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Functional modification of agarose: A facile synthesis of a fluorescent agarose–guanine derivative

Mihir D. Oza[a], Ramavatar Meena[a], Kamalesh Prasad[a], P. Paul[b], A.K. Siddhanta[a,*]

[a] Marine Biotechnology & Ecology Discipline, Central Salt & Marine Chemicals Research Institute, Council of Scientific & Industrial Research (CSIR), Bhavnagar 364 002, Gujarat, India
[b] Analytical Sciences Discipline, Central Salt & Marine Chemicals Research Institute, Council of Scientific & Industrial Research (CSIR), Bhavnagar 364 002, Gujarat, India

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ABSTRACT

A new fluorescent polymeric material was synthesized by grafting the nucleobase guanine on to the backbone of agarose. The synthesis involved a rapid water based method under microwave irradiation using potassium persulphate (KPS) as an initiator. The emission spectrum of the modified agarose recorded in 0.1 M aqueous NaOH (5×10^{-5} M) solution exhibited emission maxima ($\lambda_{em,max}$) at 340 nm by excitation at 274 nm. The emission intensity was enhanced by ca. 85% compared to that of pure guanine solution of the same concentration. When the concentration of the pure guanine solution is made equivalent to the concentration of the guanine molar component (3.63×10^{-5}) present in 5×10^{-5} M solution of modified agarose, then ca. 105% enhancement in emission intensity was observed. The remarkable fluorescent activity of the agarose–guanine derivative may have potential uses as sensor in various applications.

1. Introduction

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. Synthesis and fluorescent properties of pyrene-labeled guanine base was reported for studying the secondary structures of G-rich DNA (Okamoto, Kanatani, Ochi, Saitob, & Saito, 2004). There exist numerous reports in the literature on the modification of polysaccharides employing various strategies, e.g. grafting, cross linking, etc.

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; 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 (Fig. 1) by grafting guanine (Fig. 1) on to agarose by a water based method. This guanine modified agarose exhibited exceptionally strong fluorescent properties. Guanine is 2-amino-6-hydroxypurine, 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). To our knowledge, this agarose derivative and its effects are being reported for the first time.

2. Experimental

2.1. Materials

Agarose used in this study was extracted from the seaweed *Gracilaria dura* as described in our previous work (Meena, Siddhanta, et al., 2007). Other chemicals used in this study (e.g. sodium hydroxide, potassium persulphate (KPS) and guanine, LR grade) were purchased from S.D. Fine Chemicals Ltd., Mumbai (India).

* Corresponding author. Tel.: +91 278 2561354/7760; fax: +91 278 2567562/6970.
E-mail address: aks@csmcri.org (A.K. Siddhanta).

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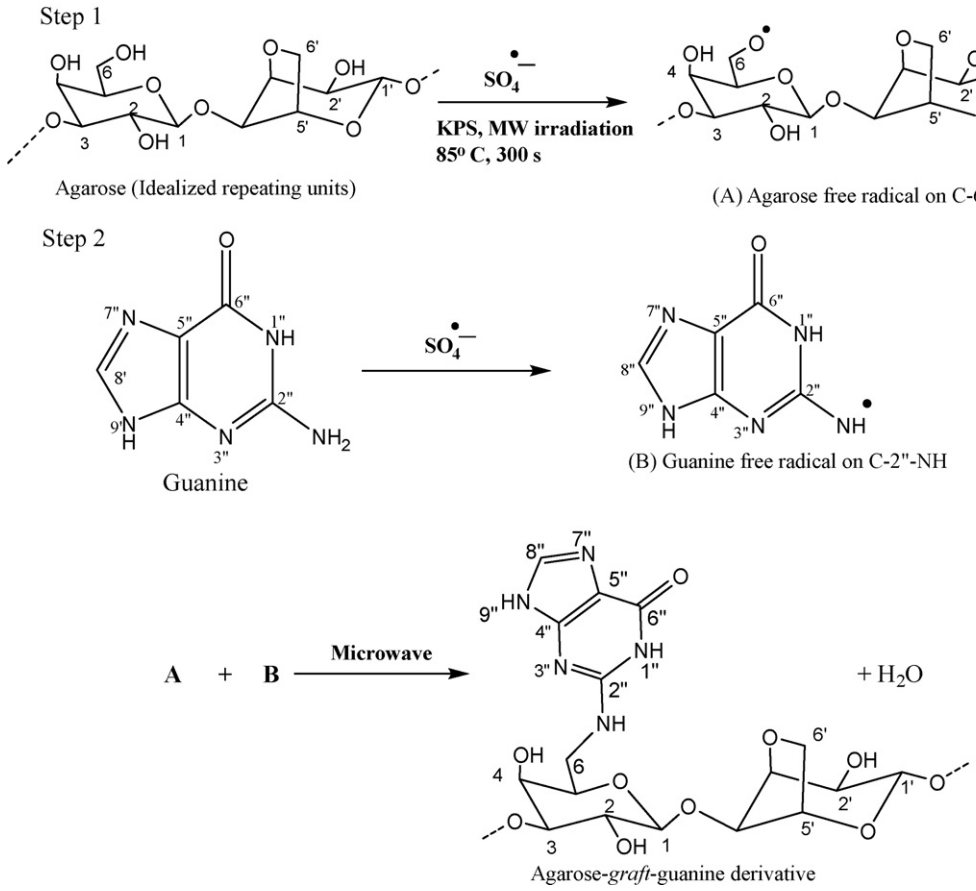

Fig. 1. Plausible mechanism of formation of agarose-*graft*-guanine.

2.2. Synthesis of agarose-graft-guanine

A known weight of agarose (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 (50 mg) of guanine was dissolved in 20 ml of 0.5 M aqueous sodium hydroxide (pH ca. 11.6), and then mixed with the hot agarose sol and KPS mixture under stirring condition followed by microwave irradiation for 5 min. The colorless reaction mixture got converted slowly into a dark yellow mixture. The yellow colored product was isolated from the reaction mixture by precipitation with isopropanol (IPA) (reaction mixture:IPA = 1:2, v/v), and was air dried. The dry product was redissolved in 0.1 M NaOH solution (ca. pH 9.5), when unreacted guanine (superimposable IR spectra) got precipitated out, leaving behind the yellow product in the solution. Unreacted guanine was filtered off. To the filtrate was added slowly dilute HCl (0.1 M) to bring the pH ca. 7.7, when onset of clouding of the solution was observed. The product was isolated by precipitation with isopropanol (IPA) (reaction mixture:IPA = 1:2, v/v) at pH 7.7. The weights of unreacted guanine and agarose-*graft*-guanine were determined.

2.3. FT-IR spectra

The non-modified and modified agarose was characterized by FT-IR analysis using a PerkinElmer FT-IR machine (PerkinElmer 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 and non-modified agarose were obtained on a Varian CARY 500 UV–vis-NIR spectrophotometer, Pittsburgh, USA. The fluorescence spectra were recorded at room temperature on a PerkinElmer Spectrofluorimeter LS-50B, USA. The fluorescence emission spectra of agarose, guanine, and agarose-*graft*-guanine were measured at a concentration 5×10^{-5} M in 0.1 M NaOH as well as additionally at a concentration of 3.63×10^{-5} M of guanine using excitation and emission slits 5.0/5.0 nm. Guanine and modified agarose were excited at 274 nm with an emission at 340 nm.

2.5. Optical rotation and circular dichroism

Optical rotations were measured on a Digipol 781 automatic polarimeter (Rudolph Instruments Inc., NJ, USA) (c 0.5%, 0.1 M NaOH) at 35 °C. Circular dichroism (CD) spectra were recorded on a 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, $[\theta]$ are reported in units of deg cm^2 $dmol^{-1}$. All measurements were performed at room temperature using 1.0 cm quartz cells.

2.6. ^{13}C NMR spectroscopy

The NMR spectra of agarose and agarose-*graft*-guanine were recorded on a Bruker AVANCE II 500 MHz spectrometer, Switzerland, at 70 °C. Agarose was dissolved in D_2O (50 mg/ml), agarose-*graft*-guanine was dissolved in D_2O and NaOH (50 mg/ml) and the spectra were recorded at 70 °C with 7000–7500 accu-

mulations, pulse duration 11.25 μs, acquisition time 1.048 s and relaxation delay 6 μs using DMSO as internal standard (ca. d 39.5). Guanine was dissolved in D_2O/NaOH (20 mg/ml) and spectra 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-guanine

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, agarose-*graft*-guanine, guanine, and the physical mixture of agarose and guanine (1:0.5, w/w). The physical mixture of agarose–guanine (1:0.5) was prepared by triturating the two compounds in a paste in presence of a few drops of water, followed by air and vacuum drying the paste, which was ground to powder. For recording SEM image, vacuum dried samples of the powder of agarose, agarose-*graft*-guanine and guanine 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.

2.8. Steric energy calculation

The steric energy was calculated by ChemBiooffice Chem3D Pro v.8.0 (2008) software (Molecular Mechanics 2 or MM2), considering the basic structure of the product given in Fig. 1.

3. Results and discussion

3.1. Yield and grafting pattern

Yield of the product was 90% which was calculated on the basis of the nitrogen content of the product (Kjeldahl's estimation) with respect to the total quantities of agarose and guanine that were used in the synthesis. Grafting percent (G%) in the product was 135%, whereas its total conversion (C%) value was 70% (cf. Meena et al., 2008).

3.2. FT-IR spectroscopy

Strong bands at 1642 for agarose (bonded H–O–H; Christiaen & Bodard, 1983; Prasad, Mehta, et al., 2006) and 1673 and 1697 cm^{-1} for guanine for amide carbonyl (Bellamy, 1957; cf. Nakanishi & Solomon, 1977) were observed in the FT-IR spectra (Fig. 2). The spectrum of agarose-*graft*-guanine exhibited strong bands at 1670 and 1695 cm^{-1} as well as a shoulder at 1642 cm^{-1}, indicating the presence of both agarose and guanine moieties in the product. In the IR spectrum of guanine, the sharp band for C-2"–NH_2 at 3335 cm^{-1} (νNH_2; cf. Nakanishi & Solomon, 1977) was not observed clearly in the spectrum of agarose-*graft*-guanine (Fig. 2), which indicated the involvement of C-2"–NH_2 in the chemical transformation to form a new compound. In the product, several bands, e.g. 1414, 1117, 687, 605 cm^{-1} were observed, whereas in guanine a relatively stronger band appeared at 1418 cm^{-1} (Fig. 1), indicating a discernible difference in the structure of the product in comparison with those of pure agarose and guanine. The band at 1414 cm^{-1} in the product and at 1418 cm^{-1} in guanine are presumably due to

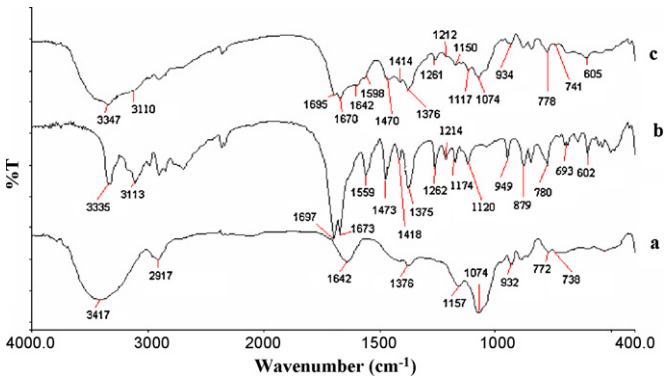

Fig. 2. FT-IR of (a) agarose, (b) guanine and (c) agarose-*graft*-guanine.

the "ring pinching mode of guanine" (Abo-riziq et al., 2007), while the bands at 1117 and 1120 cm^{-1} may be attributed respectively to C–NH–/C–NH_2 bending vibrations (Bellamy, 1957). The remaining bands at 687, 605 cm^{-1} in the product and 693 and 602 cm^{-1} in guanine may have arisen due to –CH deformations (cf. Bellamy, 1957). A broad shoulder is observed around 3110 cm^{-1} in agarose-*graft*-guanine, whereas guanine exhibited a sharp band at 3113 cm^{-1} for –NH-stretching (cf. Nakanishi & Solomon, 1977). Furthermore, characteristic bands at 934 (3,6-anhydro moiety of agarose), 778 and 741 cm^{-1} for β-skeletal bending of basic carbohydrate moieties in the IR spectrum of the copolymer indicated that during grafting reaction the agarose polymer did not get decomposed (cf. Prasad, Mehta, et al., 2006). The attribution of important IR bands of guanine and agarose (Figs. 1 and 2) is given below.

Guanine (Bellamy, 1957; cf. Abo-riziq et al., 2007; Nakanishi & Solomon, 1977): (cm^{-1}) 3335, 3113 (νNH_2, νNH); 1697, 1673 (amide C=O); 1559 (ring double bonds); 1473, 1418 (ring vibration); 1120–949 (C–NH_2 bending); 879–780 (skeletal vibration); 693, 602 (–CH deformations).

Agarose (Christiaen & Bodard, 1983; Freile-Pelegrín & Murano, 2005; Prasad, Mehta, et al., 2006): (cm^{-1}) 3417 (–OH stretching); 1642 (bonded H–O–H); 1376 (–CH_2–OSO_3^{-2} linkage at C-6, which is not shown in the idealized repeating units of agarose in Fig. 1); 1074 (C–O–C glycosidic linkage); 932 (3,6-anhydrogalactose linkage). 778 and 741 cm^{-1} (β-skeletal bending of basic carbohydrate moieties).

3.3. Thermal analysis (TGA)

The TGA patterns of the guanine grafted agarose were comparable, in fact superimposable to that of guanine in repeated experiments. The initial TGA traces of agarose and the grafted product up to 250 °C present a reversed pattern having a cross over point at ca. 250 °C whereat agarose started decomposing very fast till 500 °C. The grafted product, however, started decomposing at ca. 250 °C very slowly till ca. 460 °C. Then the TGA traces exhibited even slower decline. The thermal stability of the grafted product with respect to agarose indicated the formation of a new material with an aromatic compound, the latter being highly thermally stable.

The thermogravimetric (TGA) analysis curves of agarose, guanine and agarose-*graft*-guanine are shown in Fig. 3. The mass losses in agarose, guanine and agarose-*graft*-guanine were observed in three stages (i) mass losses were 15%, 5% and 5.5% up to 175 °C; (ii) losses were 70%, 36% and 37% up to 400 °C, respectively and (iii) losses were 65% and 67% up to 750 °C for guanine and agarose-*graft*-guanine, respectively, while agarose showing decomposition in the range 250–500 °C (Fig. 3). The first step indicated loss of bound water in the polysaccharide, which is much lower in

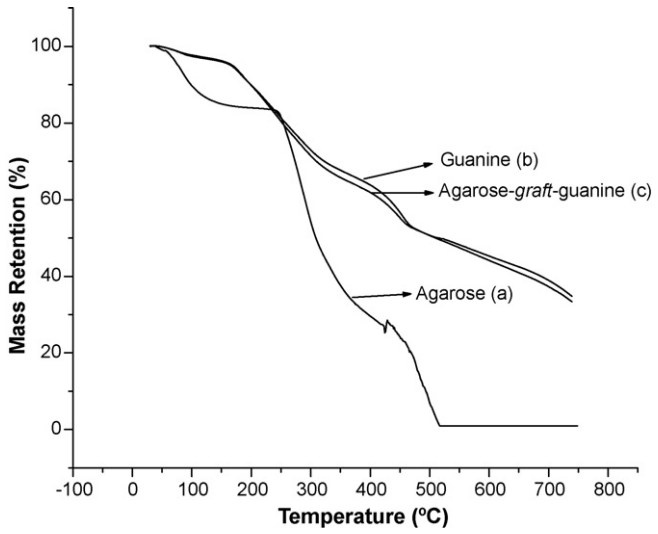

Fig. 3. TGA of (a) agarose, (b) guanine and (c) agarose-*graft*-guanine.

the grafted product presumably due to the enhanced hydrophobicity as a result of grafting of agarose reflecting the nature of guanine.

3.4. ^{13}C NMR

^{13}C NMR spectra of agarose, guanine, agarose-*graft*-guanine are given in Fig. 4a–c, having indicated the chemical shift values and the probable assignments. Five carbons of guanine appeared at 120.3, 150.5, 160.6, 162.8 and 169.3 ppm, which were assigned to C-5, C-8, C-2, C-4 and C-6 (Fig. 1), respectively. The assignments were done by comparison with the corresponding data obtained from ChemDraw v10.0. To our knowledge there exists no report of experimental ^{13}C NMR data of guanine or its derivative in the literature. The carbon chemical shifts of agarose were assigned by comparison with the data reported by Meena et al. (2007b). Of the 24 carbon resonances that were discernible in the ^{13}C NMR spectrum of agarose-*graft*-guanine (Fig. 1), containing 17 carbons, three peaks at 182.3, 76.0 and 73.6 ppm could not be assigned. The remaining 21 peaks could be assigned to any of the three possible structures of the grafted products based on agarose C-6/C-2/C-2′ and guanine C-2 NH_2 linkages (Fig. 1). Appearance of a ^{13}C NMR peak at δ 50.4 indicated a new C–N bond formation (vide mechanism in Section 3.12 below). The assignments were done by comparison with the values obtained for guanine and agarose in this study.

Table 1
Steric energy values of possible structures of the agarose-*graft*-guanine derivative.

Entry	Derivatives	Iterations	Steric energy[a] (kcal/mol)
1	Agarose C-6-guanine C-2 NH_2	661	69.6
2	Agarose C-2′-guanine C-2 NH_2	331	79.8
3	Agarose C-2-guanine C-2 NH_2	354	83.5
4	Agarose C-6-guanine N-9	452	91.6

[a] The steric energy was calculated by ChemDraw ChemBiooffice Chem3D Pro v10.0 software (Molecular Mechanics 2 or MM2), considering the basic structure of the product given in Fig. 1.

While assigning the ^{13}C NMR resonances, initially four possible structures were conceived, e.g. agarose C-6, C-2, C-2′-substituted or C-6, 2′-disubstituted products on the basis of linkages with guanine C-2 NH_2. The disubstituted product was ruled out on the basis of the nitrogen content of the product which indicated insertion of one guanine moiety on to the agarose polymer. The steric energy values were also calculated (Table 1) by ChemBiooffice Chem3D Pro v10.0 software (Molecular Mechanics 2 or MM2), considering the basic structure of the product as given in Fig. 1a (cf. Miller, Macrea, & Pycior, 2004; Seki, Ichimura, & Imamura, 1981). On the basis of the lowest calculated steric energy value of these three possible isomers it is proposed that agarose C-6 substituted one would be the most preferred structure of the product and 17 carbons of this structure could be assigned in the NMR spectrum with a reasonable degree of confidence. However, there were 4 more discernible

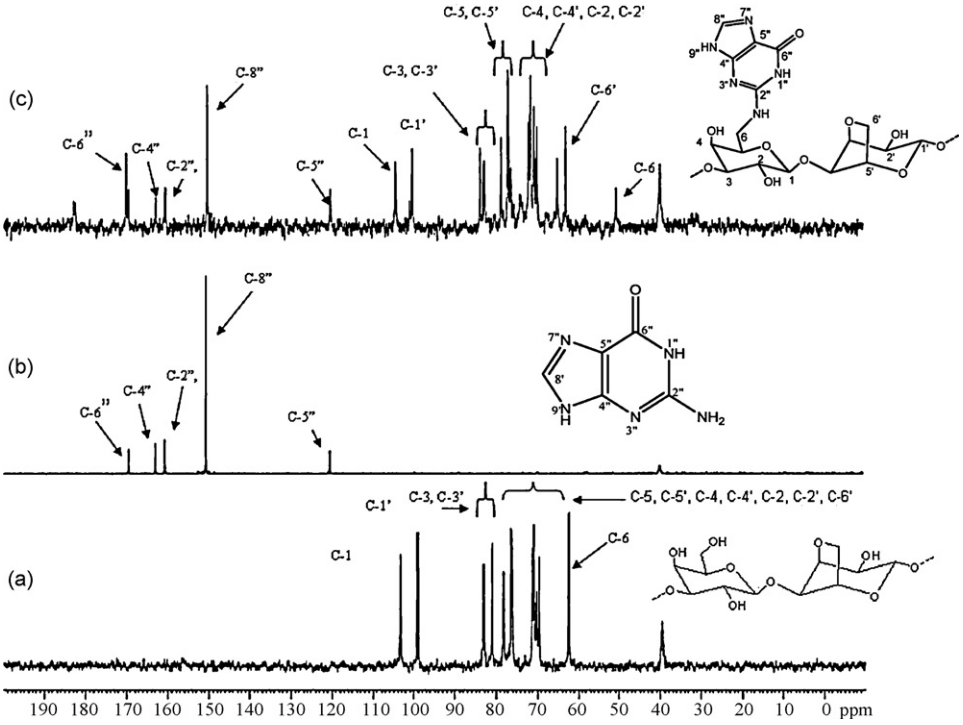

Fig. 4. ^{13}C NMR of (a) agarose, (b) guanine and (c) agarose-*graft*-guanine.

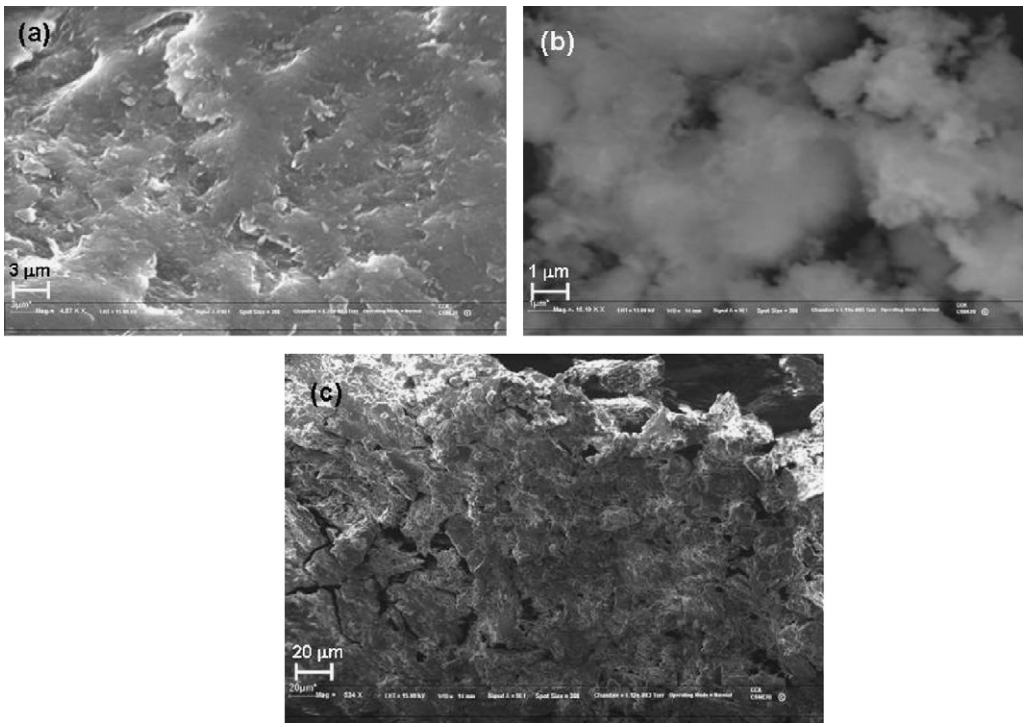

Fig. 5. SEM images of (a) agarose, (b) guanine and (c) agarose-*graft* guanine.

peaks which might have appeared due to the presence of the less preferred isomer(s) (Table 1).

Furthermore, there are possibilities that the bonding may happen between C-6 of agarose and one appropriate ring N of guanine, e.g. N-9″ (Fig. 1). However, the ^{13}C δ-values of the guanine residue of the product were in excellent agreement with those of the experimental values of guanine. Therefore, there was least perturbation on the electronic environ of the guanine residue indicating thereby the involvement of the guanine C-2″–NH_2 group in the new N–C bond formation. This was supplemented by the relatively high calculated steric energy value of agarose C-6–guanine N-9″ product indicating its low preference of formation (Table 1). The carbon resonances at 182.3, 76.0 and 73.6 were not assigned (Fig. 4).

3.5. Scanning electron microscopy (SEM)

The cloud like morphologies of parent agarose and guanine got converted into an integrated and porous structure in the agarose-*graft*-guanine in their SEM images indicated the formation of a new product (Fig. 5).

3.6. Optical rotation

The optical rotation values of parent guanine $[\alpha]_{589}{}^{30}$ (c 0.25, 0.1 M NaOH solution) and agarose $[\alpha]_{589}{}^{30}$ (c 0.25, H_2O) were +43.78° and −21.60°, respectively, while of agarose-*graft*-guanine $[\alpha]_{589}{}^{30}$ (c 0.25, 0.1 M NaOH solution) was +208.70°. The modified $[\alpha]_D$ value of agarose after grafting indicates changes in the molecular symmetry profiles as a result of functionalization with guanine.

3.7. Nitrogen content

The total nitrogen contents in agarose, guanine and agarose-*graft*-guanine were 0.105 ± 0.001%, 46.0 ± 0.50% and 10.68 ± 0.11% (Kjeldahl's estimation data), respectively, indicating addition of guanine to agarose.

3.8. X-ray diffraction analysis

The X-ray diffraction patterns of guanine, agarose and agarose-*graft*-guanine are presented in Fig. 6. The X-ray diffraction pattern of agarose indicates that agarose having mostly amorphous region in its structure, while parent guanine exhibited six sharp peaks (at $2\theta = 14°$, 25°, 26°, 28°, 31° and 32°) indicates its crystalline nature (Fig. 6). Furthermore, the XRD pattern of agarose-*graft*-guanine also showed many sharp peaks (at $2\theta = 17°$, 18°, 28°, 29°, 32°, 33°, 37°), indicating induction of crystallinity on to agarose. Enhanced crystallinity suggested ordered molecular arrangement, which was associated with a substantial change in the quantum of optical rotation values, e.g. from −21.60° (in agarose) to +208.702° (in agar-*graft*-guanine), as a result of chiroptical modification. Similar observations, i.e. enhanced crystallinity coupled with changes in the optical rotation values in the grafted product, were reported in agar-*graft*-PVP and carrageenan-*graft*-PVP blends (Prasad, Mehta, et al., 2006, Prasad, Meena, et al., 2006). The crystallinity index (C.I.) of the grafted product was determined using the following Eq. (1) described by Herman and Weidinger (1948).

$$\text{C.I.} = \frac{\text{area of crystalline peaks}}{[\text{area of crystalline peaks} + \text{area of amorphous peaks}]}. \quad (1)$$

The C.I. value calculated for the grafted product was 0.73, while the agarose polymer was amorphous.

3.9. UV–vis analysis

The absorption maxima in the UV–vis spectrum appeared at ca. 274 nm in guanine and agarose-*graft*-guanine (in 0.1 M NaOH solution), while agarose did not have any absorption bands in the UV–visible region. The product (5×10^{-5} M) exhibited absorption maxima with ε_{274} 15720 M^{-1} cm^{-1}, whereas that of guanine was 2460 M^{-1} cm^{-1} at the same concentration. At a concentration 3.63×10^{-5} M guanine exhibited molar extinction coefficient value, ε_{274} 7190 M^{-1} cm^{-1}. This concentration was equivalent to the actual molar content of guanine in the 5×10^{-5} M solution of

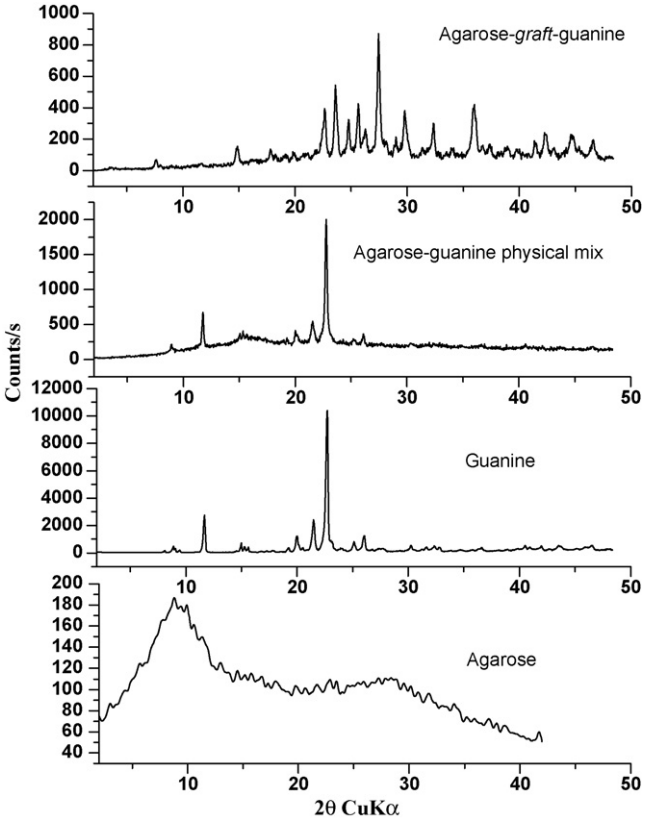

Fig. 6. XRD patterns of agarose, guanine, agarose–guanine physical mixture and agarose-*graft*-guanine.

agarose-*graft*-guanine. Hence, there was a 2.2-fold enhancement in the ε_{274} value in the latter, indicating that agarose polymer became substantially more UV sensitive in presence of guanine moiety compared to the parent agarose, which was UV transparent. This phenomenon points to the fact that there happened a transformation in the molecular make up of the polymeric architecture of the parent polysaccharide on substitution with guanine (cf. Section 3.6). The same trend of significant enhancement in the fluorescence intensity in the product compared to that of guanine was also observed in the fluorescence emission spectra (vide infra Section 3.10).

3.10. Fluorescence measurements

The fluorescence emissions (λ_{max} 340 nm) of pure guanine, agarose and the agarose-*graft*-guanine derivative were measured at 5×10^{-5} M concentration (Fig. 7). Agarose at this concentration exhibited negligibly low emissions. The emission spectrum of the modified agarose recorded in 0.1 M aqueous NaOH (5×10^{-5} M) solution exhibited emission maxima ($\lambda_{em,max}$) at 340 nm by excitation at 274 nm. The emission intensity was enhanced by ca.85% compared to that of pure guanine solution of the same concentration. When the concentration of the pure guanine solution is made equivalent to the concentration of the guanine molar component (3.63×10^{-5}) present in 5×10^{-5} M solution of modified agarose, then ca.105% enhancement in emission intensity was observed. Fluorescence yield of guanine is poor probably because of intermolecular interaction leading to quenching of emission intensity (Fig. 7) (Callis, 1979). In the product the guanine residue is placed well apart for inter-guanine molecular interaction to take place (Fig. 1). Therefore, possibly this has contributed to the enhancement of fluorescence intensity.

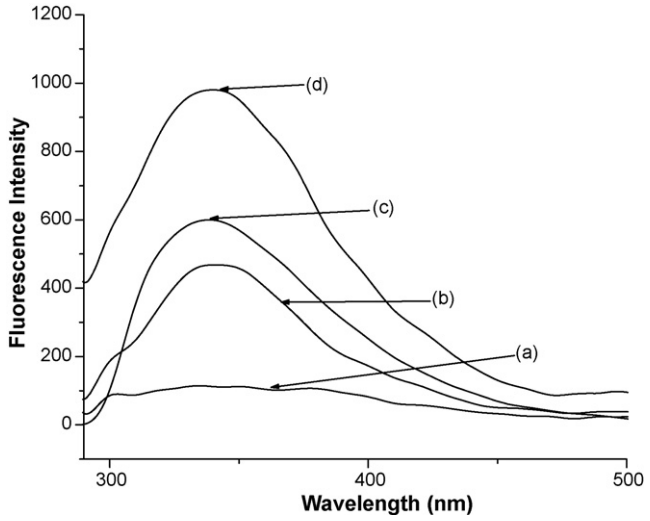

Fig. 7. Fluorescence emissions of (a) agarose, 5×10^{-5} M, (b) guanine, 3.63×10^{-5} M, containing 0.037 mM guanine, (c) guanine, 5×10^{-5} M, containing 0.051 mM guanine, (d) agarose-*graft*-guanine, 5×10^{-5} M, containing 0.037 mM guanine.

3.11. Circular dichroism (CD)

The CD curve of agarose exhibited peak [θ] −76.66 and trough [θ] −140.23 and that of guanine exhibited peak at [θ] 437.32 and at trough at [θ] −787.37. Agarose-*graft*-guanine showed intermediate behavior having peak at [θ] −612.27 and trough at [θ] −1305, indicating the insertion of guanine on to the agarose polymer. The peak-to-trough ratio of agarose 0.46 (<1), while those of guanine and agarose-*graft*-guanine were >1 (1.48 and 1.42, respectively), suggesting significant chiroptical modification of the agarose polymer (cf. Dentini, Rinaldi, Barbetta, Risica, & Skjak-Bræk., 2006; McReynolds & Gervay-Hague, 2000; Morris, Rees, Sanderson, & Thom, 1975; Morris, Rees, & Thom, 1980). This is yet another demonstration of the fact that the presence of certain molecules or substance in the parent polymer induces conformational changes in the polymeric chain under appropriate physical conditions, resulting in pronounced changes in the factor Mol. ellipticity with wavelength (cf. Morris et al., 1980).

3.12. Mechanism of formation of the grafted product

The plausible mechanism of formation of the grafted product is shown in Fig. 1. The reaction is 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 free radical on C-6 carbon (the predominant possibility) with the elimination of OH radical, as well as the guanine radical on the C-2"–NH (the most likely possibility). The two radical species thus produced subsequently got coupled to form a new C–N bond resulting in the agarose-*graft*-guanine product as evident from the ^{13}C NMR data (new peak at 50.4 ppm) and the lowest calculated steric energy value (*vide* Fig. 1 and Table 1) (cf. Prasad, Bahadur, Meena, & Siddhanta, 2008). The simple nature of the ^{13}C NMR spectrum as well as the absence of complementary carbon resonances other than those of the product shown in Fig. 1, indicated that the thermodynamically more stable product was predominantly formed in the radical coupling reaction. Chemical formation of the grafted product 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–guanine derivative has been described. This derivative exhibited substantially enhanced fluorescence emission, e.g. 105% greater than guanine at 5×10^{-5} M concentration. The remarkable fluorescent activity of the agarose–guanine derivative predisposes it for its potential uses as sensors in various applications including biomedical ones (cf. Donati, Gamini, Vetere, Campa, & 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.2010.03.062.

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