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International Journal of Pharmaceutics 356 (2008) 174–180
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Preparation and characterization of mucinated agarose: A mucin–agarose physical crosslink

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Received 24 August 2007; received in revised form 2 January 2008; accepted 7 January 2008
Available online 12 January 2008

Abstract

Efficient, biocompatible and biodegradable new polymer materials are continually being sought to meet the challenging needs of drug delivery. Mucinated agarose, a physical crosslink of mucin and agarose, which are both biodegradable natural polymers, has been successfully prepared by a temperature controlled coarcervation technique of aqueous dispersions of equal concentrations of both polymers. Some functional and physicochemical characteristics of the new polymer such as swelling, moisture uptake, mucoadhesive as well as the thermal properties were determined and compared to those of agarose and mucin alone. Turbidimetric interaction between the aqueous dispersions of mucin and agarose was used to determine the concentration ratio of optimum interaction between the two polymers. A concentration ratio mix of four parts mucin and six parts agarose was obtained as the concentration ratio of optimum interaction. A 1:1 dispersion mix was, however, used for the crosslinking process. The mucinated agarose showed characteristic swelling, mucoadhesiveness, moisture uptake and DSC thermal properties that were different from those of mucin and agarose alone. The results indicated that there was formation of a crosslink between mucin and agarose.

Keywords: Mucin; Agarose; Mucinated agarose; Temperature controlled coarcervation; Physical crosslink

1. Introduction

Novel polymer biomaterials are continually being sought for a number of biomedical applications such as drug delivery and tissue engineering (Watering et al., 2005). In the development of new polymers, the bio-compatibility and nontoxicity of the materials are important factors. Apart from these, the new material must be tailored to posses appropriate properties depending on specific use and requirement (Sousa et al., 2005). To meet the need for new polymers for the delivery of especially challenging bioactive materials and cells, different methods of preparation have been explored (Watering et al., 2005). Polymer crosslinking is one of the most common but successful methods that have been used for the development of new polymer types. Both chemical and physical crosslinking methods have been used (Wu et al., 2004; Moussa et al., 1998) to tailor polymer properties to particular needs for the delivery of challenging molecules and for delivery to specific sites (Lowman et al., 2004; Macleod et al., 1997; Watering et al., 2005).

Physical crosslinking involves the blending of two or more polymer moieties under controlled physical conditions. The resultant new polymer may have functional and physicochemical properties that are synergistic or different and superior to those of the individual component polymers (Wu et al., 2004). Some physical mixtures have shown unimolecular encapsulation behavior, while others have been used to optimize drug dissolution and modify the kinetics of release (Vudathala and Rogers, 1992).

Agarose is a hydrophilic and non-pH sensitive natural polymer (Wang and Wu, 1998) that is extensively used in the food industry because of its characteristic gelling properties. In spite of this however, it has limited use in drug delivery probably because of its high porosity and poor bioadhesiveness. Structurally, agarose is a galactose polymer in which the linkages alternate 1–4, 1–3, 1–4, 1–3. This arrangement allows two chains to join together and adopt double helices which are held together

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doi:10.1016/j.ijpharm.2008.01.006

by hydrogen bonds. The two chains have four ends and these remain as random chains that are able to linkup with other random coil ends of the other helices so resulting in extensive crosslinking (Nimmi et al., 2005).

Pure mucins are glycoproteins (Jay-Friedman, 1995) and in the presence of water form mucus gel which lines the alimentary tract and many other mucus membranes making them useful as drug delivery vehicle. Mucins are generally made up of large polysaccharides bonded too much smaller quantities of proteins. The common monosaccharides are sialic acid, galactose, glucose, fucose, manose and glucosamine (Forstner et al., 1979; Adikwu et al., 2005). It is therefore possible for the free random groups of agarose to link to the galactose moiety of mucin or to any other potentially reacting sugars thus crosslinking the glycoprotein chain to the double helical structure of the native agarose.

Considering the desirable properties of agarose and mucin, it is hoped that a novel polymer with synergistic physicochemical and functional properties will be produced by crosslinking them. Thus the objective is to produce and characterize a novel polymer through physical crosslinking of mucin and agarose by temperature controlled coarcervation.

2. Materials and methods

2.1. Materials

The materials used were agarose, porcine mucin, sodium chloride, and magnesium chloride (Sigma–Aldrich Chemie, Germany); potassium dihydrogen phosphate (May & Baker, Dagenham, England); sodium hydroxide, potassium thiocynate, potassium chloride and calcium chloride (BDH Chemicals, UK).

2.2. Methods

2.2.1. Mucin/agarose turbidimetric interaction

A 500 ml quantity of 2 mg/ml concentration of agarose and mucin were prepared separately in distilled water, phosphate buffer (PBS) pH 7.4, simulated intestinal fluid (SIF, pH 7.4) and simulated gastric fluid (SGF, pH 1.2). Agarose dispersion was made by heating in the various fluids to 90 °C with constant stirring until no solid particle was observed (Wang and Wu, 1998). The solution was filtered with a qualitative filter paper (No. 1, Whatman Ltd., England.). A 10 ml mixture of the mucin and agarose dispersion was prepared at different ratios as in Table 1. The absorbance (A) of the various mixtures were then determined after 30 min at 500 nm using a spectrophotometer (UV-160A Shimadzu, Japan). The absorbancies of agarose and mucin alone in the various fluids were also determined and used to give the theoretical values for a non-interacting system (Ping et al., 1998).

Table 1
Ratio mix of mucin and agarose for turbidimetric evaluation

Mucin	10	9	8	7	6	5	4	3	2	1	0
Agarose	0	1	2	3	4	5	6	7	8	9	10

2.2.2. Preparation of mucin–agarose coarcervate discs

100 ml quantities each of 4% (w/v) mucin and agarose dispersions were prepared in distilled water. The mucin and the agarose were mixed at predetermined ratio of 1:1 (mucin:agarose) such that the final mucin/agarose content in the dispersion was 10 g. Mixing was carried out by stirring for 30 min. The dispersions containing agarose were maintained at 40 °C throughout the duration of mixing. The agarose, mucin and mucin/agarose dispersions were precipitated with acetone maintained at −30 °C. 100 mg portions of the wet mass were then compressed into circular discs using a 2 ml syringe with one end cut open and a load of 1 kg. The discs formed were exposed to a flush of cold air (10 °C) to remove any acetone residues present, and then dried in a desiccating chamber for 7 days before use.

2.2.3. Swelling properties

The discs prepared above were each weighed (W_d) and transferred onto a glass slab (2 cm × 4 cm) of known weight. This was then placed in a Petri dish containing 60 ml of distilled water at room temperature (25 °C). At 15 min intervals the glass slabs with the hydrated discs were removed, dried by blotting with tissue paper and weighed (W_t). The weight swelling ratio (Q) was determined using Eq. (1). When the hydrated discs reached a constant weight (W_e) the swelling ratio at this point is considered to be the equilibrium swelling ratio (Q_e) and was determined according to Eq. (2) (Vervoort et al., 1998).

$$Q = \frac{W_t}{W_d} \tag{1}$$

$$Q_e = \frac{W_e}{W_d} \tag{2}$$

The mean swelling time (MST) of the polymer discs were evaluated using Eq. (3).

$$\mathrm{MST} = \left(\frac{n}{n+1}\right) K^{-1/n} \tag{3}$$

To determine the parameters (n and K) in Eq. (3), the power equation (Ritger and Peppas, 1987), Eq. (4) was used.

$$\frac{W_t}{W_d} = Kt^n \tag{4}$$

The W_t and W_d above are same as in Eq. (1), n is a kinetic constant which depends on the solvent type and is used to characterize the transport mechanism of solvent into the polymer and K is a kinetic constant incorporating characteristics of the polymeric system. The values of n and K were obtained from the plot of $\ln W_t/W_d$ versus $\ln t$ where n and K are the slope and intercept at the y-axis respectively (Vervoort et al., 1998). The equation was applied only to the initial stages of swelling (1–3 h).

2.2.4. Mucoadhesive study

The mucoadhesive characteristic of the mucinated agarose was studied by evaluating the force required to detach the hydrated agarose, mucin, and mucinated agarose polymer discs from the surface of porcine small intestine tissues using a Du Nouy tensiometer adapted for this purpose (Harding, 2003). The

polymer discs were attached to the surface of a fabricated ring of the tensiometer with glue.

2.2.5. Preparation of intestinal tissue

A portion of porcine small intestine was obtained from a freshly slaughtered male pig (from an abattoir in Federal Capital Territory, Abuja). The cut portion was placed in a phosphate buffer solution at 5 °C and conveyed to the laboratory. The tissue was then rinsed several times with cold phosphate buffer solution and cut into small pieces (each piece was ≈2 cm × 5 cm). Each of the cut pieces was attached onto a flat surface and the tissue surface irrigated with 0.5 ml of SIF before the polymer disc was placed on it. The disc was left in contact with the tissue for 10 min before the detachment process (Ravichandra et al., 1997). The experiment was repeated four times for discs prepared with agarose, mucin and the mucinated agarose. The total force in dynes required to completely detach the disc from the tissue patch was recorded. The bioadhesive force (F_b) was calculated per unit area of the polymer disc using Eq. (5).

$$F_b = \frac{F}{A} \quad (5)$$

F and A are the force applied (N) and the area of disc (m) respectively.

Unit for F_b is dynes/cm; 1 dyne = 10^{-5} N; in SI unit ∴ $F_b = \mathrm{N\,m^{-1}}$ (Martin et al., 1993).

2.2.6. Moisture sorption characteristics

Quantities of mucin, agarose and the mucinated agarose initially passed through a sieve (U.S. Standard testing sieve, No. 35, U.S.A.), were placed in a Petri dish and stored in a desiccator containing silica gel as the desiccant at 25 °C for one week to remove residual moisture from the materials. The adsorption isotherms were determined by the gravimetric method (Beristain et al., 2006). A 1 g quantity of each sample of polymer was placed in an aluminum foil and put in a desiccator with a gauze holding tray containing water or saturated solution of different salts to provide the required relative humidity (RH) (water 100%, potassium chloride 84%, sodium chloride 75%, potassium thiocynate 47% and calcium chloride 31%). The powders were weighed at 12 h intervals until equilibrium was attained. The percentage equilibrium moisture uptake is determined using Eq. (6).

$$\text{Moisture uptake} = \frac{M_e}{M_d} \times 100\% \quad (6)$$

Where M_e is the amount of moisture absorbed at equilibrium and M_d is the dry weight of the material (Lin and Chen, 2005). The moisture sorption profile of percentage weight gain versus relative humidity was then determined.

2.2.7. Differential scanning calorimetry (DSC)

DSC studies were carried out on a DSC 204 F1 (Phoenix NETZSCH) machine equipped with a thermal analysis system. Indium (156.8 °C) was used as the internal standard. Samples of approximately 1 mg were placed in an aluminum pan (25 μl) and covered with a perforated lid. Dry nitrogen was used as the purge gas (purge 20 ml $\mathrm{min^{-1}}$). The probes were heated from a start temperature of between 25 and 35 °C to 500 °C at a rate of 10 °C $\mathrm{min^{-1}}$. The solubilisation analyses of agarose and mucinated agarose were performed using a dispersion of agarose and the mucinated agarose (25%, w/w) in distilled water. These were placed in the aluminum pan. The relevant thermodynamic parameters were evaluated with the Proteus analysis software.

3. Results and discussion

3.1. Turbidimetric interactions

The turbidimetric studies provided information on the potential chemical interactions between mucin and agarose when their aqueous dispersions were mixed.

The absorbance (A) at 500 nm of agrose and mucin individually and their ratio mixtures in the various aqueous systems were measured as the reference absorbancies. The theoretical absorbance (A_T) for the mixtures was calculated from the individual absorbancies (Ping et al., 1998). The use of the various aqueous systems (SIF, SGF, PBS and H_2O) was to highlight the effect of these aqueous environments on the turbidimetric interactions. The difference in absorbance (ΔA) between the absorbance (A) and the theoretical absorbance (A_T) is regarded as the extent of interaction. Thus (ΔA) would be zero if no interaction took place (Ping et al., 1998).

The maximum turbidity resulting from the various ratio mixes of agarose and mucin dispersions occurred in water at a ratio mix of 6:4 (agarose:mucin) (Fig. 1). The intensity of turbidity is a measure of extent of interaction between agarose and mucin. There was also relatively high level of interaction in SGF (9:1), with SIF and PBS having comparatively low turbidity. The high turbidity of agarose–mucin mixture in water could be due to the water induced lowering of the ionic strength of the mucin proteins (Smith et al., 1968). It could also be due to polymer growth due to the crosslinking of mucin and agarose resulting from the interaction of the galactose moiety of the porcine mucin and that of the agarose by hydrogen bonding or van der Waals forces (Wu et al., 2004; Hassan et al., 2000a). The differences in extent of interaction due to ratio mix indicate that an optimum ratio exists at which maximum interaction

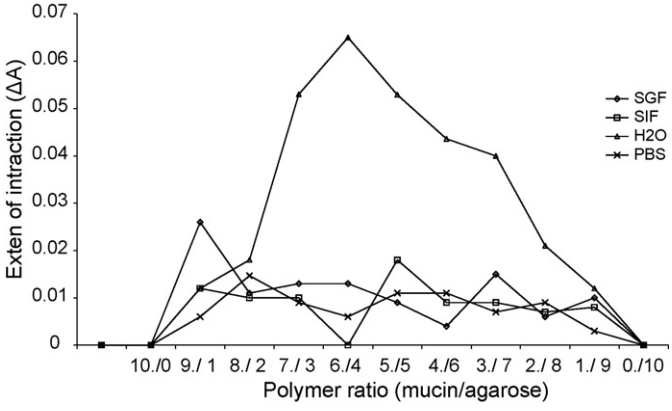

Fig. 1. Turbidimetric interaction of mucin with agarose in SGF, SIF, PBS and H_2O.

occurred as shown by Fig. 1. This was highest at a ratio mix of 6:4.

3.2. Preparation of mucinated agarose

Mucinated agarose which is an agarose–mucin coarcervate was successfully prepared from mixing of agarose and mucin dispersions in water using a technique based on temperature controlled precipitation. Mixing the aqueous dispersions of the two polymers results in the interpenetration of the copolymer network and whole precipitation with chilled acetone (−30 °C) improved the interconnectivity between the agarose and mucin molecules (Hassan et al., 2000b; Peppas et al., 2000; Wu et al., 2004). This would increase the interaction proximity between the random coils of the double helical galactose network of agarose and the galactose of the mucin which then interact by hydrogen bonding. The use of a freezing environment may also result in crosslinking by the formation of crystallites which may reduce the water solubility of the material (Peppas et al., 2000). Other non-covalent interaction sites may also contribute to the physical crosslinking. The functional groups of the amino acid backbone of the peptide chain may bind to the large glycan chain of agarose via some weak forces of interaction such as hydrogen and van der Waals forces to create a large polymer crosslink with less flexible structure.

3.3. Swelling properties of polymer discs

The swelling profile of the various discs in distilled water is shown in Fig. 2. The equilibrium swelling ratio and the time required to attain equilibrium swelling were highest for mucin, and least for agarose discs. The longer swelling time and higher swelling ratio exhibited by discs prepared with mucin may be due to its high viscosity and better gelation properties that are characterized by slow water sorption but high water holding capacity. The intermediate swelling characteristics exhibited by the mucin–agarose physical crosslink (mucinated agarose) could be due to the blocking of the large pores of the agarose polymer network by the compact mucin network crosslinked to agarose, thus reducing the movement of water across the porous double helical structure of the agarose gel network. The presence of mucin in the agarose network could have also strengthened its viscoelasticity thus preventing the easy disintegration of the polymer as compared to what was observed with agarose.

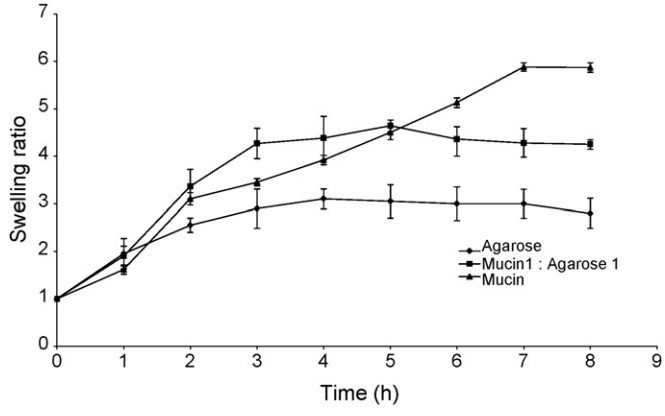

Fig. 2. Swelling profile of discs made from mucin, agarose and mucinated agarose in water.

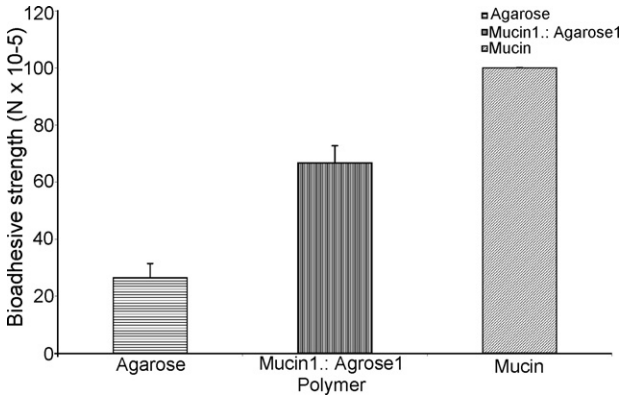

Fig. 3. Mucoadhesive strength of agarose, mucin, and mucinated agarose on pig intestine.

The MST was 0.11 ± 0.03 for agarose, 0.44 ± 0.01 for mucin and 0.23 ± 0.01 for the mucinated agarose. This indicates that the rate of swelling or water uptake was fastest in agarose, intermediate for the mucin–agarose crosslink (mucinated agarose) and slowest for mucin as seen from the swelling profiles in Fig. 2.

3.4. Mucoadhesive studies

The mucoadhesive strength of discs prepared with mucin, agarose and the mucinated agarose is presented in Fig. 3. The investigation of the bioadhesive strength of the polymers was to assess the effect of crosslinking of mucin with agarose on their mucoadhesive efficiency.

Mucoadhesive polymers adhere to the mucus membrane by interacting with the mucus gel of the mucus membrane (Harding, 2003). This is as a result of rapid reduction in the surface energy (interfacial tension) between the mucus membrane and the polymer and the formation of interfacial bonds with the mucin in the mucus layer of the tissue. The mucoadhesive interactions between the mucus layer of the pig small intestine and discs formed with the agarose, mucin and the mucin–agarose crosslink are shown in Fig. 3. The disc prepared with porcine mucin had the highest mucoadhesive strength; agarose the least, while the mucinated agarose exhibited intermediate mucoadhesive potential. The poor mucoadhesive property of agarose may be due to any or combination of the following reasons; the absence of charged groups on the agarose structure (Harding, 2003), agarose has a high onset of gelation temperature (30.6 °C). Below the gelation temperature the viscosity and elasticity of agarose is highly diminished. This results in loss of gel property and brittleness with a tendency to disintegrate in solution. Agarose also has high porosity that prevents it from retaining water for rapid hydration and maintenance of adequate wetting that is necessary for mucoadhesive interaction (Jones et al., 2002). Mucins have high wetting and water holding capacity. Crosslinking mucin with agarose increased the mucoadhesive efficiency of agarose.

3.5. Moisture uptake

The moisture uptake experiment was undertaken to assess the comparative amorphousity or crystallinity of mucin, agarose and the mucinated agarose so as to provide evidence of crosslinking between mucin and agarose in the mucinated agarose. Moisture sorption has been reported to be the most sensitive technique for assessing variation in the amorphous content of polymers (Mackin et al., 2002), as well as predicting some physicochemical (Bravo-Osuna et al., 2005; Lin and Chen, 2005) and functional (Trung et al., 2005) properties of polymers. Agarose, mucin and their physical crosslink have similar isothermic moisture uptake profiles (Fig. 4). Since the equilibrium moisture uptake of the various polymers increased with increase in RH, all the polymers can be classified as hygroscopic (Okubayashi et al., 2004). Between 0% and 80% RH range the sensitivity of the moisture uptake of the polymers in terms of amorphousity or crystallinity was not remarkable until the 100% RH (Fig. 4). The mucin took up to 86.5%, agarose 37.2% and mucin–agarose crosslink 80.8%. Moisture uptake is due to the amount of moisture taken up into the amorphous structure of the polymer powder bed as a result of the water molecules interacting with the polar groups of the polymer backbone (Bravo-Osuna et al., 2005). For similar polymeric materials the moisture uptake profile for the amorphous form exhibits a higher shift when compared to the more ordered crystalline form (Mackin et al., 2002; Burnett et al., 2006).

The moisture uptake of the agarose–mucin crosslink was quantitatively similar to that of mucin than that of agarose. The characteristic moisture uptake profile of the mucinated agarose is an evidence of crosslinking rather than an ordinary admixture as this would have exhibited a quantitative intermediate moisture uptake. Agarose is characterized by an ordered 1–4, 1–3, 1–4, 1–3 linkage forming the double helical structure. The crosslinking of the galactose moiety of mucin could have resulted in a more disordered network due to bond attractions and repulsions which would affect the galactose–galactose linkages via hydrogen bonding and van der Waals forces. The resultant creation of a disordered structure would produce a more amorphous network compared to the more ordered agarose network but less

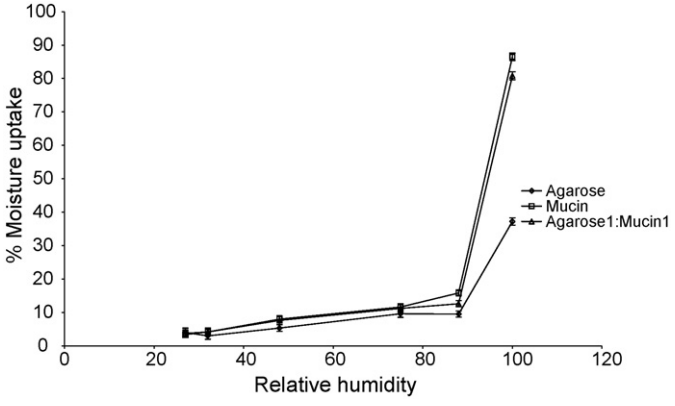

Fig. 4. Comparative moisture uptake profile of agarose, mucin and mucinated agarose.

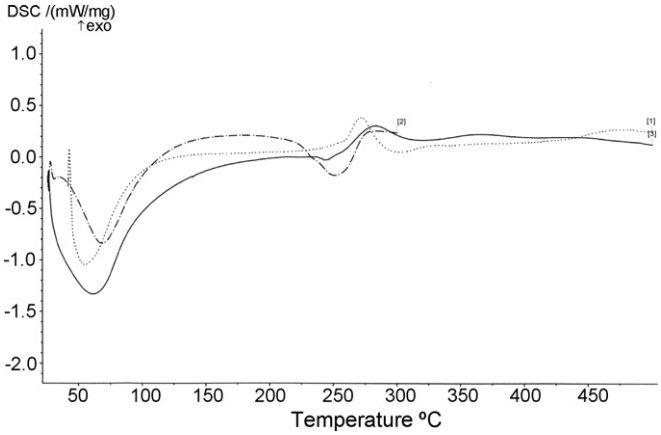

Fig. 5. Thermal characteristics of mucin, agarose, and mucinated agarose. (1) Agarose (. . .), (2) mucin (– – –) and (3) mucinated agarose (—).

than the primarily amorphous mucin. Apart from the inherent chemical nature (Maya et al., 2006) and polymorphic form of the materials, there are several other factors that could affect their moisture or water uptake. These include physical properties like surface area (Huang et al., 2005), particle size (Strange and Onwulata, 2002), porosity (Iqbal et al., 2006) and surface versus bulk sorption (Nowakowski and Hartel, 2002). However the effect of these factors was minimized by the experimental design which ensured that the particle size distribution and surface area of exposure were similar for all the batches.

3.6. Thermal properties

The DSC thermograms of the temperature controlled coarcervate of the physical mixture of mucin and agarose (1:1) and those of the individual agarose and mucin are shown in Fig. 5. The DSC was used to detect evidence of crosslinking between mucin and agarose using the temperature controlled coarcervation process.

The DSC curves showed an initial characteristic endothermic peak corresponding to the glass transition (T_g) for agarose (curve 1), mucin (curve 2) and mucin–agarose (curve 3). The summary of the thermophysical data of the three polymers is presented on Table 2. The initial endothermic peak presented by curve 1 corresponds to the glass transition (T_g = 68.4 °C). This was fol-

Table 2
Thermal properties of agarose, mucin and mucinated agarose

Parameters	Agarose	Mucin	Mucinated agarose
T_g			
Onset	49.5	40.4	62.7
End	55.6	68.6	74.6
T_m (°C)	275.1	239.2	238.6
Exothermic ΔH (J/g)	29.04		43.34
Solubilisation			
Onset (°C)	34.8		31.7
Mid (°C)	82.9		96.5
End (°C)	99.6		98
ΔH J/(g K)	781.29		261.32

T_g, glass transition temperature; T_m, melting temperature.

lowed by an exothermic peak. The exothermic peak represents the crystallization of agarose from its armophous phase. This exothermic shift was then followed by a first order transitional endothermic shift representing the melting of the crystals. Curve 2 (mucin spectra) is characterized by two endothermic peaks: the first is a second order transitional peak that represents the glass transition. The comparatively broad nature of this peak probably indicates the high amorphous nature of mucin. The second endothermic peak represents a transition peak that characterizes its melting. Curve 3 (mucinated agarose) is characterized by three endothermic peaks and a broad exothermic peak. The first endothermic peak is a second order transition peak that represents its glass transition. The second endothermic peak was small but shows a distinct minima curve that may represent melting of a residual component followed by a broad exothermic peak and finally by another transition endothermic peak that represent the final melting.

The comparative evaluation of the DSC curves indicates a possible crosslinking between agarose and mucin. The high T_g obtained for agarose–mucin coarcervate relative to that of mucin and agarose individual components is an indication of a new polymer type. Apart from the interaction of the glycan–glycan bonding of the carbohydrate moiety of mucin and the galactose of agarose, there may be other possible non-covalent interaction sites that may contribute to the physical crosslinking. Mucin is a glycoprotein made up of *N*-glycosidic linkages of peptide chains and also a carbohydrate moiety. The functional groups of the amino acid backbone of the peptide chain may bind to large glycan chain of the agarose via some weak forces of interaction such as hydrogen bonding and van der Waals forces to create a large polymer crosslink with less flexible structure. The high chain rigidity thus requires a higher temperature to cause the second order transitional change in the polymer thus resulting in the high transition temperature. The second endothermic peak is a melting peak with characteristic onset approximately equal to that of mucin. The slight shift could be due to the presence of other moieties in the system. This may represent the amount of free uncrosslinked mucin in the system. This result corroborates the result obtained for the turbidimetric interaction studies. During the preparation of the mucin–agarose coarcervate an equi ratio mixture was prepared but in turbidimetric interaction maximum interaction occurred in mucin–agarose dispersion mix of six parts of agarose to four parts of mucin. Thus the excess mucin may be responsible for the first endothermic peak of mucin–agarose coarcervate seen in curve 3. This confirms a probable optimum combination required for mucin–agarose physical crosslinking.

The onset of the crystallization of the new polymer coincides with the end of melting of the pure mucin. The exothermic curve of the new polymer is characteristically broader than that of agarose. The larger area of the peak is an indication of crosslinking of mucin and agarose (Macrogalleria Directory, Copyright, 2005). This also indicates a higher reorientation to crystalinity from amorphousity.

Agarose is soluble in hot water (Wang and Wu, 1998; Nimmi et al., 2005). The thermal spectra for agarose and mucinated agarose solubilisation are presented on Fig. 6. The evidence of crosslinking is also shown by the difference in the solubility property of agarose and the mucin–agarose physical crosslink. There was a slight reduction in the onset and end of solubilisation. Both agarose and the mucin–agarose crosslink have the same peak dissolution temperature but there was a remarkable difference in the quantity of heat per unit weight required to start and complete solubilisation. The various thermal solubility parameters of agarose and mucinated agarose are presented on Table 2.

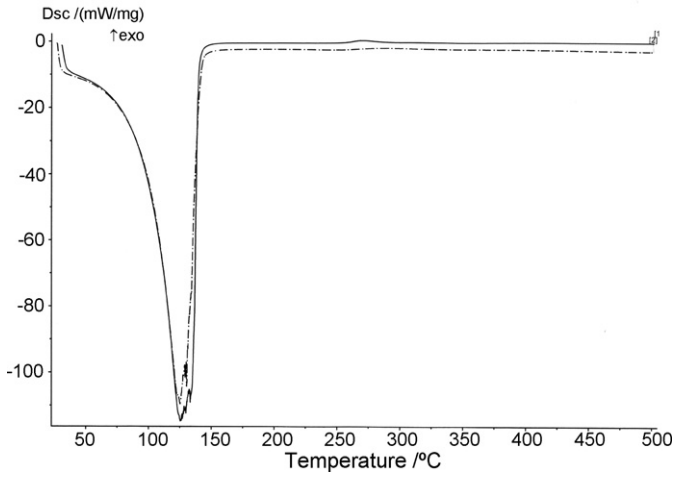

Fig. 6. DSC thermograph for the solubilisation of agarose and mucinated agarose physical crosslink. (1) Agarose (—) and (2) mucinated agarose (. . .).

4. Conclusion

A physical mixture of the water dispersion of various ratio concentrations of mucin and agarose showed evidence of interactions between the two polymers as indicated by the extent of turbidity of the dispersion mixtures. An optimum concentration ratio for interaction was obtained for mucin and agarose. A novel polymer was successfully prepared by precipitating equal concentrations of water dispersion of mucin and agarose with acetone at −30 °C. The new polymer exhibited some physicochemical and functional properties that were different from both agarose and mucin or qualitatively similar to both polymers. The DSC thermal analysis showed that the mucinated agarose has characteristic T_g, M_t and solubilisation temperatures different from that of mucin and agarose. The peak solubilisation temperature of the mucinated agarose and agarose were, however, the same. The heat change required to effect solubilisation was also different. The solubilisation characteristics were similar to those of agarose while moisture uptake, swelling and mucoadhesive properties were similar to those of mucin. This is evident of crosslinking between mucin and agarose. It can be concluded that there is selective synergism of properties which could be exploited in delivery of certain challenging molecules.

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