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Swelling and protein absorption/desorption of thermo-sensitive lactitol-based polyether polyol hydrogels

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Abstract

A series of thermo-sensitive hydrogels has been prepared from reactions of acylated poly(ethylene glycol) bis(carboxymethyl) ether (PEGBCOCl) ($M_n = 600$ dalton) with lactitol-based polyether polyols (LPEPs). These LPEP hydrogels swelled extensively in water at neutral pH and their swelling behaviors depended strongly on the PEGBCOCl:LPEP molar ratios or extent of PEGBCOCl crosslinking. A maximum swelling of 81 fold was observed on hydrogels formed with LPEP ($M_n = 4055$ dalton) at a PEGBCOCl:LPEP molar ratio of 4.25. At temperatures above 25°C, the hydrogels exhibited a phase transition and collapsed, expelling water. These super-absorbent hydrogels were stable under acidic conditions, but were sensitive to base hydrolysis. Enzyme proteins, i.e. lipase, were incorporated in the hydrogels (0.4–1.2 mg/g) by immersing the collapsed hydrogels in the protein solutions at 25°C. Protein desorption at 40°C occurred rapidly with over 90% of protein released during the first hour. The extent of protein desorption was similar among hydrogels with varying levels of absorbed proteins. The release of the lipase protein molecules is due to the structural collapse of the hydrogels and is not diffusion controlled.

Keywords: Thermo-sensitive hydrogels; Swelling behavior; Protein absorption

1. Introduction

Hydrogels are three-dimensional polymer networks, which swell significantly on contact with water. Most hydrogels are formed by copolymerization of vinyl monomers containing hydrophilic side groups with multifunctional vinyl monomers or by crosslinking reactive functional groups of hydrophilic polymers. By manipulating the chemistry of the hydrophilic segments in the polymers and the degree of crosslinking, hydrogels may be tailored to exhibit specific properties. Among these, stimuli-sensitive hydrogels have generated extensive interest. Stimuli-sensitive hydrogels display phase change or abrupt change in volume in response to the changes in the environmental (temperature, pH, solvent, etc.). The systems most studied include poly(*N*-isopropylacrylamide), poly(ethylene oxide) (PEO or PEG), poly(propylene oxide) (PPO), poly(acrylamide) (PAAm), and cellulose derivatives [1–8,14]. These materials have been reported to be very useful in applications such as controlled release of drug, controlled immobilization, immobilized catalysis, bioseparations, biosensors, and artificial tissues [7–13,15–20]. Thermal responsiveness has been engineered by varying the hydrophilic and hydrophobic contents within the network [21,22]. Incorporation of ionic groups induces pH sensitivity [22]. Furthermore, use of poly(ethylene) glycol (PEG) or its derivatives in the gel structure has been shown to reduce protein absorption and cell adhesion, producing a biocompatible material [15,23,24].

We have previously reported on the formation of thermo-sensitive polymer hydrogels by reacting acylated poly(ethylene glycol) bis(carboxymethyl) ether (PEGBCOCl) with lactitol-based polyether polyol (LPEP) [25]. The LPEPs were generated from propoxylation of lactitol. The molecular weights of these LPEPs were between 1337 and 4055 dalton by varying the propylene oxide to lactitol (PO/OH) ratios. The resulting hydrogels showed wide ranging swelling behaviors in water, absorbing up to 10 times their dry mass. The swelling behavior depends closely on the LPEP molecular weight and the extents of the

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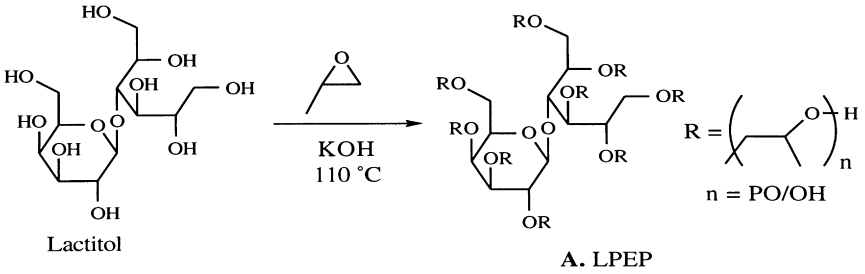
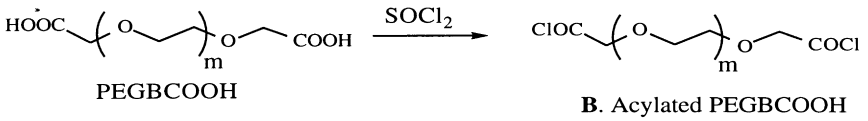


Lactitol

A. LPEP

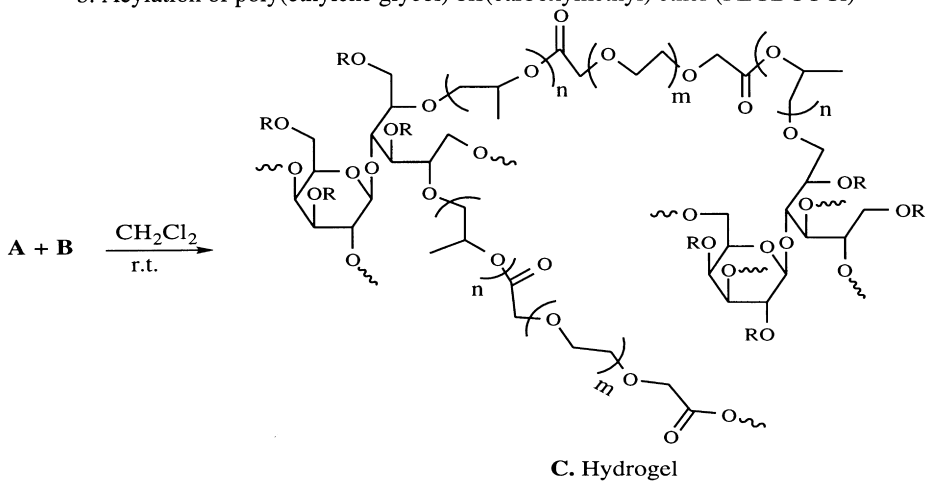
a. Synthesis of lactitol-based poly(ether polyol) (LPEP) by propoxylation of lactitol



PEGBCOOH

B. Acylated PEGBCOOH

b. Acylation of poly(ethylene glycol) bis(carboxymethyl) ether (PEGBCOCl)



C. Hydrogel

c. Formation of hydrogel

Scheme 1.

PEGBCOCl crosslinker. These LPEP-based hydrogels are in the fully swollen state at temperatures below 25°C and collapse above 30°C. This very distinct temperature sensitivity is related to the phase transition which collapses the PPO and PEO blocks between the lactitol moieties, expelling water from the gel network at temperatures between 25 and 30°C.

The aim of this study is to synthesize thermo-sensitive hydrogels with higher degrees of swelling. This can be done by varying the ratio between acylated PEGBCOCl and LPEP and the molecular weight of the LPEP. The swelling properties of these hydrogels, their responses to temperature and pH, and their ability to incorporate and expel enzyme protein molecules are examined.

2. Experimental

Methylene chloride (Fisher) was distilled over CaH_2. Poly(ethyleneglycol) biscarboxymethyl ether (PEGCOOH; $M_n = 600$ dalton; Aldrich), lipase porcine pancreas (ICN Biochemical, Inc.; 9001-62-1), methanol (Fisher), fluorescamine (Aldrich), sodium hydroxide (Fisher), and concentrated hydrochloric acid (Fisher) were used without purification.

Synthesis and characterization of the LPEP and acylated PEGCOOH have been described in our earlier paper [13]. Briefly, LPEP was prepared by reacting 150 g lactitol dihydrate with varying amount of anhydrous PO under constant stirring (800 rpm) in a 1 l high-pressure vessel (Autoclave Engineering, Erie, PA) (Scheme 1a). Water in lactitol dihydrate was treated as a di-functional initiator. Therefore, a PO/OH ratio of 1 was achieved with 13 equivalents of PO. Reactions were run at 110°C with constant stirring (800 rpm) and terminated when pressure had dropped and stabilized. The crude LPEP was dissolved in deionized water, neutralized with Amberlite IR-120 (H^+), filtered, concentrated, and then azeotroped with benzene to remove water. LPEP was dried at room temperature under high vacuum for 24 h.

The M_n and the average number of PO repeating units in each branch (i.e. PO/OH) of LPEP were determined via ^1H NMR (GE-300) of trifluoroacetyl (TFA) derivatives of LPEP according to ASTM 4273-83. Assuming that every

hydroxyl of lactitol acquires PO moieties according to this ratio, the number average molecular weight (M_n) is calculated as:

$$M_n = 344.32 + 9\,(PO/OH)\,58.08 \quad (1)$$

PEGBCOCl was synthesized by acylation of PEGBCOOH (4 g) with $SOCl_2$ (3 ml) at room temperature under constant stirring for 24 h (Scheme 1b). The excess $SOCl_2$ was removed by vacuum evaporation at 40°C for 8 h.

Hydrogels were prepared from crosslinking LPEP with PEGBCOCl (Scheme 1c). The PEGBCOCl:LPEP molar ratios were varied between 3 and 6. CH_2Cl_2 was added to PEGBCOCl to decrease its viscosity; and the PEGBCOCl solution was poured into a CH_2Cl_2 solution of LPEP, stirred, and allowed to crosslink for 24 h in a desiccator. The hydrogel was cut into $1 \times 1 \times 0.4$ cm^3 pieces, weighing approximately 1.5 g per piece. These hydrogel pieces were washed with CH_3OH to extract CH_2Cl_2, neutralized with 0.1 N NaOH, and finally washed with deionized water until neutral. Hydrogels were immersed in a water bath held at a designated temperature for 4 h, blotted dry with tissue paper, and weighed to obtain the wet weight (W_w). The hydrogel was then dried under vacuum at 100°C for 48 h to obtain the dry weight (W_d). The swelling degree (SD, %), was calculated as

$$SD = (W_w - W_d)/W_d \times 100 \quad (2)$$

For studying the effect of pH on swelling, the hydrogels were first collapsed in 50°C water and immersed in 25°C deionized water at a specific pH. Aqueous solutions, with pH ranging from 2 to 11, were obtained using proper amounts of either NaOH or H_2SO_4. The ionic strength was kept constant by adding NaCl. After immersions in the solutions of varying pHs, the samples were blotted dry, weighed (W_w), and weighed again after drying under vacuum at 100°C (W_d).

The cyclic swelling was observed in response to low-and-high temperature cycles. Samples were repeatedly collapsed in an elevated temperature water bath for 4 h and allowed to reswell at 25°C for an additional 4 h. The reswollen hydrogels were then blotted dry and weighed after each cycle. Thermal behavior of the hydrogels was studied using in nitrogen from −20 to 12°C at 2°C/min.

The lipase porcine pancreas (ICN Biochemical, 9001-62-1) was used to study the absorption and desorption of protein molecules in these hydrogels. Fully hydrated hydrogel pieces ($0.75 \times 0.75 \times 0.4$ cm), weighing approximately 1 g per piece, were collapsed via immersion in a 50°C water bath for 4 h. The collapsed hydrogels were then placed in 10 ml lipase solution at 25°C for 4 h to induce swelling and absorption of the lipase protein. The final concentrations of the lipase solutions, along with the swollen hydrogel weights, were used to calculate the amount of enzyme absorbed. The lipase-incorporated swollen samples were then placed in 10 ml of 50°C water for 4 h to induce the collapse of the hydrogels and release of the protein. A desorption profile was obtained by monitoring the lipase concentration of the desorption bath at specific time intervals.

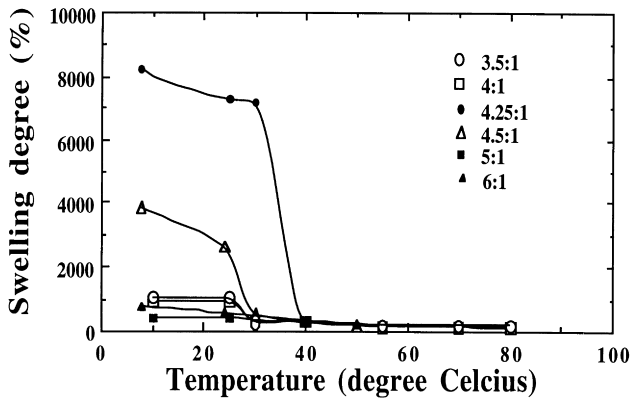


Fig. 1. Relationship of swelling degree vs. temperature for PEGBCOCl:LPEP ratios ranging from 3.5 to 6.0 (polyol $M_n = 4055$ dalton).

A fluoremetric assay for the detection of lipase was employed to study absorption and desorption of the hydrogel [26]. This assay measures the fluorescent product of fluorescamine and the free amino groups of the lipase proteins. Calibration was done using known amounts of lipase in 2 ml aqueous solutions buffered with sodium phosphate at pH between 8 and 9, the basicity necessary for the production of fluorescent products. While vortexing, 1 ml of a 3/10 v/v fluorescamine/dioxane solution was quickly added via a syringe. Fluorescence measurement (Perkin Elder LS 50B Luminescence Spectrometer) was taken 60 s after the addition using excitation and emission wavelengths of 390 and 475 nm, respectively.

3. Results and discussion

Ring opening polymerization of PO initiated by the hydroxyl groups of lactitol gives LPEP containing 9 PPO branches (Scheme 1a). The number average molecular weight (M_n) of LPEP in the branches was determined by ^{1}H NMR to be 1337 to 4055 dalton. These M_n translates to an average of 2.3–7.1 PPO units per branch. The two acyl chloride groups of PEGBCOCl offer high reactivity towards the OH groups of the LPEP to form ester linked three-dimensional crosslinked structures (Scheme 1c). These gel networks consist of lactitol moieties connected with PPO and PEO blocks. The crosslinks between the lactitol moieties are fixed in length, i.e. PPO-b-PEO-b-PPO block is the summation of the 11 ethylene oxide (EO) units plus the 7 PO units in each of the LPEP. The degree of crosslinking is controlled by varying the PEGBCOCl/LPEP molar ratio. When the PEGBCOCl/LPEP ratio was 3 or less, the hydrogels formed were soft and difficult to handle. Therefore, higher PEGBCOCl/LPEP molar ratios in the range of 3.5–6 were employed.

These hydrogels swelled at temperatures at and below

25°C and collapsed at and above 40°C (Fig. 1). The swelling measured at 25°C showed the highest swelling at 8100% on hydrogels formed at 4.25 PEGBCOCl/LPEP ratio. A 4.5 PEGBCOCl/LPEP ratio gave the next highest SD of 2600%, while all others fell to 1000% and below. The hydrogels exhibiting the highest SDs were synthesized from very close to equal molar ratio of the COCl/OH functional groups. The 4.25 PEGBCOCl/LPEP ratio is equivalent to 8.5/9 COCl/OH ratio and 4.25 PEGBCOCl/LPEP ratio gives equal COCl/OH ratio. The swelling degrees of hydrogels formed at 5 and 6 PEGBCOCl/LPEP ratios, on the other hand, were much lower than those at 3.5 and 4 PEGBCOCl/LPEP ratios. These higher PEGBCOCl/LPEP ratios represent excess of the crosslinker than the polyol, i.e. 10/9 and 12/9 COCl:OH ratios. The hydrogels produced from lower PEGBCOCl/LPEP ratios of 3.5 and 4 swell slightly better than those with higher PEGBCOCl/LPEP ratios of 5 and 6. The swelling behavior seems to be better for those hydrogels with slightly excess hydroxyl groups (LPEP) than acyl groups (PEGBCOCl).

It is apparent that the integrity of the hydrogels depends on the level of crosslinking. As stated earlier, the hydrogels are strong enough to be handled when formed at PEGBCOCl/LPEP ratios of 3.5 and above. However, when the crosslinking exceeds a certain level, in this case, a PEGBCOCl/LPEP of 5, the highly crosslinked gel network is too dense to hold a significant amount of water molecules. Since the hydrophilic contents in the hydrogels with PEGBCOCl:LPEP ratios between 4.0 and 4.25 are not that different, the sharp increased SD in the hydrogel with a 4.25 PEGBCOCl:LPEP ratio may reflect a significant change in the network structures of the hydrogels. The pore structure or morphology of the hydrogels is being further studied in this laboratory.

At 40°C, the swelling degrees of all hydrogels fell to the vicinity of 250–300%. The volume transition with increasing temperature from 25 to 40°C was quite dramatic, particularly for the one with a 4.25 PEGBCOCl:LPEP ratio. Between 30 and 40°C, the hydrogels with a 4.25 ratio expelled approximately 80 times their own weights of water. While translucent in the swollen state, the hydrogels became opaque when collapsed, evident of a phase transition. The swelling of all other hydrogels reduced significantly at between 25 and 30°C, lower temperature range than the one with a 4.25 PEGBCOCl:LPEP ratio. Temperature sensitivity of the hydrogels with 4.25 and 4.5 PEGBCOCl/LPEP ratios is higher than the hydrogels formed at other ratios. The varying swelling degrees and levels of temperature sensitivities of these hydrogels show potential for thermal sensor applications.

Exposing the hydrogel with a 4.25 PEGBCOCl:LPEP ratio through swelling/collapse cycles demonstrates that the volume-phase transition is reversible (Fig. 2a). The hydrogels exhibited a slight increase in SD and expelled slightly less water upon collapsing with each additional cycle. Hydrogels lost their cubic shape and became

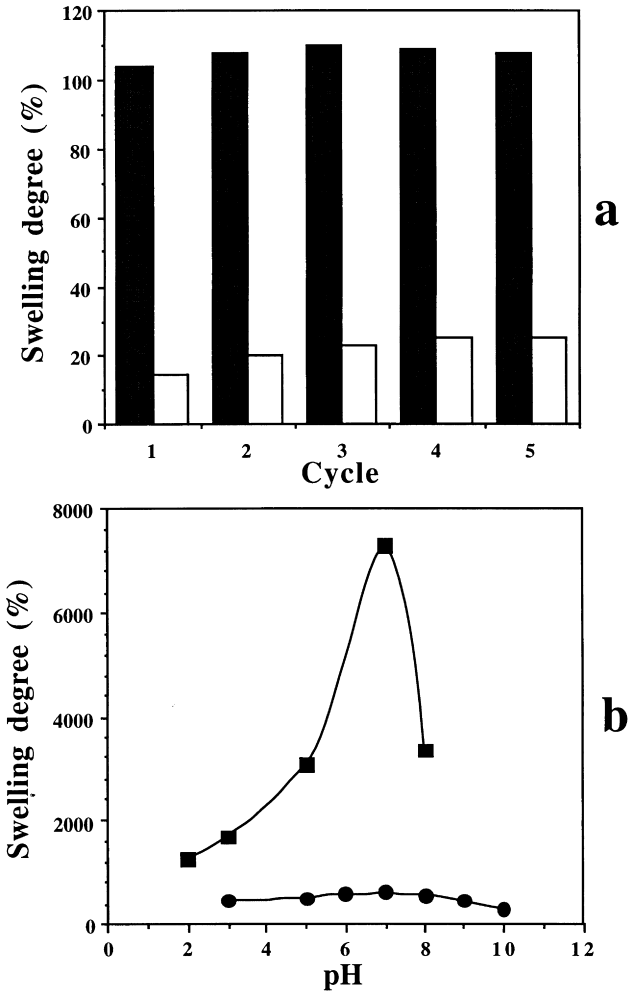


Fig. 2. (a) Effect of cyclic swelling/collapsation on the degree of swelling of the hydrogels with polyol $M_n = 1337$ dalton and a 4.25 PEGBCOCl:LPEP ratio (after equilibration at 25°C water for 4 h: ■ swollen state; □ collapsed state. (b) Effect of pH on swelling degree of hydrogels at a 4.25 PEGBCOCL:LPEP ratio: ■ polyol $M_n = 4055$ dalton; ● polyol $M_n = 1670$ dalton.

globular. We attribute this to the high degree of network mobility during the collapse-swelling cycles; the structure gradually opened up, allowing the matrix to imbibe more water. Eventually, after five cycles, the hydrogels broke into several pieces with no apparent mass loss. The disintegration of these hydrogels is possible when the hydrogels contain a low level of physical entanglement, which could be lost from repeated stress cycles.

We have also observed that hydrogels with lower SDs tended to exhibit better stability in water. For instance, a hydrogel, which exhibited a SD of 600% at room temperature and a SD of 75% at 50°C, did not change their physical forms even after five cycles. It appears that the accessibility of water into the gel network not only determines the SD but also influences the physical integrity of the gel. The results thus far show that the less water-absorbing LPEP-based

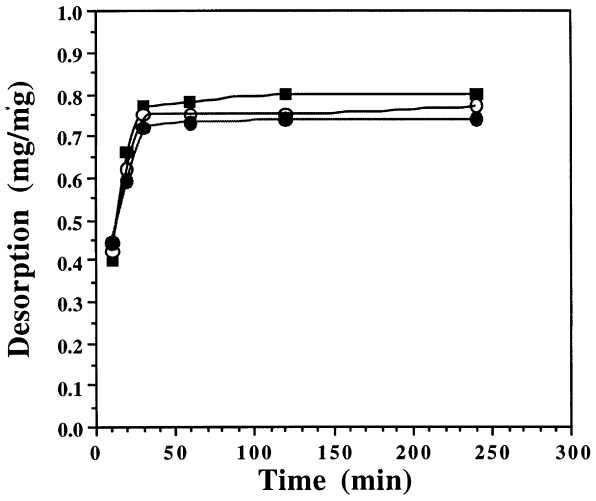


Fig. 3. Desorption profile for release of lipase from hydrogels (polyol M_n = 4055 dalton and PEGBCOCl:LPEP ratio = 4.25) : ■ 0.5; ○ 1.0; and ● 2.0 g/l lipase concentrations.

hydrogels retain their physical integrity better in cyclic transitions than the more absorbent ones.

The effects of pH on SD of these hydrogels were studied at a constant ionic strength (Fig. 2b). At room temperature, reswelling was maximized at neutral pH and gradually decreased with increasing acidity and basicity. This is expected since there are no ionizable groups within the polymer matrix to bias the swelling maximum. In addition, hydration of ionic species is favored over that of PEG chains [15]; hence, as the ionicity increases, a greater number of bound water molecules is lost to solvation of ionic species, causing the polymer matrix to pack closer together and reducing the degree of swelling. At pH 8, the SD was reduced to 46% of its maximum value, above which the hydrogel fully degraded via base hydrolysis. At pH 2, the SD was reduced to 19% of the neutral value. However, the gels stayed intact and degradation did not appear to be responsible for the reduced swelling. Furthermore, hydrogels synthesized by LPEP of lower molecular weights were less sensitive to the changing pH. These hydrogels swelled to lesser degrees and the pH effects were similar to those observed during cyclic swelling/collapsing.

Absorption of enzyme protein was studied using the hydrogels with a 4.25 PEGBCOCl/OH ratio. The absorption of lipase increased linearly from 0.4 to 1.2 mg/g with increasing enzyme concentrations (0.5–2.0 g/l). Desorption of lipase proteins from the hydrogels followed similar profiles among these three protein loading levels (Fig. 3). Over 90% of the total amount of protein were released during the first 60 min. Since these hydrogels collapse significantly within this time period, the rapid release appears to be a result of the expulsion of lipase protein during volume-phase transition. Hence the release of lipase within the first 60 min is not believed to be diffusion controlled. Beyond 60 min, lipase release was extremely slow. Since the inherent sensitivity in the fluorescent measurements is within the same magnitude as the fluctuations in release within this region, the measurements of actual enzyme release are less accurate at the lower levels. Nevertheless, the release of enzyme was almost negligible after the hydrogel had collapsed significantly, i.e. after 60 min.

The transition of the hydrogel in response to temperature changes was observed with thermal analysis. Once the phase transition occurs inside the water-swollen hydrogel, the temperature and heat of phase separation can be determined. Fig. 4 shows the DSC diagram of fully hydrated hydrogels synthesized from LPEPs at a 4.25:1 PEGBCOCl:LPEP

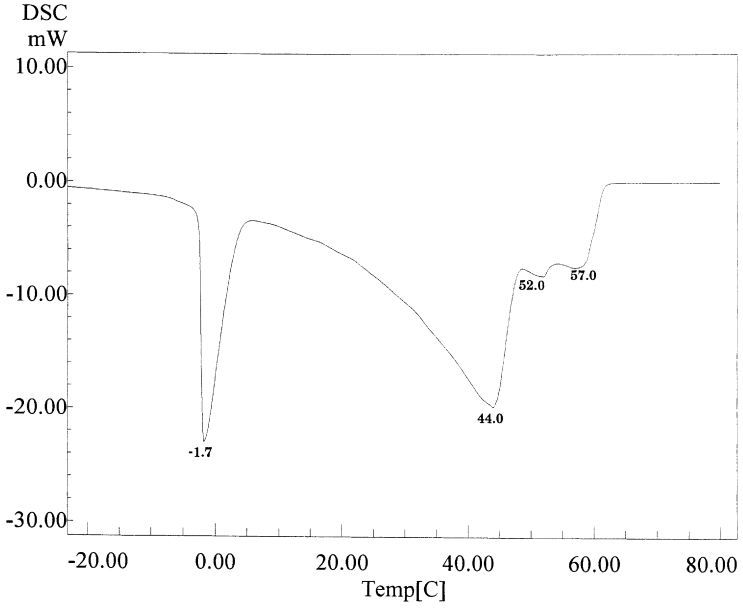


Fig. 4. DSC of hydrogels (polyol M_n = 4055 dalton and PEGBCOCl:LPEP ratio = 4.25).

ratio. Melting of water in the hydrogel was observed at −1.7°C, followed by a large and broad endotherm with a peak temperature of 44°C and then two smaller endotherms at 52 and 57°C. Similar observation on other thermo-sensitive hydrogels has been attributed to the collapse temperatures of different chain segments [15]. The three endothermic peaks between 44 and 57°C are thought to be associated with the different phase changes of the PEO and PPO segments in these lactitol-based hydrogels. The exclusion of water associated with the transition of the less hydrophilic PPO is thought to occur at the lower temperature, and that of the more hydrophilic PEO occurs at the higher temperature. The fact that there are three endotherms suggests that the phase transitions may also depend on whether the PEO and/or PPO segments are bonded or not. However, the exact sources of these three endothermic peaks during phase transitions are not clear at this point.

4. Conclusion

A series of new thermo-sensitive hydrogels has been produced from reactions between acylated PEGBCOCl and LPEP. We have previously shown that the swelling behavior of these gels can be controlled by the PPO length in the LPEP or the molecular weight LPEP and the extent of crosslinking, or the PEGBCOCl:LPEP ratio. Evidently longer PPO spacers in the LPEP provide a greater free volume for the more hydrophilic PEO chains to bind water and to swell. This study further investigates the effects of the extents of crosslinking, i.e. PEGBCOCl:LPEP ratios. The highest SD (8100%) was observed on the hydrogel with a 4.25 PEGBCOCl:LPEP ratio, followed by one at a 4.5 PEGBCOCl:LPEP ratio. However, hydrogels with lower SDs tended to exhibit better stability in water. Swelling degrees of these hydrogels are highest at neutral pH. These super-absorbent hydrogels were stable under acidic conditions, but were sensitive to base hydrolysis. Lipase enzyme protein molecules were incorporated by immersing the collapsed hydrogels in the protein solutions at a temperature below 25°C. The amounts of lipase absorbed increased with lipase protein concentrations. These hydrogels expelled water when the temperature was raised between 25 and 40°C. Protein desorption has been found to be similar among hydrogels with various levels of absorbed proteins. Over 90% of protein were released during the first hour. The rapid release of protein appears to be a result of volume-phase transition and is not diffusion controlled. The wide ranging swelling properties coupled with the thermo-sensitivity exhibited by these hydrogels make them excellent candidates for controlled release systems.

Acknowledgements

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