Formulation and characterization of gellan–DEAE-dextran polyelectrolyte complex hydrogels

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ABSTRACT

Background: There is a need to formulate hydrogels via direct physical interaction to circumvent the use of crosslinker which can affect the integrity of the entrapped drug in the hydrogel.

Objective: The aim of this study is to formulate and characterise plain polyelectrolyte complex (PEC) hydrogels prepared by physical interaction between gellan gum and oppositely charged DEAE-Dextran.

Method: Gellan-DEAE-Dextran (GDD) PEC hydrogels were prepared by polyelectrolyte complexation of gellan and DEAE-Dextran. The GDD-PEC hydrogels were characterized using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). Their swelling kinetics and rheological properties were also evaluated.

Results: Morphological characteristics analysed by SEM of plain gellan (G) hydrogel showed strand-like smooth compact structured surface while the GDD-PEC hydrogels exhibited crinkled rough surfaces. The shifts and disappearances in peaks shown in the FTIR spectra, suggested an interaction between gellan and DEAE-Dextran. DSC showed that the gellan and DEAE-Dextran merged into a single endothermic melting peak between 99.8 and 115.3 $^{\circ}$C in the GDD-PEC hydrogels. Their TGA showed that the plain gellan (G) hydrogel was able to withstand more heat when compared to the GDD-PEC hydrogels. The GDD-PEC hydrogels exhibited pseudoplastic and elastic characteristics based on the concentration of DEAE-Dextran. The addition of DEAE-Dextran to GDD-PEC hydrogels significantly increased ($p < 0.05$, $n = 4$) their swelling ratio when compared to the plain gellan (G) hydrogel.

Conclusion: GDD-PEC hydrogels were successfully formulated and characterised. This formulation has the potential for use in transdermal drug delivery systems.

Keywords: gellan, DEAE-Dextran, binary, polyelectrolyte complexation, hydrogels

Formulation et caractérisation des hydrogels complexes poly-électrolytes Gellan–DEAE-Dextran

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Résumé

Contexte: Il est nécessaire de formuler des hydrogels par interaction physique directe pour contourner l'utilisation de réticulant qui peut affecter l'intégrité du médicament encapsulé dans l'hydrogel.

Objectif: Le but de cette étude est de formuler et de caractériser les hydrogels complexes de poly-électrolyte brut (PEC) préparés par interaction physique entre la gomme de gellane et DEAE- Dextran de charge opposée.

Méthode: Les hydrogels gellane - DEAE- Dextran (GDD) PEC ont été préparés par complexation poly-électrolyte de gellane et DEAE- Dextran. Les hydrogels GDD - PEC ont été caractérisés par microscopie électronique à balayage (MEB), spectroscopie infrarouge à transformée de Fourier (IRTF), calorimétrie différentielle à balayage (DSC) et analyse thermogravimétrique (TGA). Leur cinétique de gonflement et les propriétés rhéologiques ont également été évaluées.

Résultats: Les caractéristiques morphologiques analysées par MEB de l'hydrogel de gellane brut (G) a montré une surface structurée compacte lisse sous forme de brin tandis que les hydrogels GDD-PEC ont exposé des surfaces plissées rugueuses. Les déplacements et les disparitions des sommets indiqués dans les spectres FTIR, suggèrent une interaction entre Gellane et DEAE-Dextran. DSC a montré que Gellane et DEAE-Dextran ont fusionné en un seul sommet de fusion endothermique entre 99,8 et 115,3°C dans les hydrogels GDD-PEC. Leur ATG a montré que leur hydrogel de gellane brut (G) est capable de supporter plus de chaleur par rapport aux hydrogels GDD PEC. Les hydrogels GDD-PEC ont présenté des caractéristiques pseudo-plastiques et élastiques en fonction de la concentration de DEAE-Dextran. L'addition de DEAE-Dextran aux hydrogels GDD-PEC a augmenté de façon significative ($p < 0,05$, $n = 4$), leur rapport de gonflement par rapport à l'hydrogel gellane brut (G).

Conclusion: Les hydrogels GDD-PEC ont été formulés et caractérisés avec succès. Cette formulation a le potentiel pour une utilisation dans des systèmes de livraison transdermique de médicaments.

Mots-clés: gellane, DEAE-Dextran, binaire, complexation poly-électrolyte, hydrogels

INTRODUCTION

Hydrogels are three-dimensional hydrophilic polymeric networks with tendency to absorb large quantity of water or biological fluids and thus resembling biological tissues.[1] The polymeric networks are made up of homopolymers or copolymers and the presence of chemical or physical crosslinks renders them insoluble. They exhibit thermodynamic compatibility with water which aids their swelling in aqueous media.[1] They are classified based on the nature of their side groups as neutral or ionic and based on the physical structures of their networks as amorphous, semi-crystalline, hydrogen bonded or super-molecular structures.[1,2] Other categories include: physiologically responsive hydrogels whose polymer complexes can be broken or networks swollen due to changing external environment such as pH, ionic strength, changes in concentration of glucose, temperature and electromagnetic radiation.[3] Environmental sensitive hydrogels are also referred to as 'intelligent' or 'smart' hydrogels. [4] They are ideal candidates for developing self regulated drug delivery systems.[5] The disadvantage of these smart hydrogels is their slow response time; therefore, fast acting gels are necessary.

Hydrogels may require crosslinking to prevent matrix erosion which may affect their solubility. Chemical hydrogels are formed by irreversible covalent crosslinking. Crosslinking reagents in trace quantities are toxic (glutaraldehyde, epichlorohydrin) and if drug is added before the cross linking step, the crosslinker can affect the integrity of the drug entrapped.[6] This has led to the crosslinker being replaced by polyelectrolyte complexes since they crosslink by ionic linkages or direct physical interactions.[7] Various forces such as van der Waals, electrostatic attraction and hydrogen bonding have been reported to be responsible for the physical interaction of polymer chains. These physical hydrogels or polyelectrolyte complex hydrogels are formed by the interaction of positively charged polymer with an oppositely charged polyelectrolyte. Positively charged chitosan has been used to form polyelectrolyte complex gels by electrostatic interaction with negatively charged polyelectrolytes.[8] In our earlier studies, ternary chitosan-ibuprofen-gellan nanogels were prepared using a combination of electrostatic nanoassembly and in situ ionic gelation.[9] Ionized ibuprofen species were adsorbed onto cationic polysaccharides via their hydrophilic carboxylic groups giving rise to hydrogen bonding, electrostatic or hydrophobic interactions (conjugation).[10-12]

Gellan gum is a linear anionic polysaccharide gelling agent produced by Sphingomonas elodea. It consists of tetrasaccharide repeating units of glucose, glucuronic acid and rhamnose in the 2:1:1 ratio.[13] Gellan can form solid transparent gels at concentrations as low as 0.1%.[14] Gelation of gellan is either temperature dependent or cation induced. As temperature decreases, there is a coil to double helix transition forming a gel based on pH and ionic strength of the solution. The process of gelation starts with the gellan coil molecules forming double helices with the reduction in temperature; and helices aggregate subsequently forming junction zones, leading to system gelation.[15] Diethylaminoethyl-Dextran (DEAE-Dextran) is a water soluble polycationic derivative of Dextran produced by reacting diethylaminoethyl chloride with Dextran. There is no report showing that DEAE-Dextran forms gels or films.

Little attention has been paid to the preparation and characterisation of gellan gum and DEAE-Dextran PEC hydrogel as a carrier for drugs. Therefore, the purpose of this research was to develop and characterise a novel polyelectrolyte complex hydrogel from gellan gum and DEAE-Dextran with a potential for transdermal delivery of drugs.

METHODS

Materials

Gellan gum (Phytagel®), DEAE-Dextran hydrochloride and sodium hydroxide (Sigma-Aldrich, UK) were used as supplied.

Procedure

Preparation of binary polyelectrolyte hydrogels

Gellan reference hydrogel (G) was prepared by dispersing 2% gellan powder in distilled water while under magnetic stirring (Jenway 1000 hotplate and stirrer, UK) until homogeneous and heated up to 90 °C. The pH was adjusted to 6 with sodium hydroxide (NaOH) (Mettler Toledo pH meter, UK) and left to cool.The gellan solution was transferred into DEAE-Dextran solution at different mixing ratios (2:0.125, 2:0.25, 2:0.5 and 2:1) at the same temperature under continuous magnetic stirring. The resulting composite polyelectrolyte complex hydrogels were adjusted to pH 6 with NaOH and further left to cool. These samples will be referred to GDD 2:0.125, GDD 2:0.25, GDD 2:0.5 and GDD 2:1 presented in Table 1. The samples were stored at room temperature for further analysis.

Table 1 Composition of plain gellan and gellan-DEAE-Dextran hydrogels.

Sample	Gellan (%w/v)	DEAE-Dextran (%w/v)
G	2.0	-
GDD 2:0.125	2.0	0.125
GDD 2:0.25	2.0	0.25
GDD 2:0.5	2.0	0.5
GDD 2:1	2.0	1.0

All samples were made up to 100 g with distilled water.

Scanning electron microscopy (SEM)

The surface morphologies of the PEC hydrogels were examined by a scanning electron microscope (Carl Zeiss SEM EVO HD 15, Germany) using variable pressure technology at low voltages with beam deceleration and high definition backscattered electron (BSE) imaging. The hydrogel samples were mounted on double sided carbon tabs that were previously secured to aluminium stubs and then analysed at different magnifications and a pressure of 10 Pa. The accelerating voltage was 10 KV with probe current of 400 pA.

Fourier transform infra-red spectroscopy (FTIR)

The spectra of hydrogel samples were acquired by a Precisely Spectrum One FTIR spectrophotometer (Perkin Elmer, USA) fitted with a Universal Attenuated Total Reflectance (ATR) Sampling Accessory. The hydrogels were mounted directly on the diamond surface and the arm was placed over it by applying enough pressure in the range of 100 to 120 units. The spectrum was recorded in the wavelength region of 4000 to 650 cm^{-1}. All spectra were then collected at an average of 16 scans at a resolution of 4 cm^{-1}. All measurements were taken in replicates of four determinations.

Differential scanning calorimetry (DSC)

DSC was performed using Perkin Elmer Jade DSC machine in conjunction with a Perkin Elmer Intracooler SP cooling Accessory (Perkin Elmer, USA) to study the thermal characteristics of PEC hydrogels. The hydrogel samples of mass 3 mg (±0.5 mg) were heated in hermetically sealed aluminium pans under nitrogen flow (40 mL/min) at a scanning rate of 20°C/min from 30 to 300°C. Empty aluminium pan was used as a reference pan. Zinc and indium were used as the standard reference materials to calibrate the DSC instrument. All measurements were an average of four determinations and expressed as mean ± S.D.

Thermogravimetric analysis (TGA)

Mass changes as a function of temperature in a controlled chamber were studied using Pyris 1 Thermogravimetric Analyser (Perkin Elmer, USA). The weight of the empty reference pan placed in the crucible was zeroed and then removed. Samples of known weight 17 mg (±3 mg) were placed in aluminium pans and measurements performed at a scanning rate of 10°C/min in the range of 25 to 500°C. All measurements were an average of four determinations and expressed as mean ± S.D.

Oscillatory rheological measurements

Dynamic rheological properties of the plain and drug loaded polymeric complex hydrogels were evaluated using a Physica MCR501 rheometer (Anton Paar, Austria) using cone and plate geometry (CP50 with 1° angle) in oscillatory mode at 32°C. Tests were conducted in controlled strain amplitude performed within the linear viscoelastic region (LVR) so that the storage (G') and loss (G") moduli were independent of strain. A strain sweep measurement between 0.1 and 100% was conducted first at a frequency of 1 Hz for plain gellan and gellan-DEAE-Dextran PEC hydrogels; at a strain value which was within the linear viscoelastic region for both samples was selected for the subsequent frequency sweeps. The strain value selected for all the PEC hydrogel samples was 1%. Frequency sweeps at this constant strain were then conducted between 0.01 to 50 Hz and a solvent trap was used to prevent sample dehydration during the test. All tests were done in replicate of four determinations using fresh sample for each test and expressed as mean ± S.D. The rheological parameters examined were processed with pre-installed Physica-software provided with the rheometer. The flow test was also conducted.

Swelling kinetics

A 500 mg of PEC hydrogel was weighed and dried in an oven at 60 °C to a constant weight. The dried polymeric gel was accurately weighed and immersed in 5 mL of Phosphate Buffer Saline (PBS) (pH 7.4) at 37 °C (B Braun Certomat WR Shaker Waterbath, Germany). At

predetermined intervals, swollen gels were taken out, and the excess surface water removed by blotting with filter paper. This was then weighed on a balance. The following equation (equation 1) was used to determine the swelling ratio of the gel.

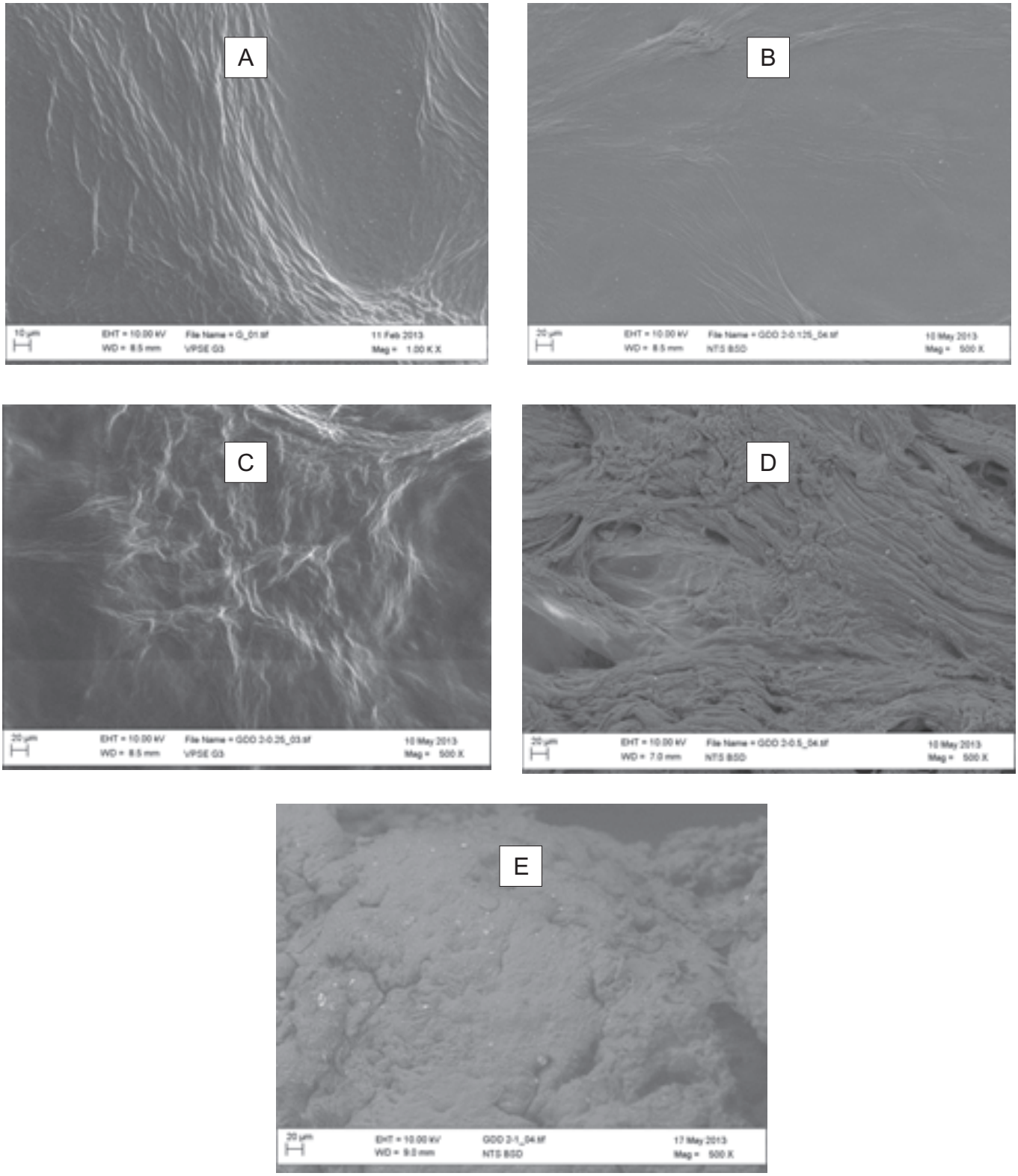

Figure 1 Scanning electron micrograph of G and GDD-PEC hydrogels
(A) plain gelan G, (B) GDD 2:0.125, (C) GDD 2:0.25, (D) GDD 2:0.5 and (E) GDD 2:1

Fourier transform infra-red (FTIR) spectroscopy

The FTIR spectra of gellan gum, DEAE-Dextran and the complex hydrogel formulations obtained are shown in Figure 2. Gellan showed significant peaks at 3306 cm^{-1}, 1602 cm^{-1}, 1408 cm^{-1} and 1020 cm^{-1}. While pure DEAE-Dextran showed significant peaks at 3295 cm^{-1}, 2921 cm^{-1}, 1641 cm^{-1}, 1342 cm^{-1} and 1007 cm^{-1}. The peaks at 3318 cm^{-1}, 2163 cm^{-1}, 2080 cm^{-1} and 1601 cm^{-1} in pure gellan shifted to 3285 cm^{-1}, 2214 cm^{-1}, 2108 cm^{-1} and 1638 cm^{-1} in the plain gellan G hydrogel. The peaks at 2897 cm^{-1}, 2324 cm^{-1}, 2018 cm^{-1}, 1974 cm^{-1}, 1403 cm^{-1}, 1295 cm^{-1}, 1198 cm^{-1}, 1021 cm^{-1}, 891 cm^{-1}, 836 cm^{-1} and 806 cm^{-1} in gellan powder disappeared in the plain gellan G hydrogel.

The -OH stretching peak at 3318 cm^{-1} in pure gellan and at 3309 cm^{-1} in DEAE-Dextran shifted to a range of 3277 to 3285 cm^{-1} in the GDD-PEC hydrogels. The N-H deformation peak at 1644 cm^{-1} in DEAE-Dextran and carboxyl group peak at 1601 cm^{-1} in gellan shifted to a range of 1631 to 1638 cm^{-1} in GDD-PEC hydrogels. Additional peak at 1491 cm^{-1} was exhibited in GDD 2:0.125 and GDD 2:0.25 hydrogels. The C-C stretching peak at 1456 cm^{-1} in DEAE-Dextran shifted to 1491 cm^{-1} in the hydrogels with lower concentrations of DEAE-Dextran. The peak at 1491 cm^{-1} disappeared in GDD PEC hydrogels with high concentrations of DEAE-Dextran in GDD 2:0.5 and GDD 2:1 hydrogels. Features of DEAE-Dextran became increasingly prominent in the complex hydrogels with increasing concentration of DEAE-Dextran. GDD 2:0.5 and GDD 2:1 showed additional peaks at 1071 cm^{-1}, 1238 cm^{-1}, 1293 cm^{-1}, 1370 cm^{-1} and 1411 cm^{-1} contributed by gellan and DEAE-Dextran powders. The C-H stretching peak at 2922 cm^{-1} in DEAE-Dextran shifted to 2929 cm^{-1} and 2941 cm^{-1} in GDD 2:0.5 and GDD 2:1 hydrogels.

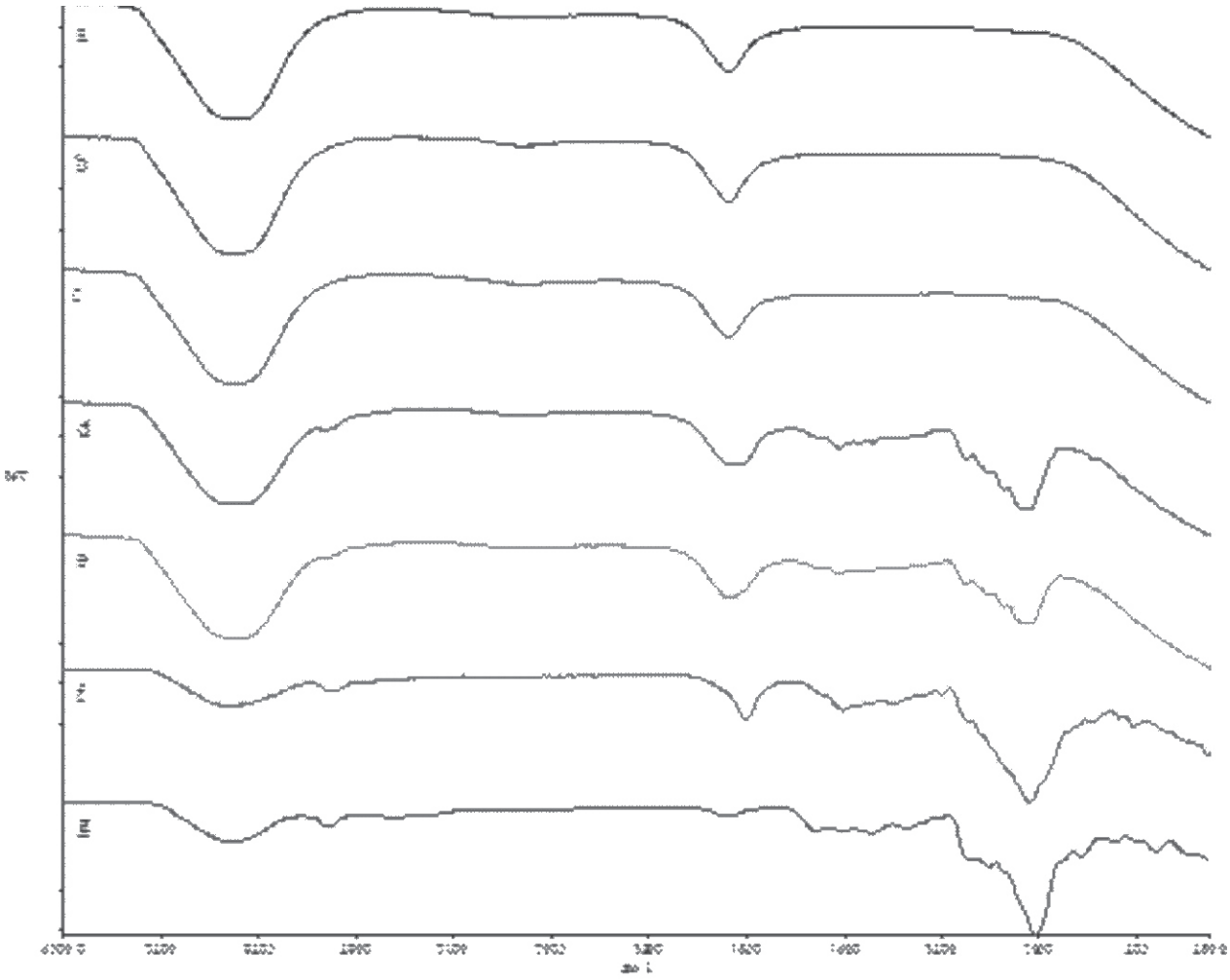

Figure 2 The FTIR spectra G and GDD-PEC hydrogels (a) plain gellan gel G (b) GDD 2:0.125 (c) GDD 2:0.25 (d) GDD 2:0.5 (e) GDD 2:1 (f) gellan powder-reference (g) DEAE-Dextran powder-reference.

Differential scanning calorimetry (DSC)

The DSC thermal characteristics of plain (G) and binary (GDD-PEC) hydrogels were compared with pure gellan and DEAE-Dextran and presented in Figure 3. Pure gellan powder exhibited a broad melting peak at 127.86 °C and exothermic decomposition peak at 254.84 °C. DEAE-Dextran showed a small peak at 57.26 °C which indicated its glass transition endotherm (Tg); a broad endothermic peak at 124.05 °C due to its amorphous characteristic; a small peak at 201.21 °C and a final peak at 268.50 °C attributed to the decomposition of DEAE-Dextran.

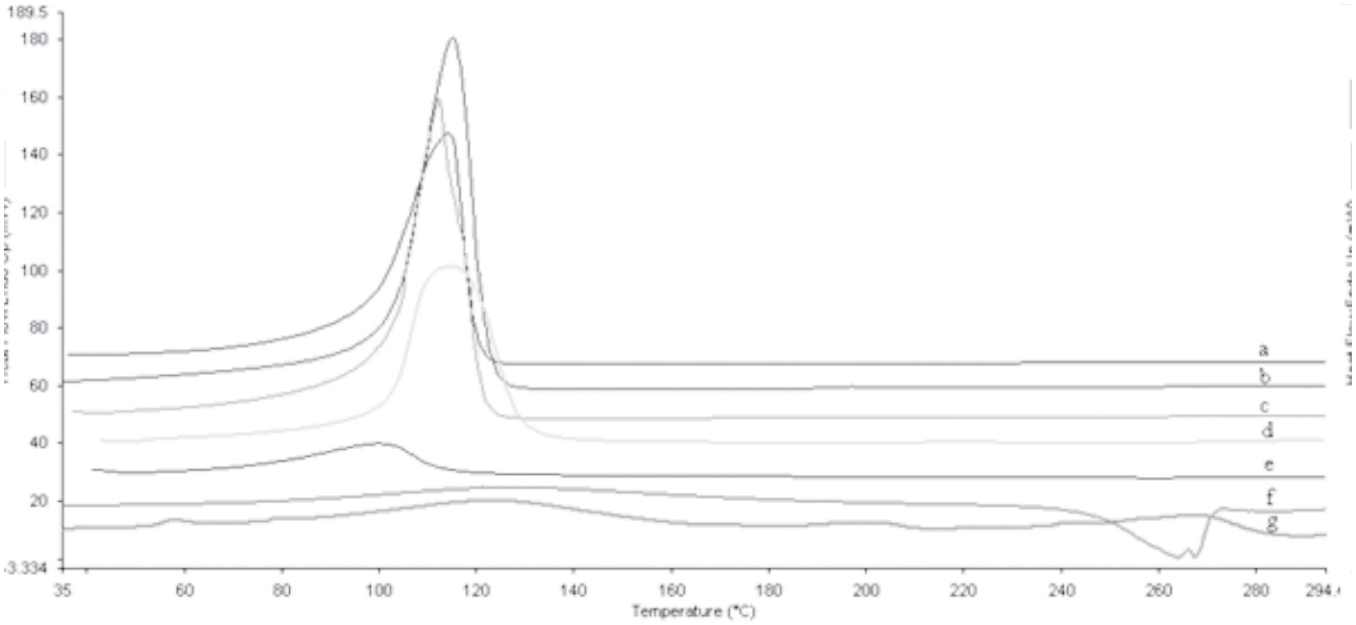

Figure 3 DSC thermograms of G and GDD-PEC hydrogels (a) plain gellan G (b) GDD 2:0.125 (c) GDD 2:0.25 (d) GDD 2:0.5 (e) GDD 2:1 (f) gellan powder-reference (g) DEAE-Dextran powder-reference.

Thermogravimetric analysis (TGA)

The thermograms from the TGA analyses conducted to examine the thermal stability of the GDD-PEC hydrogels are presented in Figure 4. Gellan powder showed two steps of inflection at 90.4 °C and 264.9 °C with corresponding weight losses of 7.9% and 59.7%. DEAE-Dextran showed two steps of inflection at 77.47 °C and 272.15 °C with corresponding weight losses of 6.94% and 67.61%. The plain gellan reference G hydrogel showed two steps of inflection at 100 °C and 257 °C with corresponding weight losses of 97.17%, and 2.14% respectively. The GDD-PEC hydrogels also exhibited two steps of inflection ranging from 106.5 °C to 108.7 °C and 251.8 °C to 258.3 °C; with corresponding weight losses ranging from 97.20 to 97.70% and from 2.00 to 2.80%. The total weight loss exhibited by G hydrogel was 99.30% and for GDD gels was in the range of 99.60 to 100%.

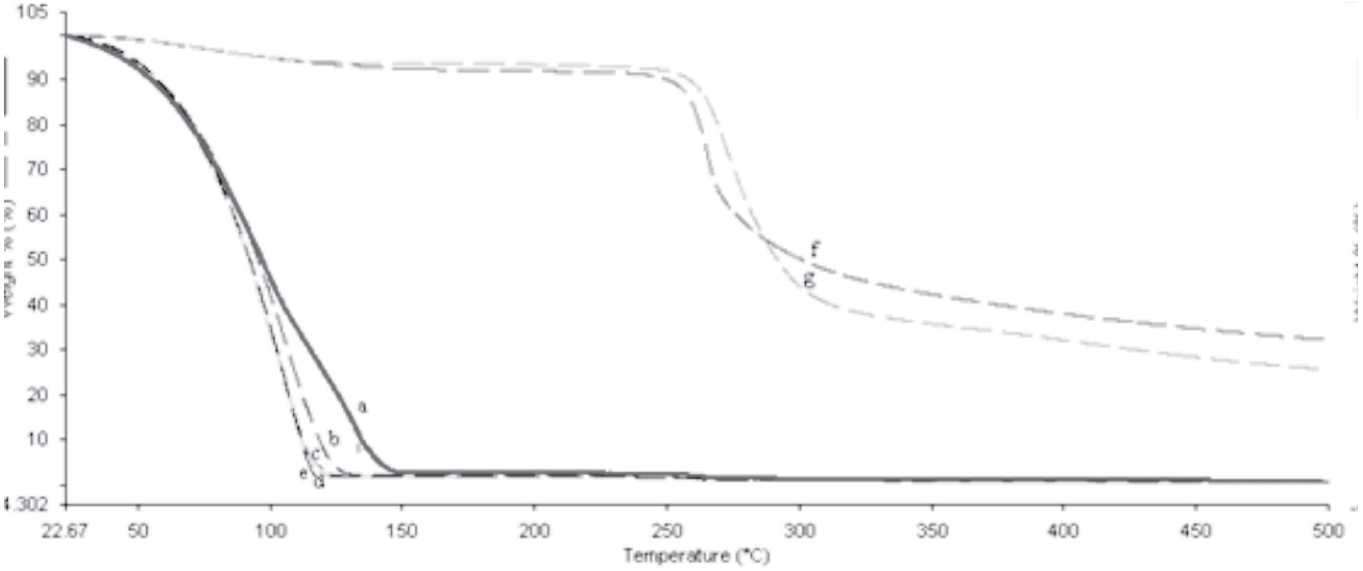

Figure 4 TGA thermogram of G and GDD-PEC hydrogels (a) plain gellan G (b) GDD 2:0.125 (c) GDD 2:0.25 (d) GDD 2:0.5 (e) GDD 2:1 (f) gellan powder-reference (g) DEAE-Dextran powder-reference.

Oscillatory rheological measurements

The viscoelastic response of the gels to an applied strain showed a linear viscoelastic region (LVR) observed in Figure 5 where G' and G" are both linear across the strain range 0.1 to 50%

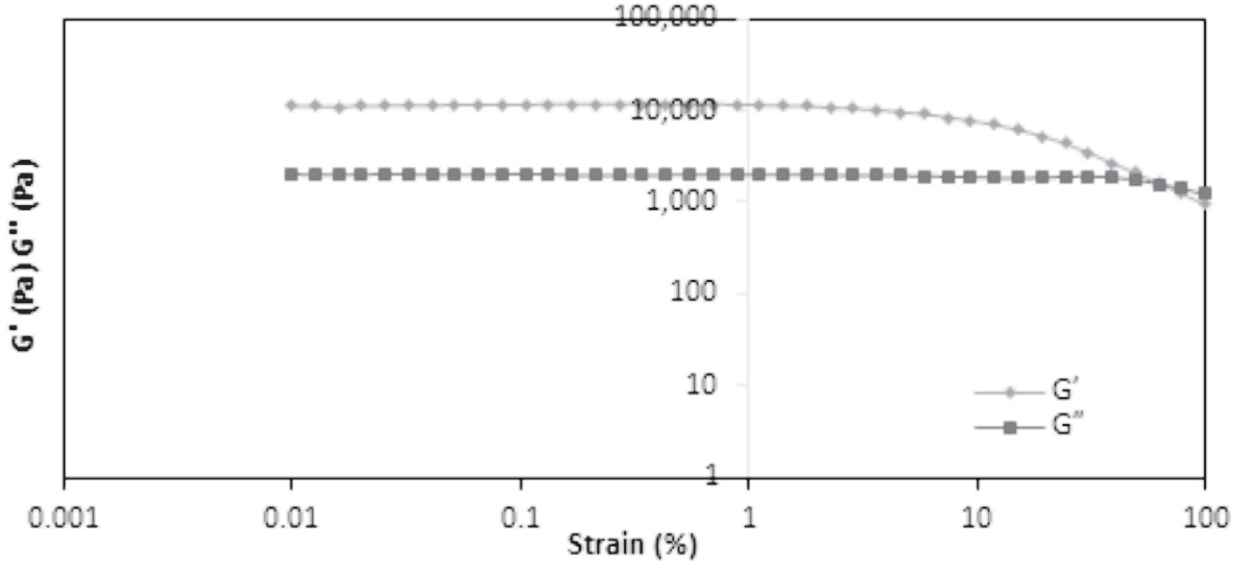

Figure 5 Strain amplitude sweeps of G' and G" with linearity at strain of 1% for plain gellan reference G hydrogel.

The storage modulus – elastic part (G'), loss modulus - viscous part (G"), complex viscosity (η*) and loss tangent (δ) were measured in oscillation mode. Figures 6 and 7 showed the behaviour of viscoelastic parameters G', G", η* and tan δ when subjected to frequency oscillation sweep across a frequency range of 0.1 to 50 Hz at a strain of 1% at 32 °C for plain gellan G and GDD-PEC hydrogels. Figure 6A and 6B showed an increase in G' and G" of the hydrogels as the frequency increased across the frequency range. The storage modulus G' solid-like characteristics of all the hydrogels tested; predominated over the loss modulus G" liquid-like characteristics.

The GDD complex gels exhibited an increase in G' (elastic modulus) in ascending order as follows G < GDD 2:1 < GDD 2:0.125 < GDD 2:0.25 < GDD 2:0.5 with GDD 2:0.5 exhibiting the highest G' value (elasticity) at frequency of 1 Hz mid way through the frequency sweep shown in Figure 6A. Across the frequency range, G' increased sharply from 0.05 to 1 Hz (at low

frequency) and slowly increased till 50 Hz for G, GDD 2:0.125 and GDD 2:0.25 gels; while G' increased steadily across the frequency range for GDD 2:0.5 and GDD 2:0.1. The plain gellan G, GDD 2:0.125 and GDD 2:0.25 (lower concentrations of DEAE-Dextran) hydrogels required a higher frequency to assume the solid-like state characterized by the sharp increase in G' to achieve a similar response when compared to the GDD 2:0.5 and GDD 2:0.1 (higher concentrations of DEAE-Dextran) hydrogels.

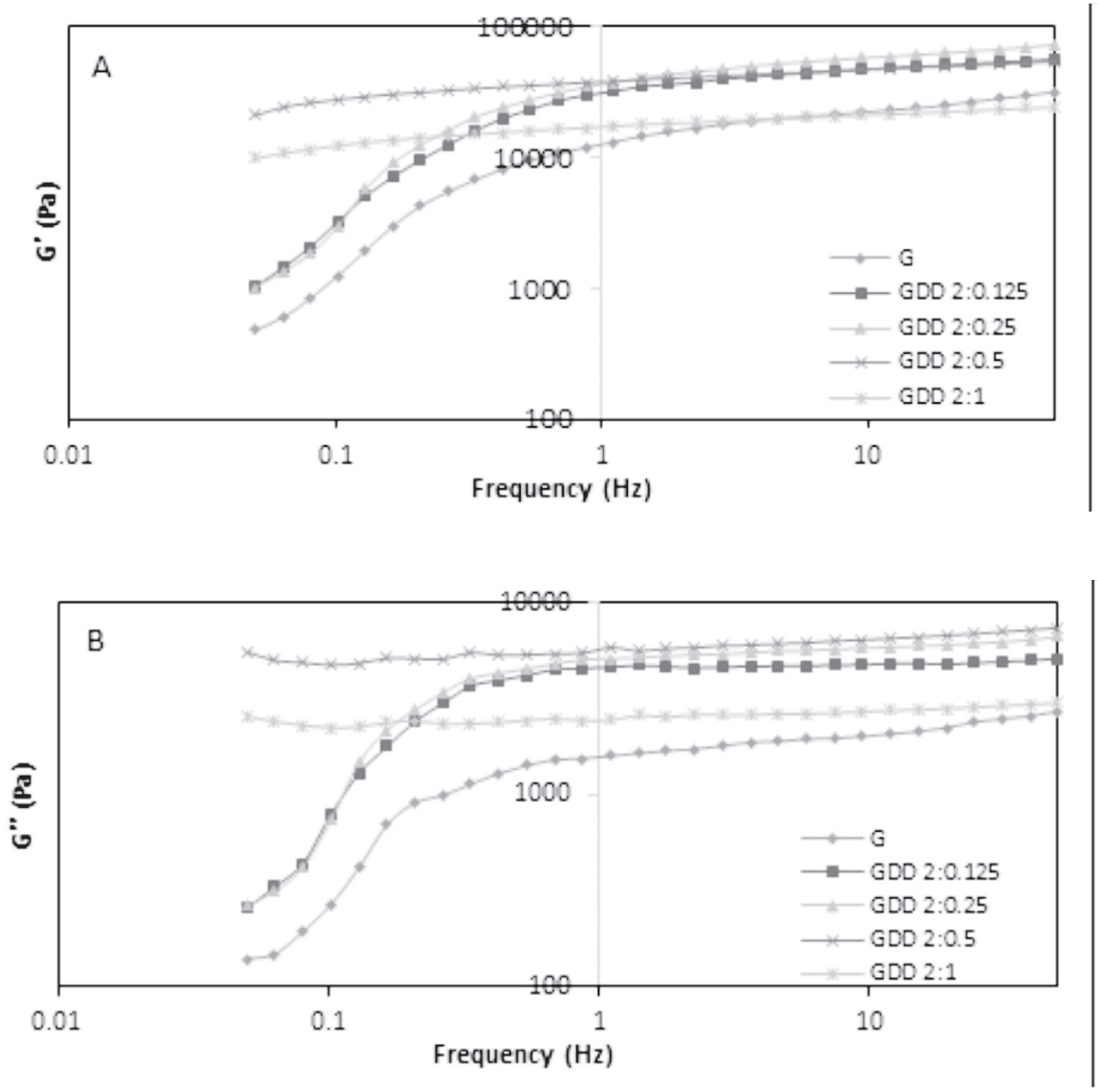

Figure 6 (A) Storage modulus profiles across a frequency range of 0.01 to 50 Hz at 32°C for plain gellan G and GDD-PEC gels and (B) Loss modulus profiles across a frequency range of 0.01 to 50 Hz at 32 °C for plain gellan G and GDD-PEC hydrogels. Each data point represents mean ± S.D (n=4).

The GDD-PEC hydrogels exhibited an increase in G" in ascending order through G < GDD 2:1 < GDD 2:0.125 < GDD 2:0.25 < GDD 2:0.5; with GDD 2:0.5 exhibiting the highest G" value (viscous) at frequency of 1 Hz shown in Figure 6B. Across the frequency range, G" increased sharply from 0.05 to 1Hz and slowly increased till 50 Hz for G, GDD 2:0.125 and GDD 2:0.25 hydrogels; while G" increased steadily across the frequency range for GDD 2:0.5 and GDD 2:1.

The flow behaviour of the hydrogels in Figure 7A showed that for G, and low concentrations of DEAE-Dextran GDD 2:0.125 and GDD 2:0.25 hydrogels, there was an initial increase in complex viscosity followed by a decrease across the frequency range while complex

viscosity for GDD 2:0.5, GDD 2:1 hydrogels decreased steadily across the frequency, suggesting pseudoplastic behaviour.

With reference to GDD-PEC hydrogels, complex viscosity increased in ascending order through G < GDD 2:1 < GDD 2:0.125 < GDD 2:0.25 < GDD 2:0.5; with GDD 2:0.5 exhibiting the highest complex viscosity trend shown in Figure 7A. These results showed an increase in the complex viscosity frequency sweep profiles as DEAE-Dextran content was increased.

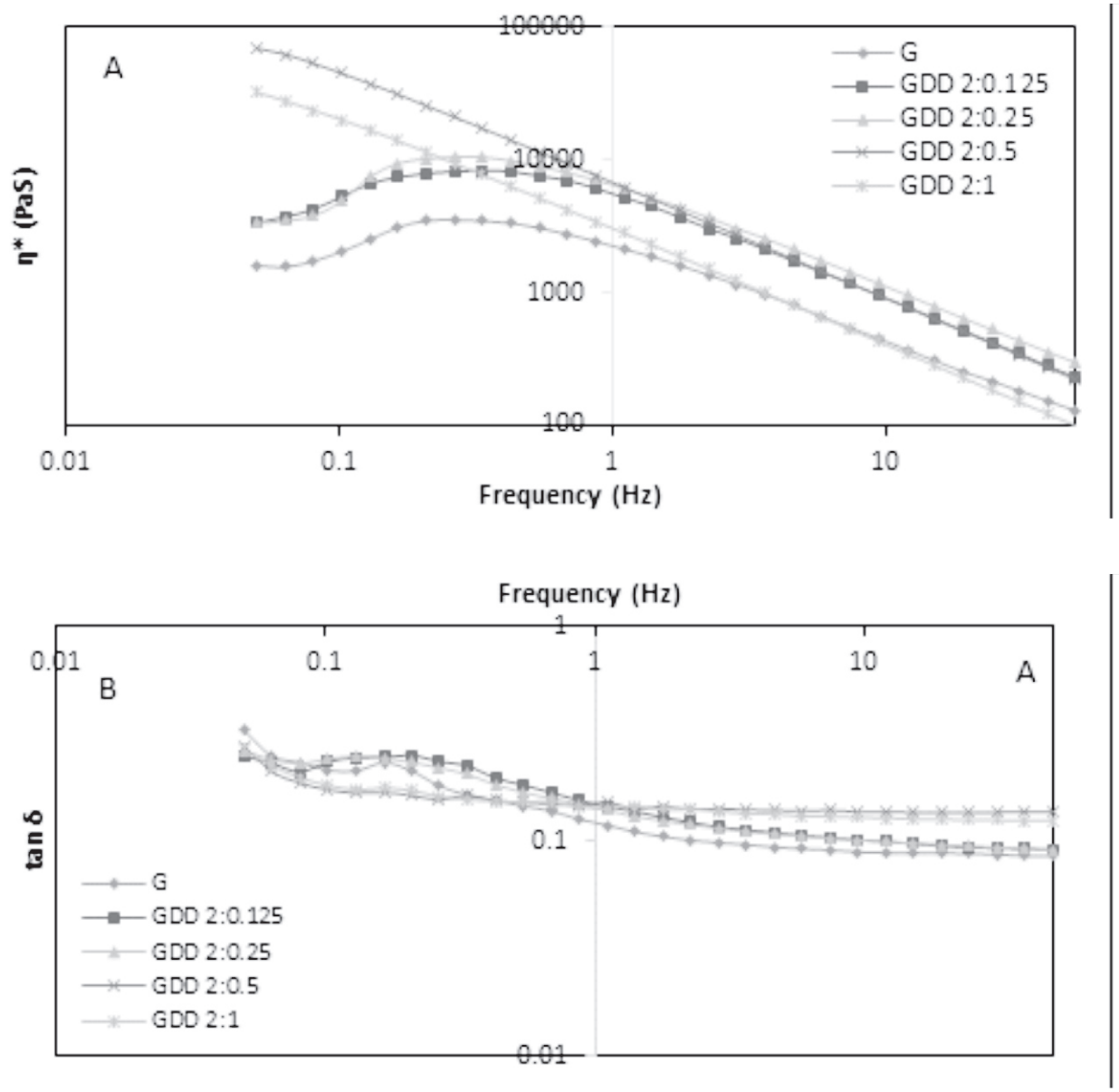

Figure 7 (A) Complex viscosity profiles across a frequency range of 0.01 to 50 Hz at 32 °C for plain G and GDD-PEC hydrogels and (B) Tan delta profiles across a frequency range of 0.01 to 50 Hz at 32°C for plain G and GDD-PEC hydrogels.

Each data point represents mean ± S.D (n = 4).

The tan δ frequency sweep profiles for all the formulations were below 1 shown in Figure 7B. Tan δ remained almost constant near 0.1 for all the plain GDD-PEC hydrogels. The flow behaviour of plain gellan G hydrogel exhibiting hysteresis is shown in Figure 8.

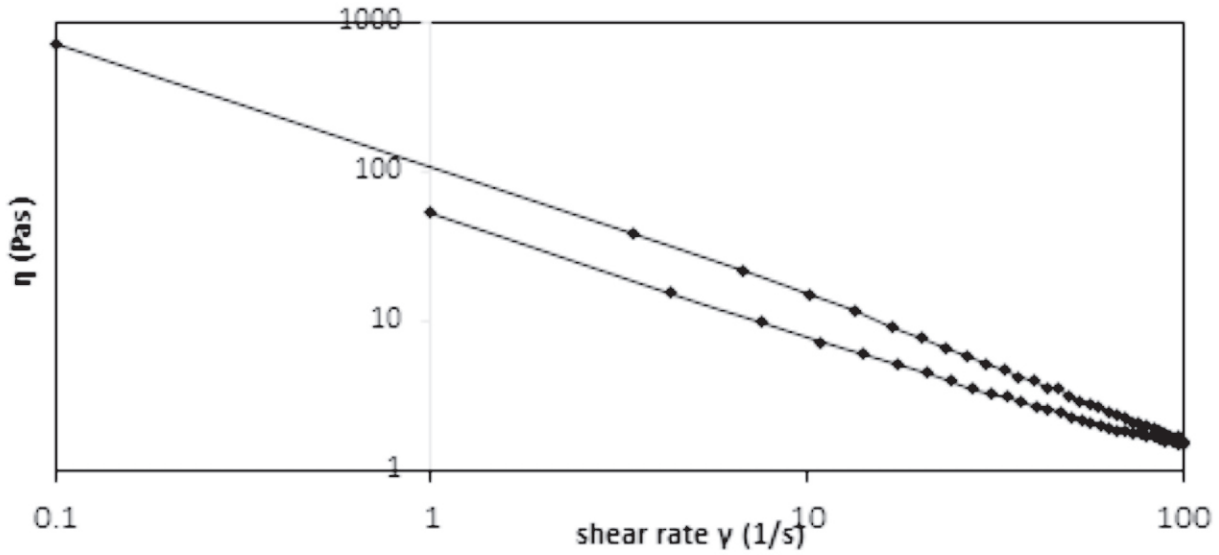

Figure 8 Viscosity curve against shear rate with hysteresis (thixotropy) for plain gellan G hydrogel.

Swelling kinetics

Plain gellan G hydrogel increased in swelling with increasing contact time in PBS pH 7.4 up to 0.47 times its dried weight without losing its physical integrity at the end of 48 h (Figure 9). The general trend is that the swelling increased with time and the swelling ratio of the gels reached equilibrium at 6 h. The swelling ratio ranged from 0.53 to 0.59 times its dried weight in PBS pH 7.4 at 37 °C for the GDD-PEC hydrogels. The addition of DEAE-Dextran to GDD-PEC hydrogels significantly increased ($p < 0.05$, $n = 4$) their swelling ratio when compared to the plain gellan G hydrogel.

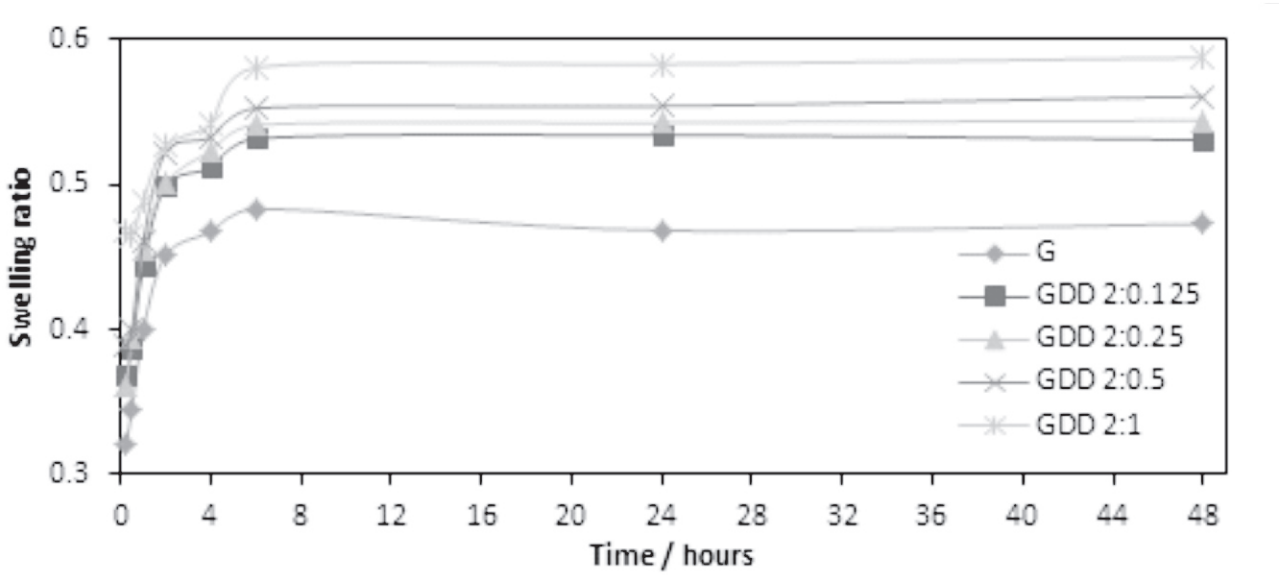

Figure 9 The swelling kinetics of G and GDD-PEC hydrogels. Each data point represents mean ± S.D (n = 4).

DISCUSSIONS

Polymer concentration has been reported to influence the helix content in gellan solutions with increased concentration forming stronger physical crosslinking.[16] Gellan gum has the ability to undergo physical gelation. It swells when dispersed in water and on application of heat up to 90 °C; forming a clear and transparent solution (sol). Subsequent decrease in temperature transformed the sol to gel, thus gellan gel was formed. When the temperature of the gellan solution is decreased, the gellan chains undergo a conformational thermo-reversible change from random coil to double helices.[17] The aggregation of the double helices forms a three dimensional network by hydrogen bonding with water. DEAE-Dextran solution, a positively charged polymer, was added to gellan solution (negatively charged polymer) at 90 °C to form polyelectrolyte complex GDD-PEC hydrogels of different mixing ratios. It was observed that after the addition of DEAE-Dextran, the GDD-PEC hydrogels were still formed in response to temperature decrease. The GDD-PEC hydrogels were free from grittiness important for any topical formulation.

Sudhamani and his co-workers have also described the FTIR peak assignments of gellan.[18] The peak at 3306 cm^{-1} is assigned to hydroxyl stretching. Gellan gum has hydroxyl and carboxyl groups in its structure capable of undergoing a nucleophilic substitution reaction with DEAE-Dextran as described for the methacrylation of gellan gum (GG-MA) with the most favourable reaction being with the carboxyl group.[19] The peaks at 1602 cm^{-1} and 1408 cm^{-1} are assigned to the characteristic absorption band of carboxyl group in gellan gum.[20] The peak at 1020 cm^{-1} is assigned to C-O stretching. Pure DEAE-Dextran showed N-H deformation vibration at 1641 cm^{-1}. This peak is assigned to the free N-H group present on DEAE-Dextran. The peaks at 2921 cm^{-1} and 3295 cm^{-1} are assigned to N-H stretching and C-H stretching vibrations. The peaks at 1342 cm^{-1} and 1007 cm^{-1} are assigned to methyne C-H bending and C-N stretching respectively. The shifts and disappearances in peak highlighted in the results suggest an interaction between gellan and DEAE-Dextran.

The DSC thermograms of hydrogels exhibited one endothermic melting peak for plain gellan hydrogel (G) at 114.2 °C and the GDD-PEC hydrogels ranged from 99.8 to 115.3 °C shown in Table 3 which indicated that the two polymers merged into one peak. There was a shift to lower values from the melting peak shown by gellan powder reference at 127.9 °C, DEAE-Dextran powder reference at 124.1 °C suggested the miscibility or compatibility of gellan and DEAE-Dextran.

The first weight loss below 150 °C in the TGA thermograms of gellan and DEAE-Dextran powders was attributed to loss of free or loosely bound water while the second weight loss was due to the decomposition of the polymers. The hydrogels lost their moisture content faster when compared to the individual polymer powders. It showed that the hydrogels' main constituent was water. The plain gellan G hydrogel was able to withstand more heat when compared to the GDD-PEC hydrogels.

Rheology is useful in the monitoring of gelation (crosslinking) and microstructural changes in a material allowing materials to be probed when 'at rest' conditions without disruption of the microstructure.[21] Rheological properties are used to describe the flow characteristics and textural behaviour of materials. The storage or elastic modulus is the measure of how structured a material is; and its ability to store energy. The loss or viscous modulus is the ability of the material to dissipate energy. The loss tangent is the ratio of energy lost to energy stored, in the cyclic deformation (the ratio of G"/ G'). When tan $\delta < 1$ (because G' > G") means a prevalent elastic behaviour and or gel state. The strain amplitude values were verified to ensure that all the rheological measurements were performed within the viscoelastic range, with the storage (G') and the loss (G") moduli being independent of the strain.[22] The LVR in Figure 5 suggested that a strain of 1% could be used for all gellan hydrogel formulations measurements. G' and G" are linear up to about 50% as evidenced by Chenite and co-workers. Beyond the linear viscoelastic (LVE) range, the G' and G" abruptly decreased indicating that a structural breakdown occurred as a consequence of large deformations imposed in line with Moura and co-workers.[23] The results showed that G'> G" for all samples in the LVE range exhibiting prevalence of the elastic behaviour over the viscous behaviour. The addition of DEAE-Dextran increased the elasticity of the hydrogels as the concentration of DEAE-Dextran increased which suggests the enhancement of their elasticity and a clear indication of the improvement on the three dimensional network stability. The formulations with lesser G' showed a less solid-like behaviour, hence are less elastic in viscoelastic character. A near plateau was achieved for all the hydrogels towards the end of the frequency range (50 Hz) suggesting a stable polymer network. Tan delta (δ) is the ratio of energy loss to energy stored which gives the overall viscoelasticity of the sample.[24] Therefore as tan δ becomes smaller, the

elasticity of the material increases, but the viscous behaviour is reduced.[25] Tan δ values for the hydrogels were below 1, indicating that all samples were gels with dominant elastic (solid like) behaviour.[26] Addition of DEAE-Dextran slightly increased the tan δ values of plain G and GDD-PEC hydrogels as the concentration of DEAE-Dextran increased. This indicated a more solid-like state resulting in a decrease in tan δ. Generally, frequency sweeps of the tested GDD-PEC hydrogels showed typical gel behaviour with G' greater than G" and tan δ well below 1.[27] The viscosity of G hydrogel decreased with increase in shear rate which indicated a pseudoplastic behaviour (shear thinning). The plain gellan G gel gave a flow like behaviour with increasing shear rate exhibiting hysteresis (thixotropy) on return, this suggests that the structures of the samples were broken and did not fully recover to the original state shown in Figure 8. The increase in shear rate permanently destroyed the sample structures. This was observed in all the batches including the reference gel formulations. The area between the curves is an indication of the extent of thixotropy. The larger the curve, the more thixotropic the sample is. Thixotropic materials lose their structure during shear, and rebuild it on standing. This behaviour is an important parameter in the ability of the gel to be easily applied to a surface (through structure breakdown in spreading) and then rebuild its structure and viscosity so that it does not drip and run.

The swelling behaviour of the hydrogels was assessed by immersing the hydrogels in PBS (pH 7.4) at 37 °C. Hydrogels are crosslinked macromolecular network with swelling capabilities in aqueous media or biological fluids. Due to the high water content of the hydrogel formulations and their ability to retain great quantities of water, the gels were initially weighed and dried to constant weight before starting the swelling tests. The swelling behavioural pattern showed a steady increase in swelling ratio in the GDD-PEC hydrogels as the concentration of DEAE-Dextran increased. Therefore, the swelling ratio was increased by the addition of DEAE-Dextran.

CONCLUSION

Novel polyelectrolyte complex hydrogels were prepared by ionic gelation method via in situ interaction between gellan gum and different concentrations of oppositely charged DEAE-Dextran. Our studies showed that the DSC thermograms exhibited a single melting peak suggesting an interaction between the polymers. This was confirmed by the FTIR spectra, with shifts and disappearances in peaks. The TGA of the hydrogels showed that the plain G hydrogel had higher decomposition temperature than the GDD-PEC hydrogels suggesting greater thermal stability. This correlates with the rheological findings which showed pseudoplastic behaviour in the GDD-PEC hydrogels formulated. Addition of DEAE-Dextran increased the swelling ratio of the GDD-PEC hydrogels. These novel binary GDD-PEC hydrogels has potential applications in the transdermal delivery systems.

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