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Agarose based multifunctional materials: Evaluation of thixotropy, self-healability and stretchability

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ABSTRACT

This paper reports a microwave assisted one pot facile synthesis of ester derivatives of agarose (Agr-GA_{Est}) through chemical reaction of agarose (Agr) with gallic acid (GA), an organic acid found in many plants employing carbodiimide chemistry. Agr-GA_{Est} was characterised by FT-IR, ^{13}C NMR spectroscopy, thermogravimetric analysis (TGA), DMA measurements, scanning electron microscopy (SEM), UV–vis spectrophotometry and rheometry. The native agarose was insoluble in ethylene glycol, but Agr-GA_{Est} (obtained at an optimized molar ratio of 1: 0.5) formed good quality gel ($\tan\delta \sim 0.1$) at 4% (*w/v*) concentration. The gel thus obtained exhibited substantial degree of thixotropy (hysteresis loop area = 38.73%), rapid self-healing ability (12 min) upon complete cleavage of the gel and excellent stretching ability (>20 times of its original length). These types of multifunctional gels would find applications in food and personal health care industries.

1. Introduction

Multi-functional materials that show reversible properties such as self-healing are known as dynamic materials and are inspired by biological systems, where any damage got self-repaired (Phadke et al., 2012). These type of materials often has superior mechanical properties and finds multiple applications (Lehn, 2005). Most of the polymer based gel materials show dual properties such as liquid like flow and elastic behaviour and during their physical interactions, suitable polymeric entanglements lead to the solid like properties (Urban, 2012). These remarkable properties of gels make them very good candidates to induce tailor made functional properties such as self-healing or solvent responsive healing (Sharma, Mondal, Mukesh, & Prasad, 2013). Cleavage of the macromolecular chains leads to the formation of free radicals or functional groups such as —C=C—, —COOH, —NH_2, —OH, —Si—O, *etc*., and chain mobility or diffusion in the segments bring reactive groups in contact with each other resulting repairing of physical network (Yang & Urban, 2013). There are several reports on self-healing polymeric gel systems fabricated mainly by cross-linking with groups containing —NH—CO— moiety capable of forming reversible covalent bonds upon cleavage (Hager, Greil, Leyens, van der Zwaag, & Schubert, 2010; Kushner, Vossler, Williams, & Guan, 2009). In oppose to the conventional approach of introducing groups capable to induce self-healing, Imato, Nishihara, Kanehara, Amamoto, Takahara, and Otsuka (2012) have prepared a polymer gel by the reversible formation of diarylbibenzofuranone cross-linker from the dimerization of stable arylbezofuranone, which self-healed without any external stimuli (Imato et al., 2012). Although different kinds of interactions have been exploited to design polysaccharide-based physical networks such as hydrogen bonds (Braccini, Rodríguez-Carvajal, & Pérez, 2005), ionic (Chung et al., 2002), host–guest recognition (Charlot, Auzély-Velty, & Rinaudo, 2003), non-covalent interactions in polysaccharide ion gels (Sharma, Mondal, Mukesh, & Prasad, 2013), but there are no report on the studies on non-covalent bond triggered self-healing ability of functionalized polysaccharide including agarose based materials and healing induced by solvents.

Highly stretchable hydrogels are of great interest in tissue engineering and for bio-medical applications. Ionically crosslinked alginate and covalently crosslinked polyacrylamide gels are reported to have stretchability more than 20 times of its original length (Sun et al., 2012). The prior arts revealed that there are no reports on the multifunctional materials based on seaweed derived polysaccharides. These encourage us to take the study on

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natural polymers to produce materials for multi-utility. Herewith we report synthesis of agarose (a seaweed polysaccharide extracted from red seaweed having repeating disaccharide units made up of D-galactose and 3, 6-anhydro-L-galactopyranose) derivative capable of forming thixotropic, highly stretchable material in ethylene glycol, and having healing ability with or without any external stimuli. Solvent (ethylene glycol) used in this study for obtaining multi utility materials is an organic polar solvent and can be used over wide range of temperatures for various polymers (Nemat-Nasser & Zamani, 2004).

2. Experimental

2.1. Materials

Agarose (gel strength = 950 g cm^{-2} in 0.5% gel and sulphate content = ≤0.25%) was extracted from the red seaweed *Gracilaria dura* of Indian waters following the method reported in our previous work (Meena et al., 2014). Gallic acid was purchased from TCI fine chemicals (India) Pvt. Ltd, Chennai, India. Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from M/s Spectrochem Pvt. Ltd., Mumbai, India and other chemicals were of AR grade and were used as received. 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 reaction.

2.2. Synthesis of agarose derivatives

Agarose powder (306 mg *i.e.*, 0.2 mol per repeating disaccharide unit) was dissolved in N, N-dimethylformamide (DMF) at 90 °C for 5 min in microwave reactor under vigorous stirring condition. To the solution, pre-solubilized mixture of gallic acid (170 mg i.e., 0.2 mol), DCC (412 mg i.e., 0.4 mol) and DMAP (30.5 mg i.e., 0.05 mol) in DMF was added under stirring and the reaction was carried out under microwave irradiation at 90 °C for 12 min applying 400 W power. After the completion of reaction, the product was isolated by precipitation in isopropyl alcohol (reaction mixture: IPA, 1: 2 *v/v*). The precipitated product was further washed with IPA (×4) to remove excess unreacted reagents followed by vacuum drying.

2.3. Characterizations

FT-IR spectrum was recorded on a Perkin-Elmer FTIR machine (Spectrum GX, USA) on 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. For this, agarose (Agr) was dissolved in D_2O (60 mg mL^{-1}), Agr-GA_{Est} was dissolved in DMSO-d_6 and spectra were recorded at 70 °C, using DMSO-d_6 as internal standard (ca. δ 39.5 ppm). ^{13}C spectrum of gallic acid was recorded at ambient temperature. The surface morphology of the parent agarose, gallic acid and Agr-GA_{Est} were analysed by scanning electron microscopy (SEM) on a Carl-Zeiss Leo VP 1430 instrument (Oxford INCA) employing acceleration voltage of 5 kV and magnification 200×. The UV–vis absorption spectra of the modified and parent agarose, gallic acid 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–vis spectrophotometry. Thermogravimetric analysis (TGA) was carried out using Mettler Toledo Thermal Analyser, (TGA/SDTA 851e, Switzerland).

2.4. Dynamic mechanical analysis (DMA)

DMA analysis was carried out to measure the elastic (E') modulus and loss factor (Tan delta) as a function of time on DMA 242E (NETZSCH, Germany). Compression test was carried out using 3.0×2.0 mm/mm^2 dimension of gel sample, at frequency of 1 Hz, at 25 °C under nitrogen atmosphere.

2.5. Tensile analysis

Stretching ability of gel was measured by universal tensile testing machine (UTM, Zwick/Roell—Z2.5, Germany). For the measurement 4% *w/v* gel of Agr-GA_{Est} (dimension L × W of 1.5 cm and thickness 0.4 cm) prepared with 0.5 mole of gallic acid was fixed with the jaws of the sample clamp and measurement was carried out at ambient (25 °C) temperature with test speed 10 mm/min.

2.6. Differential scanning calorimetry (DSC) measurements

DSC measurements were carried out using NETZSCH DSC 204 F1 Phoenix®, Germany, in the temperature range of −20 °C to 30 °C at the heating rate of 5 °C/min under nitrogen atmosphere. About 20 ± 1 mg of gel sample was sealed in to the 40 μL DSC pan. For each sample, an empty 40 μL pan was used as a reference pan.

2.7. Rheological measurements

Rheological measurements were carried out on an Anton Paar Physica MCR 301 rheometer, USA, using parallel plate PP50/P-PTD200 geometry (50 mm diameter; 0.1 mm gap). The temperature was maintained at 25 ± 1 °C by a Viscotherm VT2 circulating water bath during the measurements. For thixotropic measurements, the shear rates applied were varied from 0.1 to 1000 s^{-1} in 5.0% (*w/v*) concentrations of the materials in ethylene glycol for 1000 s in an upward sweep immediately following up by a downward sweep. Areas under the upstream (A_{up}) and the downstream data points (A_{down}) as well as the hysteresis (thixotropic) area ($A_{up} - A_{down}$) were obtained using rheoplus software. The percentage of relative thixotropic area (A_r) was calculated using the Eq. (1) as described by Dolz, González, Delegido, Hernández, and Pellicer (2000).

$$A_r = (A_{up} - A_{down}/A_{up}) \times 100 \quad (1)$$

The time dependency of storage (G') modulus and loss (G'') modulus were also carried out using the same instrument at 25 °C to show the gel structure of the ester agarose derivative obtained under optimised conditions with 1: 0.5 molar ratios.

3. Results and discussion

Optimization studies revealed that microwave (MW) irradiation (400 W) for 12 min at 90 °C led to the formation of ester bonds between carboxy termini of gallic acid and hydroxyl groups of agarose using N, N′-dicyclohexylcarbodiimide (DCC) chemistry as reported in the literature (Heinze, Liebert, & Koschella, 2006; Kalaskar et al., 2010; Mehta, Kondaveeti, & Siddhanta, 2011; Kondaveeti, Mehta & Siddhanta, 2014). Further the resulting by product N, N′-dicyclohexylurea (DCU) was removed through repeated washings of isopropyl/ethyl alcohol. Yield of the ester derivative (Agr-GA_{Est}) was decreased with increasing the molar ratio of gallic acid. The highest yield (92%) was obtained with 0.5 mol of gallic acid, whereas 2 mol of gallic acid resulted lowest yield of product (82%) (Table 1). However, the degrees of substitution (DS) were increased from 0.45 to 1.10 (Table 1). This study revealed that beyond 2 mol concentration of gallic acid, the DS got saturation, may be due to the non-availability of active sites on the agarose backbone for further esterification reaction. Furthermore the decrement in the product yields may be due to the higher concentration of gallic acid used for obtaining the product with higher DS, which may cause depolymerization of agarose polymer (Mehta et al., 2011).

Table 1
Yield (%) and solubility of agarose and agarose derivative.

Agarose derivatives (Agr/GA, molar ratio)	Yield (%)	DS	Solubility data			
			DMF	DMSO	Ethylene glycol	Water
Agarose	NA	NA	+	+	–	+
Agr-GA_{Est} (1:0.5)	92	0.45	+	+	+	–
Agr-GA_{Est} (1:1)	85	0.62	+	+	+	–
Agr-GA_{Est} (1:2)	82	1.10	+	+	+	

DS = degree of substitution, NA = not applicable; + = Soluble (10 mg mL^{-1}); – = insoluble.

The solubility profile of the parent agarose was changed completely after modification (Table 1). Ester derivatives of agarose were found to be soluble in ethylene glycol upon heating and formed gel upon cooling, while the parent agarose was soluble in water at elevated temperature and formed gel on cooling. This indicates that the native agarose has been modified during esterification reaction. The native agarose and ester derivatives of agarose are completely soluble in DMF and DMSO on heating at 80 °C and formed viscous solutions, but gel formation was not observed on cooling to room temperature. Further, the formation of Agr-GA_{Est} was confirmed by FT-IR, ^{13}C NMR spectroscopy, thermogravimetric analysis (TGA), scanning electron microscopy (SEM) and UV–visible spectrophotometry.

Characteristic IR band at 1728 cm^{-1} in the FT-IR spectrum of Agr-GA_{Est} confirmed the formation of ester bond as a result of the reaction between hydroxyl group of agarose and carboxyl termini of gallic acid (Mehta et al., 2011). FT-IR of the product also showed the characteristic IR bands of agarose at 3438, 1629, 1162, 1073 and 932 cm^{-1} indicated that the backbone of agarose remained largely intact during the esterification reaction (Fig. S1). ^{13}C NMR spectra showed characteristic chemical shift for anomeric carbon of agarose at δ 103.13 ppm (Fig. S2) and other characteristics bands of agarose can be also seen in the spectra (Mehta et al., 2011). Appearance of chemical shifts due to gallic acid in the δ range of 109.26 to 162.14 and down field shifting of the signal due to C-6 (from 62.19 to 60.01) further confirmed the formation of the ester derivative of agarose with gallic acid (Fig. S2).

The ester prepared with agr: gallic acid molar ratio 1: 0.5 gave formation of gels at 4% *w/v* in ethylene glycol exhibits self-healing, stretchability and excellent thixotropic properties. On the other hand, it was also difficult to prepare the gels at concentrations higher than 4% *w/v* due to the rapid coagulation of the structure during dissolution (Fig. S3).

The modification of agarose was also confirmed by UV–vis (Fig. S4). UV spectrum of the native agarose was not exhibited any absorption maxima in UV–vis region, while UV spectra of gallic acid and Agr-GA_{Est} exhibited absorption at λ_{max} (ethylene glycol, nm): 216, 270 (ε 3007.67, 997.78 L mol^{-1} cm^{-1}), and λ_{max} 218, 280 nm (ε 2438.75, 1040.05 L mol^{-1} cm^{-1}), respectively. The appearance of absorption maxima at λ_{max} 218 nm and 280 nm in Agr-GA_{Est} indicated insertion of gallic acid on the agarose backbone (Fig. S4).

The thermal stability of the native agarose, gallic acid, Agr-GA_{Est} was investigated by TGA (Fig. S5). Agarose, gallic acid, Agr-GA_{Est} exhibits 9.28%, 18.22% and 26.00% mass retention up to 600 °C. TGA analysis showed that Agr-GA_{Est} has the greatest thermal stability (mass retention 26.00%) compared to the native agarose and gallic acid. This increment in thermal stability of Agr-GA_{Est} also confirmed that modification of agarose has taken place.

SEM images showed that the morphology of the native agarose has been modified significantly after esterification of agarose with gallic acid (Fig. S6). These results indicate that the morphology of the product was highly ordered, compact and smoother compared to the native agarose (cloudy) and gallic acid (rod like) morphologies.

The 4% *w/v* Agr-GA_{Est} gel (1: 0.5) was cleaved into two segments as shown in Fig. 1. Gel segments were kept at close vicinity at room temperature (25 °C) for 15 min, and were found to be tightly attached to each other and could not be detached manually. Similarly healing of Agr-GA_{Est} gel was also obtained in polar solvents such as ethyl acetate (t_{heal} = 12 min) and ethyl lactate (t_{heal} = 20 min) (triggered healing property). However, the polar protic solvents such as alcohols could not induce healability in the gel. In contrast, agarose did not soluble in ethylene glycol; but soluble in water and formed hydrogel in 1–4% (*w/v*) concentration on cooling. Bisection of agarose gel is easy, but gel on cleavage was not healed with or without any external stimuli up to 2 h under identical conditions (Fig. S7). In addition on stretching of the native agarose gel it breaks immediately. Result of this study also revealed that the native agarose gel was unable to heal and stretch.

It is well known that autonomous self-healing typically accomplished in polymers having low glass transition temperature (T_g) (Urban, 2012). For such systems the network mobility is generated due to the accession of the free volume by the molecular chains. In the dynamic scanning calorimetric (DSC) study, the T_g of the native agarose gel was observed at −8.9 °C, while that of ethylene glycol and gallic acid showed T_g at −7.8 and −9.2 °C, respectively (Fig. S8a–c). The gel of Agr-GA_{Est} exhibited slightly lower glass transition at −9.1 °C compare to the native agarose gel, while the specific heat capacity (*C*p) of the Agr-GA_{Est} gel was identical to the native agarose gel and was lower compared to gallic acid (Fig. S8d). The shifting of the T_g from −8.9 (for parent agarose gel) to −9.1 (for Agr-GA_{Est} gel) indicated phase changes during modification of agarose. Further, the Agr-GA_{Est} gel obtained with 0.5 mol gallic acid having healing and stretching ability showed two T_g values at −1.3 °C and −9.1 °C respectively as because all the $-CH_2OH$ of agarose did not take part in the ester formation (DS was 0.45) (Fig. S8e). The Cp of

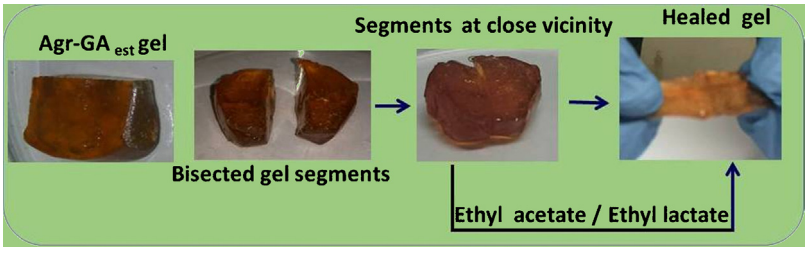

Fig. 1. Schematic out-lay of self-healing and solvent responsive healing ability of Agr-GA_{Est} (1:0.5) gel prepared in ethylene glycol.

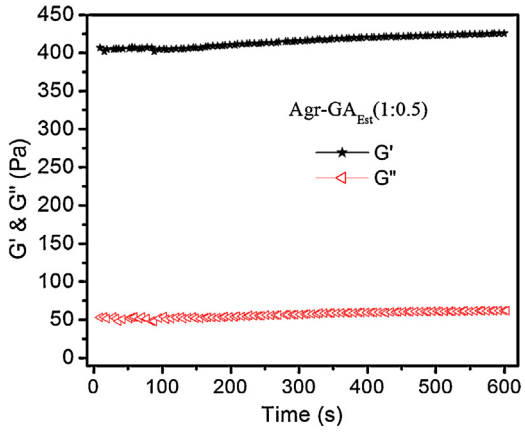

Fig. 2. Evaluation of storage modulus (G') and loss modulus (G'') as a function of time for Agr-GA$_{Est}$ gel.

T_g (1) was much higher in comparison to agarose, gallic acid and ethylene glycol. This too supports presence of two phases in gel of Agr-GA$_{Est}$. Further, lower T_g may be responsible for the chain mobility of the Agr-GA$_{Est}$ gel prepared in ethylene glycol. Since in gel networks, non-covalent hydrogen bonding interactions also plays important role in healing process (Cordier, Tournilhac, Soulie-Ziakovic, & Leibler, 2008) in the above gel systems perhaps these types of interactions also responsible for the healing. In order to understand the solvent responsive healing, the FT-IR of ethylene glycol, ethyl acetate, ethyl lactate and their mixture was recorded. FT-IR vibrational frequency band due to —OH was appeared at 3403 cm^{-1} and 1043 cm^{-1} respectively (Fig. S9a) and that of ethyl acetate showed ester band at 1742 cm^{-1} (Fig. S9b). In the mixture of ethylene glycol and ethyl acetate, the band due to ester of ethyl acetate completely disappeared and shifting in the —OH band of ethylene glycol was also observed indicating participation of ester —C=O of ethyl acetate and —OH of ethylene glycol in the hydrogen bond formation (Fig. S9c). Similarly, ethyl lactate showed vibrational frequency bands at 3464 and 1738 cm^{-1} due to —OH and —C=O (Fig. S9d). After mixing with ethylene glycol, the intensity of —C=O substantially reduced and shifting in the —OH band also observed indicating interaction of ethylene glycol with EL (Fig. S9e). These interactions perhaps responsible for the induction of chain mobility in the gel systems eventually leading to the development of polymer chain entanglements suitable for healing.

Fig. 2 showed time dependent viscoelastic measurements of Agr-GA$_{Est}$ (1:0.5) gels. Result of this study indicates typical viscoelastic behaviour of gel material with predominance of storage modulus (G') and low tan $\delta \sim 0.1$. It should be noted that, the storage modulus was more predominant over the loss modulus (G'') during the experiment and having less time dependency, indicating more solid like behaviour for Agr-GA$_{Est}$ gel. Fig. S10 shows dynamic mechanical (DMA) analysis of the parent agarose and Agr-GA$_{Est}$ gel samples. Result of DMA analysis also confirmed that gel prepared from the native agarose (tan $\delta \sim 0.40$) and Agr-GA$_{Est}$ (tan $\delta \sim 0.1$) shows properties like viscoelastic materials. This result also revealed that Agr-GA$_{Est}$ based gel is more elastic compared to the native agarose gel.

Self-healing and thixotropicity are interrelated phenomena and evaluation of thixotropicity of materials can give important insights of the self-healing phenomenon (Mukhopadhyay et al., 2010). The thixotropic properties of 4% *w/v* gel of the agarose ester derivative obtained with three different molar ratios of agarose to gallic acid were determined by rheometry employing hysteresis loop and relaxation test. For the evaluation of thixotropic behaviour by hysteresis loop, the samples were subjected for thinning under increasing shear rate (upward curve) immediately followed by dropping the applied shear rate (downward curve) (Fig. 3). The area of the loop thus formed gave an idea of the extent of thixotropy present in the samples calculated employing Eq. (1). The rheological study confirmed that the thixotropic area in the ester derivative (Agr-GA$_{Est}$) was decreased with increasing the Agr/GA molar ratio (Fig. 3a). Agr-GA$_{Est}$ with 1:0.5 molar ratio shows highest thixotropic area (38.73%) which decreased up to 7.0% in Agr-GA$_{Est}$ obtained with 1: 2 molar ratio. Shear stress is dependent on the shear rate in all the gel materials prepared in this study. Agarose ester derivative with 2 mol gallic acid showed greatest shear dependency compared to the other agarose ester derivatives (Fig. 3a). Furthermore, no thixotropic area was observed during rheological study of the native agarose gel confirmed non-thixotropic behaviour (Fig. 3b).

Furthermore, the samples were subjected to continuous subsequent ramp cycles and the shape of the resulting hysteresis loops was monitored. A lowering in the hysteresis loop area after each cycle was observed for each samples, the upward and backward curved completely overlapped for at 16th cycle in Agr-GA$_{Est}$ (1:0.5), 9th cycle in Agr-GA$_{Est}$ (1:1), 6th cycle in Agr-GA$_{Est}$ (1: 2), and at 3th cycle in ethylene glycol (Figs. S11–S14 respectively). Therefore, it can be concluded that insertion of gallic acid into the agarose moiety induced thixotropicity in the agarose based ester derivatives. Perhaps the phenomenon of induction of thixotropy was due to the change in the molecular arrangements of the native agarose due to the ester linkage, having a reinforcing stability of the helical structure through the carboxyl group participation and additional

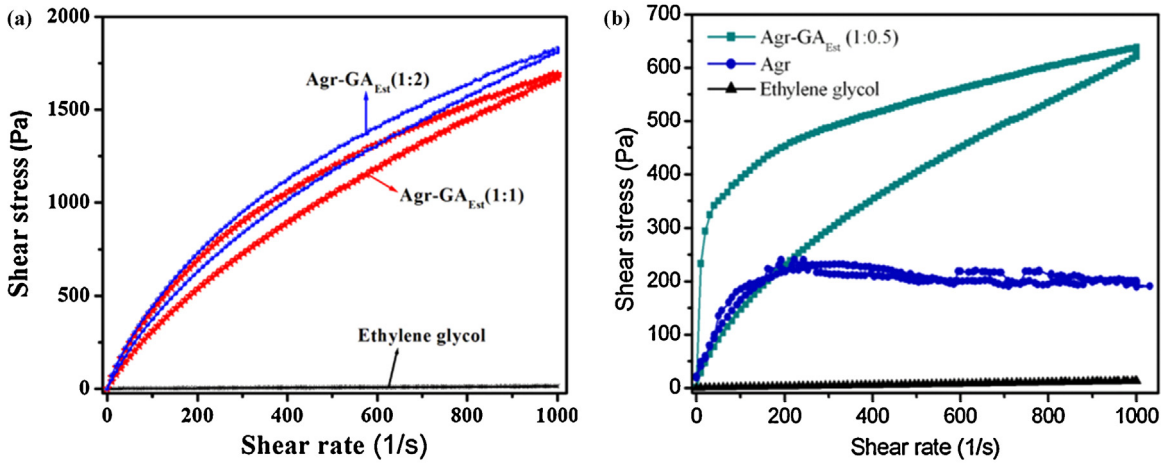

Fig. 3. Evaluation of thixotropic behaviour by hysteresis loop measurements for the native agarose and Agr-GA$_{Est}$ gel systems.

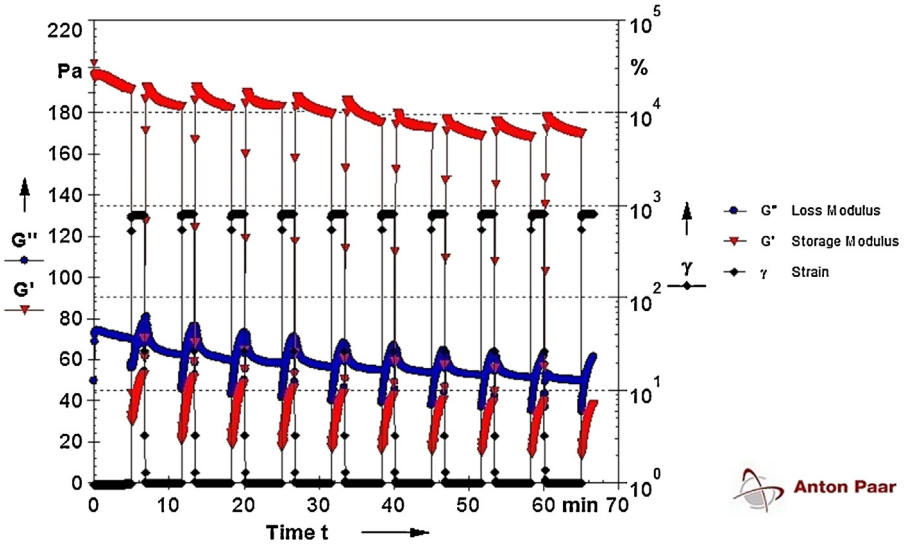

Fig. 4. Self-healing in Agr-GA_{Est} (1:0.5) gel as characterized by dynamic recovery of the storage modulus (G') upon relaxation.

hydrogen bond formation eventually leading to self-healing of the gel.

In order to investigate recovery of storage modulus (G') of the 4% *w/v* ester gels upon relaxation, the gel was fractured employing high strain followed by relaxation. The gel was initially subjected to 1% strain at 1 Hz frequency for 300 s and G' and G'' were monitored during the process followed by fracturing of the gels employing very high strain at 1 Hz for 100 s. It was observed that G' of the gel upon forced deformation recovered to almost original value upon relaxation during the deformation-restoration cycles (Fig. 4). However, the G' plateau decreased mainly after first break cycle, we assumed that insignificant network disturb at high strain values and required detailed study. The cycles could be repeated for more than ten consecutive times without significant decrement in the modulus values except in the first cycle. Similar observation during recovery test has been reported in the literature (Jay, Langheinrich, Hanson, Mahalingam, & Kiser, 2011). This experiment further support the self-healing nature of the gel (Terech et al., 2013). However, further studies to understand the molecular level interactions leading to self-healing in these novel systems is required and presently the endeavour is going on.

Stretching ability was performed on universal tensile testing (UTM) machine (Fig. 5). The result of UTM measurement shows that the gel prepared from Agr-GA_{Est} (1: 0.5 molar ratios) showed excellent stretching ability (over 20 times of its original length) (Fig. 5). The same observation was obtained by manual stretching experiment as also shown in Fig. 5 and Video File 01. Gel prepared from the native agarose was unable to stretch manually and breakdown on stretching may be due to the brittleness nature of this gel and was not tested on the UTM.

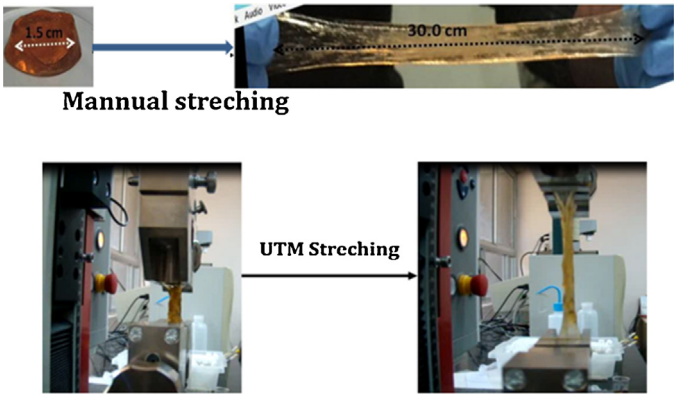

Fig. 5. Schematic out-lay of the physical state of agarose ester derivative gel prepared in ethylene glycol upon manual stretching and mechanical stretching on a universal tensile testing machine (UTM).

4. Conclusions

We report a rapid synthesis and characterisation of agarose ester derivative (Agr-GA_{Est}). The main finding of this study is that Agr-GA_{Est} (1: 0.5, Agr: GA) capable of forming good quality gel in ethylene glycol. The gel thus obtained exhibits excellent stretching ability, self-healing and thixotropic property. These types of multifunctional gel materials may find applications in sensors/actuators, bio-medical and tissue engineering applications. Additionally, this type of material having ability to autonomous repair after an induced damage are useful as longer-lasting, fault-tolerant products and components in many applications such as coatings, electronics, transportation, and energy (Burnworth et al., 2011).

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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.2014.08.024.

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