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Facile synthesis of fluorescent polysaccharides: Cytosine grafted agarose and κ-carrageenan

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ABSTRACT

New fluorescent polymeric materials were synthesized by grafting the nucleobase cytosine on to the backbone of agarose and κ-carrageenan, employing a rapid water based method under microwave irradiation using potassium persulphate (KPS) as an initiator. The emission spectrum of the modified agarose and κ-carrageenan recorded in aqueous solution (5×10^{-5} M) exhibited emission maxima ($\lambda_{em,max}$) at 348 nm by excitation at 266 nm. The emission intensity was enhanced by ca. 104% and 60% compared to that of pure cytosine solution of the same concentration. When the concentration of the pure cytosine solution is made equivalent to the concentration of the cytosine molar component (3.09×10^{-5}) and (3.5×10^{-5}) present in 5×10^{-5} M solution of modified agarose and κ-carrageenan, respectively, then ca. 143% and 81% enhancement in emission intensity was observed. The remarkable fluorescent activity of the agarose–cytosine derivative may have potential uses as sensor in various applications.

1. Introduction

The fluorescence phenomenon was harnessed to study agarose gelling system by Hayashi, Kinoshita, and Yasueda (1980). Polysaccharide conjugates were prepared with fluorescein to distinguish underivatized polysaccharides as well as for localizing and quantifying cell surface proteins in cell biology research (Glabe, Harty, & Rosen, 1983). Other fluorescent polysaccharides and their conjugates were prepared with an eye to identifying biomolecules, sensing pH as well as preparing cellulose based organic light emitting diode (Karakawa et al., 2007; Kobayashi, Urayama, & Ichishima, 1990; Qiu, Xu, Zhu, & Qiu, 2005; Schulz et al., 2009; Suizhou et al., 2003). Urreaga and De la Orden (2007) have reported modification of cellulose with amino compounds and their fluorescence properties. A facile synthesis of a fluorescent agarose–guanine derivative has been reported by Oza, Meena, Prasad, Paul, and Siddhanta (2010). Cytosine excited state dynamics was studied by femtosecond fluorescence (Sharonov, Gustavsson, Carre, Renault, & Markovitsi, 2003). There exist numerous reports in the literature on the modification of polysaccharides employing various strategies, e.g. grafting and cross linking.

In an ongoing program of our laboratory on modification of seaweed polysaccharides for preparing new materials with improved functional properties (Meena, Prasad, Mehta, & Siddhanta, 2006; Meena, Prasad, & Siddhanta, 2006; Meena, Prasad, & Siddhanta, 2007; Meena, Chhatbar, Prasad, & Siddhanta, 2008; Oza et al., 2010; Chhatbar, Meena, Prasad, Chejara, & Siddhanta, 2011; Prasad, Siddhanta, Rakshit, Bhattacharya, & Ghosh, 2005; Prasad, Trivedi, Meena, & Siddhanta, 2005; Prasad, Mehta, Meena, & Siddhanta, 2006; Prasad, Meena, & Siddhanta, 2006), we report herein functional modification of agarose and κ-carrageenan (Figs. 1and 2) by grafting cytosine on to agarose and κ-carrageenan (Figs. 1and 2) by a water based method. This cytosine modified agarose exhibited exceptionally strong fluorescent properties than cytosine modified κ-carrageenan. Cytosine is 4-amino 1H pyrimidine 2-one, which is one of the four nitrogenous bases found in nucleic acids (Finar, 2004). Agarose is a hydrophilic polymer and is widely used in biomedical applications and bioengineering. The basic disaccharide repeating units of agarose consists of (1,3) linked β-D-galactose (G) and (1,4) linked α-L-3,6-anhydrogalactose (A) (Fig. 1) (Rochas & Lahaye, 1989). Carrageenans represent yet another prominent class of gelling polysaccharide obtainable from red seaweeds known for its versatility envisaging wide areas of applications which include food, feed, pharmaceuticals and agri-horticulture. Major carrageenans are termed ι-, κ-, λ-carrageenans. Structurally, these carrageenans are consisted of sequences of: D-galactose-4-sulphate and 3,6-anhydro-D-galactose-2-sulphate (ι-carrageenan), D-galactose-4-sulphate and 3,6-anhydro-D-galactose (κ-carrageenan). D-Galactose-2-sulphate and D-galactose-2,6-disulphate (λ-carrageenan) (Araki, Arai, & Hirase, 1967). To our knowledge, these fluorescent agarose and κ-carrageenan derivatives are being reported for the first time.

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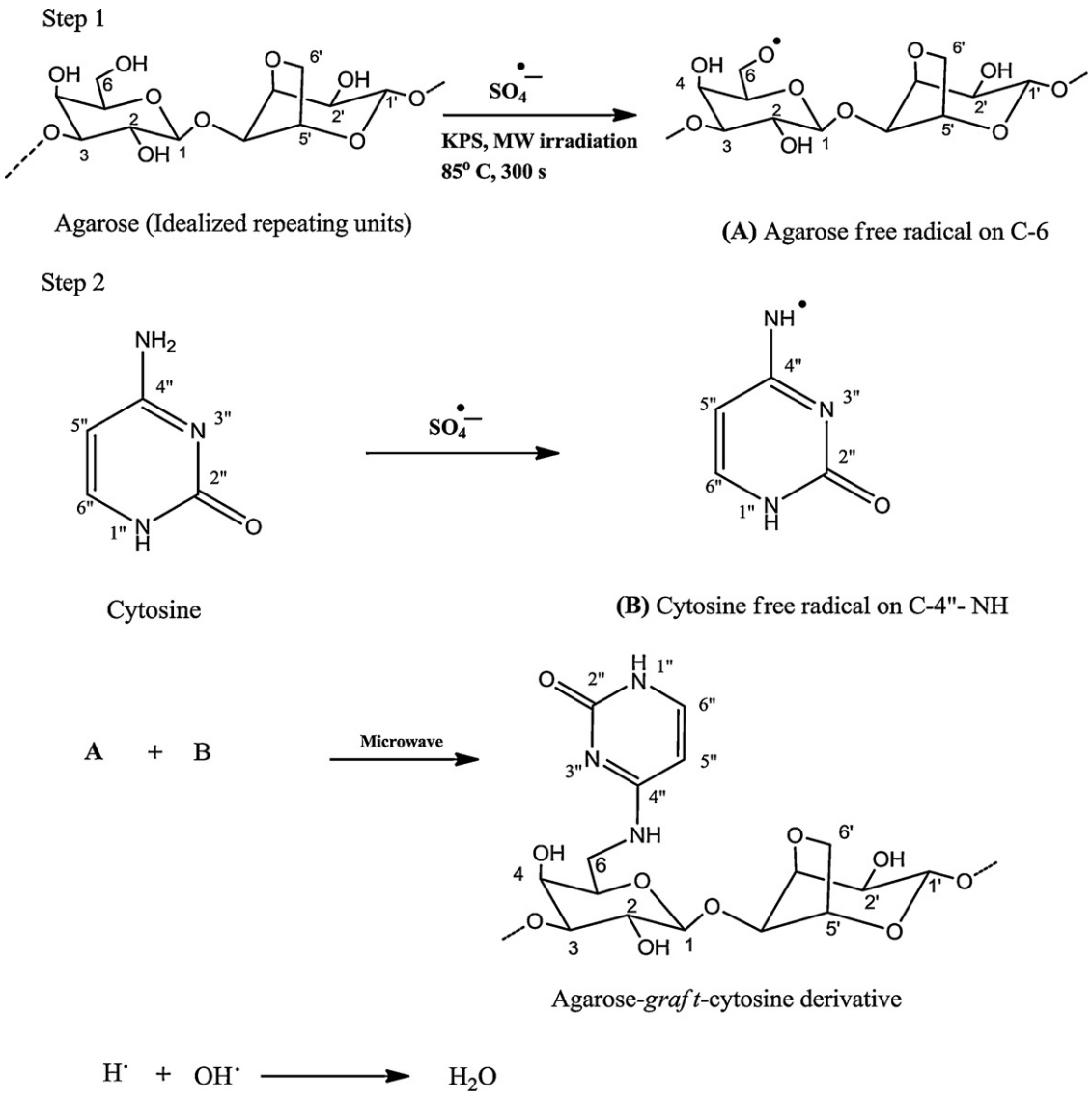

Fig. 1. Plausible mechanisms of formation of agarose-*graft*-cytosine.

2. Experimental

2.1. Materials

Agarose used in this study was extracted from the seaweed *Gracilaria dura* as described by Meena, Siddhanta, et al. (2007). κ-Carrageenan (refined carrageenan) used in this study was extracted from the seaweed *Kappaphycus alvarezii* (Craigie & Leigh, 1978). Other chemicals used in this study (e.g. potassium persulphate (KPS) and cytosine, LR grade) were purchased from M/s S.D. Fine Chemicals Ltd., Mumbai, India.

2.2. Synthesis of agarose-graft-cytosine and carrageenan-graft-cytosine

A known weight of polysaccharide (agarose or κ-carrageenan) (100 mg) was dissolved in 20 ml of hot water, to which 10.0 mg (0.738 mM) of KPS was added and mixed well. In a beaker, a known weight (100 mg) of cytosine was dissolved in 20 ml of hot water, and then mixed with the hot agarose or carrageenan sol and KPS mixture under stirring condition followed by microwave irradiation for 2 min. The colorless reaction mixture got converted slowly into a yellow solution. A yellow colored product was isolated from the reaction mixture by precipitation with isopropanol (IPA) (reaction mixture:IPA = 1:2, v/v). The precipitated product was washed with IPA (90%) to remove unreacted cytosine followed by drying under vacuum.

2.3. FT-IR spectra

The non-modified and modified polysaccharide (agarose or κ-carrageenan) were characterized by FT-IR analysis using a Perkin-Elmer FT-IR machine (Perkin-Elmer Spectrum GX FT-IR System, USA), by taking 10.0 mg of sample in 600 mg of KBr. All spectra were average of two counts with 10 scans each and resolution of 4 cm^{-1}. IR spectra were recorded as KBr pellets.

2.4. UV–vis and fluorescence spectroscopy

The UV–vis absorption spectra of the modified, non-modified agarose and non-modified carrageenan were obtained on a Varian CARY 500 UV-VIS-NIR spectrophotometer, Pittsburgh, USA. The fluorescence spectra were recorded at room temperature on a Perkin-Elmer Spectrofluorimeter LS-50B, USA. The fluorescence

emission spectra of agarose, carrageenan, cytosine, agarose-*graft*-cytosine and carrageenan-*graft*-cytosine were measured at a concentration 5×10^{-5} M in distilled water as well as of cytosine solution at 3.09×10^{-5} M and 3.5×10^{-5}, the molar equivalents present in the grafted products respectively, using excitation and emission slits 5.0/5.0 nm. Cytosine, modified agarose and carrageenan were excited at 266 nm with an emission at 348 nm.

2.5. Optical rotation and circular dichroism

Optical rotations were measured on a Digipol 781 automatic polarimeter (Rudolph Instruments Inc., NJ, USA) (ca. 0.5%, distilled water) at 35 °C. Circular dichroism (CD) spectra were recorded on JASCO model J-815 CD Spectrometer, Tokyo, Japan, in the range 190–250 nm using sample concentration of ca. 0.8 mg/ml (800 ppm). Molar ellipticity values, [θ] are reported in units of° cm^2 $dmol^{-1}$. All measurements were performed at room temperature using 1.0 cm quartz cells. The ratio of peak height to trough depth was calculated using below given equation described by Morris, Rees, and Thom (1980) and Chhatbar et al. (2009)

$$\frac{\text{Peak}}{\text{trough ratio}} = \left(\frac{\theta_{\text{trough}} - \theta_{\text{peak}}}{\theta_{\text{trough}}}\right) \quad (1)$$

2.6. ^{13}C NMR spectroscopy

The NMR spectra of agarose, carrageenan, agarose-*graft*-cytosine and carrageenan-*graft*-cytosine were recorded on a Bruker AVANCE II 500 MHz spectrometer, Switzerland, at 70 °C. These samples were dissolved in D_2O (50 mg/ml) and the spectra were recorded at 70 °C with 7000–7500 accumulations, pulse duration 11.25 μs, acquisition time 1.048 s and relaxation delay 6 μs using DMSO as internal standard (ca. d 39.5). Cytosine was dissolved in D_2O (20 mg/ml) and spectrum was recorded at room temperature with 2000 accumulations, pulse duration 9.40 μs acquisition time 1.048 s and relaxation delay 6 μs using DMSO as internal standard.

2.7. Other characterizations of agarose-graft-cytosine and carrageenan-graft-cytosine

Thermogravimetric analysis (TGA) was done on a Mettler Toledo Thermal Analyzer, model TGA/SDTA 851e, Switzerland. Powder X-ray diffractions were recorded on a Philips X'pert MPD X-ray Powder Diffractometer, The Netherlands, in the 2θ range 10–60° for vacuum dried samples of the powder of agarose, carrageenan, agarose-*graft*-cytosine, carrageenan-*graft*-cytosine, cytosine, and the physical mixture of agarose–cytosine and carrageenan–cytosine (1:1, w/w). The physical mixture of agarose–cytosine and carrageenan–cytosine (1:1) were prepared by triturating the two compounds in a paste in presence of a few drops of water, followed by successive air and vacuum drying of the paste, which was ground to powder. For recording SEM image, vacuum dried samples of the powder of agarose, carrageenan, agarose-*graft*-cytosine, carrageenan-*graft*-cytosine and cytosine were mounted on a sample holder, coated with gold and the micrographs were recorded on a scanning electron microscope (Carl-Zeiss), model LEO 1430 VP, Germany, at an accelerating voltage of 20 kV and 202× magnification. Total nitrogen was estimated by Kjeldahl method on a KEL PLUS-KES 201 Digestion unit attached to a KEL PLUS-CLASSIC DX Distillation unit (M/s PELICAN equipments, Chennai, India). Crude protein content was calculated multiplying the nitrogen content by the factor 6.25; the results were calculated as means ± SD of four replicates.

3. Results and discussion

3.1. Yield and grafting pattern

Yields of the agarose-*graft*-cytosine and carrageenan-*graft*-cytosine were 92% and 89% which were calculated on the basis of the nitrogen content of the products (Kjeldahl's estimation) with respect to the total quantities of agarose, carrageenan and cytosine that were used in the synthesis. Grafting percent (G%) in the agarose-*graft*-cytosine was 160% and carrageenan-*graft*-cytosine was 146%, whereas its total conversion (*C*%) value was 60% and 46% respectively (cf. Meena et al., 2008).

3.2. FT-IR spectroscopy

Strong bands at 1642 cm^{-1} for agarose and 1643 cm^{-1} for carrageenan (bonded H–O–H; Christiaen & Bodard, 1983; Prasad, Mehta, et al., 2006) and 1655 cm^{-1} for cytosine for amide carbonyl (Bellamy, 1957; cf. Nakanishi & Solomon, 1977) were observed in the FT-IR spectra (Figs. 3 and 4). The spectra of agarose-*graft*-cytosine and carrageenan-*graft*-cytosine exhibited strong bands at 1651 and 1666 cm^{-1} as well as a shoulder at 1642 cm^{-1}, indicating the presence of both polysaccharides as well as that of cytosine moiety in the respective products. In the IR spectrum of cytosine, the sharp band for C-4″-NH_2 at 3383 cm^{-1} (νNH_2; cf. Nakanishi & Solomon, 1977) was not observed clearly in the spectrum of agarose-*graft*-cytosine (Fig. 3) and carrageenan-*graft*-cytosine (Fig. 4) which indicated the involvement of C-4″-NH_2 in the chemical transformation to form a new compound. Cytosine exhibits an IR band (shoulder) at ca. 1730 cm^{-1} as a result of C2=O8···HN1, with a stronger intermolecular interaction being implemented by the hydrogen bonds between the C2=O8 and N1 atoms (Ten & Baranov, 2005). In the present study, the IR spectra of pure cytosine exhibited a very weak shoulder (at 1724 cm^{-1}) of the broad band at ca. 1655 cm^{-1}. Both the agarose and carrageenan derivatives of cytosine exhibited this band rather clearly at a lower frequency (ca. 1728 and 1732 cm^{-1} respectively), compared to that of pure cytosine suggesting thereby the formation of the respective new compounds (Figs. 3 and 4). Several other bands, e.g. 1458 cm^{-1}, 682 cm^{-1}, 599 cm^{-1} for agarose-*graft*-cytosine and 1452 cm^{-1}, 696 cm^{-1}, 601 cm^{-1} for carrageenan-*graft*-cytosine were observed, whereas in cytosine a relatively stronger band appeared at 1464 cm^{-1}, demonstrating discernible differences in the structure of the products in comparison with those of pure agarose, carrageenan and cytosine. The remaining bands at 682 cm^{-1}, 599 cm^{-1} and 696 cm^{-1}, 601 cm^{-1} in the products and 698 cm^{-1} and 600 cm^{-1} in cytosine may have arisen due to –CH deformations (cf. Bellamy, 1957). A broad shoulder was observed around 3178 cm^{-1} in agarose-*graft*-cytosine, whereas cytosine exhibited a sharp band at 3170 cm^{-1} for –NH– stretching (cf. Nakanishi & Solomon, 1977). Furthermore, characteristic bands at 931 cm^{-1} (3,6-anhydro moiety of agarose), 790 cm^{-1} and 784 cm^{-1} for β-skeletal bending of basic carbohydrate moieties in the IR spectrum of the copolymer suggested that during grafting reaction the agarose and carrageenan polymer did not get decomposed (cf. Prasad, Mehta, et al., 2006). The important IR bands of cytosine, agarose and carrageenan (Figs. 3 and 4) are given below.

Cytosine (Bellamy, 1957; cf. Nakanishi & Solomon, 1977; Ten & Baranov, 2005): (cm^{-1}) 3383 cm^{-1}, 3170 cm^{-1} (νNH_2, νNH); 1655 cm^{-1} (amide C=O); 1538 (ring double bonds); 1464 cm^{-1} (ring vibration); 966 cm^{-1} (C–NH_2 bending); 820–789 cm^{-1} (skeletal vibration); 693 cm^{-1}, 602 cm^{-1} (–CH deformations).

Agarose (Christiaen & Bodard, 1983; Freile-Pelegrín & Murano, 2005; Prasad, Mehta, et al., 2006): (cm^{-1}) 3417 cm^{-1} (–OH stretching); 1642 cm^{-1} (bonded H–O–H); 1376 cm^{-1} (–CH_2–OSO_3^{-2} linkage at C-6, which is not shown in the idealized repeating

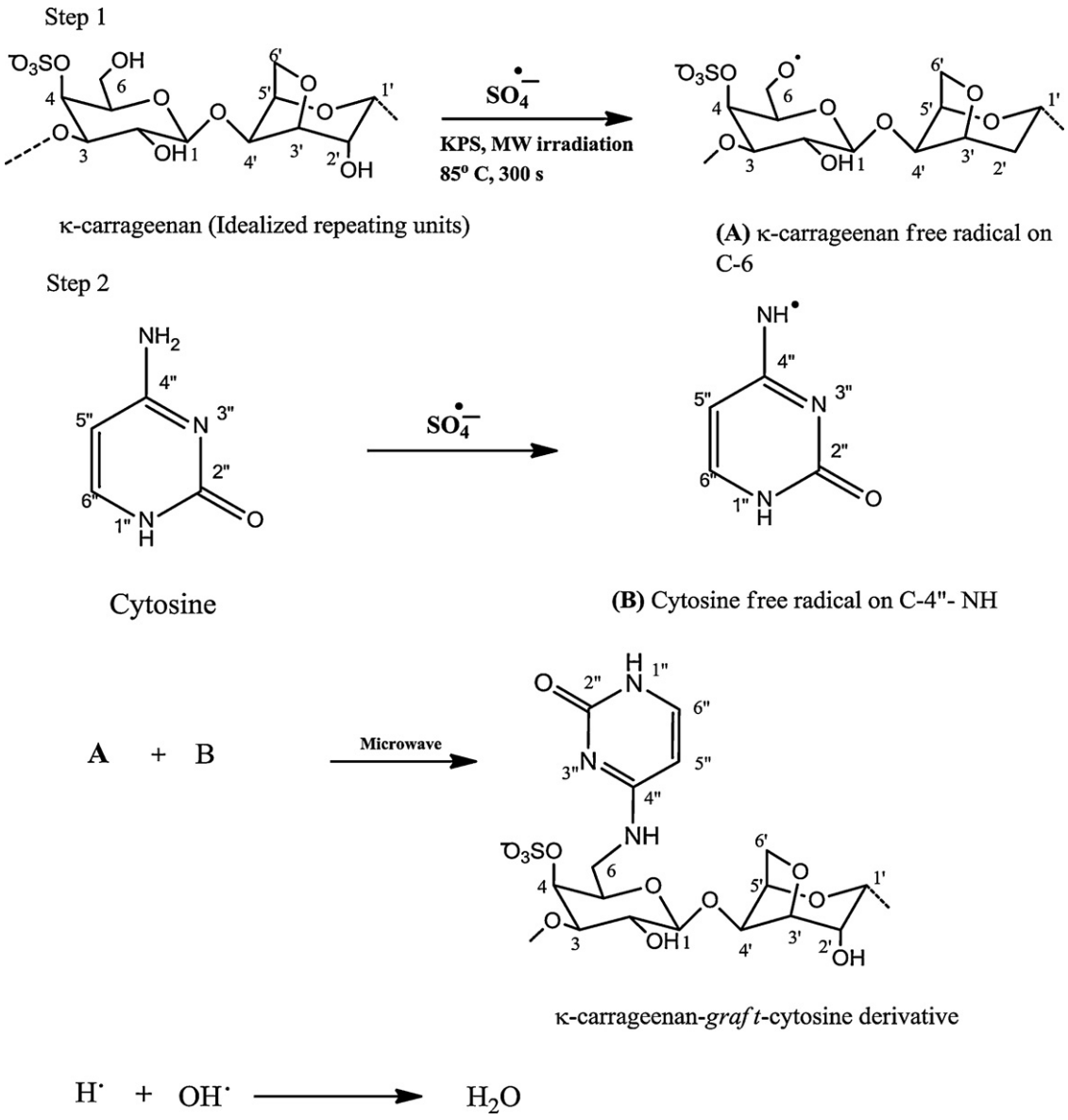

Fig. 2. Plausible mechanism of formation of carrageenan-*graft*-cytosine.

units of agarose in Fig. 1); 1074 cm^{-1} (C–O–C glycosidic linkage); 932 cm^{-1} (3,6-anhydrogalactose linkage). 778 cm^{-1} and 741 cm^{-1} (β-skeletal bending of basic carbohydrate moieties).

κ-Carrageenan (Prasad, Mehta, et al., 2006): (cm^{-1}) 3392 (–OH stretching); 1643 cm^{-1} (bonded H–O–H); 1376 cm^{-1} ($-CH_2-OSO_3^{-2}$ linkage at C-6, which is not shown in the idealized repeating units of κ-carrageenan in Fig. 4); 1072 cm^{-1} (C–O–C glycosidic linkage); 929 cm^{-1} (3,6-anhydrogalactose linkage). 848 and 737 cm^{-1} (β-skeletal bending of basic carbohydrate moieties).

3.3. Thermal analysis (TGA)

The TGA patterns of the cytosine grafted agarose and carrageenan were comparable, in fact superimposable to that of cytosine in repeated experiments (Fig. 5). The initial TGA traces of agarose and the grafted product up to 250 °C presented a reversed pattern having a cross over point at ca. 290 °C where agarose started decomposing very fast till 500 °C. The grafted product, however, started decomposing at ca. 290 °C very slowly till ca. 495 °C. The initial TGA traces of carrageenan and the grafted product up to 190 °C present a reversed pattern having a cross over point at ca. 290 °C where carrageenan started decomposing quickly till 400 °C. The grafted product, however, started decomposing at ca. 270 °C very slowly till ca. 400 °C. Then the TGA traces exhibited even slower decline. The greater thermal stability of the grafted products with respect to the parent polysaccharides demonstrated the formation of new materials with an aromatic compound, the latter being highly thermally stable.

The thermogravimetric (TGA) analysis curves of agarose, κ-carrageenan, cytosine, agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine are shown in Fig. 5. The mass losses in agarose, κ-carrageenan, cytosine, agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine were observed in three stages (i) mass losses were 7%, 12%, 0%, 15% and 9% up to 175 °C; (ii) losses were 80%, 74%, 45%, 55% and 45% up to 400 °C, respectively; and (iii) losses were 77%, 65%, 88 and 72% up to 750 °C respectively, except for agarose, which showed decomposition in the range 250–500 °C (Fig. 5). The first step indicated loss of bound water in the polysaccharide, which is much lower in the grafted product presumably due to the reduced hydrophilicity as a result of grafting involving the –OH groups of the polysaccharides.

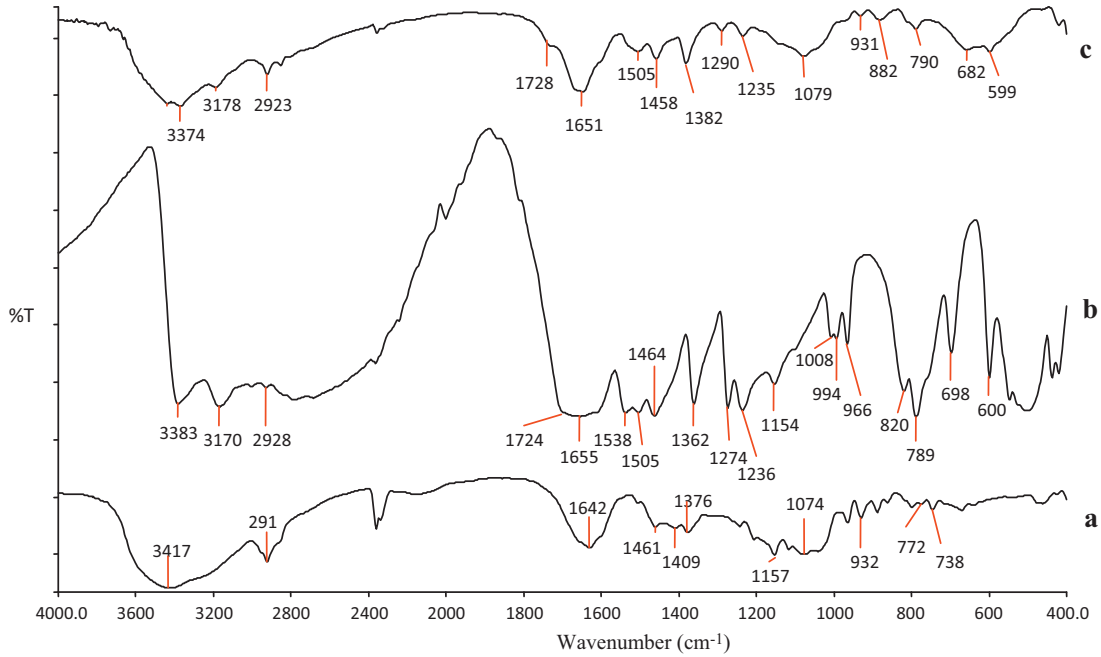

Fig. 3. FT-IR of (a) agarose, (b) cytosine and (c) agarose-*graft*-cytosine.

3.4. ^{13}C NMR

^{13}C NMR spectra of agarose, κ-carrageenan, cytosine, agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine are depicted in Figs. 6 and 7, having indicated the chemical shift values and the probable assignments. Four carbons of cytosine appeared at 96.6, 144.8, 160.7, and 168.9 ppm, which were assigned to C-5″, C-6″, C-2″, and C-4″ (Fig. 1) respectively. The assignments were done by comparison with the corresponding data obtained from ChemDraw v12.0. The carbon chemical shifts of agarose were assigned by comparison with the data reported by Meena, Siddhanta, et al. (2007). Sixteen carbon resonances were discernible in both the ^{13}C NMR spectra of agarose-*graft*-cytosine (Fig. 6) and κ-carrageenan-*graft*-cytosine (Fig. 7). These 16 peaks could be assigned to any of the three possible structures of the grafted products based on agarose/κ-carrageenan C-6/C-2/C-2′ and cytosine C-4″ NH_2 linkages (Figs. 1 and 2), having assigned the peaks at δ 49.5 and 49.6 to the newly formed C–N bonds respectively (*vide* mechanism in Section 3.12 below). The assignments were done by comparison with the values obtained for cytosine, agarose and κ-carrageenan in this study.

While assigning the ^{13}C NMR resonances, initially four possible structures were conceived C-6, C-2, C-2′ substituted or C-6, 2′-disubstituted on the basis of linkages with cytosine C-4″ NH_2.

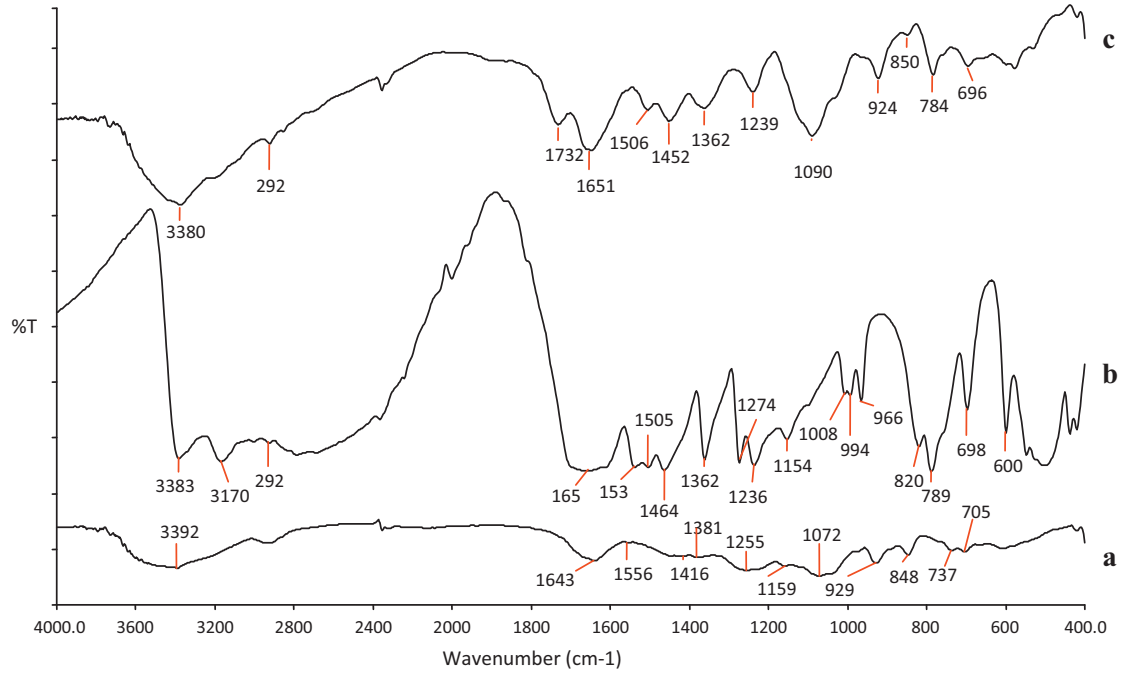

Fig. 4. FT-IR of (a) κ-carrageenan, (b) cytosine and (c) κ-carrageenan-*graft*-cytosine.

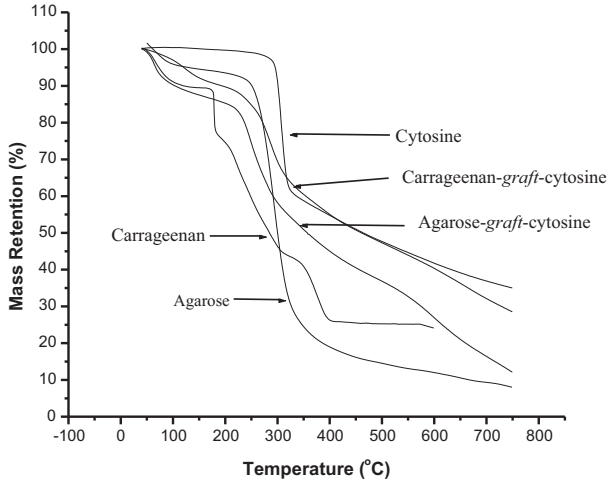

Fig. 5. TGA of (a) agarose, (b) κ-carrageenan, (c) cytosine, (d) agarose-*graft*-cytosine and (e) κ-carrageenan-*graft*-cytosine.

The di-substituted product was ruled out on the basis of the nitrogen content of the products which indicated insertion of only one cytosine moiety on to the agarose/carrageenan polymers. On the other hand, the possibilities of bond formation involving C-2/2′ were also ruled out on the basis of their carbon resonances, which remained almost unchanged (*vide* S2 in ESI). Thus the new C–N bond formation happened between C-6 and C-4″-NH_2.

Furthermore, there are possibilities that the bonding may happen between C-6 of agarose/κ-carrageenan and one appropriate N of cytosine molecule (C-4″-NH_2 or N-1″) (Figs. 1 and 2). However, the ^{13}C δ-values of the cytosine residue of the products were in excellent agreement with those of the experimental values of cytosine. Therefore, there was least anisotropic perturbation on the cytosine residue indicating thereby the involvement of the cytosine C-4″-NH_2 group in the new –C–N bond formation, ruling out the possibility of the ring nitrogen N-1″ being involved in the grafting reaction. The downfield shifts of C-6′ were presumably due to the influence of the deshielding zone of the protruding cytosine residue. Similar observation was encountered in the agarose–guanine derivative reported earlier (Oza et al., 2010). All the 17 carbons of this structure could be assigned in the NMR spectrum with a reasonable degree of confidence (Figs. 6 and 7).

3.5. Scanning electron microscopy (SEM)

The morphologies of parent agarose, κ-carrageenan and cytosine, having cloud like morphologies got converted into an integrated and rod like structures in the grafted products as exhibited in their SEM images indicating formation of new compounds (*vide* ESI Fig. S5).

3.6. Optical rotation

The optical rotation values of parent cytosine $[\alpha]^{30\,^{\circ}C}_{589\,nm}$ (c 0.25, H_2O), agarose $[\alpha]^{30\,^{\circ}C}_{589\,nm}$ (c 0.25, H_2O), κ-carrageenan $[\alpha]^{30\,^{\circ}C}_{589\,nm}$ (c

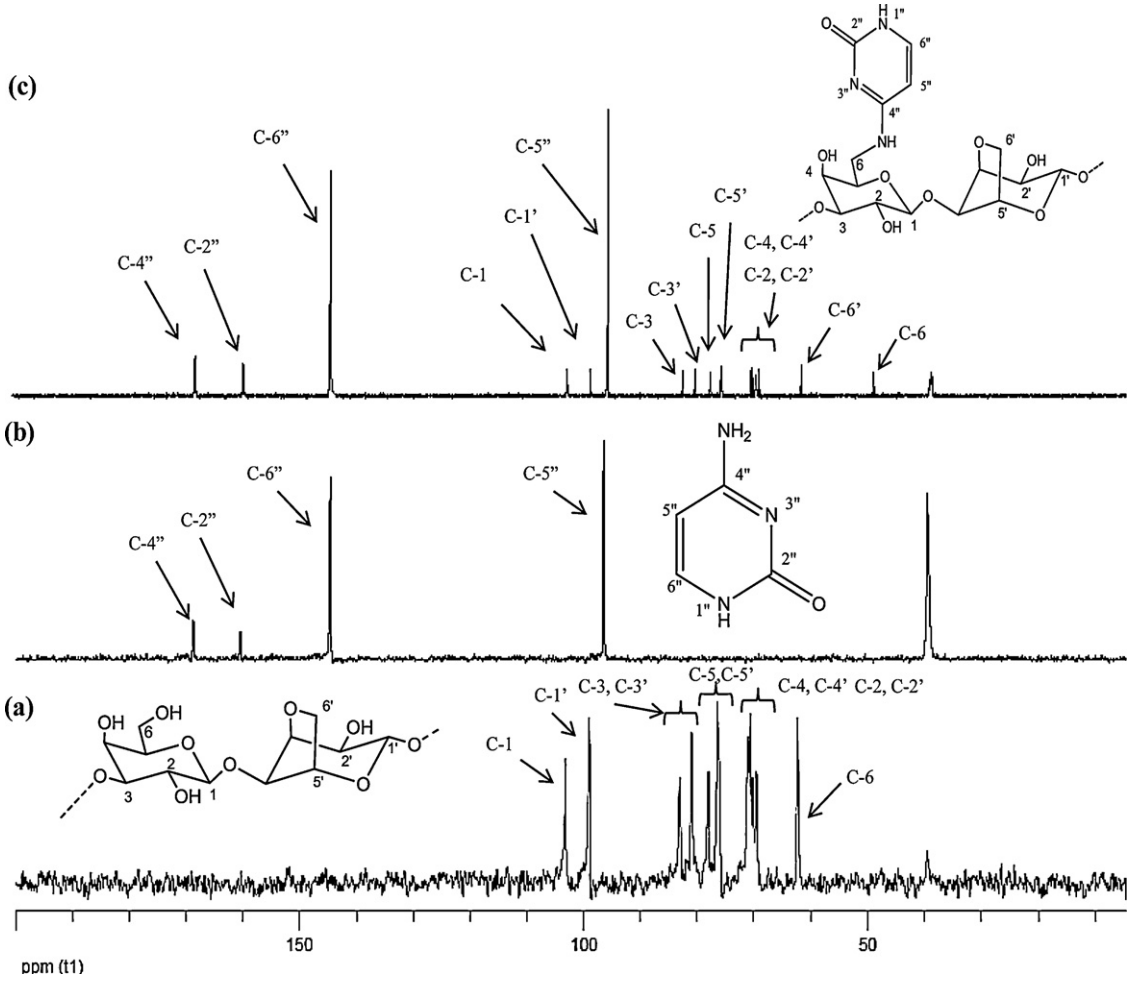

Fig. 6. ^{13}C NMR of (a) agarose, (b) cytosine and (c) agarose-*graft*-cytosine.

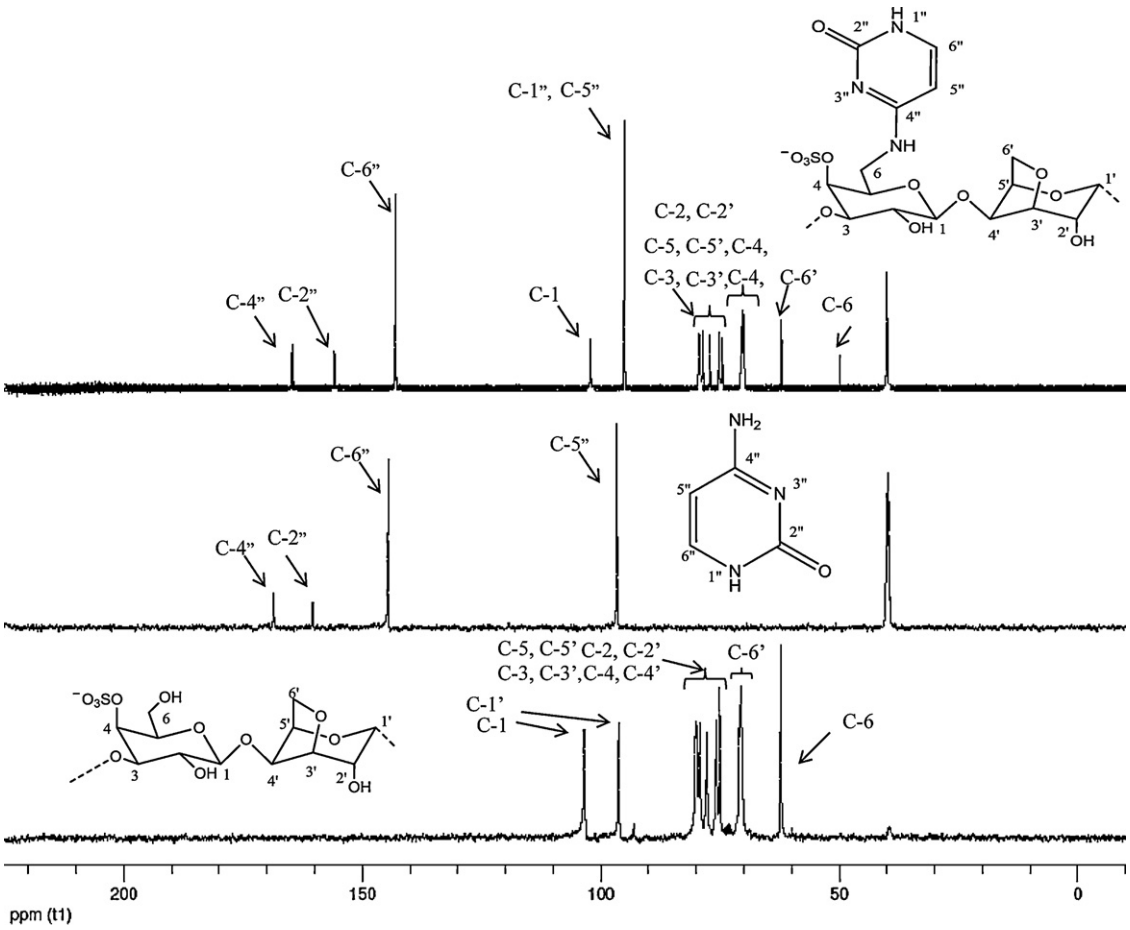

Fig. 7. ^{13}C NMR of (a) κ-carrageenan, (b) cytosine and (c) κ-carrageenan-*graft*-cytosine.

0.25, H_2O) were +767.484°, −21.6°, +67.64°, whereas those of agarose-*graft*-cytosine $[\alpha]^{30\,^\circ C}_{589\,nm}$ (c 0.25, H_2O) and κ-carrageenan-*graft*-cytosine $[\alpha]^{30\,^\circ C}_{589\,nm}$ (c 0.25, H_2O) were +82.23° and +106.64° respectively. The significantly modified $[\alpha]_D$ values of agarose and κ-carrageenan after grafting suggested changes in the molecular architecture as a result of functionalization with cytosine.

3.7. Nitrogen content

The total nitrogen contents (Kjeldahl's estimation data) in agarose, κ-carrageenan, cytosine were 0.105 ± 0.001%, 0.21 ± 0.001%, 36.92 ± 0.5%, and those of agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine were 6.65 ± 0.1%, 5.16 ± 0.1%, respectively, indicating addition of cytosine to agarose and carrageenan.

3.8. X-ray diffraction analysis

The X-ray diffraction patterns of cytosine, agarose, κ-carrageenan and their respective grafted products are presented in Figs. S6 and S7 (*vide* ESI). The X-ray diffraction pattern of agarose and κ-carrageenan indicated that agarose and κ-carrageenan were largely amorphous, while parent cytosine exhibited nine sharp peaks (at 2θ = 10°, 12°, 15°, 22°, 22.4°, 23°, 25°, 26.5° and 29°) indicating its crystalline nature (*vide* ESI Figs. S6 and S7). Furthermore, the XRD pattern of agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine also showed many sharp peaks (at 2θ = 9.6°, 19°, 23°, 28.6°) and (at 2θ = 10.6°, 17°, 20°, 22°, 24°, 26.5°) indicating induction of crystallinity on to agarose and κ-carrageenan respectively. Enhanced crystallinity suggested ordered molecular arrangements, which were associated with a substantial change in the quantum of optical rotation values, e.g. from −21.60° (in agarose) to +82.23° (in agarose-*graft*-cytosine) and from +67.64° (in κ-carrageenan) to +106.64° (in κ-carrageenan-*graft*-cytosine), as a result of chiroptical makeover. Similar observations, i.e. enhanced crystallinity coupled with changes in the optical rotation values in the grafted products, were reported in agar-*graft*-PVP and carrageenan-*graft*-PVP blends (Prasad, Mehta, et al., 2006; Oza et al., 2010). The crystallinity index (C.I.) of the grafted products were determined using the following Eq. (1) described by Herman and Weidinger (1948).

$$\text{C.I.} = \frac{\text{area of crystalline peaks}}{\text{area of crystalline peaks} + \text{area of amorphous peaks}}. \quad (2)$$

The C.I. value calculated for the grafted products were 0.50 and 0.53, while the agarose and carrageenan polymers were amorphous.

3.9. UV–vis analysis

The absorption maxima in the UV–vis spectrum appeared at ca. 266 nm in cytosine, agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine (in distilled water), while agarose and κ-carrageenan did not have any absorption bands in the UV–vis region. The grafted products (5×10^{-5} M) exhibited absorption maxima with ε_{266} 2478 $M^{-1}\,cm^{-1}$, whereas that of cytosine was 2272 $M^{-1}\,cm^{-1}$ at the same concentration. At a concentration 3.09×10^{-5} and 3.5×10^{-5} M pure cytosine exhibited molar extinction coefficient values, ε_{266} 4003 $M^{-1}\,cm^{-1}$ and 3534 $M^{-1}\,cm^{-1}$. These

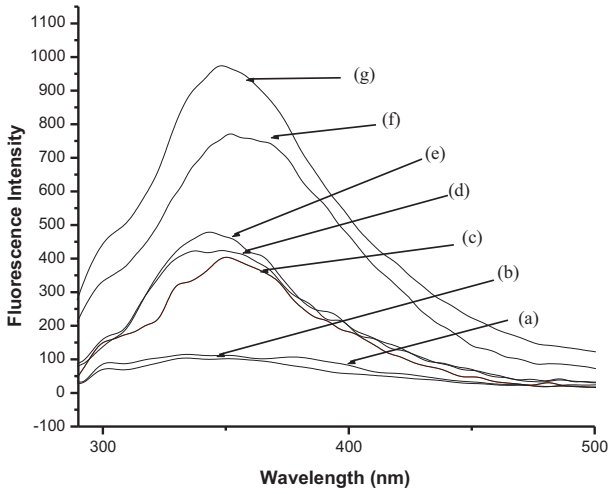

Fig. 8. Fluorescence emissions of (a) agarose, 5×10^{-5} M; (b) κ-carrageenan, 5×10^{-5} M; (c) cytosine, 3.09×10^{-5} M, containing 0.030 mM cytosine; (d) cytosine, 3.5×10^{-5} M, containing 0.035 mM cytosine; (e) cytosine, 5×10^{-5} M, containing 0.048 mM cytosine; (f) agarose-*graft*-cytosine, 5×10^{-5} M, containing 0.030 mM cytosine; (g) κ-carrageenan-*graft*-cytosine, 5×10^{-5} M, containing 0.035 mM cytosine.

concentrations were equivalent to the actual molar contents of cytosine in the 5×10^{-5} M solution of agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine respectively. It may be noted that there were decrements in the ε_{266} values in the grafted products when compared to those of pure cytosine at the concentrations mentioned above. This phenomenon points to the fact that there happened a transformation in the molecular make up of the architecture of the parent polysaccharides on substitution with cytosine (cf. Section 3.6). A reverse trend, however, was registered in the fluorescence intensity of the grafted product under the similar circumstances (*vide* infra Section 3.10).

3.10. Fluorescence measurements

The fluorescence emissions (λ_{max} 348 nm) of pure cytosine, agarose, κ-carrageenan, the respective grafted products were measured at 5×10^{-5} M concentration (Fig. 8). Agarose and κ-carrageenan at this concentration exhibited negligibly low emissions. The emission spectrum of the modified agarose and κ-carrageenan recorded in distilled water solution (5×10^{-5} M) exhibited emission maxima ($\lambda_{em,max}$) at ca. 348 nm by excitation at 266 nm. At this concentration, the emission intensities of grafted agarose and κ-carrageenan products were enhanced by ca. 104% and 60% respectively, compared to that of pure cytosine solution. At the concentration of the pure cytosine solution, which was equivalent to the molar contents, e.g. 3.09×10^{-5} and 3.5×10^{-5} M, present in 5×10^{-5} M solution of modified agarose and κ-carrageenan then ca. 143% and 81% enhancement in the emission intensities were observed, respectively. The relatively poorer fluorescence yield of cytosine moiety in the products in their higher concentration was probably because of stronger intermolecular interactions leading to quenching of emission intensity (Fig. 8) (Callis, 1979). Further, the enhancement of fluorescence intensity in less concentrated solution may also be attributed to the fact that the cytosine residue in the grafted products occurred well apart for inter-cytosine molecular interactions to take place (Figs. 1 and 2).

3.11. Circular dichroism (CD)

The CD spectrum of non-modified agarose was fully in the positive region with a peak value of [θ] 70.86 at 190 nm while non modified κ-carrageenan showed a negative trend having the peak value of [θ] −22.00 at 180 nm and trough [θ] −155.00 at 190 nm. The CD spectrum of cytosine showed positive peak values [θ] 17.04 at 187 nm and 1.31 at 264 nm. Agarose-*graft*-cytosine showed a trough at [θ] −1.76 at 208 nm, while carrageenan-*graft*-cytosine showed peak at [θ] 27.50 at 195 nm (*vide* Fig. S4), suggesting significant chiroptical changes in the nucleobase grafted polymers (cf. Dentini, Rinaldi, Barbetta, Risica, & Skjak-Bræk, 2006; McReynolds & Gervay-Hague, 2000; Morris, Rees, Sanderson, & Thom, 1975; Morris et al., 1980). Furthermore, it can also be explained on the basis of peak/trough ratios of the parent and modified polymeric materials. The peak-to-trough ratios of cytosine and agarose were 54.25 and 70.86, respectively (>1), while that of grafted product agarose-*graft*-cytosine was −1.26 (<1). Similarly peak-to-trough ratios of κ-carrageenan was 0.86 (<1) and that of grafted product κ-carrageenan-*graft*-cytosine 27.5 (>1), confirming major chiroptical makeover of the agarose and κ-carrageenan polymers (cf. Chhatbar et al., 2009; Morris et al., 1975, 1980; Oza et al., 2010).

This further exemplifies that the presence of certain molecules or substance in the parent polymers induces conformational alterations in the polymeric chain under appropriate physical conditions, resulting in pronounced changes in the factor mol. ellipticity against wavelength (cf. Morris et al., 1980).

3.12. Mechanism of formation of the grafted products

The plausible mechanisms of formation of the grafted products are shown in Figs. 1 and 2. The reactions are proposed to take place via free radical mechanism where sulphate anion radical is formed first from KPS under microwave irradiation conditions. Then the radical ion generated the agarose and the carrageenan free radicals on the C-6 carbon (the predominant possibility) with the elimination of OH radical (A), as well as the cytosine radical (B) on the C-4″-NH (the most likely possibility). The OH radical from the polymer and the H radical from cytosine, which were generated during the radical formation process reacted with each other giving rise to the water molecule (Figs. 1 and 2). The two radical species (A and B) thus produced subsequently got coupled to form a new C–N bond resulting in the agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine products as evident from the ^{13}C NMR data (new peak at 49.5 and 49.6 ppm) (*vide* Figs. 1 and 2) (cf. Prasad, Bahadur, Meena, & Siddhanta, 2008). The simple nature of the ^{13}C NMR spectrum in which all the 16 carbon resonances could be assigned to the 17 carbons of the respective grafted polymers indicated that the products were formed exclusively in the radical coupling reaction (Figs. 1 and 2). Chemical formation of the grafted products was thus confirmed by the FT-IR, ^{13}C NMR spectral data, which were supplemented by the XRD pattern, CD spectrum and thermal behavior as well as UV and fluorescence spectra.

4. Conclusion

A facile water based synthesis and characterization of agarose-*graft*-cytosine and κ-carrageenan-*graft*-cytosine have been described. At a concentration of 5×10^{-5} M these products exhibited good fluorescence emissions, wherein the molar contents of cytosine were 3.09×10^{-5} and 3.5×10^{-5} M respectively. At the latter concentrations of the grafted agarose and κ-carrageenan products, significant enhancements (143% and 81%) in the fluorescence emissions were registered. These fluorescent polysaccharides may be of potential utility in the domain of various sensor applications including the biomedical ones (cf. Donati, Gamini, Vetere, Campa, and Paoletti (2002)).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.10.004.

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