



Antioxidant Properties of Indole-3-Acetic Acid based Biopolymeric Hydrogels

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A series of novel indole-3-acetic acid based hydrogel was synthesized by condensation polymerization without cross-linker. The polymeric hydrogel was prepared using citric acid, indole-3-acetic acid and triethylene glycol with equimolar composition. The hydrogel revealed a pH-sensitive swelling behaviour, with increased swelling in acidic media and then decreased swelling in basic media. The biopolymeric hydrogels were characterized by Fourier transform infrared (FT-IR) spectroscopy, ¹H NMR, ¹³C NMR and TGA-DTA. The antioxidant activity has evaluated by DPPH and NO radical assay at different concentration compared with ascorbic acid, which has shown excellent antioxidant property.

Keywords: Hydrogel, Indole-3-acetic acid, Swelling equilibrium, Antioxidant activity.

INTRODUCTION

Hydrogels are three dimensional network and used for various applications. The first hydrogel was reported in 1960, Wichterle and Lim [1] prepared poly(2-hydroxyethyl methacrylate) for contact lenses. Then, use of hydrogels has expanded to various applications because of their high swelling ratio, excellent hydrophilic properties and biocompatibility. Hydrogels have been broadly used in biomedicine, agriculture, biosensors, tissue engineering, antibacterial materials, removal of heavy metals [2,3]. Recently, natural resource based material contributed for the synthesis of biopolymeric hydrogels. Normally, indole-3-acetic acid present in auxin plant hormone [4]. Indole derivatives were applicable important properties such as anti-cancer [5,6], antioxidant [7], antirheumatoid and anti-HIV [8,9]. Our research group reported on the synthesis and applications of indole-3-acetic acid with ethylene glycol and diethylene glycol hydrogels with biological applications [10,11]. The interesting group of heterocyclic compound of indole derivatives, which have been their exclusive chemical structure and biological properties [12].

In recent years, some undesirable effects, the insist for natural and synthetic antioxidants to make stronger the endogenous suspicion has grown. Antioxidants must react with radicals and other reactive species faster than biological compound, thus protecting biological targets from oxidative damage [13,14].

The antioxidant radical must have a high stability and interrupt a chain reaction [15]. The research on free radicals provides more information about medicinal development *in vitro* methods. Based on literature survey already reported aliphatic hydrogels but the present study is focused on the synthesis of indole-3-acetic acid (aromatic heterocyclic hydrogel) based biocomposite hydrogels. The structural characterization, thermal studies and morphological analysis of biocomposite hydrogels deliberated by various instrumentation technique like Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (¹H NMR, ¹³C NMR) and TGA-DTA. Swelling equilibrium in various pH solutions in the range of 3-10. Furthermore, antioxidant activity of biocomposite hydrogel has also been analyzed.

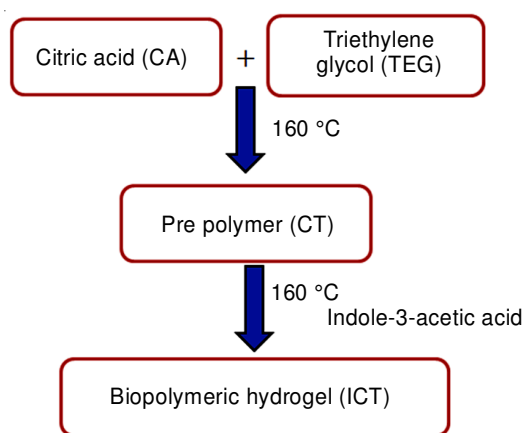
EXPERIMENTAL

Anhydrous citric acid, triethylene glycol and indole-3-acetic acid were purchased from Sigma-Aldrich (Bangalore, India). Ethanol was purchased from Merck (India). Demineralized water used for preparation of the buffer solutions.

FT-IR spectra of hydrogels were recorded by using Shimadzu spectrophotometer-8400S (Japan) in KBr medium at room temperature in the region of 4000-500 cm⁻¹. NMR spectra was recorded with a Bruker NMR (China) spectrometer at the frequencies of 250 MHz (¹H) and 62.5 MHz (¹³C) using DMSO as a

solvent in 5 mm outside diameter tubes. Both curves of TGA and DTA were measured by using SDT Q 600 (USA) in the temperature range of ambient to 800 °C at a heating rate of 10 °C/min under a N₂ atmosphere.

Preparation of biocomposite hydrogel: Citric acid (0.025 mol) was dissolved in ethanol (5 mL) using a round bottom flask closed with guard tube and stirred with magnetic stirrer. Triethylene glycol (0.025 mol) was added dropwise and the mixture was stirred for 1 h in silica oil bath at 160 °C. The formation of white colour sticky gel was formed named as pre-polymer. In addition, indole-3-acetic acid (0.025 mol) which has dissolved in 10 mL ethanol, added into pre-polymer and then heated continuously for 3 h in nitrogen atmosphere. The high viscous reddish brown glassy gel was formed. Further, the gel was immersed in distilled water for 24 h to remove the unreacted monomers (**Scheme-I**). Then the sample was dried in an oven for 48 h at 35 °C [10].



Scheme-I: Preparation of biopolymeric hydrogel (ICT)

Swelling equilibrium studies: The hydrogel weighed and dipped in various pH (phosphate buffer solution - PBS) for 48 h in the various pH from 3 to 10. Further, gel absorbed some amount of buffer solution then remaining solution drained thoroughly and weighed. The same procedure was repeated for the solutions with different pH values. The percentage of swelling equilibrium was calculated using eqn. 1:

$$S_{eq}(\%) = \frac{W_{eq} - W_d}{W_d} \times 100 \quad (1)$$

where W_d and W_{eq} are the weight of the sample in before swollen state, swollen at equilibrium, respectively.

Free radical scavenging activity of hydrogel on DPPH:

The antioxidant activity of the sample was determined by radical scavenging ability, using stable radical DPPH [16]. The sample at various concentrations (100-500 µg) was taken and the volume was adjusted to 125 µL with DMSO. Then added methanolic solution of DPPH[•] and allowed to stand for 20 min at 27 °C. The absorbance can be measured at 517 nm. Percentage radical scavenging activity of the sample was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A - B}{A} \times 100 \quad (2)$$

where, A = Control optical density, B = Sample optical density.

Nitric oxide radical scavenging activity of hydrogel:

The nitric oxide scavenging activity of the sample was measured

according to the reported method [17]. The samples were prepared at different concentrations (100-500 µg) then incubated at room temperature for 2 h. After incubation time, 0.5 mL of Griess reagent (1 % sulfanilamide and 0.1 % naphthyl ethylenediamine dihydrochloride in 2.5 % phosphoric acid) was added. The absorbance measured at 546 nm. The percentage of NO radical scavenging activity of the sample was calculated using eqn. 2.

RESULTS AND DISCUSSION

FT-IR: The FTIR spectra of biocomposite hydrogel is shown in Fig.1, the peak at 1735 cm⁻¹, which is due to C=O stretching [18]. The C-O stretching vibration observed at 1124 cm⁻¹ [19]. It is clearly certified that the formation of ester by utilization of citric acid and diol units through poly condensation reaction. The -CH₃, -CH₂ and -C-H symmetric stretching vibration occurred at 2363.72 cm⁻¹. The broad stretching peak at 3174 cm⁻¹ can be attributed to either the hydrogen bonded -OH in diol or -NH bond or both (overlapped) [20,21]. The new absorption peaks observed at 1401 cm⁻¹ that can be assigned to stretching vibration of -COO⁻ [22,23]. The peak at 1636 cm⁻¹ was due to the bending vibration of -NH bond in biocomposite hydrogel. These results confirmed that there was no change of -NH group in biocomposite hydrogel, this results contributed from indole-3-acetic acid. The peaks at 747 cm⁻¹ was attributed -CH- out of plane bending of aromatic ring. These results confirmed the incorporation of aromatic moieties presents in biocomposite hydrogel. In Fig. 1, indole-3-acetic acid, -OH stretching frequency at 3388.28 cm⁻¹ has been disappeared in biocomposite hydrogel. While incorporating indole-3-acetic acid, there was no change in indole and -NH groups but changed stretching frequency of third position of acid group which has observed in Fig. 1.

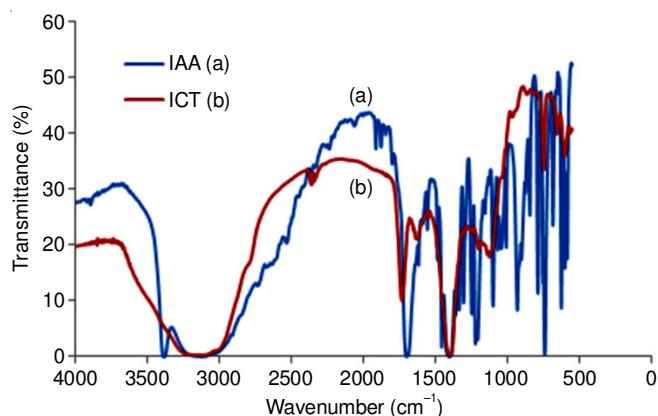
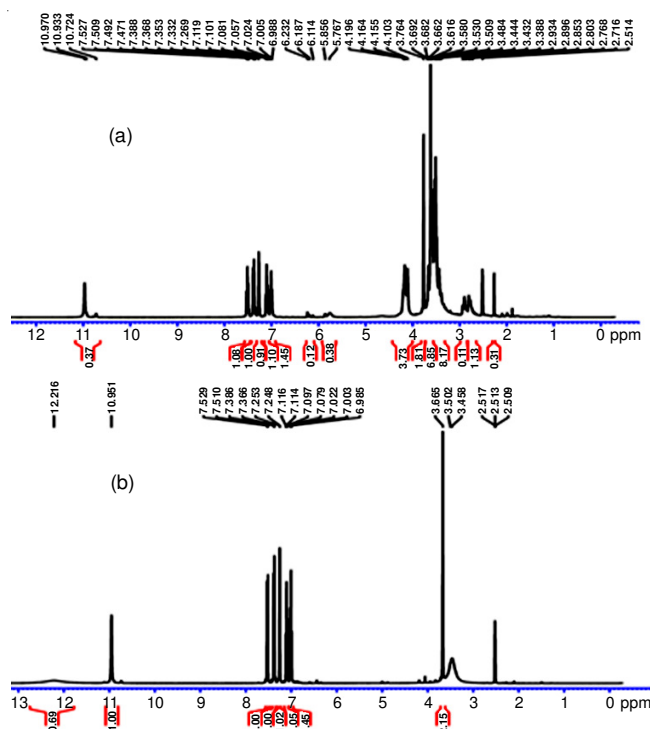


Fig. 1. FT-IR spectroscopy of polymeric hydrogel (ICT)

¹H NMR: Fig. 2a illustrated the ¹H NMR spectra of biocomposite hydrogel with respect to dimethyl sulphoxide. The alcoholic group (-OH) from diol appeared at 4.107-4.196 ppm which has considered as -OH peak from diol [24]. The departure of centre carbon hydroxyl group from citric acid as proved as vanished peak of 4 ppm [25]. The multiple peaks of -CH₂ proton contributed from citric acid observed in the range of 2.514-2.934 ppm. The peaks at 3.388-3.764 ppm contributed the proton signal of -OCH₂CH₂- units from diol [26]. The multiple peaks around 7.005-7.527 ppm were attributed indole ring from

Fig. 2. ^1H NMR spectroscopy of polymeric hydrogel

indole-3-acetic acid [27], which has aromatic moieties present in polymeric product. This polymeric hydrogel was observed -NH peak at 10.724-10.970 ppm appeared from indole-3-acetic acid (Fig. 2b) which has proved there was no change in -NH group in this hydrogel. However, -COOH group from indole-3-acetic acid at 12.21 ppm was also disappeared due to formation of polyester. The sharp peak at 3.5 ppm indicated the presence of DMSO [28].

^{13}C NMR: The ^{13}C NMR signals of biocomposite hydrogel at 31.45 ppm appeared as $-\text{CH}_2$ group from indole-3-acetic acid (Fig. 3a). The peaks were from 107.41 to 136.56 ppm related to aromatic carbons of indole ring which has proved the aromatic moieties present in hydrogel compound (Fig. 3b). The methylene group of citrate was raised at 43.32 and 43.63. The quaternary center carbon atom of citric acid has followed 72.800 to 73.516 ppm. The signals were 63.95-68.78 ppm marked as a carbon atom of $-\text{CO}-\text{O}-\text{CH}_2-\text{CH}_2-$ present in the polyester network [29]. The ester carbonyl carbon peaks were 170.17-173.70 ppm [30]. The signals between 39.462 and 40.420 ppm were indicated as DMSO [31].

Equilibrium swelling studies of hydrogel: The equilibrium swelling studies has been performed by various buffers with pH value ranging from 3 to 10 at room temperature. At each of the pH values, the equilibrium swelling was calculated based on eqn 1. The results of swelling equilibrium at various pH of citric acid, indole-3-acetic acid and triethylene glycol based hydrogel are shown in Fig. 4. The Seq % value of biocomposite hydrogel at pH 3.0, 4.0, 6.0, 7.0, 9.0 and 10.0 were 1450, 1355, 1342, 1200, 800 and 740 % due to protonation of amino groups fall-out to the high osmotic pressure and electrostatic repulsion between protonated amino groups (-NH) of polymeric chains, resulting to increased swelling of hydrogel. On the other hand, at higher pH the protonated amino groups transformed into unionized amino groups due to hydrogen bonding. Similarly,

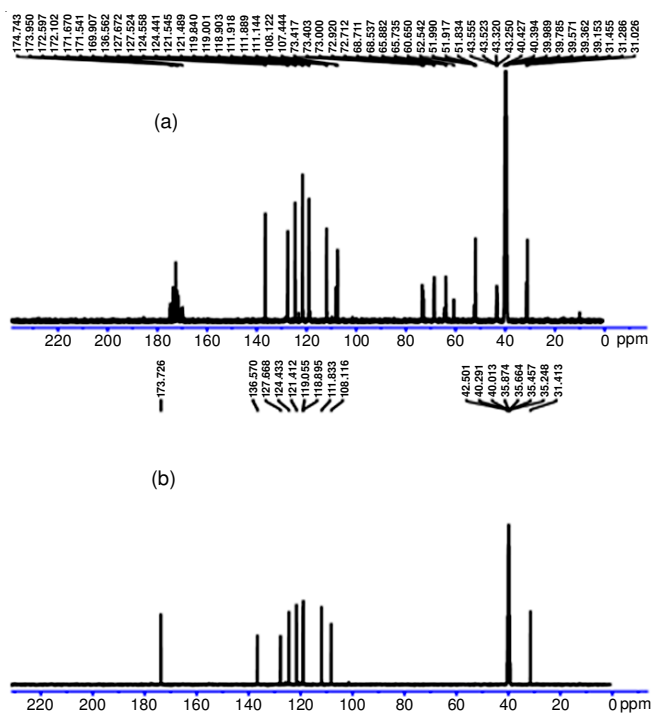
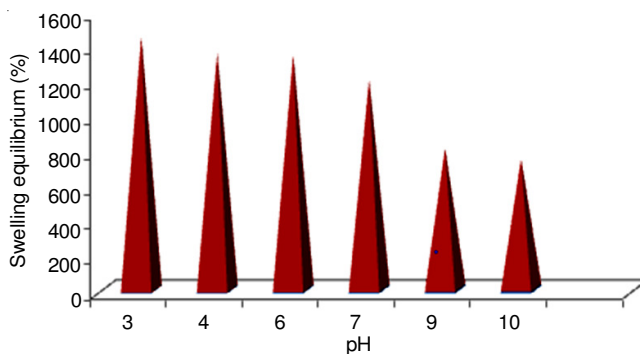
Fig. 3. ^{13}C NMR spectroscopy of polymeric hydrogel

Fig. 4. Swelling equilibrium (%) of polymeric hydrogel

at higher pH the protonated amino groups altered into unionized amino groups due to hydrogen bonding [32]. Hence, the dilapidated of osmotic pressure and electrostatic repulsion between protonated amino groups (-NH) which leads to decrease in swelling in basic media of hydrogel [33]. This biocomposite hydrogel was less swelled when compared with ethylene glycol and diethylene glycol based hydrogels [10,11].

Thermal analysis: Thermogram of biocompatible hydrogel (Fig. 5) has shown in two stages of decomposition. The first stages of decomposition consider as loss of moisture and cleavage of side chain were observed at 200-253 $^{\circ}\text{C}$ with 28 % weight loss. Second stage of decomposition was observed in the range between 253-335 $^{\circ}\text{C}$ with 48 % weight loss due to breakage of polyester chain in polymeric hydrogel [10]. DTA results are also supported with TGA results and swelling equilibrium. This biocompatible hydrogel was less thermal stable than ethylene glycol and diethylene glycol based hydrogels due to complexation of hydrogel [10,11].

Antioxidant activity: DPPH radical scavenging activity increased from 35.31, 49.55, 55.70, 62.21 and 70.02 % when increase the concentration from 200-1000 μg compared with

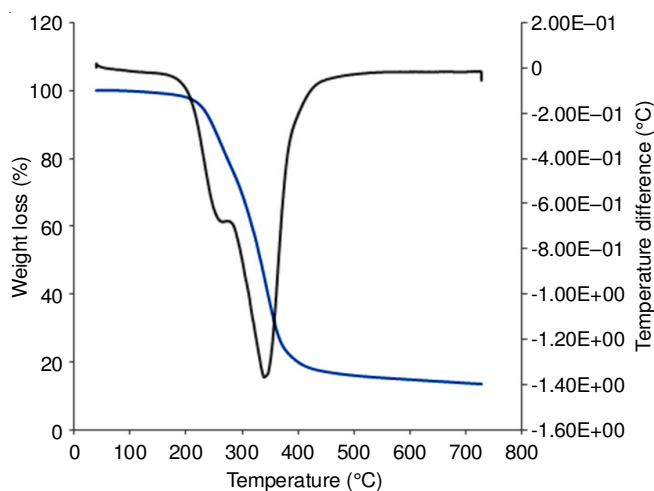


Fig. 5. TGA-DTA studies of polymeric hydrogel

respect to ascorbic acid. NO radical scavenging activity was 20.60, 38.70, 49.89, 61.39 and 69.12 %. At the same time, IC_{50} values of DPPH and NO radicals were 489 and 644 $\mu\text{g/mL}$ (Table-1) in which high value of IC_{50} leads to less radical scavenging activity [34]. Furthermore, DPPH had more active than NO Radicals, compete with oxygen to react with NO and thus more inhibit the generation of the nitrite and peroxy nitrite anions [35].

TABLE-1
ANTIOXIDANT ACTIVITY OF
BIOPOLYMERIC HYDROGEL (ICT)

Conc. (μg)	DPPH radical scavenging activity		Nitric oxide radical scavenging activity	
	Activity (%)	IC_{50} ($\mu\text{g/mL}$)	Activity (%)	IC_{50} ($\mu\text{g/mL}$)
200	35.31	489	20.60	644
400	49.55		38.70	
600	55.70		49.89	
800	62.21		61.39	
1000	70.02		69.12	

Conclusion

Indole-3-acetic acid based aromatic functionality containing biocomposite hydrogels synthesized by polycondensation successfully. The formation and identification of biocomposite hydrogel confirmed by FT-IR, ^1H NMR and ^{13}C NMR, respectively. The hydrogels have exhibited a maximum of swelling at acidic pH, while the swelling ratio decreases with increase alkalinity. The thermogram supported the thermal stability and behaviour of hydrogels. The antioxidant of biocomposite hydrogel increased from lower concentration to high concentration; comparatively antioxidant activity of DPPH radical was better than NO radical.

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