° . The diffraction peaks associated with CMCh are very weak indicating low crystallinity. This is in a good agreement with literature (Costa et al. 2008). However diffraction peaks of pure PAN is located at 17° . This peak is strong and intense indicating the high crystalline structure of PAN. On the other hand, the X-Ray investigations showed that semi-IPN hydrogels based on CMCh/PAN contained two diffraction peaks occur at $2\theta \sim 16^\circ$ and 28° . These peaks intensity decreased with increasing CMCh content. Therefore,

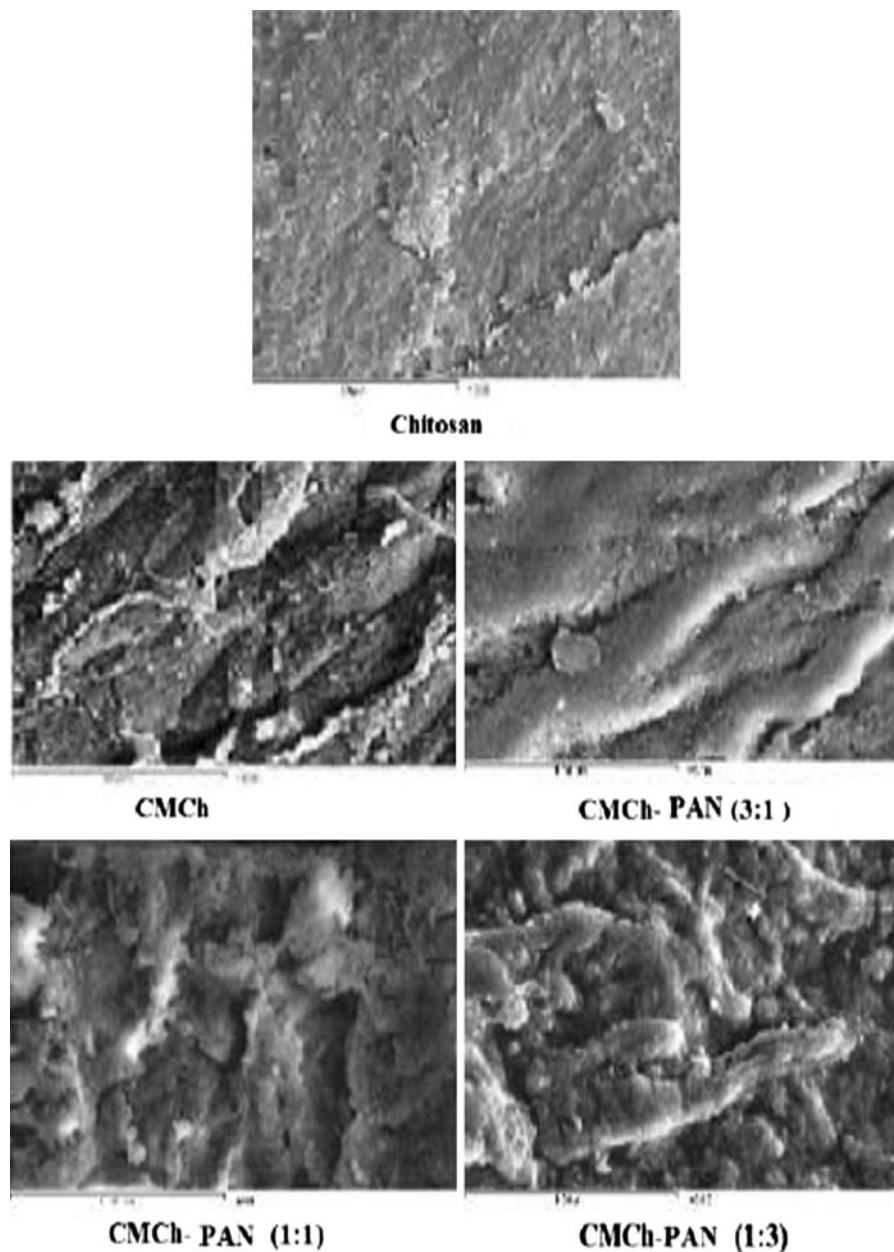


Fig. 2 SEM micrographs for chitosan, CMCh and CMCh/PAN with different ratios

addition of CMCh suppresses the ability of PAN to crystallize into the hydrogel.

Thermogravimetric analyses (TGA)

TGA analyses of CMCh, PAN and the blend hydrogels formed between them are all illustrated

in Fig. 4 and tabulated in Table 1. Thermal stability of CMCh/PAN hydrogels is improved compared to CMCh itself. This may be attributed to the formation of conjugated cyclic systems consisting of $-C=N-$ groups from the addition reaction of adjacent nitrile groups in PAN (Grassie 1977). Moreover, the initial decomposition temperature (IDT) values also show higher values as compared to IDT of CMCh due to

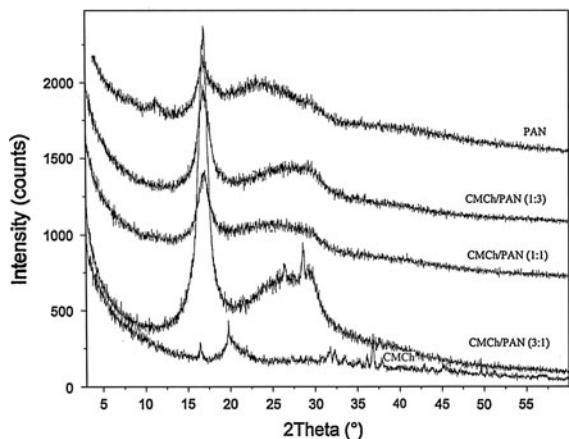


Fig. 3 XRD for PAN, CMCh and CMCh/PAN hydrogels with different ratios

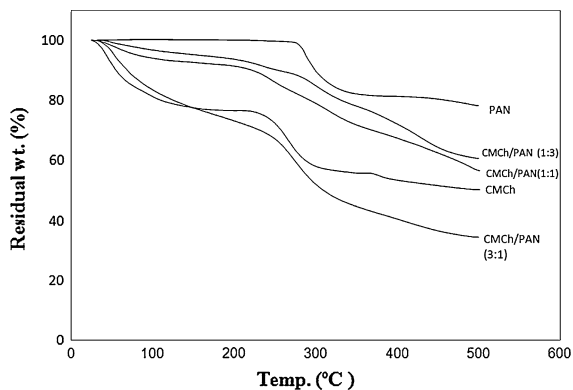


Fig. 4 TGA curves for PAN, CMCh and CMCh/PAN hydrogels with different ratios

the increase of PAN content in the hydrogel—Table 1.

At 300 °C, thermal degradation data for CMCh/PAN hydrogel (3:1) showed weight loss of almost 45%, while as for CMCh/PAN hydrogels (1:1), the weight loss was only 20%. Finally as for CMCh/PAN

hydrogel (1:3)—with highest PAN content—showed the least weight loss of 12%.

Gel fraction

PAN interpenetrates into the crosslinked CMCh by using agar. In this study, the gel formation (%) was calculated for pure CMCh crosslinked by agar as CMCh is one of the polysaccharides which are able to form hydrogels (Zhao et al. 2003). Also gel formation was calculated for pure PAN and it was found almost nil (only 4%) and was calculated for the prepared CMCh/PAN hydrogels. It was found that the gel formation (%) was increased with the increase in PAN content in the blend hydrogels—Table 2. This indicates the formation of semi-IPN CMCh/PAN hydrogels.

As an evidence from the FTIR charts of the CMCh/PAN hydrogels—irrespective to their blend ratio— the appearance of the –CN band at 2,246 cm⁻¹ indicating that PAN doesn't contribute in the crosslinking process of CMCh by agar.

A schematic representation for CMCh/PAN hydrogels maybe illustrated as follows:

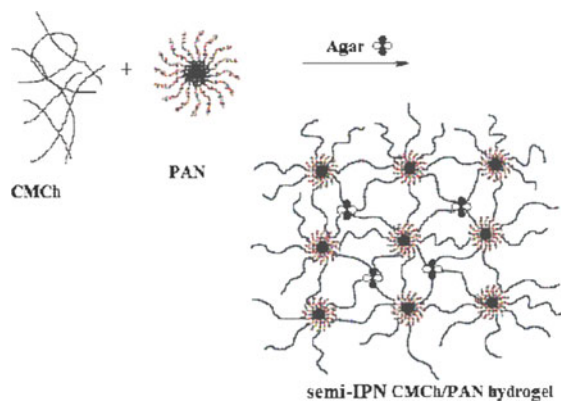


Table 1 Initial decomposition temperature (IDT) of CMCh, PAN, CMCh/PAN hydrogels in ratio (3:1), (1:1), (1:3)

Sample	IDT (°C)	Wt. loss % at 200 °C	Wt. loss % at 250 °C	Wt. loss % at 300 °C	Wt. loss % at 400 °C
CMCh	240	20	25	40	45
CMCh/PAN (3:1)	256	22	30	45	60
CMCh/PAN (1:1)	270	8	10	20	30
CMCh/PAN (1:3)	276	5	8	12	23
PAN	275	0	0	8	18

Table 2 Gel formation (%) of CMCh/PAN hydrogels

Sample	Gel fraction (wt%)
CMCh	62
CMCh/PAN (3:1)	88
CMCh/PAN (1:1)	91
CMCh/PAN (1:3)	95
PAN	4

Swelling behavior

All swelling experiments were reported as the average of three trials. All hydrogels swelled rapidly and reached equilibrium within 24 h. The hydrogel that contained the highest content of CMCh, showed the highest swelling percentage, Table 3. CMCh is an amphoteric macromolecule containing both carboxyl and amino groups. CMCh swelled at low pH (pH = 4.0) and also at high pH (pH = 9.0); however, deswelling occurred in the range of pH 7:0. CMCh forms a network with oppositely charged structures which change the charge state of the ionic groups varying with pH value.

In case of low pH, the dominant charges on the gel are the protonated amino groups. In case of high pH, the dominant charges on the gel are the carboxylate anions. The hydrogel CMCh/PAN (3:1) had the highest swelling percentage, while the swelling percentage of CMCh/PAN (1:3) is the least (Table 3). The swelling percentage increased with increasing the content of hydrophilic carboxymethyl chitosan in the hydrogel. This behavior might be mainly due to the formation of more loosely crosslinked network with the increase in the more hydrophilic CMCh content. As the content of CMCh increases, crosslinking of CMCh/PAN hydrogel decreases and molecular entanglement between CMCh and PAN is weakened, which results in improvement of its water absorbing ability. Moreover, CMCh is a hydrophilic polymer in contrary to PAN and consequently, the swelling percentage

increased with increasing the content of the hydrophilic groups of CMCh in the hydrogel (Mucha and Pawlak 2005). This pH-responsive swelling behavior was demonstrated in Table 3. The hydrogels show a lower swelling ability in pH 7 as compared with the acidic and alkaline pH. It is known that a high concentration of charged ionic groups in the hydrogel increases swelling due to osmosis and charge repulsion. Thus, when the degree of ionization of the hydrogel bound groups is decreased, swelling decreases (Mucha and Pawlak 2005). Since the swelling process of hydrogels involves the ionization of amino groups in the acidic buffer solution, the acid would be attached to the hydrogels by ionic bonds. Therefore, the weight of the hydrogels increased in the acidic buffer. Also at high pH, the swell ability of the hydrogel increased due to the presence of $-\text{COOH}$ groups in the hydrogel. This could be attributed to the electrostatic attraction or repulsion between ionic groups of CMCh in different pH environments (Abou-Aiad et al. 2006). It was considered that high pH sensitivity was induced mainly by CMCh. Poly (acrylonitrile) has been widely applied in the preparation of separation membranes and hydrogels. These hydrogels have received much attention in the field of water treatment (Hyo-Sik et al. 2003).

Metal uptake

For CMCh/PAN hydrogels used in this study, it may be expected that adsorption might be due to many available chelating sites on the hydrogels. The adsorption sites (chelation centers) for metal ions; Cd^{2+} and Co^{2+} are at both the $-\text{NH}_2$ and $-\text{C}=\text{O}$ groups in CMCh and the $-\text{CN}$ groups in PAN. Also, there is another possibility for metals adsorption which could be done by salt formation on the $-\text{COOH}$ group of CMCh.

Table 3 Swelling behavior of CMCh/PAN hydrogels in different buffered solutions

pH	Weight (%)				
	PAN	CMCh/PAN (1:3)	CMCh/PAN (1:1)	CMCh/PAN (3:1)	CMCh
4	17	22	109	148	154
7	15	19	62	106	122
9	18	22	124	335	591

Table 4 Adsorption of Cu (II), Co (II) and Cd (II) ions by CMCh/PAN hydrogels

Sample	Cu(II) (ppm)	Co (II) (ppm)	Cd (II) (ppm)
CMCh	3,000	3,290	2,600
CMCh/PAN (3:1)	4,665	2,256	2,210
CMCh/PAN (1:1)	4,659	3,981	4,095
CMCh/PAN (1:3)	3,211	4,876	4,757
PAN	3,024	5,331	5,081

Table 4 represents the equilibrium adsorption amounts of Cd^{2+} , Co^{2+} and Cu^{2+} ions at 24 h. It was found that the adsorption of Co (II) and Cd (II) ions increased with the increase in concentration of PAN content as the chelating centers increase. For example, the adsorption of CMCh/PAN (3:1) hydrogel for Co (II) and Cd (II) cations is less than CMCh/PAN (1:1) hydrogel which is less than CMCh/PAN (1:3) hydrogel.

The chelating centres of the crosslinked CMCh with agar maybe involved into the crosslinking process and they are not free and available for chelating more metal ions, so the chelating centre in PAN via $-CN$ groups is more pronounced into the hydrogels.

However, for the adsorption of Cu (II) ions, it decreased with the increase in PAN concentration in the blend hydrogel, this behavior is due to the selectivity of the hydrogel to the metal ion nature.

This property would enable the use of these hydrogels for the treatment of waste water derived from factories which use heavy metals ions in their processes.

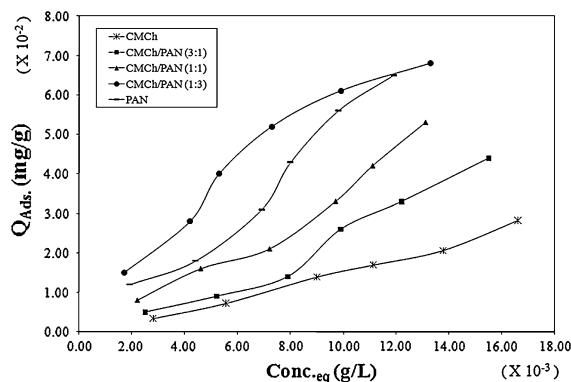
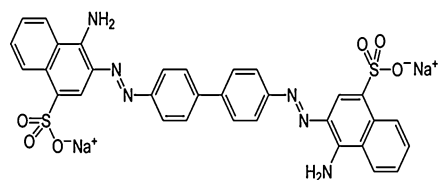


Fig. 5 Variation of congo red dye adsorbed by CMCh/PAN hydrogels

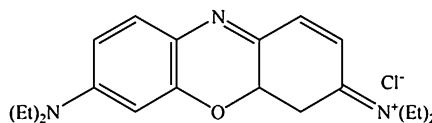
Dye uptake

Dyes of different classes are selected for this study. Among these dyes, Congo Red Dye which is an acidic dye, bears two cationic sites (amino groups) and two anionic sites (sulfonate groups).

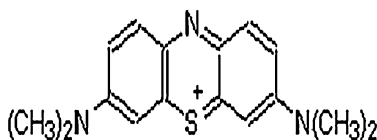
The adsorption of this dye increases by the decrease of CMCh content in the hydrogel due to the acidic character of the dye, so CMCh/PAN (1:1) hydrogel adsorbs much more Congo red dye than CMCh/PAN (3:1) which is more acidic in character, while CMCh/PAN (1:3) hydrogel adsorbs much more dye than all other hydrogels as shown in Fig. 5.



Congo Red Dye



Maxiolo Blue Dye



Methylene Blue Dye

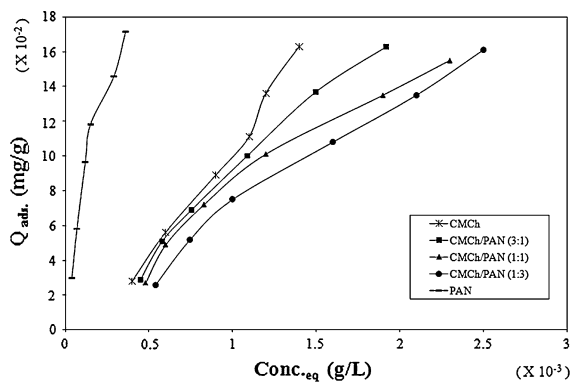


Fig. 6 Variation of maxilon blue dye adsorbed by CMCh/PAN hydrogels

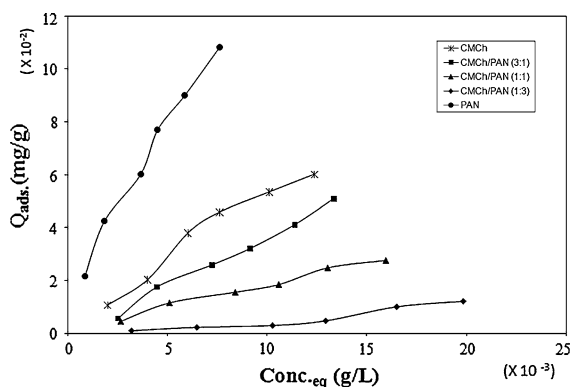


Fig. 7 Variation of methylene blue dye adsorbed by CMCh/PAN hydrogels

Table 5 Biological activity of CMCh/PAN hydrogels against *E. coli*

Samples	CFU ml ⁻¹ (× 10 ⁷)	(% Inhibition)
Untreated	–	0
CMCh	1	24
CMCh/PAN (3:1)	2	71
CMCh/PAN (1:1)	5	34
CMCh/PAN (1:3)	8	4
PAN	–	0

Another behavior was noticed for both Maxilon Blue dye (cationic dye)—Fig. 6 and Methylene blue dye (basic dye)—Fig. 7. The adsorption of these two dyes increases with the increase in the acidic –COOH groups in the hydrogels due to the basic nature of both dyes, and consequently the dye uptake for these two kinds of dye increases with the increase in CMCh ratio

in the hydrogel. This property would enable the use of these semi-IPN hydrogels for the treatment of waste water coming from dyestuff factories (Figs. 6, 7).

Antimicrobial activity

Table 5 shows the effect of exposure of Gram negative *E. coli* to different CMCh/PAN hydrogels. These hydrogels caused a decrease in viable cell counts of *E. coli*.

PAN itself has no clear effect on the viable cell count of *E. Coli*. It was observed that increasing CMCh content was much effective in decreasing the viable cell count of *E. coli* and increasing their inhibition (%) till 71% at the ratio CMCh/PAN (3:1), as CMCh itself has some inhibiting effect against *E. coli*.

If CMCh/PAN hydrogels are used in disinfection treatment of water, there will be a great variety of advantages, such as strong antibacterial activity, without residues, without disinfection by-products and avoiding recontamination of water.

Conclusions

Semi-IPN CMCh/PAN hydrogels were synthesized in different ratios and several analyses were done on those hydrogels. Some important obtained results could be summarized as follows:

1. Thermogravimetric analysis indicates that both the IDT and the thermal stability of the hydrogels increase due to the increase in PAN content in the hydrogel.
2. Gel formation (%) increased with the increase in PAN content in the CMCh/PAN blend hydrogels.
3. Swell ability of hydrogels increased due to the addition of CMCh, and it increases as CMCh content increases. The hydrogel CMCh-PAN (3:1) had the highest swelling percentage, while CMCh/PAN (1:3) had the least.
4. Adsorption of Congo Red dye was increased by the increase of PAN content in the hydrogel, while different behavior was noticed for Maxilon Blue dye (cationic dye) and Methylene blue (basic dye); adsorption decreases with increase in PAN content in hydrogels.
5. Adsorption of Co (II) and Cd (II) ions was increased by the increase in PAN content in the

hydrogel as the chelating centers increase, while as for the adsorption of Cu (II) ions, it was decreased with the increase in PAN content in the blend hydrogels.

6. Increasing CMCh content was much effective in decreasing the viable cell count of *E. coli* and increasing the inhibition (%) of the hydrogels till 71% at the ratio CMCh/PAN (3:1).

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