

Characterization of soft gelatin capsules by thermal analysis

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Abstract

Thermal analysis of soft gelatin capsule was used as a diagnostic tool to evaluate the effect of temperature and humidity stress conditions as well as formulation water, propylene glycol and ethanol on the softening of the gelatin shells. Results obtained using modulated and conventional DSC given as ΔT_m (change in gel–sol transition temperature) were compared with the results obtained using manual hardness tester given as % hardness loss. No difference between the two methods was observed in their ability to determine the extent of softening due to formulation water, propylene glycol and ethanol content. Thermal analysis and shifts in the reversible heat flow determined using MDSC provided additional insight into the structural changes and extent of deformation within the gelatin network upon exposure to formulation ingredients, temperature and humidity. Modulated thermal analysis is, therefore, a useful tool for screening the variables influencing the hardness of gelatin capsules. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Soft gelatin capsules (SGC) have been available since the nineteenth century. Since then they have been used to dispense a variety of liquids and solids (Stanley, 1986) with multitude of advan-

tages over the conventional dosage forms (Shah et al., 1992). Studies on the physicochemical properties of the gelatin gels have been reported (Flory and Garrett, 1958; Djaburov and Papon, 1983; Tseretely and Smirnova, 1992; Reich, 1995, 1996; Watanabe et al., 1997; Kuijpers et al., 1999); however, little has been done to investigate the physical properties of the gelatin shells as in SGC. Some studies investigated the brittleness of gelatin capsule as a function of relative humidity (Kontny and Mulski, 1989). Others studied the effects of different shell composition (Shah et al., 1992) and addition of water insoluble powder (Samura et al., 1993) on the strength of the capsules. The effect

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of temperature, humidity and formulation variables on the softening of the gelatin capsules has, however, not been adequately studied. Hardness tester is often used in industry to determine relative softening of capsules under various conditions. This method, while easy to apply, does not provide any insight into structural changes occurring in gelatin capsule. This paper describes an alternative approach, e.g. thermal analysis using modulated and conventional DSC, to investigate softening of the gelatin capsules. This study is based on our understanding of gelatin and collagen structures. Collagen chains consist of three helical polypeptide chains held together by hydrogen bonds (Belitz, 1987). The thermoreversible transformation of collagen to gelatin is interpreted as the disintegration of these helical structures into random coils (Watenabe et al., 1997; Kuijpers et al., 1999). Upon cooling, random coils undergo a conformational coil to helix transition (Kuijpers et al., 1999) during which they attempt to reform the original collagen structure (Mackie et al., 1998). Depending on the temperature and time at which the random coils are allowed to cool, they form less organized gelatin gel. In this sense, gelatin is prepared by complete thermal denaturation of collagen (Mackie et al., 1998) followed by partial renaturation through nucleation and growth of crystalline links (Chabala et al., 1994; Reich, 1996). The resulting three-dimensional network is responsible for the strength and integrity of the gelatin gel. Only a fraction of the gelatin macromolecules, however, comprises the crystalline network. The space between the fibrils is composed of disordered amorphous polypeptide chains (Chabala et al., 1994) plasticizers and water providing the elasticity to the soft gelatin capsules.

The objectives of the present study are to gain an understanding of the structural changes of SGC when exposed to exaggerated temperature and humidity conditions by observing the reversible heat flow using the modulated thermal analysis, and to correlate the thermal data with the hardness loss data on the effects of water, propylene glycol and ethanol on softening of SGC.

2. Materials and methods

2.1. Materials

Hydrophilic oval airfilled soft gelatin capsules (size 18) were supplied by RP Scherer North America (St. Petersburg, FL). The following chemicals and raw materials were purchased and used as supplied: Cremophor EL (BASF Corp Parsippany, NJ), Ethanol (McCormick Distilling Co, Weston, MO), Propylene Glycol (Aldrich Chemical Co, Milwaukee, WI), Magnesium Chloride (Sigma Chemicals St. Louis, MO) and Sodium Chloride (Mallinckrodt Inc, Paris, KY). Water was deionized and filtered with Millipore Milli-Q water system.

2.2. Sample preparation

Formulations of Cremophor EL with water, alcohol or propylene glycol were prepared on the weight (%w/w) basis by mixing the ingredients into a clear solution. Cremophor EL was used as the base in this study because of its common use as a non ionic surfactant in self microemulsified oral delivery systems. Protrusions of the elongated empty shells were cut with a sharp blade and 1 g of the formulation was injected using a syringe fitted with 15G needle. Openings of the capsules were sealed with a hot metal spatula. Fifteen capsules of each formulation were then placed in two 20-ml clear glass vials. Each vial was capped and placed in a constant humidity desiccator. Humidity conditions, 33 and 75%RH, were maintained by a saturated solution of magnesium chloride and sodium chloride, respectively. Twenty control airfilled capsules were placed in an open glass dish and stored in humidity-controlled desiccators. Desiccators were then stored in a preset incubator at 26, 37 °C or in a refrigerator at 4 °C.

2.3. Differential scanning calorimetry

Thermal behavior and melting temperatures were determined with a 2920 differential scanning calorimeter (TA Instruments New Castle, DE). Samples for thermal analysis were prepared as

follows. SGC was cut with a sharp blade allowing the fill formulation to be removed. Inner surface of the capsule was then carefully wiped with a lint-free tissue to remove any residual contents. Accurately weighed 5 mg sample of the shell was then hermetically sealed into aluminum DSC pans, and the equipment was operated in modulation mode. The scan rate was 5 °C/min over an appropriate temperature range. Modulation amplitude was 1 °C/60 s. Indium was scanned by the MDSC to verify the calibration of the instrument. Refrigerated Cooling System (RCS) was used to ensure proper temperature cycling.

It is important to note herein that one of the limitations imposed on the conditions of operating MDSC is that at least six modulations are required throughout the duration of each thermal event (Coleman and Craig, 1996). This is not achieved by utilizing a scan rate of 5 °C/min with a modulation amplitude of 1 °C/60 s. However, these parameters were selected based on our initial studies on the effect of scan rate on the resolution of the thermal events associated with gelatin samples. No difference was observed between a scan rate of 2 and 5 °C/min, thus a scan rate of 5 °C/min was selected in performing the experiments reported in this study.

2.4. Hardness test

Manual PTC Instruments, type A durometer model 306L (Pacific Transducer Corp, Los Angeles, CA) was used to measure the hardness of SGC. Capsule samples were placed on a hard, horizontal surface, where the durometer was held vertically and the presser foot was applied to the shell as rapidly as possible without shock, keeping the foot parallel to the surface of the capsule. Sufficient force was applied to obtain firm contact between the presser foot and the sample. Hardness of the capsule was then obtained by reading the ancillary hand.

2.5. Critical swelling ratio

Critical swelling ratio could be used to demonstrate the existence of a kinetic equilibrium between the moisture within the shell of a soft gelatin capsule and the water content in the fill material. Swelling ratio is the ratio of capsule weight in the presence of water to the weight before swelling. Critical swelling ratio (S^*) is the maximum amount of water that can bind to the gelatin network without exerting an ice melting endotherm at 0 °C. S^* could be defined as the swelling ratio below which water does not crystal-

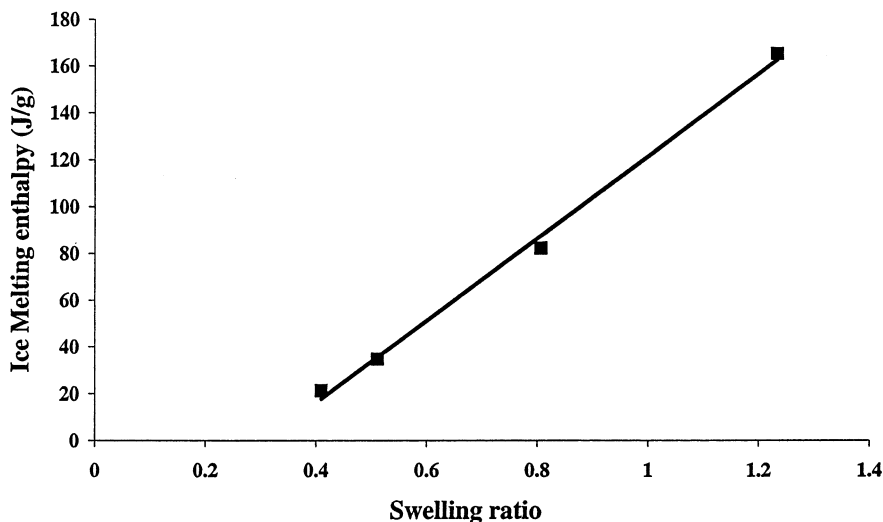


Fig. 1. Ice melting enthalpy as a function of water gain.

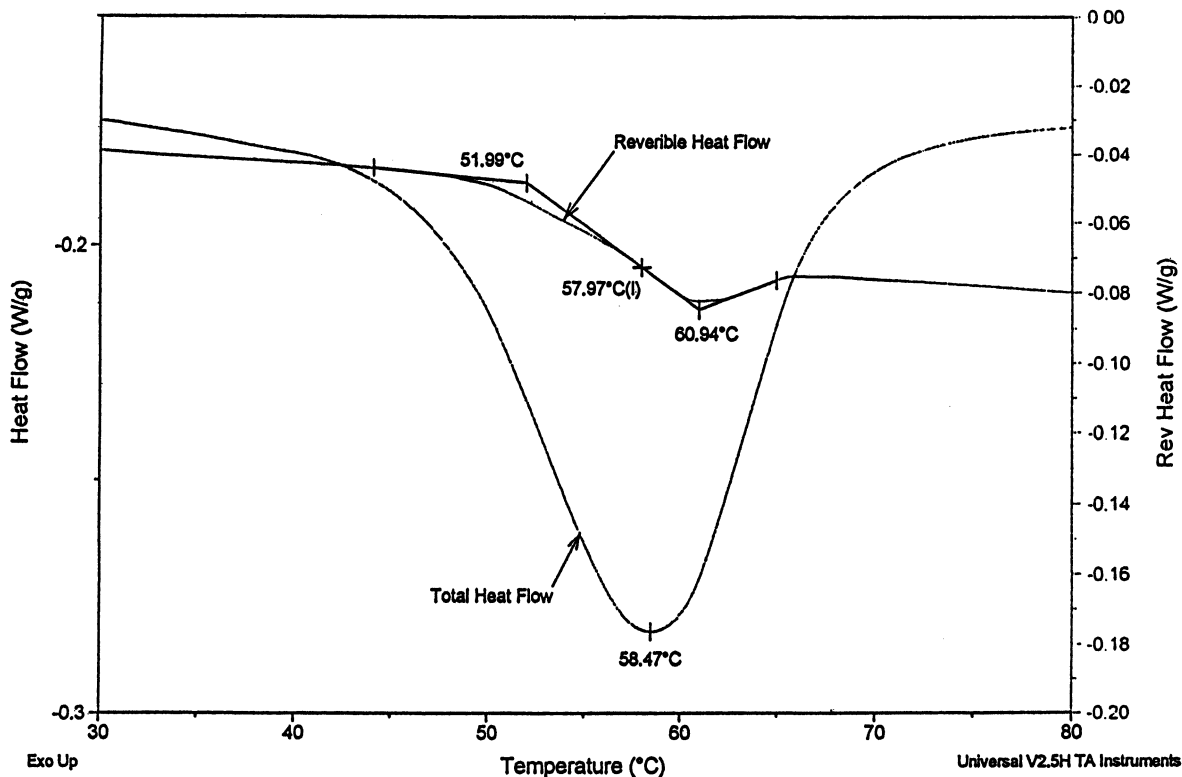


Fig. 2. Modulated thermal plot of an airfilled SGC.

lize and only exists as bound molecules to the gelatin chains (Apostolov et al., 1999).

S^* was determined as follows. Sealed airfilled SGC were completely immersed in 20 ml of pure distilled water at 22 °C. At 5-min intervals for 20 min, a sample was weighed after gentle surface wiping using lint-free tissue and analyzed with a differential scanning calorimeter. Ice melting enthalpy (ΔH_m) of the free unbound (crystalline) water was plotted versus the swelling ratio of the capsules (Fig. 1). Intercept of the line with the horizontal axes yields the critical swelling ratio, S^* .

3. Results and discussion

3.1. Storage temperature and relative humidity

To examine the effect of temperature, humidity or formulation factors on SGC, we first analyzed

fresh airfilled SGCs to establish a baseline. Shown in Fig. 2 is a representative modulated thermal plot of an airfilled SGC. The broad endotherm given by the total heat flow at 58.5 °C corresponds to the liquefaction or gel–sol transition (Flory and Garrett, 1958) of the gelatin shell. This transition is a result of the conformational change due to the melting of the ordered crystalline structure of the native gelatin network within the gelatin shell of the SGC (Kuijpers et al., 1999). The maximum of the curve, referred to as the shell transition temperature (T_m), is unique for the particular gelatin formulation.

The reversible heat flow, the top curve in Fig. 2, corresponds to the transition where the gelatin system undergoes a transformation from a system with a higher heat capacity to a system with a lower heat capacity. The midpoint (~ 58 °C) between the onset and the end-point of this transition is close to T_m . As we will show below, total

heat flow curves generated by the conventional DSC sometimes showed multiple endothermic peaks owing to the structural heterogeneity of the gel. The structural heterogeneity can arise from exposure to various environmental conditions. The reversible heat flow curve is more consistent between samples since it reflects true gel–sol transition and therefore, a better measure of physical changes of SGC.

Next, we examined air-filled SGC samples stored for 1 week at the following three conditions: 37 °C/75%RH, 37 °C/33%RH and 4 °C/75%RH. These conditions were chosen because they represent severe conditions that can be encountered during storage and usage despite whatever labeling would have recommended. As is shown in Fig. 3, the total heat flow curves showed two endothermic peaks for SGC samples stored at 37 °C/75%RH and 37 °C/33%RH while only one peak for samples stored at 4 °C/75%RH. The multiple endothermic peaks are a result of the melting of the original gelatin network and the

melting of the re-generated fine crystallites of the molten chains. Indeed, it has been reported that molten chains would partially reform fine crystallites (i.e. coil to helix transition) at room temperature, with a lower gel–sol transition temperature (Vassileva et al., 1999). The reformation of the fine crystallites can occur within a short time such as between sample removal from the incubator and their analysis with the DSC (Vassileva et al., 1999). Hence, for samples stored at 37 °C/75%RH, a lower transition endotherm at ~ 38 °C_{Tm} reflected the reformed crystallites while the transition endotherm at a temperature range between 48 and 63 °C corresponded to the original gel network remnants. This thermal pattern is similar to the pattern observed for samples stored at 37 °C/33%RH (Fig. 3), only that the later had a smaller peak at ~ 38 °C_{Tm} but a larger peak around 48–53 °C. This is expected as at a constant temperature, less gelatin helices melt at a lower humidity. Consequently, relatively more ‘original’ gelatin network remained and thus a

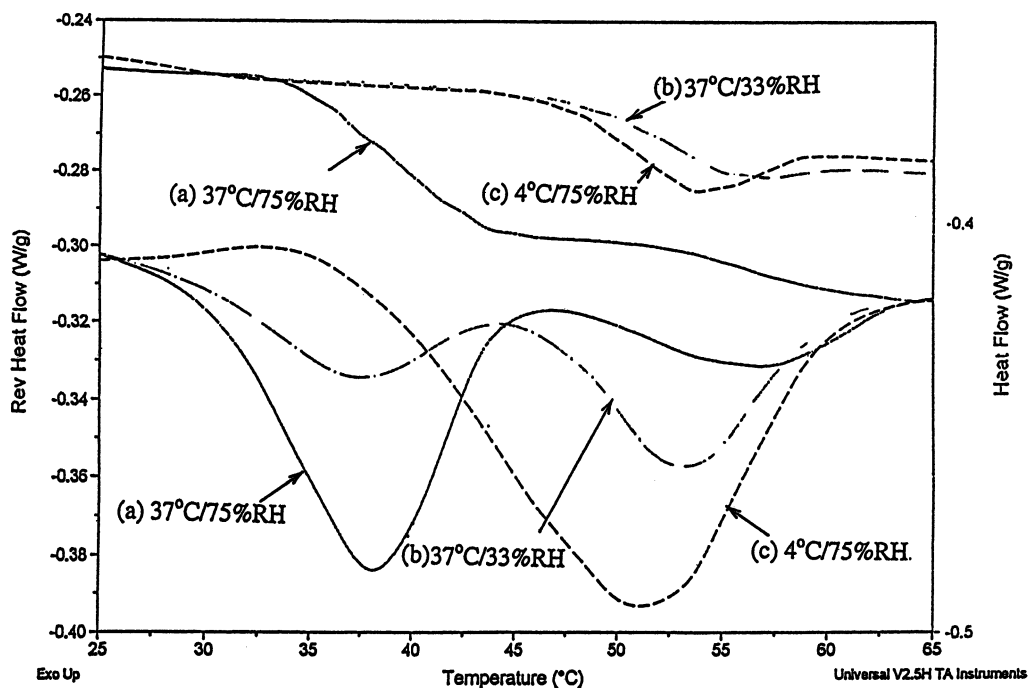


Fig. 3. Modulated thermal plot of SGC exposed to (a) 37 °C/75%RH, (b) 37 °C/33%RH and (c) 4 °C/75%RH. The top three lines represent reversible heat flow and the bottom ones represent total heat flow.

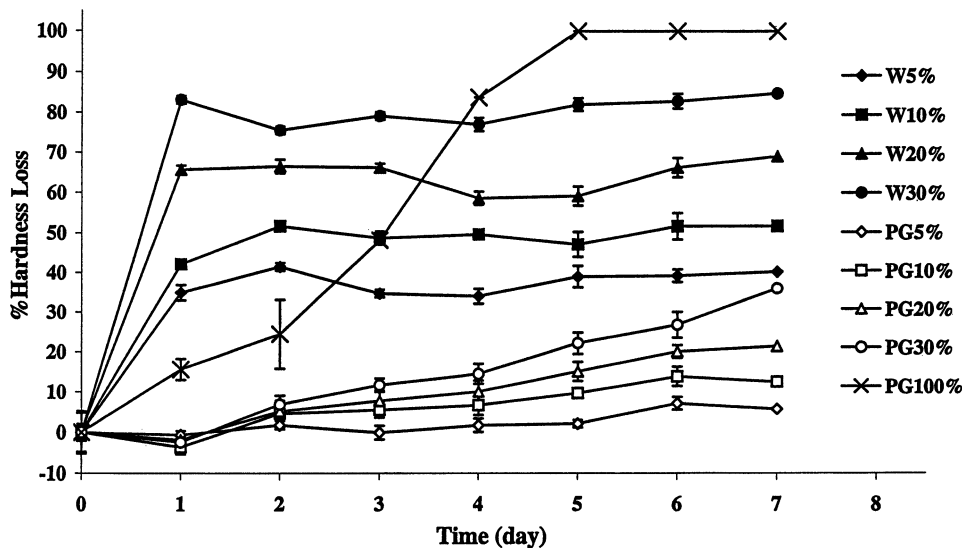


Fig. 4. Effect of formulation water (W) and propylene glycol (PG) on SGC hardness ($n = 3$).

corresponding larger endothermic peak. For samples that were stored at 4 °C/75%RH, moderate moisture uptake due to high relative humidity softened the gelatin and resulted in a broader endotherm with a shift of the transition temperature to ~ 52 °C (Fig. 3) from the reference at 58.5 °C (Fig. 2). The mid-points of the reversible heat flow were 38, 54 and 50.7 °C for samples stored at 37 °C/75%RH, 37 °C/33%RH and 4 °C/75%RH, respectively (Fig. 3), a shift from the reference of 58 °C (Fig. 2). These results demonstrate that temperature and humidity have significant impact on the physical property of the gelatin capsule tested. This impact can be detected by modulated DSC in a week, long before visual examination is able to detect. Thus modulated DSC may be useful not only for monitoring stability of drug-containing SGC but also for screening gelatin formulations.

3.2. Formulation variables

3.2.1. Formulation water content

Water in low amount is used as a cosolvent in the fill formulation for some SGC dosage forms. To evaluate the effect of water in fill formulation on the softness of the gelatin capsules, four formulations with varying water content (5, 10, 20

and 30% w/w in Cremophor EL) were prepared and injected into the capsules as described earlier. Cremophor EL was shown in our earlier studies to be inert to the capsule shells and did not compromise the seal of these systems over an extended period of time. Effect of water on the hardness of the capsules was measured using manual hardness tester and the differential scanning calorimetry. Percentage hardness loss measured with the hardness tester as a function of time at various formulation water contents is given in Fig. 4. Similarly, hardness loss measured as ΔT_m , the difference in transition temperature between the reference and the sample, as a function of time is given in Fig. 5. Both methods demonstrate similar softening patterns. Gelatin shell softening occurred in the first day after fill and remained relatively constant thereafter regardless of water content in the fill formulation. Hence, the effect of water content in the fill formulation on the long-term physical stability of SGC can be predicted in a relatively short period of time. Any subsequent softening in the capsules, during usage or storage, may not be due to the formulation water but rather due to heat, humidity or other ingredients in the fill material. In addition, ice-melting enthalpy for SGC filled with 30% of water was absent after a storage period of 2 weeks (data not

shown), indicating that there were no free water molecules in the gelatin shell and that the softening of the capsules is the result of the bound water molecules to the gelatin chains within the shell network. As previously described in Section 2.5, the maximal amount of bound water a gelatin capsule can absorb without expressing a melting endotherm at 0 °C is the critical swelling ratio (S^*). This value was determined to be 0.3 (Fig. 1). This suggests that the amount of bound water an average 320 mg airfilled capsule can absorb is about 99 mg. This value correlates very well with that for un-crosslinked gelatin gels (Tseretely and Smirnova, 1992) and crosslinked gelatin samples (Apostolov et al., 1999). Although the actual water content in the shell was not determined, the absence of free water in the shells containing 30% or 300 mg of water as a fill material and a constant hardness over a period of 2 weeks suggest that the amount of water absorbed by the shell did not exceed 99 mg. This further suggests that a kinetic equilibrium due to moisture uptake was achieved within the first sampling period. To further elucidate the mechanism by which water exerts its effect on gelatin shells, a DSC plot of

the filled SGC is given in Fig. 6. In general, a broad transition endotherm is indicative of a wide distribution of size and degree of gelatin aggregates (Djaborurov and Papon, 1983). These aggregates are the crystalline bundles of triple alpha helices and the two hydrogen-bonded peptide repeat units that are part of the adjacent gelatin chains located in the amorphous domain (Schott, 1992). Water uptake by gelatin gels has been reported to cause a break in the hydrogen bonds and consequently to reduce the overall density of the cross links (Kuijpers et al., 1999), thus plasticizing the amorphous region of the gelatin network (Schott, 1992). This effect was observed by the gradual shift of the transition endotherm to a lower temperature. Hence, higher water concentrations in the fill material decreased T_m to a greater extent as observed in Fig. 6. Bound water molecules embedded between gelatin chains, however, may partially facilitate regeneration of some ordered triple helices that are further stabilized by intramolecular hydrogen bonds (Djaborurov and Papon, 1983). These intramolecular bonds may account for the relatively constant heats of fusion (Q_m).

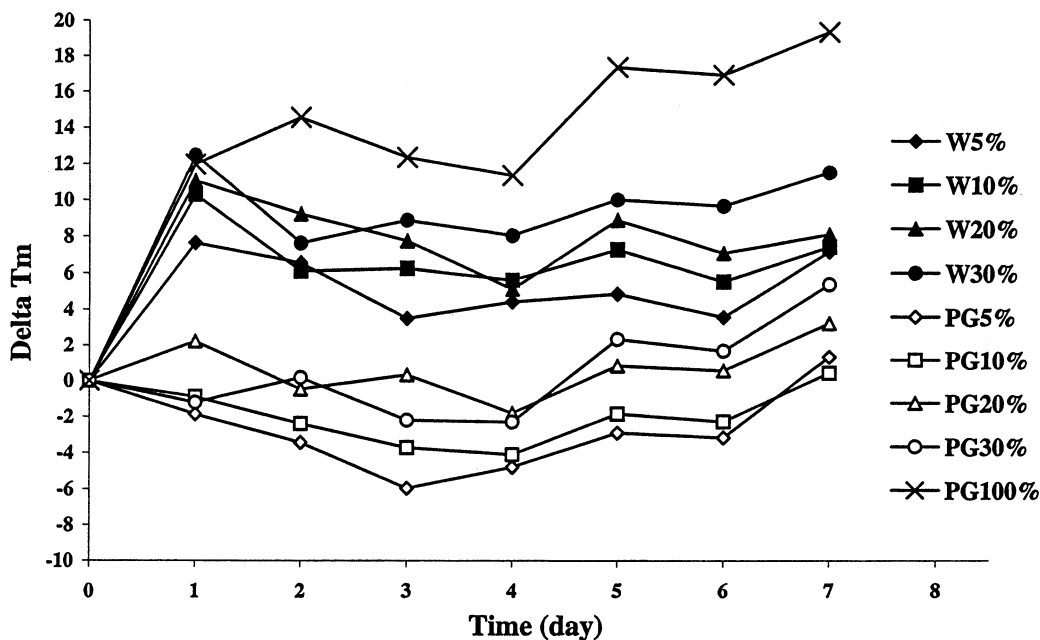


Fig. 5. Effect of formulation water (W) and propylene glycol (PG) on depression in melting temperature (ΔT_m).

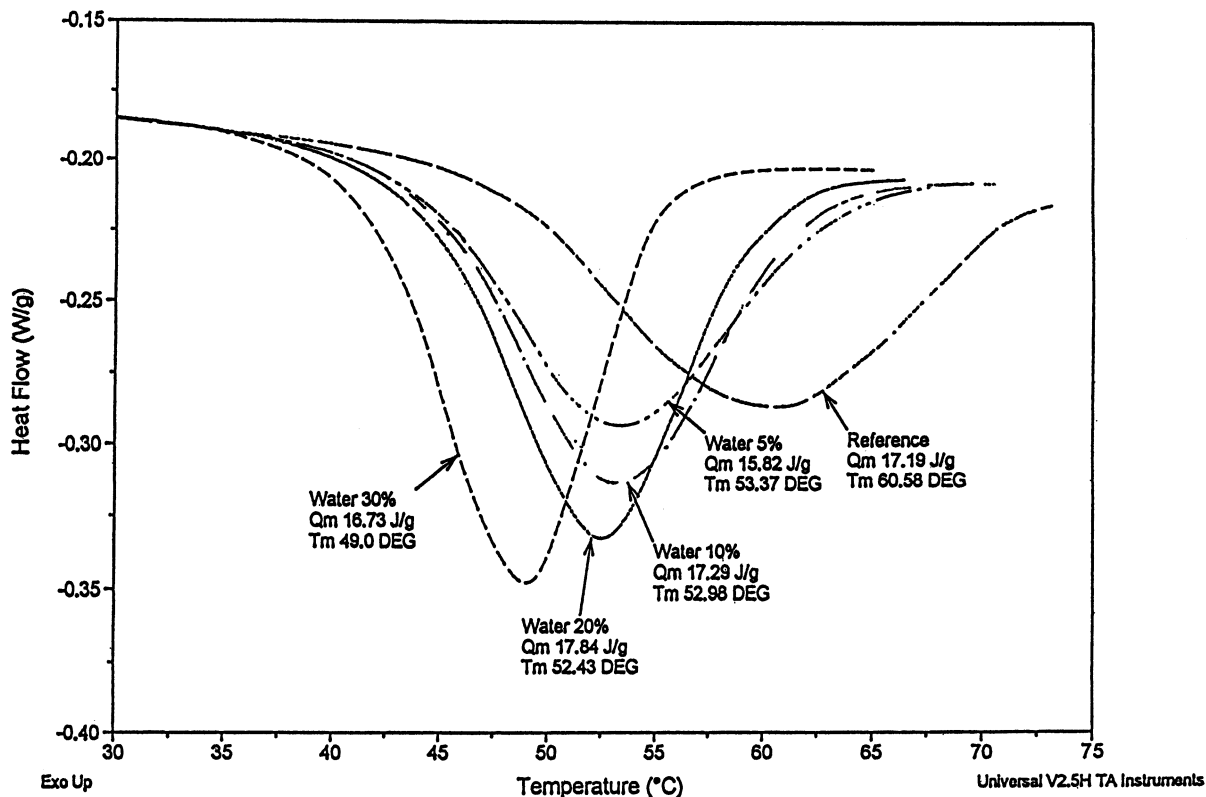


Fig. 6. Melting endotherms (after 7 days) for a reference and SG filled with 5, 10, 20, and 30% water based fill formulations.

3.2.2. Propylene glycol (PG)

To investigate the effect of PG on the structure and softening of the gelatin capsules, five formulations, 5, 10, 20, 30 and 100% w/w in Cremophor EL were prepared and injected into the capsules. Over a period of 1 week, hardness as a function of PG concentration was measured using manual hardness tester (Fig. 4). Similarly, depression in the transition temperature (ΔT_m) was measured with the DSC (Fig. 5). Higher PG concentration tended to lower shell hardness and T_m to a greater extent. Effect of PG concentration on SGC hardness, however, was more evident using hardness tester. A slower, less dramatic change in the hardness and T_m was observed in PG filled capsules than in the water-filled capsules. This could be due to the slower diffusion of PG through the gelatin network. To further elucidate the effect of PG on gelatin shells, DSC plots for the filled capsules after a period of 1 week is given in Fig.

7. Softening effect of 5 and 10% formulations might be due to the plasticizing effect of PG without interference with the gelatin structure. Thus, similar endotherms compared with that of the reference were observed. It was only at 20, 30 and 100% PG when irregularities in the thermal plots appeared, which might be a result of the plasticizing effect and the physical strain of PG on the helices in the crystalline domain. This explains the gradual decrease in T_m , Q_m and hardness values for capsules with PG concentrations greater than 20%.

3.2.3. Ethanol

Ethanol is a common cosolvent used in pharmaceutical formulations. Diffusion and subsequent evaporation of alcohol through the gelatin shell of the soft elastic capsules may cause physical incompatibilities within the fill formulation. Moreton (Moreton and Armstrong, 1998) investi-

gated the diffusion of ethanol through soft gelatin films and provided alternative gelatin formulations that may retard diffusion and facilitate manufacturing of capsules for alcohol containing fill formulations. In our studies, no difference in hardness and thermal behavior over a period of 1 week was observed for 5, 10, 20 and 30% ethanol filled SGC compared with that of the reference. Thus, 30% ethanol filled products were used as a representative sample in our subsequent discussion. To illustrate how ethanol might interfere with gelatin network, thermal plot for two formulations, 30 and 100% w/w in Cremophor EL, after a period of 1 week, is given in Fig. 8. Shape deformation and decrease in T_m and Q_m with 100% ethanol filled SGC were observed. And these could be due to ethanol evaporation as well as to the loss and displacement of the bound water. As bound water stabilizes the triple helical

structure, its displacement by the diffusing alcohol (Moreton and Armstrong, 1998) destabilizes the triple helical structure leading to gradual deformation. Despite of the visible deformation at 100% ethanol level, similar thermal behavior between the reference (Fig. 2), and that of 30% ethanol filled SGC was observed, suggesting that the physical stability of the capsules could be maintained, at least within a reasonable time frame, for formulations with ethanol concentrations as high as 30%.

4. Conclusion

Thermal analysis of SGC provides valuable data on the physical changes of gelatin capsules that may be caused by external factors or formulation ingredients. Such thermal events are corre-

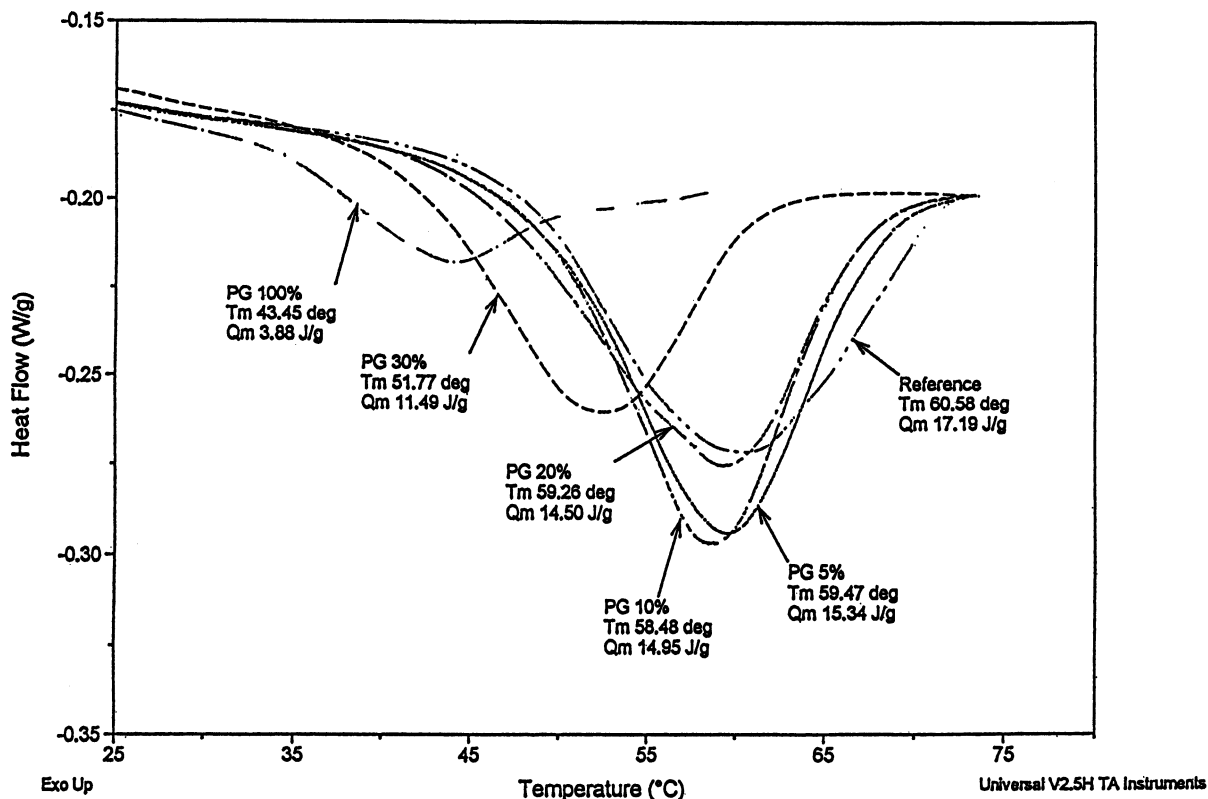


Fig. 7. Melting endotherms (after 7 days) for a reference and SGC filled with 5, 10, 20, 30, and 100% propylene glycol based fill formulations.

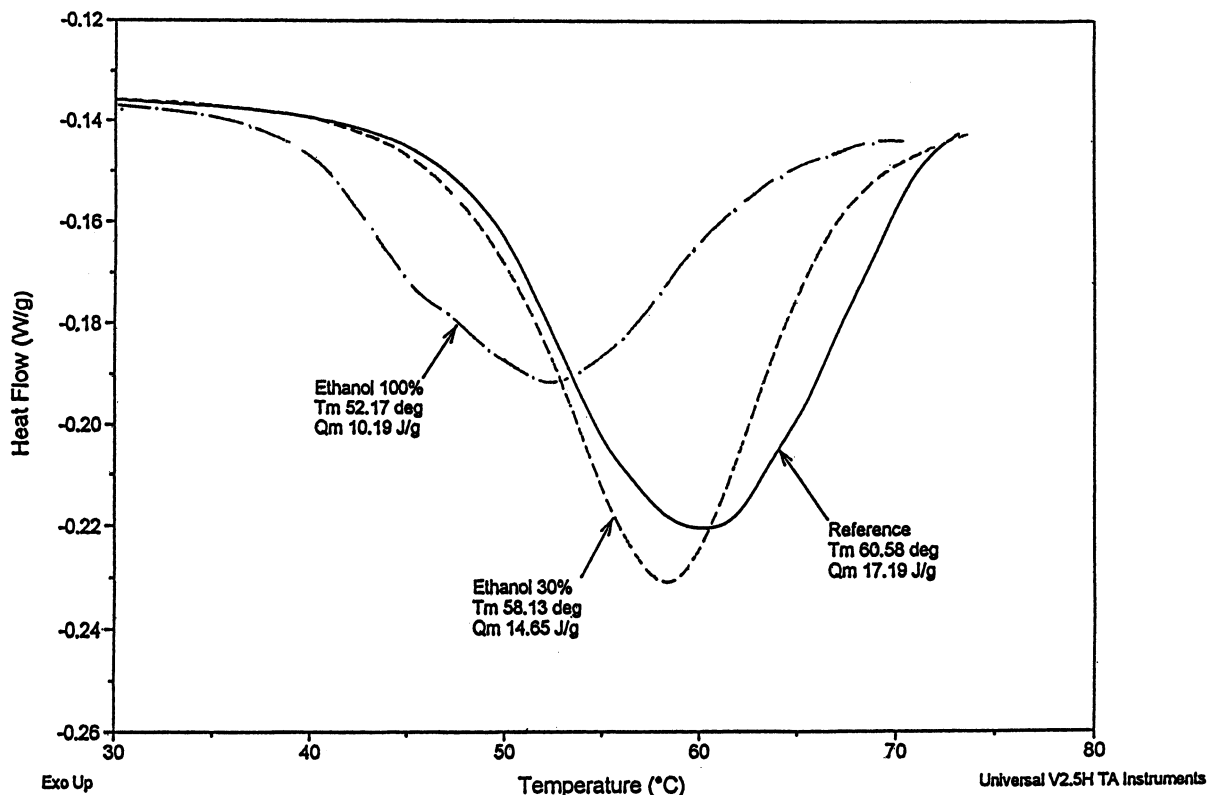


Fig. 8. Melting endotherms (after 7 days) for a reference and SGC filled with 30 and 100% ethanol based fill formulations.

lated with the hardness data and could be used to predict the extent of shell softening. Reversible heat flow is particularly useful when a conventional thermal analysis reveals multiple endotherms. While two types of crystallites may coexist when the samples are stored at elevated temperatures, only the endotherm that correlates with the transition midpoint of the reversible heat flow is responsible for maintaining the integrity of the gelatin shell. High relative humidity at low temperatures causes moderate softening due to low chain mobility. There is a good correlation between the shift in the transition endotherm and hardness loss for SGCs containing water and PG as part of the fill formulation. Water is found to attain equilibrium within a short period of time as predicted from the critical swelling ratio. Due to the destructive effect of water on the gelatin network and partial regeneration of some helices, the transition endotherms associated with water are

shifted to lower temperatures with characteristic peak narrowing and a constant Q_m . Propylene glycol, on the other hand, has a slower plasticizing effect that causes a gradual shift in the transition endotherms with characteristic peak broadening and a decrease in Q_m . Ethanol had little effect on shell hardness. The effect of ethanol on the physical deformation in the gelatin shell could be due to ethanol evaporation and bound water displacement in the aqueous filled pores.

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