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Synthesis, characterization and reversible transport of thermo-sensitive carboxyl methyl dextran/poly (*N*-isopropylacrylamide) hydrogel

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Abstracts

A thermo-sensitive hydrogel based on a copolymer of carboxyl methyl dextran (CM-dextran) and poly(*N*-isopropylacrylamide)-NH_2 (PNIPAAm-NH_2) has been synthesised using 1-Ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS). FTIR spectra supported the formation of gel network. The responsive properties of the hydrogels were characterized in terms of structural changes using SEM, and the effect of temperature on their balance of hydrophobicity using turbidity measurements. Composite pore filled membranes formed by impregnating glass fiber filters disc with the polymer mixture prior to gelation were used to determine the transport changes in response to temperature, using riboflavin (MW: 376), reactive red 120 (MW: 1470) and lysozyme (MW: 14300). Clear correlation was found between changes in morphology, turbidity and hydrogel transport as the temperature was increased from 24 to 37 °C. The transport profile of reactive red 120 through hydrogel composite membrane with thermal cycling shows the transport of reactive red 120 acted as reversible change in response to temperature.

Keywords: Thermo-sensitive hydrogel; Poly (*N*-isopropylacrylamide); Carboxyl methyl dextran

1. Introduction

Thermo-sensitive materials are attractive candidates as tools to solve biological problems such as bioseparation, drug delivery, tissue engineering, and protein folding [1]. One of the most intensively studied systems is based on poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels, which exhibit a temperature-induced volume phase transition in water upon heating above 32 °C [2]. PNIPAAm hydrogels are the most commonly studied temperature sensitive materials mainly because of their sharp phase transition, the proximity of their lower critical solution temperature (LCST) around 32 °C, and the ease with which the LCST can be adjusted by co-polymerization with other molecules [3–15].

Biodegradable polymers, either synthetic or natural polymers such as polysaccharides and proteins, have been extensively used and investigated for hydrogel preparation. Dextran is the most abundant and naturally occurring biodegradable polymer, it is relatively inert and non-toxic, and has been widely studied for biomedical applications [16–18]. Due to its biocompatibility and biodegradability, dextran and its derivatives have been used as blood substitutes and drug carriers [19,20]. A great deal of work has been carried out on the properties of dextran via various chemical modifications for specific applications, such as polymeric drug carriers and hydrogels [21,22].

To attain thermo-sensitive hydrogels, Zhang et al. [23] recently reported dextran–maleic anhydride (Dex–MA)/poly(*N*-isopropylacrylamide) hybrid hydrogels synthesized by UV photocrosslinking. The dextran-based precursor (Dex–MA) was prepared by substituting the hydroxyl groups in Dex for MA. They found that these hybrid hydrogels were responsive to the external changes in temperature as well as pH. The magnitude was found to depend on the relative composition of the two precursors. Another temperature sensitive dextran hydrogel reported by Kumashiro et al. [24] and Huh et al. [25] was based on the grafting of a temperature sensitive polymer (poly(NIPAAm-*co*-*N*,*N*-dimethylacrylamide) (*co*-polyNIPAAm-DMAAm))

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on a dextran precursor followed by chemical crosslinking of the grafted dextran precursor with 1,6-hexamethylenediamine crosslinker. The degradation rates of this hydrogel by dextranase were reduced as the grafted copolymer length increased.

PNIPAAm hydrogels are usually formed by the covalent crosslinking of PNIPAAm chains and another monomer, such as methylcellulose, with a commercial crosslinking agent, like *N*,*N*′-methylenebisacrylamide (MBAAm) [14,26]. Another type can be designed from an alloy composed of two independent interpenetrating polymer networks (IPNs), where one crosslinked network is intertwined with another, like gelatin, and chitosan [27,28]. However, considerable improvements in mechanical strength, and biodegradability are needed before these hydrogels using the traditional crosslink methods can be usefully employed in biomedical applications. Also some applications need hydrogel gelation in situ [29–31].

Over the past few years, different stimulus-responsive hydrogels were fabricated in our lab. Carbodiimide chemistry was used for cross-linking carboxy methyl dextran (CM-dextran) to prepare various kinds of hydrogels which can be responsive to either D-glucose or pH [32,33]. In this study, the thermo-sensitive hydrogel having CM-dextran as a backbone network was prepared using carbodiimide chemistry. Huh et al. [25] reported graft copolymers consisting of dextran as a main chain and poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (poly(NIPAAm-co-DMAAm)) as graft chains, but the resultant is conjugates and inter-crosslinking in CM-dextran was not indicated. The aim of this study is to attain a hybrid hydrogel of CM-dextran and PNIPAAm-NH_2. The hydrogel can be produced by intermolecular formation of ester bonds between the hydroxyl and carboxyl groups in the CM-dextran and also amide bonds from PNIPAAm-NH_2 with CM-dextran. The consequential hydrogel was characterized by Fourier transform infrared spectroscopy (FT-IR), NMR, SEM with cryofixation and cryofracturing techniques, phase transition studies, and reversible transport.

2. Experimental

2.1. Materials

Dextran and lysozyme were obtained from Sigma-Aldrich, UK. All other chemicals were of analytical grade and obtained from Lancaster Synthesis Ltd, UK.

2.2. Hydrogel synthesis

CM-dextran preparation is as described in previous reports [32,33]. The final COOH concentration in CM-dextran was then calculated by the means of acid titration. In this case, the ratio of COOH groups per dextran molecule is 1-COOH:65-glucose residues used for hydrogel.

N-Isopropylacrylamide (NIPAAm,) was purified by recrystallization from *n*-hexane. Poly(NIPAAm) with one terminal amino group was synthesized by free-radical polymerization as described in the literature [21,34,35]. The dried polymer was purified by precipitation in hot distilled water (>40 °C), with washing at this temperature prior to dissolving in distilled

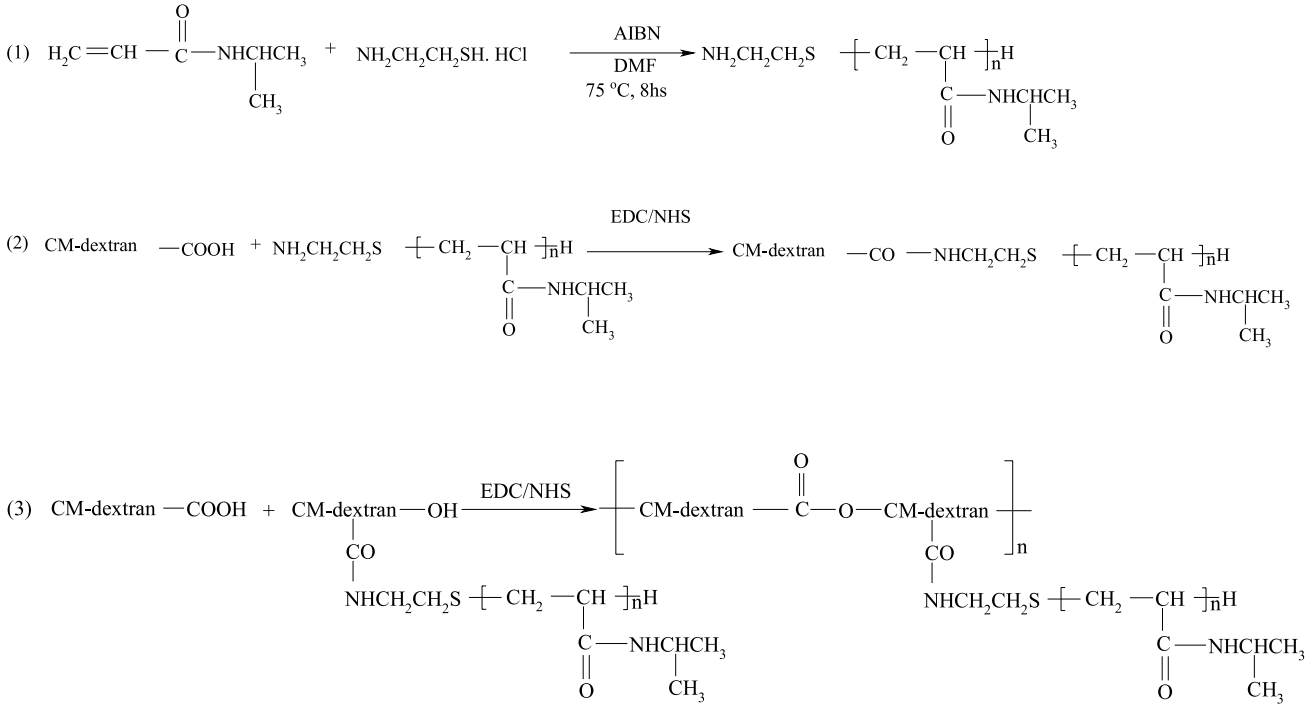


Fig. 1. Chemistry of CM-dextran-PNIPAAm hydrogel.

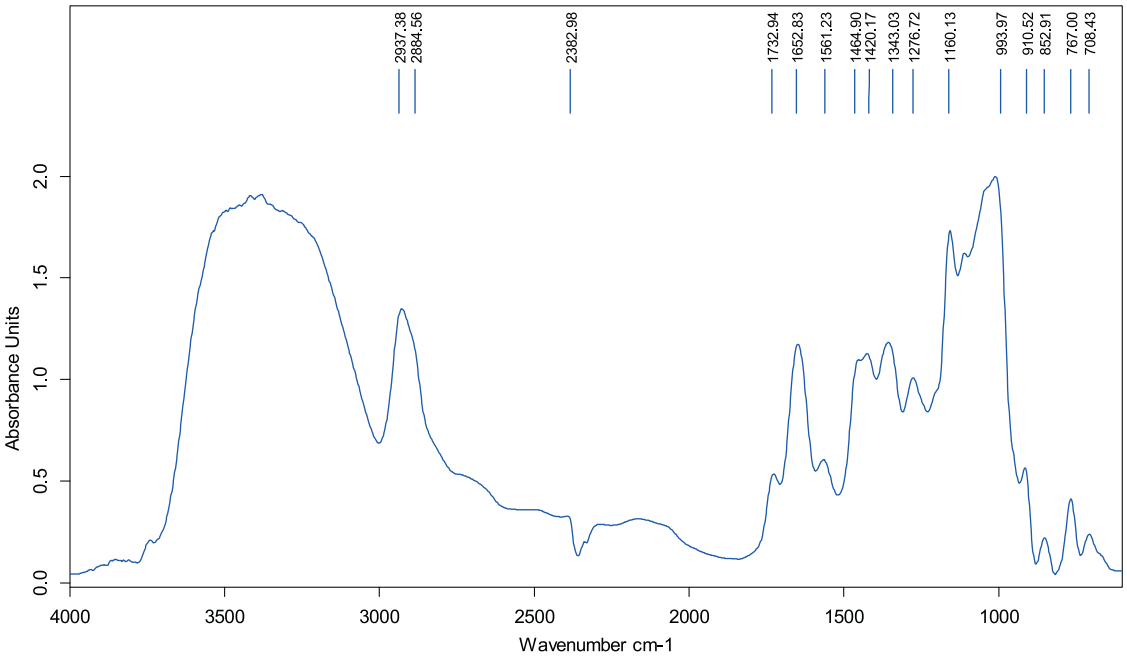


Fig. 2. FTIR spectra of CM-dextran/PNIPAAm hydrogel.

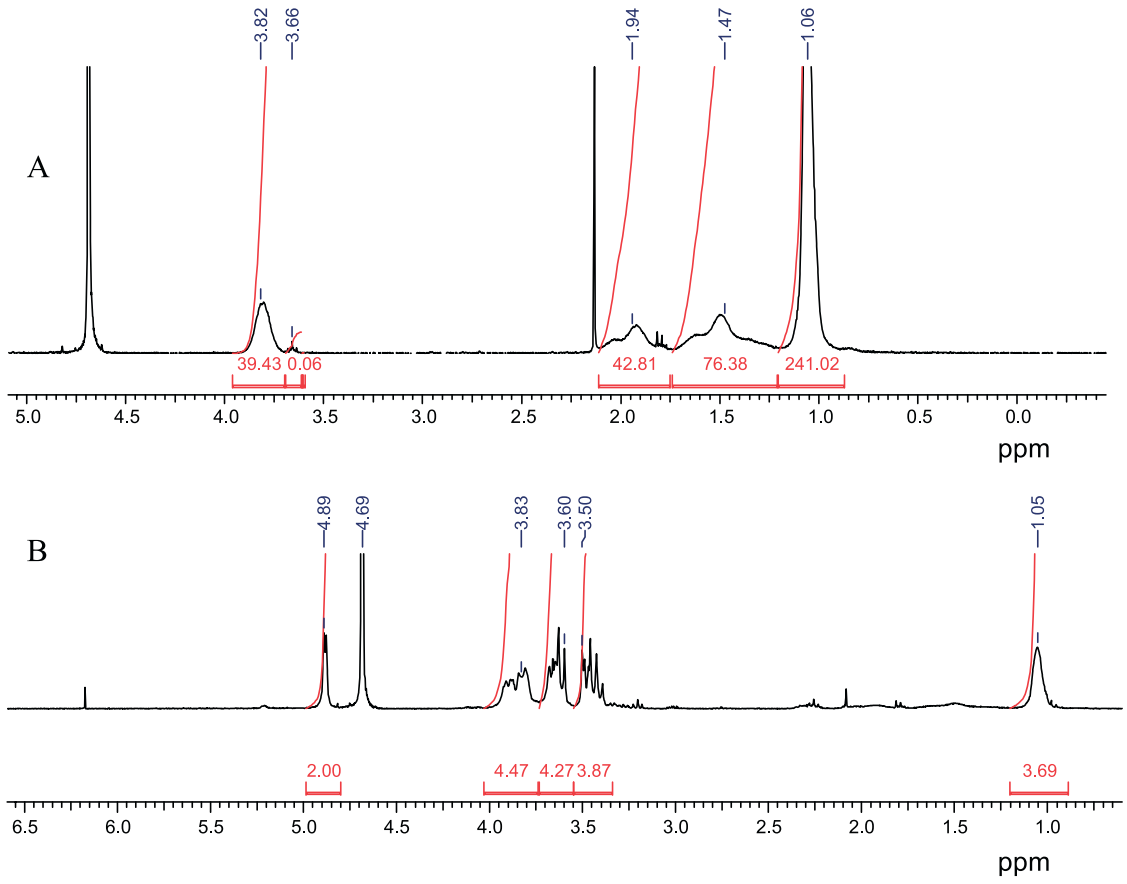


Fig. 3. ^{1}H NMR spectra of PNIPAAm (A) and CM-dextran/PNIPAAm hydrogel (B).

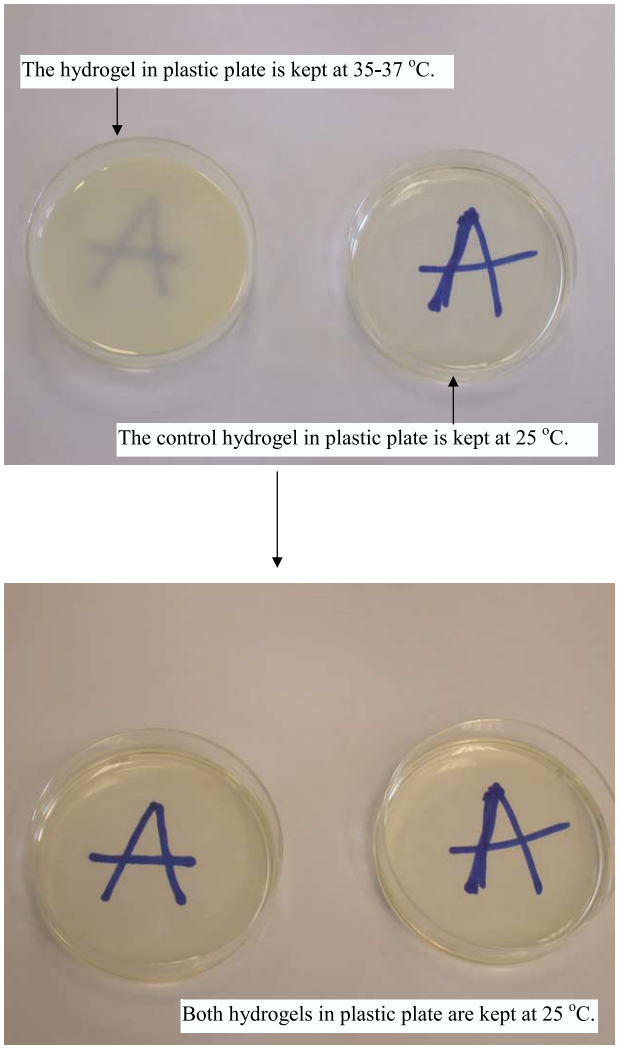
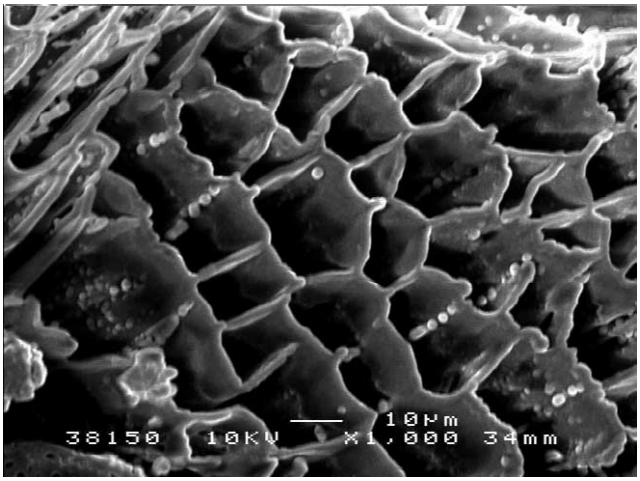
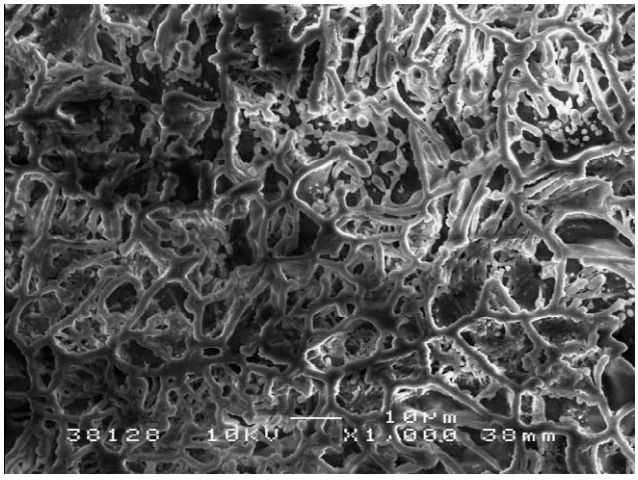


Fig. 4. Photographs of CM-dextran/PNIPAAm hydrogel at 25 and 37 °C.



(A) hydrogel in 25 °C



(B) hydrogel in 37 °C

Fig. 5. SEM pictures of hydrogel at 25 °C (top) and 37 °C (bottom).

water at 25 °C. The final polymer preparation was recovered from the solution by freeze-drying.

To fabricate the thermal-sensitive hydrogel at room temperature (about 25 °C), 1 g CM-dextran was dissolved in 4 ml of distilled water with stirring, the resulting solution was then degassed. Six hundred milligram 1-ethyl-(3-3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) and 100 mg *N*-hydroxysuccinimide (NHS) dissolved in 2 ml distilled water was added to the CM-dextran solution and stirred for 10 min. Then 500 mg PNIPAAm- NH_2 dissolved in 4 ml distilled water was poured into the reaction solution with continuous stirring for 20 min at which point it was cast and allowed to form a gel as required for subsequent analysis.

Infrared spectroscopy was performed on a Bruker-equinox 55 FT-IR spectrometer. Freeze-dried gel samples were mixed with potassium bromide powder and pressed into tablets under vacuum. For each sample 100 scans were recorded from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1}.

The ^1H NMR spectra of PNIPPAm-NH_2 was taken on a Bruker AVANCE 300 (300.13 and 75.47 MHz, respectively) spectrometer using D_2O as solvents. The composition of the grafted PNIPPAm in hydrogel was also estimated from the NMR, The hydrogel was dissolved in 0.1 mM NaOH and then freeze-dried prior to NMR analysis.

2.3. Hydrogel morphology

The morphology of the hydrogel without any support was examined using a Jeol 6310 SEM equipped with a cryo-stage and energy-dispersive X-ray (EDX). The hydrogel was incubated in 25 and 37 °C with 20 mM Tris buffer (pH 7.4) before a sample of the hydrogel was rapidly frozen in liquid nitrogen then introduced into the SEM-chamber pre-cooled to a temperature of ca. −160 °C. At this stage the sample was heated to a temperature of ca-80 °C to sublime

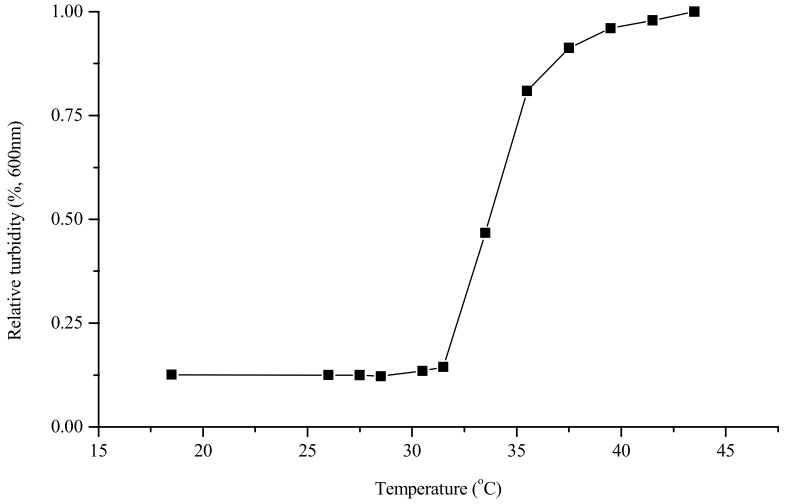


Fig. 6. Effect of temperatures (18–43 °C) on the absorbance of a CM-dextran/PNIPAAm hydrogel slab at 600 nm in 20 mM Tris buffer (pH 7.4).

the surface water. After cooling to −160 °C, the sample was gold sputtered for 3 min. The sample was scanned at a magnification of 1000×.

2.4. Phase transition studies

The phase transition behaviour of the synthesised hydrogels was studied by determining the optical transmittance of the system as a function of temperature; studies were conducted at 600 nm over the temperature range of 20–40 °C, using a UV-Visible spectrophotometer (Shimadzu 1601) with a hydrogel slab mounted in a 5 mm glass cuvette. The hydrogels in cuvette were placed in the water bath with controlled temperature for at least 30 min to reach equilibrium before the optical transmittance analysis. From the point of inflection of the turbidity curve, LCST of hydrogel can be determined.

2.5. Membrane transport experiments

Hydrogel composite membrane discs were prepared by cross-linking the CM-dextran poly(NIPAAm) hydrogel within the pores of sintered glass filter discs (Millipore, Catalogue number FDR-215-040G, particle retention 2.7 μm, thickness 0.98–1.04 mm, diameter 47 mm). Dry sintered glass filter membrane discs were submerged into the polymerisation mixture. The discs were left in the polymerisation mixture for 6 h. The discs were removed from the polymerisation mixture and placed in distilled water, which was replaced daily for one week to remove any unreacted compounds. Any hydrogel that formed on the surface of the discs was removed by gentle scraping. The dried hydrogel inside disc is about 260 mg in each disc.

Trans-membrane transport was investigated using riboflavin (0.05 mg/ml), Reactive Red 120 (2 mg/ml) and

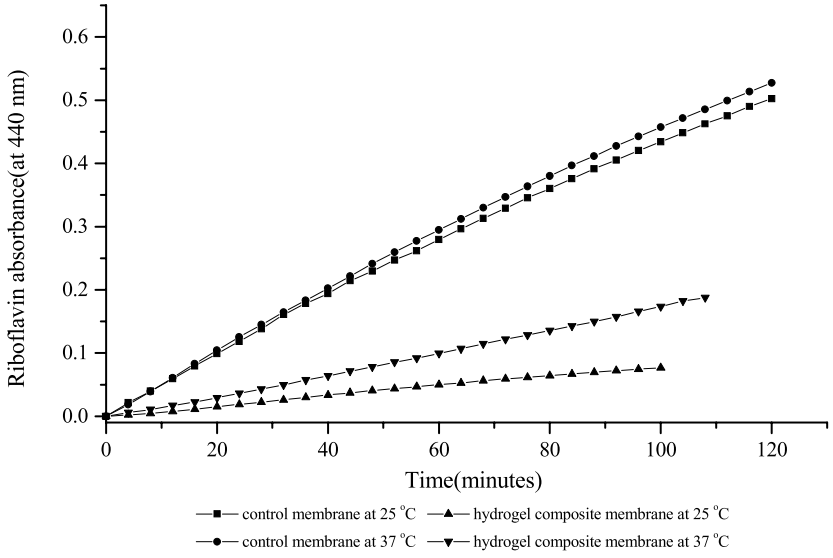


Fig. 7. Transport of riboflavin through the pore filled hydrogel membrane and a control unfilled glass filter membrane at 25 and 37 °C.

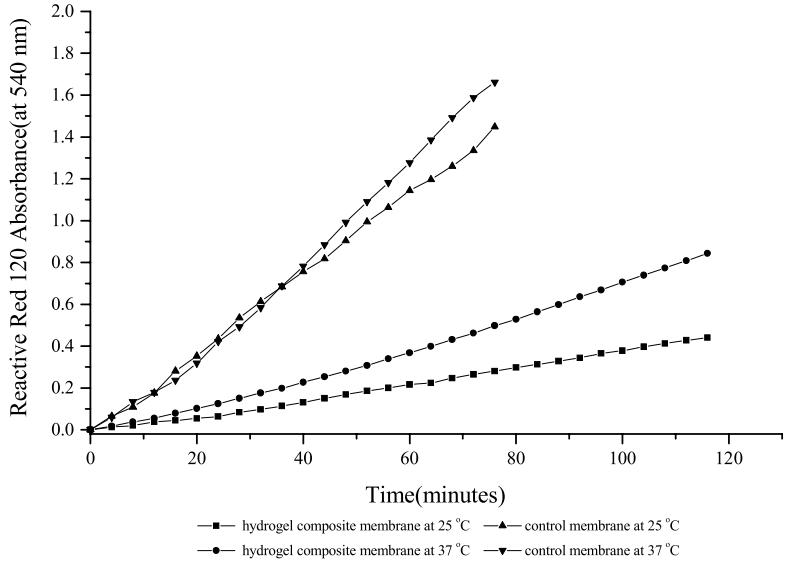


Fig. 8. Transport of reactive red 120 through the pore filled hydrogel membrane and a control unfilled glass filter membrane at 25 and 37 °C.

lysozyme (2 mg/ml) as test molecules and was studied using a diffusion cell consisting of donor and receptor chambers of equal volumes of 4.4 ml as described previous report [22]. Briefly hydrogel membranes with a surface area of 4.5–4.6 cm^2 were mounted between the two chambers, both chambers were filled with 20 mM Tris buffer (pH 7.4, I = 0.1 M). The donor chamber was connected to the test molecule's reservoir via a pump. The receptor chamber was connected to a UV–Visible spectrophotometer (Shimadzu 1601), to allow test molecule transport across the membrane to be automatically monitored and logged from optical density changes (riboflavin at 440 nm, Reactive Red 120 at 450 nm and lysozyme at 280 nm). The effect of temperature was investigated by varying the temperature of the water bath containing the diffusion cell and solute reservoir.

3. Results and discussion

To prepare the thermal-sensitive hydrogel, carbodiimide chemistry was employed to cross-link carboxylic hydroxyl with hydroxyl groups in CM-dextran and with amino group in PNIPAAm-NH_2. According to the expected cross-linking mechanism shown in Fig. 1, PNIPAAm-NH_2 was synthesized by free radical chain-transfer polymerization using 2-aminoethanethiol hydrochloride (AESH) as a chain transfer reagent and 2,2′-azobis-butyronitrile (AIBN) as the initiator. It was observed by Lu et al., [9] that the higher the concentration of AESH, the lower the molecular weight of PNIPAAm and that the concentration of initiator did not have a significant effect on the molecular weight of the PNIPAAm. Thus, the activated ester bond in CM-dextran can react with amino acid group on the PNIPAAm and is

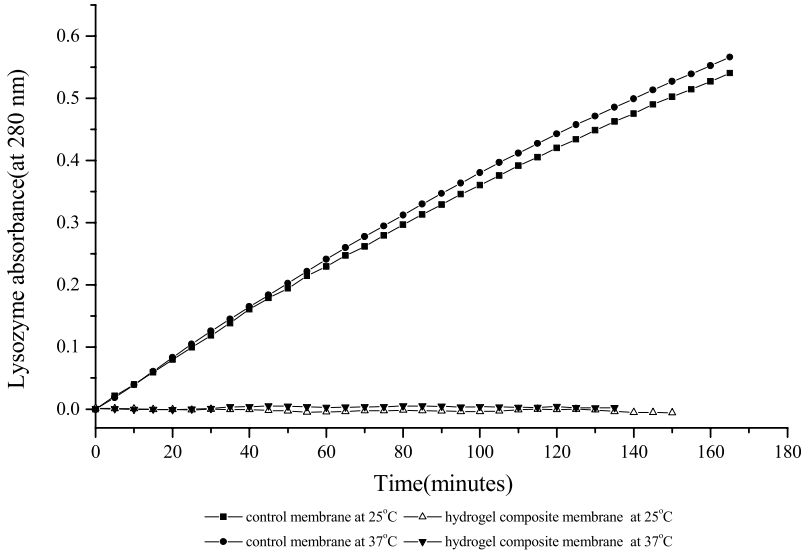


Fig. 9. Transport of lysozyme through the pore filled hydrogel membrane and a control unfilled glass filter membrane at 25 and 37 °C.

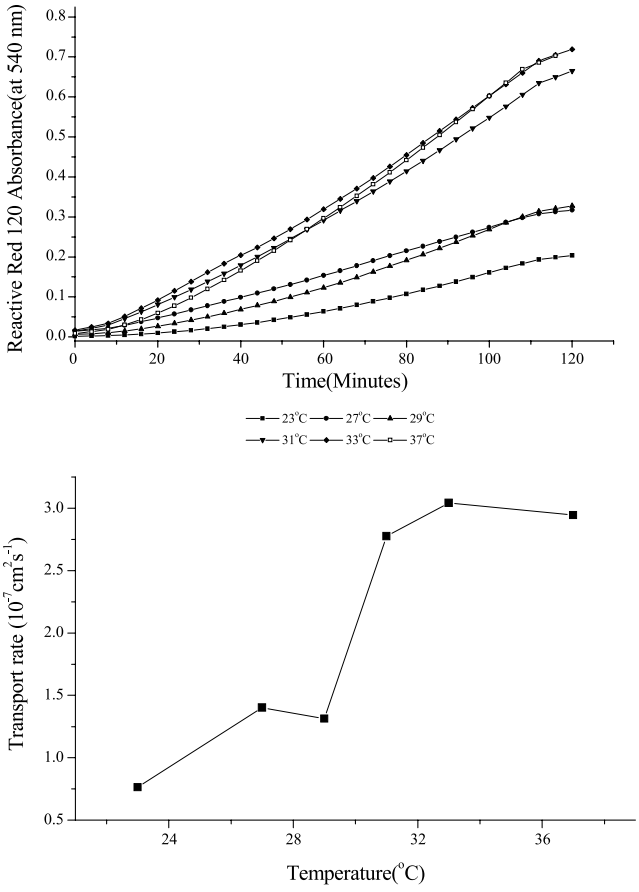


Fig. 10. Transport of reactive red 120 through the pore filled hydrogel membrane at different temperatures.

also intercrosslinked with hydroxyl group in CM-dextran in the presence of 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) [36,37].

The FT-IR absorption spectra of the CM-dextran/PNIPAAm hydrogel samples are shown in Fig. 2. The IR spectra show a broad band in the range of 3700–3100 cm^{-1} resulting from O–H (from CM-dextran) and N–H (from PNIPAAm) stretching vibrations. The typical amide I and II bands in PNIPAAm are confirmed at 1652 and 1561 cm^{-1}. The successful incorporation of the COOH group into dextran is demonstrated by the presence of a carboxylic FT-IR band from the carbonyl (C=O) stretching of CM-dextran at 1733 cm^{-1}.

The number-average molar masse of PNIPAAm was estimated to be 5156 from its NMR, methylene group (δ 3.66 ppm, 2H) and the methyl groups (δ 1.06 ppm, 6H per unit) as seen in Fig. 3. The composition of the grafted PNIPAAm in hydrogel was also estimated from its NMR by comparing the methyl groups (δ 1.05 ppm, 240 H per PNIPAAm) and the C^1-protons of the Dextran main chain (δ 4.88 ppm, 2H per unit). The molecular weight of PNIPAAm (5156) and Dextran (500,000) was used in the calculation. There are 20 PNIPAAm units at each dextran. So the conversion of the grafting PNIPAAm is about 45% and the composition ratio of PNIPAAm in the final hydrogel is 17%(W/W) by calculating the NMR results of hydrogel. Base on the results of swollen ratio experiment [38], this hydrogel has a cross-linking density of 1.97×10^{-5} mol/cm^3.

LCST is a critical temperature at which polymer solution undergoes phase transition from soluble to insoluble state when the temperature is raised. It has been widely reported that the lower critical solution temperature (LCST) of PNIPAAm is about 32 °C, individual PNIPAAm chains grafted in hydrogel collapse prior to aggregation, increasing the scattering of light in the solution and causing cloudiness [39]. The cloud point is followed by the appearance of two phases; one is composed of collapsed gel that has expelled most of its associated water and the other is the water itself. Thus, this hydrogel undergoes a phase transition from a hydrophilic to a hydrophobic structure. It was found to be transparent at 25 °C and cloudy at 37 °C, turbidity can be reversed by simply varying the temperature in Fig. 4.

SEM pictures show the structures of CM-dextran/PNIPPAm hydrogel hydrate to form expanded structures in buffer at 25 °C, while at 37 °C they become dehydrated forming compact structures, as seen in Fig. 5.

The LCST of the hydrogel was determined by turbidity measurements. LCST determination was performed by UV/Vis spectro-photometer (Shimadzu 1601). A cuvette packed with one slab of hydrogel was placed in a water bath where the temperature was raised from 20 to 40 °C and the absorbance at 600 nm was measured. The results in Fig. 6 illustrate the LCST of this hydrogel is 33 °C in comparison with PNIPAAm oligomer which exists between 31 and 32 °C, and matches the LCST of a poly(*N*-isopropylacrylamide)-dextran derivative conjugate reported by Anastase-Ravion et al., [21].

Hydrogel transport was quantified using composite membranes with hydrogel synthesised in situ within the pores of a sintered glass filter to provide a robust membrane for use in transport studies. The hydrogel within the pores of the filter membrane, which appeared swollen below its 33 °C, acts as a barrier for molecular transport. In contrast, the shrunken hydrogel in fixed pores of the filter membrane enhances the transport of test molecules.

Figs. 7–9 show results of the trans-membrane transport of riboflavin (0.05 mg/ml) Reactive Red 120(2 mg/ml), and lysozyme (2 mg/ml) at 25 and 35 °C through the pore filled hydrogel membrane and a control unfilled glass filter membrane. The transport of test molecules through a control unfilled glass filter membrane showed no effect of temperature on trans-membrane flux. However, riboflavin and reactive red 120 transport faster at 37 °C than at 25 °C. Unlike the small molecules, in the case of the pore filled hydrogel membrane lysozyme is completely rejected at 25 and 37 °C possible because lysozyme (pI = 11) positively charged can be absorbed by CM-dextran with extra COO^- at pH 7.4, thus the resultant can block the pore of membrane.

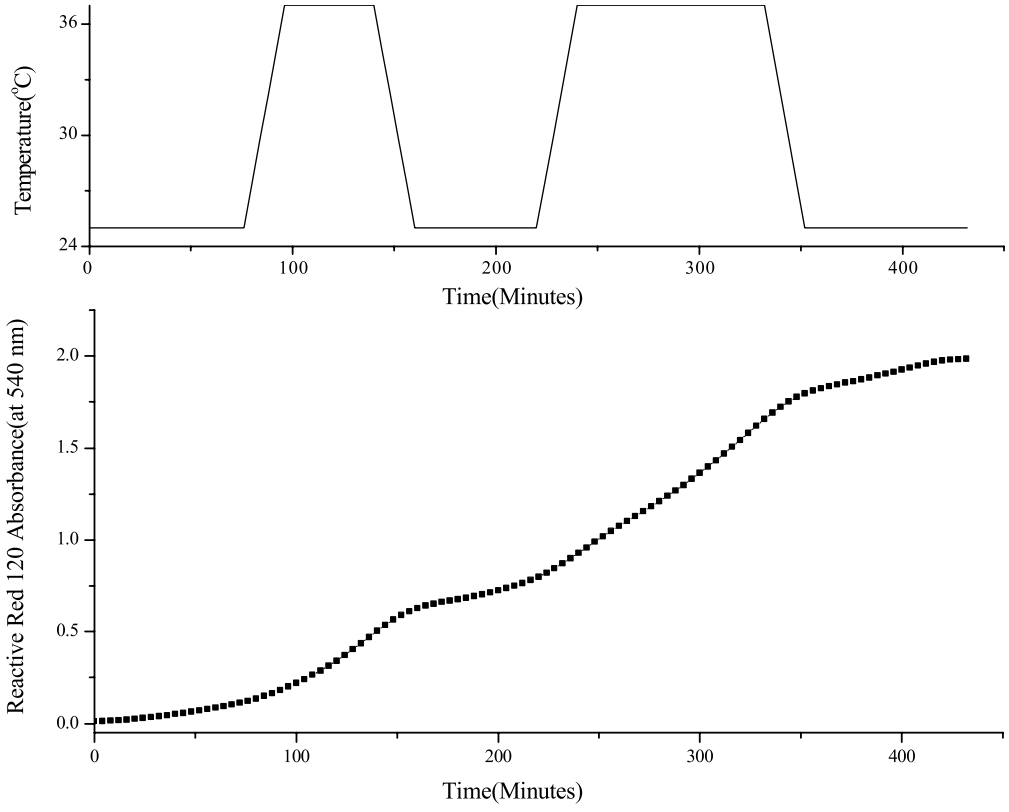


Fig. 11. Transport of reactive red 120 through the pore filled hydrogel membrane with thermal cycling.

The transport of reactive red 120 was tested at various temperatures as shown in Fig. 10. The results demonstrate that temperatures below the 32 °C have a negligible effect on the transport of tested molecules, while considerable transport is found above 32 °C. Obviously, the PNIPAAm phase change plays a key role in the molecular reversible transport in response to external temperature.

Fig. 11 shows the transport profile of reactive red 120 through the pore filled hydrogel membrane with thermal cycling. The figure also shows the profile of the thermal cycling sequence. These transport profiles clearly showed that the transport of reactive red 120 was reversible in response to temperature.

The CM-dextran/PNIPAAm hydrogel studied is simple and gelation in situ in contrast to the report by Zhang et al. [23] or Huh et al. [25]. In contrast to conventional cross-linking agents EDC/NHS does not chemically bind to the networks of the hydrogel [36,37], and a water-soluble urea derivative produced has a far lower cytotoxity and is easily washed out. The in situ fabrication of this thermo-sensitive hydrogel will have an advantage in biosensor or micro-fludics applications [40].

4. Conclusion

A simple protocol is reported the preparation of a CM-dextran/PNIPAAm hybrid hydrogel using EDC and NHS. The method is the first step toward the development of thermo-sensitive hydrogels acted as reversible transport of molecule.

Acknowledgements

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