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A facile one-pot synthesis of a fluorescent agarose-*O*-naphthylacetyl adduct with slow release properties

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ABSTRACT

A microwave assisted facile synthesis of a fluorescent 6-*O*-naphthylacetyl agarose (NA-agarose) employing carbodiimide chemistry (dicyclohexylcarbodiimide/4-dimethylaminopyridine) has been described. NA-agarose was characterized by TGA, GPC, UV spectrophotometry, fluorescence spectroscopy, FT-IR, ^{1}H and ^{13}C NMR spectra, exhibiting that in NA-agarose the naphthylacetyl group was attached to the backbone of the agarose polymer. The hydrolysis of NA-agarose in heterogeneous aqueous phase showed that the 1-naphthyl acetic acid (NAA), a plant growth regulator, got released in a controlled manner, the release rate being dependent on the hydrophilicity of the polymer adduct as well as on pH and temperature. The fluorescence emission (λ_{max} 332 nm) of NA-agarose (1×10^{-3} M) in ethylene glycol was significantly higher (ca. 82%) than that of the molar equivalent of NAA content in the product i.e. 0.08 mg in 1×10^{-3} M solution. The resulting polymer would be of potential utility as a sustained release plant growth regulator and sensory applications.

1. Introduction

Agrochemicals such as, plant growth regulators (PGRs), pesticides, fertilizers and herbicides are very useful for agriculture. Controlled release polymers are becoming increasingly important in a variety of agricultural field applications because low-molecular agrochemical substances dissolve quickly and lose their efficiency when applied by standard methods (Martin, Sánchez-Chaves, & Arranz, 1999). For the preparation of the polymer-bioactive compound conjugates, conversion of the polymer into a reactive derivative is usually required. Controlled release polymers are of great value in the domains of industry, agriculture and daily life for facilitating release of active substances such as nutrients, pesticides, drugs and flavoring agents (Fernández-Pérez, Villafranca-Sánchez, Flores-Céspedes, & Daza-Fernández, 2011; Shaviv, 2000). Polysaccharides have attracted increasing interest as the drug carriers because they are commercially available at low cost, which can be readily modified by simple chemical reactions for specific applications, less reactivity at low temperature with the bioactive compound, better biodegradability, biocompatibility, thus exhibiting a broad range of physicochemical properties. Thus, the development of new, highly reactive functional groups in the carrier molecule or in the bioactive agent involves coupling reactions creating covalent bonds (Sa'nchez-Chaves &Arranz, 1997). With this in view, agarose polymer by virtue of its unique physicochemical properties would be a feasible carrier candidate, and so this has been used for the preparation of polymer-bioactive agent adducts in the present investigation.

Agarose, the red seaweed polysaccharide is widely used in biomedical and bioengineering applications. 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) (Araki, 1966). 1-Naphthylacetic acid (NAA), one of the well known plant growth regulators (PGRs) belonging to the auxin family. NAA is widely used in agriculture for rooting of cuttings, inhibition of fruit-set, fruit abscission, and induction and control of flowering (Qiu et al., 2012).

Various kinds of slow or controlled release polymers containing NAA were synthesized which were based on both synthetic and natural polymers (Herrmann, 2007; Jantas, 2007; Tao, Pang, Chen, Ren, & Hu, 2012). Natural polymers such as dextran, starch, cellulose, chitosan, sodium alginate, lignin and their derivatives are more promising than synthetic polymers in food and biomedical applications due to their better biodegradability and biocompatibility (Fernández-Pérez et al., 2011; Kurita, Ikeda, Yoshida, Shimojoh, & Harata, 2002; Ma, Yang, Kennedy, & Nie, 2009; Sánchez-Chaves, Rodríguez, & Arranz, 1997; Zhang, Jin, Liu, & Du, 2001). A variety of methods have been described to transform the polysaccharide carrier into a reactive derivative that originally lacked the appropriate functionality (Molteni, 1985; Schacht, Vermeersch, Vandoorne,

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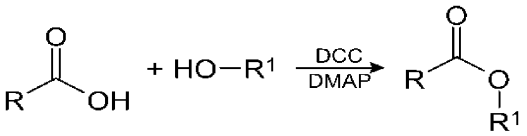
Vercauteren, & Remon, 1985). Microwave assisted esterification is one of the facile ways to modify polysaccharides. This technique has been used in synthesis to prepared polysaccharide based materials (Biswas, Shogren, Kim, & Willett, 2006; Biswas et al., 2007, 2008; Mehta, Kondaveeti, & Siddhanta, 2011). Synthesis of polysaccharide ester conjugates using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as activating agents for carboxylic acid have been studied widely (Heinze, Liebert, & Koschella, 2006; Kalaskar et al., 2010; Kapus'niak, Ciesielski, Kozioł, & Tomasik, 1999) as given in the following equation.



We report herein the synthesis of a novel fluorescent *O*-naphthylacetyl agarose (NA-agarose) through a reaction of 1-naphthylacetic acid with agarose in presence of dicyclohexylcarbodiimide/4-dimethylaminopyridine (DCC/DMAP), exhibiting sustained hydrolytic release of NAA. The motivation of the work is to modify agarose for transforming it into a functional polymer, being reported for the first time to our knowledge.

2. Experimental

2.1. Materials and instruments

Agarose was extracted from red seaweed *Gracilaria dura* of Indian waters following the method reported in the literature (Meena et al., 2007). The gel strength, sulphate and ash contents of the agarose were 950 g cm^{-2} (0.5% gel), ≤0.25% and 0.9%, respectively. 1-Naphthylacetic acid (NAA) was purchased from M/s. Loba Chemie Ltd., Mumbai, India. Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from M/s Spectrochem Pvt Ltd, Mumbai, India. Milestone Start-S (Italy) programmable microwave reactor (Model Start-S; Terminal T260; Line Voltage 230 V; Magnetron S.N. 131528; Frequency 2450 Hz) was used for the reaction. The characterizations were done by FT-IR analysis using a Perkin-Elmer FT-IR machine (Spectrum GX, USA) on a KBr disc (2 mg sample in 600 mg KBr). ^{13}C NMR spectra were recorded on a Bruker Avance-II 500 (Ultra shield) Spectrometer, Switzerland, at 125 MHz, while ^{1}H NMR spectra were recorded at 500 MHz. Samples (agarose) were dissolved in D_2O (60 mg mL^{-1}) and spectra was recorded at 70 °C, using DMSO as internal standard (ca. δ39.5 ppm) (Rees, 1969), The ^{13}C NMR spectra of NA-agarose was dissolved in DMSO at ambient temperature. The ^{13}C NMR spectra of 1-naphthylacetic acid were recorded at ambient temperature. The ^{1}H NMR spectra of agarose, NA-agarose and 1-naphthylacetic acid were recorded in 10 mg mL^{-1} of D_2O, DMSO respectively at ambient temperature. The UV–vis absorption spectra of the modified and non-modified agarose were recorded on a Varian CARY 500 UV-VIS-NIR Spectrophotometer, employing a concentration of 1×10^{-3} M in ethylene glycol. The degree of substitution was measured by the UV spectrophotometry. The fluorescence spectra of agarose, NA-agarose and 1-naphthylacetic acid (1×10^{-3} M in ethylene glycol) were recorded at room temperature on a Perkin-Elmer Spectrofluorimeter LS-50B, USA, using excitation and emission slits 1.0/1.0 nm. 1-naphthylacetic acid, NA-agarose was excited at 280 nm with an emission at 332 nm.

The molecular weights (M_n, M_w) and polydispersity index (PDI) were measured by gel permeation chromatography (GPC). The GPC was performed on a Waters Chromatograph equipped with an ERC-7515A refractive index detector and Styragel columns (HR 0.5 DMF, HR 4E DMF and HR 5 DMF). The mobile phase was DMF, delivered at a flow rate of 0.8 mL min^{-1} using a Waters 515 isocratic pump. The MW calibration curve was based on eight polystyrene standards. Apparent viscosity was measured (1%, w/v sample in DMF) on a Brookfield viscometer (DV-II $^{+}$Pro), using SC4-18 spindle at 60 rpm and room temperature. The solubility of agarose and its derivates was evaluated in organic solvent such as dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), chloroform, toluene, and ethylene glycol at the concentration of 10 mg mL^{-1} at room temperature. Thermograms of agarose, NA-agarose and 1-naphthylacetic acid samples were recorded on a Mettler Toledo TGA system (Switzerland), using a temperature program from 30 °C to 600 °C at a heating rate of 5 °C/min in nitrogen atmosphere.

The 1-naphthylacetic acid releases from NA-agarose tests was carried out by hydrolytic method in heterogeneous aqueous phase, since NA-agarose was insoluble in the medium, using freshly prepared aqueous buffer solutions having pH 4.2, pH 9.0, and pH 12.0. For studying the release of the active ingredient (1-naphthylacetic acid), partially modified agarose samples containing 1-naphthylacetate groups were used, which were insoluble in water. Finely powdered polymer samples (0.1 g) each was placed in small dry tea-bags, which were entirely permeable to water. These tea-bags were placed in Pyrex conical flasks with stopper, each one containing 25 mL of an aqueous buffer solutions of different pH (∼4.2, 9.0 and 12.0) The flasks were then immersed in a thermostated bath at 37 °C. Periodically samples were assayed by removing the tea-bag, stirring the solution and pipetting out a 1 mL aliquot of sample from the conical flask, the pipetted out volume in each flask having been topped up by an equivalent volume of fresh solvent before placing the tea bag back in again. This was factored in, in the subsequent steps for calculation of concentration in the flasks. The set of experiments were repeated with temperature as a function using fresh samples having incubated at 20 °C, 40 °C and 60 °C, separately. The concentration of 1-naphthylacetic acid released was determined by UV spectrometry at 281 nm.

2.2. Synthesis of NA-agarose

In a typical batch, the dried agarose (306 mg, 1 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) at 80 °C for 3 min in a microwave reactor under stirring. To this was added a ca. 20 mL DMF solution, a pre-solubilized mixture of 1-naphthylacetic acid (186 mg, 1 mmol), DCC (808 mg, 4 mmol), and DMAP (30.5 mg, 0.25 mmol). The reaction mixture was irradiated with microwave at 90 °C for 10 min applying 300 W power (Scheme S1). The reaction temperature and time were optimized on the basis of the highest yield of the product. After the completion of reaction under optimized conditions, the product was isolated by precipitation using iso-propyl alcohol (reaction mixture-IPA, 1:2, v/v). The precipitated product was washed with IPA (10 mL 4×) under stirring (20 min each) to remove excess of the reagents/unreacted substances followed by drying under vacuum.

2.3. Characterization

Characteristic IR bands of NA-agarose: ν_{max} (cm^{-1}) 3438*br* (—OH, stretching vibration), 2921*w* (—CH_2, stretching vibration), 1733*s* (—C═O, stretching vibration of ester), 1645*w* (H—O—H, stretching vibration of bound water), 1511*w* (naphthalene ring), 1372*w* (methylene group), 1260*w* (covalent sulphate group), 1160, 1074*br* (C—O—C, stretching vibration of glycosidic linkage), 932*m* (3,6-anhydro galactose), 893s (1,4-substituted ring), 783*w* (—CH_2, rocking vibration), 671*w*,*br* (benzene ring) (Fig. 1a).

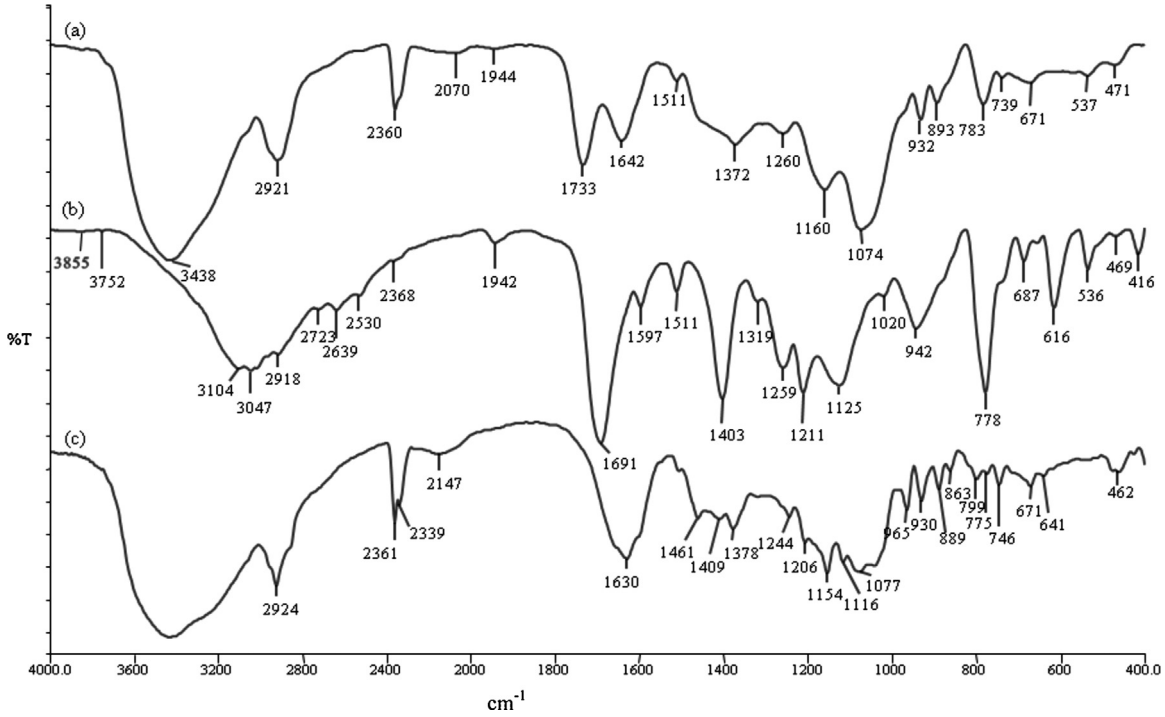


Fig. 1. FT-IR spectra of (a) NA-agarose, (b) 1-naphthylacetic acid and (c) agarose.

Characteristic IR bands of 1-naphthylacetic acid: ν_{max} (cm^{-1}) 3752*br* (—OH, stretching vibration) 3047*br* (—CH, stretching vibration), 1942*w* (C=C, stretching vibration), 1691s (C=O, anti-symmetrical stretching vibration), 1597*s* (naphthalene ring), 1403 (CH_2 bending vibration), 1372 (C=C bending vibration, aromatic ring), 1259*m* (—OH bending vibration), 1127*m* (—C—C, stretching vibration), 1211*w* (—CH_2, wagging vibration), 1023*m* (C–O stretching), 778s (—CH_2, rocking vibration), 687*br* (benzene ring), 471*br* (C=C bending vibration, aromatic ring) (Fig. 1b) (Nagabalasubramanian, Karabacak, & Periandy, 2011).

Characteristic IR bands of agarose: ν_{max} (cm^{-1}) 3434br (O—H, stretching vibration), 2924w (C—H, stretching vibration), 1630w (H—O—H, stretching vibration of bound water), 1461w (CH_2, bending vibration), 1244w [$S(=O)_2$], 1154, 1077br (C—O—C, bending vibration of glycosidic linkage), 930m (3,6-anhydro galactose), [band intensities: br = broad; m = medium; w = weak] (Fig. 1c) (Christiaen & Bodard, 1983).

^{13}C NMR of agarose (125 MHz, D_2O, DMSO-d_6 as internal standard): δ 103.1 [C-1′], 98.9 [C-1], 82.9 [C-3′], 80.8 [C-3], 78.0 [C-4], 76.3 [C-5], 76.08 [C-5′], 71.0 [C-2′], 70.6 [C-2], 70.0 [C-6], 69.5 [C-4′], 62.1 [C-6′] (Fig. 2). These values were in good agreement with those reported in the literature (Meena et al., 2007; Rees, 1969).

^{13}C NMR of 1-naphthylacetic acid (125 MHz, D_2O, DMSO-d_6 as internal standard): δ 172.8 [C-a], 133.3 [C-d], 131.9 [C-e], 131.7 [C-g], 128.5 [C-h], 128.0 [C-l], 126.2 [C-f], 126.2 [C-j], 125.7 [C-i], 125.5 [C-k], 124.0 [C-c], 38.5 [C-b] (Fig. 2) (Arranz & Sánchez-Chaves, 1995).

^{13}C NMR of NA-agarose (125 MHz, D_2O, DMSO-d_6 as internal standard): δ 171.1 [C-a], 133.2 [C-d], 131.7 [C-e], 130.7 [C-g], 128.4 [C-h], 128.0 [C-l], 127.6 [C-i], 126.2 [C-f], 125.7 [C-j], 125.5 [C-k], 123.8 [C-c], 101.4 [C-1′], 96.9 [C-1], 79.4 [C-3′, C-3, C-4], 74.2 [C-5, C-5′], 68.0 [C-2′, C-4′, C-2, C-6], 63.5 [C-6 linked with —OH], 60.1 [C-6′ linked with 1-naphthylacetic acid], 37.8 [C-b] (Fig. 2).

3. Result and discussion

3.1. Physicochemical properties

Optimization studies revealed that microwave irradiation for 10 min at 90 °C led to the formation of ester bond between carboxyl termini of 1-naphthylacetic acid and hydroxyl groups of agarose in good yields, having molar ratios (agarose:NAA) 1:0.5, 1:1 and 1:2 affording 90%, 86%, 82% yields respectively. The respective degrees of substitution (DS_{est}) were determined by UV spectroscopy, based on the extinction coefficient of the highest peak in the UV absorption spectra of the polymers concerned, λ_{max} 280 nm (ε 5000 L mol^{-1} cm^{-1}) (Nowakowska, Zapotoczny, Sterzel, & Kot, 2004). The respective degrees of substitution (DS_{Est}) were 0.38, 0.62, and 0.83 (Table 1). At all the DS_{Est} (0.38, 0.62, and 0.83) the agarose ester (NA-agarose) became insoluble in

Table 1
Yield (%) and physico-chemical properties of agarose and agarose derivative.

Samples	1-NAA/agarose	Isolated yield (%)	Degree of esterification (DS_{Est})	Apparent viscosity (cP)[a]	Molecular weights (KDa)		Poly dispersity index (PDI)
					M_w	M_n	
Agarose	NA	NA	NA	7.6 ± 1	127.571	45.690	2.792
NA-agarose	0.5:1	90	0.38	9.4 ± 1	125.695	46.698	2.691
NA-agarose	1:1	86	0.62	10.8 ± 1	120.364	44.105	2.729
NA-agarose	2:1	82	0.83	14.3 ± 1	116.325	43.658	2.664

[a] Apparent viscosity (cP) was measured in DMF solvent 1% (w/v) at room temperature.

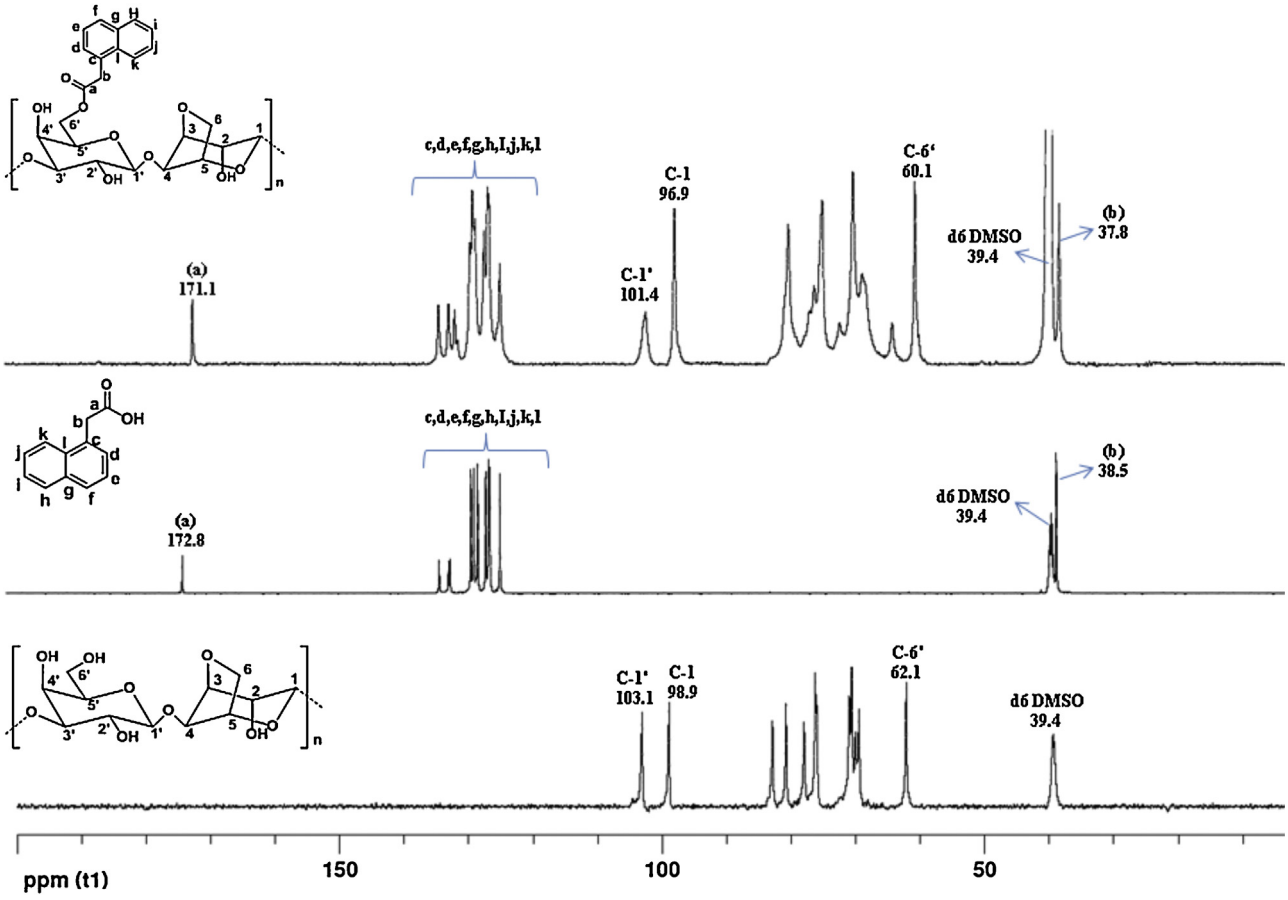


Fig. 2. ^{13}C NMR spectra of agarose, 1-naphthylacetic acid, and NA-agarose.

water but they were soluble in DMSO, DMF and ethylene glycol at room temperature, apparently due to the relatively greater degree of hydrophobization of agarose polymer caused by the aromatic moiety (Table 2). The parent polysaccharide agarose was dissolved in aqueous solution and *N*,*N*-dimethylformamide with difficulty at 70 °C and the resulting agarose derivatives exhibited an enhanced affinity for organic solvents, thus the solubility of NA-agarose was significantly improved, which was soluble in tetrahydrofuran (THF), ethylene glycol, dimethylsulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF), because of the naphthyl acetyl ester moiety on the agarose backbone, imparting lipophilicity (Table 2). The apparent viscosities of NA-agarose derivatives with different degrees of substitution 0.38, 0.62, and 0.83 were measured to be 9.4 ± 1 cP, 10.8 ± 1 cP, and 14.3 ± 1 cP, in DMF respectively. This reflected a linear relationship of DS and apparent viscosity presumably because of enhanced intermolecular π–π interactive forces (Table 1). The weight average molecular mass (M_w), number average molecular mass (M_n) and polydispersity indices (PDI) of agarose and NA-agarose are given in Table 1. The M_w and PDI data of NA-agarose indicated that the agarose biopolymer backbone remained largely intact during the esterification reaction by microwave irradiation.

3.2. Spectral characterization of NA-agarose

The NA-agarose was characterized by FT-IR and ^{13}C NMR spectrometry. Formation of ester bond as a result of the reaction between hydroxyl group of agarose and carboxyl termini of 1-naphthylacetic acid was confirmed by the appearance of the band at 1733 cm^{-1} in the FT-IR spectrum of NA-agarose. The characteristic absorption bands of agarose were 3438, 1642, 1160, 1074 and 932 cm^{-1} and the band at 1511 cm^{-1} was assigned to the naphthalene ring, indicating that the backbone of agarose remained largely intact during the esterification reaction (Fig. 1). The ^{13}C NMR spectra of NA-agarose exhibited a peak at 171.1 ppm, confirming the presence of ester carbonyl group, which in NAA appeared at 172.8 ppm, besides one at 37.8 ppm due to the methylene carbons of the 1-naphthylacetate residue, showing a little upfield shift from 38.8 ppm NAA (Arranz & Sánchez-Chaves, 1995). The anomeric carbons of NA-agarose appeared at 101.4 and 96.9 ppm as opposed

Table 2
Solubility of agarose and agarose derivative.

Samples	1-NAA/agarose	Solvents[a]					
		DMF	DMSO	Ethylene glycol	Toluene	Chloroform	Water
Agarose	NA	–	–	–	–	–	+
NA-agarose	0.5:1	+	+	+	–	–	–
NA-agarose	1:1	+	+	+	–	–	–
NA-agarose	2:1	+	+	+	–	–	–

[a] +, soluble (10 mg mL^{-1}); –, insoluble.

103.1 and 98.9 ppm in agarose, indicating insertion of NAA on agarose polymer backbone. Further, the carbon resonance of the C-6 of agarose at 62.1 ppm exhibited an upfield shift to 60.1 in NA-agarose, which clearly indicated that the esterification took place involving the C-6 hydroxyl group of agarose. Similar observation of modification at C-6 position of agarose was reported in the literature by Oza, Meena, and Siddhanta (2012). The peaks that appeared in the range 133.2–123.8 ppm were attributed to the carbons of the naphthalene ring in NA-agarose, compared to the slightly upfield group (133.3–124.0 ppm) of the aromatic carbons in NAA (Fig. 2) (Arranz & Sánchez-Chaves, 1995). The group of resonances for the methylne and methane carbons of NA-agarose in the range 79.4–63.5 ppm showcased a different pattern than that of agarose in the range 82.9–62.1 ppm, corroborating again the formation of a new ester derivative. The assignments of carbon resonances were done by comparison with literature values as well as with data obtained with ChemBioDraw Ultra 11.0 (Arranz & Sánchez-Chaves, 1995; Meena et al., 2007; Oza et al., 2012).

The proton NMR spectra of agarose, 1-naphthylacetic acid and NA-agarose are depicted in the Supplemental Data (Fig. S1a–c). The ^{1}H NMR spectrum of NA-agarose exhibited two sets of peaks of anomeric protons at 5.23–5.08 and 4.87–4.68 ppm indicating a pervasive anisotropy in the molecular structure of NA-agarose with DS 0.38 (Fig. S1c), as against the two anomeric proton resonances of agarose appearing at 5.42 and 4.91 ppm. The remaining signals in the range 4.51–3.79 ppm were attributed to the methylene and methine protons of NA-agarose, while those of agarose appeared in the range 4.55–3.58 ppm (Ducatti, Colodi, Gonçalves, Duarte, & Noseda, 2011). The keto-methylene protons of NA-agarose appeared at 3.41 ppm, while the same in NAA appeared at 4.06 ppm (Arranz & Sánchez-Chaves, 1995). The aromatic protons of NA-agarose appeared as two sets of multiplets at 7.93–7.86 and 7.54–7.46 ppm, whereas these protons in NAA appeared at the two downfield ranges e.g. 8.00–7.84 ppm and 7.57–7.45 ppm (Fig. S2a–c). This body of data confirmed the formation of NA-agarose in the synthetic reaction.

3.3. Thermal behavior and release study

The thermal stability of the agarose and NA-agarose was investigated by using TGA technique. The TGA curves of agarose, NA-agarose and 1-naphthylacetic acid are shown in Fig. S3. The thermograms of agarose and NA-agarose indicated greater thermal stability of the latter than the parent agarose. The mass losses in agarose, NA-agarose and 1-naphthylacetic acid were observed in three stages – mass losses of (i) 7%, 8%, and 0% up to 175 °C; (ii) 85%, 73%, and 99% up to 400 °C, and (iii) 91%, 82% and 99% up to 600 °C, respectively. The mass losses in NA-agarose (Fig. S3a), 1-naphthylacetic acid (Fig. S3b) and agarose (Fig. S3c), were measured to be 8%, 0% and 7%, up to 175 °C, indicating losses of bound water in the polysaccharides. Agarose started decomposing very fast till 500 °C. The greater thermal stability of the NA-agarose with respect to the parent polysaccharides demonstrated the formation of new material involving an aromatic residue, the latter being highly thermally stable.

To study the release profile of NAA, the polymeric derivatives were hydrolyzed in aqueous media having pH 4.2, 9.0 and 12.0, wherein NA-agarose was insoluble. Commonly, the release behavior of bioactive agents from polymer-bioactive compound adducts depends on a number of factors, the hydrophilic character of the compound, the incubation temperature and the pH value of the medium (Arranz & Sánchez-Chaves, 1995). The typical profiles of the hydrolysis at 37 °C of an agarose-1-naphthylacetic acid adduct in the three different pH media (~4.2, 9.0, 7.0 and 12.0) showed that the rate of hydrolysis was greatly affected by the pH of the medium (Fig. 3). Hydrolysis of the polymer adduct having DS 0.38 at pH 9.0

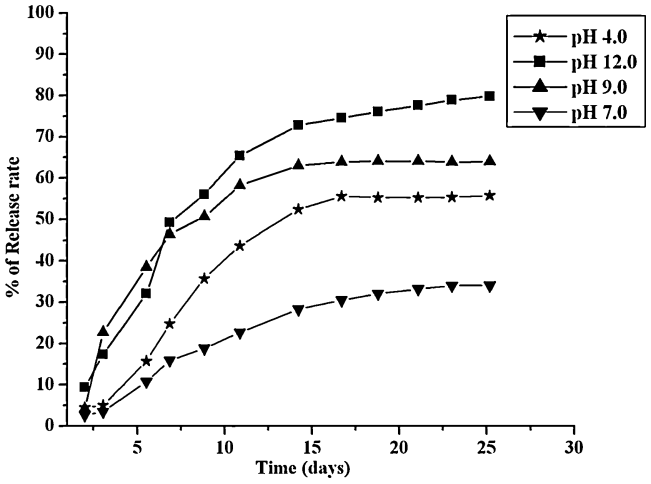


Fig. 3. The release behavior of NAA on hydrolysis at different pH values at 37 °C, DS = 0.38.

and 12.0, showed 75% and 80% release respectively (Figs. 3 and 4). The rates were relatively faster during 0–15 days releasing 70% and 75% of NAA respectively; thereafter it got plateaued over till 25 days releasing the remaining amount. These data indicated that the release were favored at alkaline pH, since at pH 4.0 the greatest release recorded was 50%. The hydrolysis of polymer adduct with DS 0.83 at 60 °C and pH 9.0 the release amount was 65%, while affording a lower extents of release at lower temperatures e.g. at 40 °C (55%) and 20 °C (28%), indicating a linear temperature dependence of release rate (Fig. 5). At 60 °C a 55% release was recorded during 0–10 days, releasing the remaining amount over 25 days in a plateaued fashion. Keeping the overall performance profiles of the polymer adducts in view, it appears that the one with DS 0.38 exhibited superior total release (75–80%) to that (65%) having DS 0.83, corroborating the fact that relative hydrophilicity of the former facilitated the hydrolytic release process, as mentioned above (Ignatova, Manolova, & Rashkov, 1998). This was presumably because of the greater swelling of the polymer adduct leading to a facile nucleophilic attack of catalyst at the reaction site (Ignatova et al., 1998). This series of studies exemplify that a material with appropriate DS and conditions can deliver dosage schedules of choice (Figs. 3–5). The profiles of the release rates of low molecular weight bioactive compound (NAA) from the agarose polymer upon hydrolysis of the ester groups were in good agreement with those reported in the literature (Eynard, Gay, Occelli, Dolci, & Martini, 1986; Tsatsakis et al., 1995).

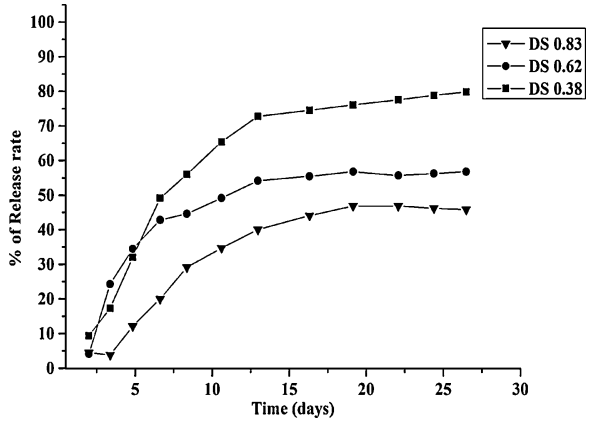


Fig. 4. The release behavior of NAA on hydrolysis at different DSs at 37 °C, pH 9.0.

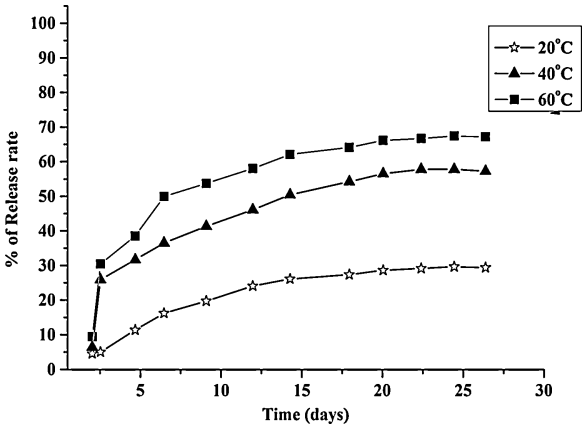


Fig. 5. The release behavior of NAA on hydrolysis at different temperatures at pH 9.0, DS = 0.83.

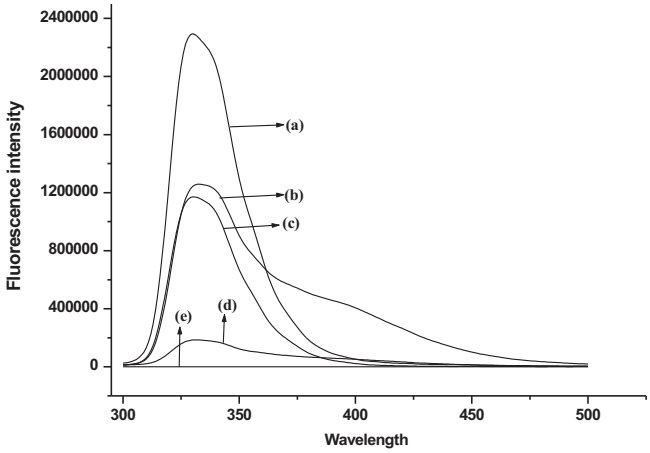


Fig. 6. Fluorescence emissions of (a) NA-agarose, 1×10^{-3} M; (b) 1-naphthylacetic acid, 0.08 mg in 1×10^{-3} M (c) NA-agarose, 1×10^{-4} M; (d) NA-agarose, 1×10^{-5} M; (e) agarose, 5×10^{-5} M.

3.4. UV–vis and fluorescence spectra

The non-modified agarose did not exhibit any absorption maxima in the UV–vis region, while 1-naphthylacetic acid and NA-agarose exhibited absorption at λ_{max} (ethylene glycol, nm): 282 (ε 288.8 L mol^{-1} cm^{-1}), λ_{max} 280 nm (ε 5000 L mol^{-1} cm^{-1}) respectively. The appearance of absorption maxima at λ_{max} 280 nm in NA-agarose indicated insertion of 1-naphthylaceticacid moiety on the agarose backbone (Fig. S4). The fluorescence emissions (λ_{max} 332 nm) of pure agarose, 1-naphthylacetic acid, and the NA-agarose derivative were measured at 1×10^{-3} M concentration (Fig. 6). Parent agarose at this concentration exhibited very low emissions. The fluorescence emission of the NA-agarose derivative in 1×10^{-3} M solution was significantly higher (ca. 82%) than that of pure 1-naphthylacetic acid in 0.08 mg in 1×10^{-3} M solution, the molar equivalent of NAA present in the 1×10^{-3} M solution of NA-agarose. This enhancement in fluorescence emissions may be attributed to certain molecular arrangements in the naphthylacetic acid pendant agarose polymer derivative facilitating intermolecular transition processes including the π–π ones (Oza, Meena, Prasad, Paul, & Siddhanta, 2010; Oza et al., 2012).

4. Conclusions

Functionalization of agarose has been achieved through the synthesis of a novel fluorescent 6-*O*-naphthylacetyl agarose derivative (NA-agarose), wherein the plant growth regulator NAA group can get released in controlled manner under hydrolytic conditions. The release rate was dependent on pH, temperature as well as hydrophilicity of the polymer adduct, which were optimized, greater release rate having been obtained at higher temperature (60 °C), pH (12.0) and lower DS (0.38). The fluorescence emission (λ_{max} 332 nm) of the agarose derivative (1×10^{-3} M) in ethylene glycol was significantly higher (ca. 82%) than that of the molar equivalent of NAA i.e. 0.08 mg present in the 1×10^{-3} M solution. This modified agarose derivative may be of potential utility in the domains of agrochemicals as well as sensory applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.06.046.

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