Research paper

Preparation of a novel freeze thawed poly(vinyl alcohol) composite hydrogel for drug delivery applications

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Abstract

We describe a drug delivery system based on a physically cross-linked poly(vinyl alcohol) (PVA) hydrogel for the release of Theophylline (TH). A composite was created by freezing an aqueous solution of PVA/NaOH onto a PVA/poly(acrylic acid) substrate. This formed a strong interface and demonstrated greater physical strength than the hydrogel alone. Such systems have potential for a variety of localised controlled drug delivery applications, for example, as coatings for implantable devices. Importantly, the results suggest that a versatile synthetic platform is possible that may provide different functional materials or combination of such. The resultant samples were characterised using optical microscopy, modulated differential scanning calorimetry (MDSC) and dissolution testing. The microstructure of the gels was examined using micro-thermal analysis (μTA) which is a combination of atomic force microscopy and thermal analysis. TH was found to have an effect on the crystalline structure and dissolution showed a Fickian release, suggesting that swelling and crystallinity were the controlling mechanisms.
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Keywords: Micro-thermal analysis; Drug release; Poly(vinyl alcohol); Freeze–thaw cycle

1. Introduction

The term hydrogel is used to describe materials that are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water [1,2]. Hydrogels have important applications in the areas of controlled drug delivery, as coatings in pharmaceutical applications and as dissolution and binding agents in tablets [3,4].

One method of producing a hydrogel, without utilisation of chemical cross-links, is freeze/thaw processing [5,6]. PVA hydrogels prepared using freeze/thaw cycling are excellent candidates for biomaterials as they exhibit a high degree of swelling in water, a rubbery elastic nature,

are non-toxic, non-carcinogenic and can be readily accepted in the body [6]. In order to improve the functionality, PVA is often combined with poly(acrylic acid) (PAA) [7,8]. Pure PVA hydrogels are insensitive to pH changes and the addition of PAA in the freeze/thaw process results in pH sensitive gels [9]. PAA polymers have good adhesive properties, but a tendency to cause irritation has limited their application as buccal bioadhesives [10].

The versatility of PVA hydrogels makes them a material of choice for many biomedical applications, as matrices for cell immobilization and for the controlled release of drugs [11–13]. Drug-release applications have been investigated which employ the bioadhesive nature of PVA gels. Morimoto et al. examined the controlled release of several drugs from cross-linked PVA gel carriers for rectal administration [14].

The diffusion of macromolecules through cross-linked PVA networks was studied by Peppas [15]. Drug release rates from PVA hydrogels tend to be relatively high and can be varied in accordance with the pore size, extent of

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cross-linking, and the nature of the incorporated drug and typically follow first-order kinetics [16,17]. Shaheen et al. discussed the use of PVA/NaCl/H_2O systems for the delivery of TH [18,19]. The drug release behaviour showed an irregular Fickian diffusion. Typically, the degree of PVA hydrogel cross-linking, which is intimately linked with the mechanical properties and water content, influences drug release properties [6].

The drug TH, used in the research, may be classified according to the biopharmaceutics classification system as a Class 1 compound which has a high solubility, high permeability and is very well absorbed [20]. Szepes et al. found that a freeze-casting technique was suitable for the formulation of porous dosage forms using TH as an active pharmaceutical ingredient (API) and potato starch as a filler. The physical properties of TH, such as poor flowability and thermal stability under freeze process conditions, allow TH to be a good candidate for this technique [21].

The characterisation of drug systems may be performed with a range of methods. Amongst the different techniques is microthermal analysis (μTA). For μTA, the conventional atomic force microscopy (AFM) tip is replaced, with a miniature heater thermometer which enables a surface to be visualised, according to its response to the input of heat [22]. On rastering over the sample surface, the topography is obtained in an identical manner to that found for AFM (albeit at lower resolution due to the large size of the tip). The probe can be utilised as an ultra miniature differential thermal analyser, allowing a comparison of the electrical energy supplied to the probe and the surface [22].

In previous work in our laboratory we have studied the effect of the addition of sodium chloride, sodium hydroxide and hydrochloric acid on the production of freeze-thawed PVA gels and we found that gels with enhanced properties were possible [23]. We presently report the development of a composite (PVA–NaOH hydrogel/PVA PAA film) TH drug delivery system, which exhibits Fickian diffusion. Biomedical applications of such systems are biomedical membranes or coatings of in-dwelling medical devices. Furthermore, a detailed characterisation of the thermal, micro-structure properties of the composite, as well as a drug release study were undertaken.

2. Materials and methods

2.1. Preparation of samples

The preparation of the composite consists of casting an aqueous solution onto a substrate, all of which is then subsequently frozen.

2.1.1. Preparation of aqueous solution component

Poly(vinyl alcohol) used in this study was supplied by Aldrich and had a weight average molecular weight of 146,000–186,000 and a saponification value of 98–99%. TH was supplied by Aldrich with a molecular weight of 180.2 and a melting point of between 270 and 274 °C.

Solutions were prepared by mixing polymer powder (1 g) with distilled water (40 ml) and 0.025 M NaOH and 0.3 g of TH. Dissolution was achieved by heating the mixture to 80 °C for 90 min, while slowly stirring. When solids were no longer apparent and the mixture was clear, the beaker containing the solution was placed in an ultra sonic bath at 70 °C for 5 min to remove all bubbles.

2.1.2. Preparation of film component

PAA with a weight average molecular weight of 3,000,000 was supplied by Aldrich. Solutions were prepared by mixing 66% PVA and 34% PAA in 300 ml of distilled water. Dissolution was achieved by heating to 80 °C, while slowly stirring for about 90 min. When the polymers were no longer apparent the solution was placed in an ultra sonic bath at 70 °C for 5 min to remove all bubbles. The solution was then cast onto a Teflon coated glass basin and left in an oven at 80 °C for 24 h.

2.1.3. Preparation of composite

The dried PVA/PAA film was placed in a beaker and the aqueous solution containing PVA/NaOH/H_2O was added. This beaker was placed in a trough and approximately 500 ml of liquid nitrogen was added to the trough over a period of 10 min. The solidified solution was allowed to thaw at room temperature for 24 h resulting in a composite of hydrogel and film. Fig. 1 shows a picture of the film aspect of the composite, while Fig. 2 shows a picture of the hydrogel aspect of the composite.
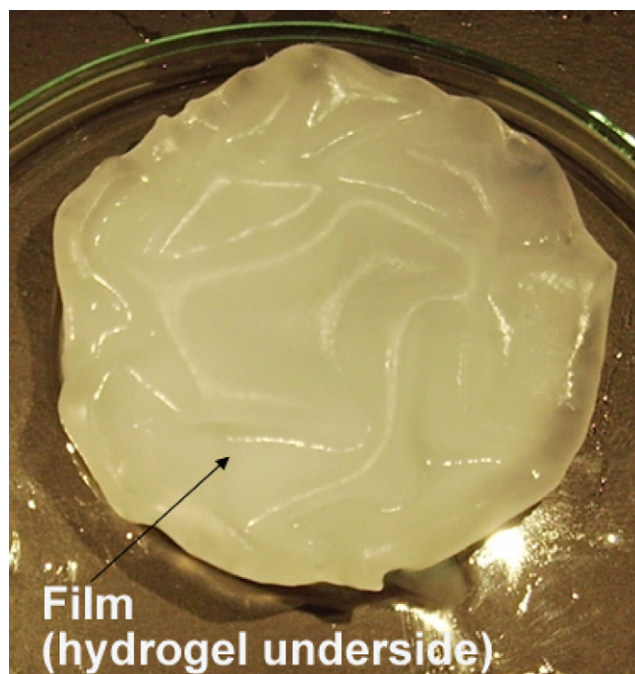

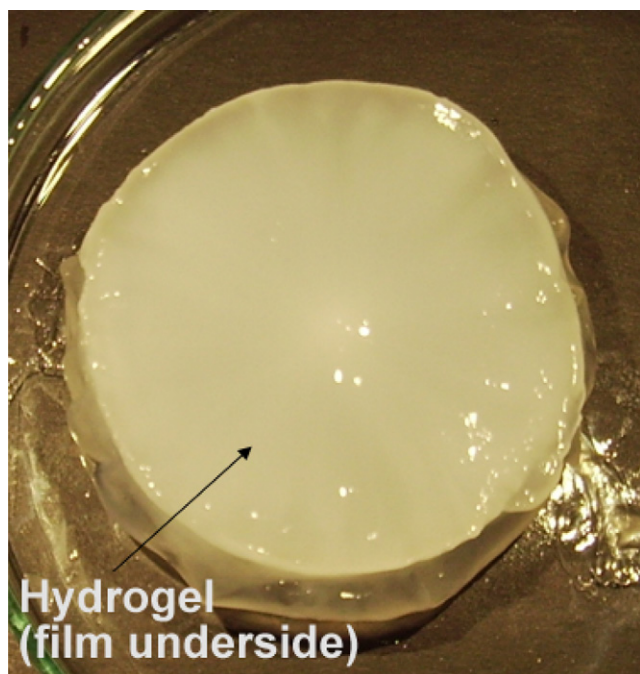Fig. 1. Composite with film on the upper surface (hydrogel underside).

Fig. 2. Composite with hydrogel on the upper surface (film underside).

2.2. Optical microscopy

Optical analysis was carried out on samples to examine the interface created. An 'Olympus BX60' microscope with a magnification of 10× was used to characterise the coating at a microscopic level.

2.3. Modulated differential scanning calorimetry

Modulated differential scanning calorimetry (MDSC) was performed using a DSC 2920 MDSC from TA instruments on samples that had been dried under atmospheric conditions for a minimum of 7 days. The dried samples contained negligible amounts of water. A sample of between 10 and 11 mg was tested in sealed aluminium pans. The samples were cooled to 25 °C, the modulation was ±1.00 °C every 60 s and the temperature was ramped from 25 to 285 °C and then ramped down to 25 °C.

2.4. Dissolution studies

Dissolution testing was evaluated using a Sotax AT7 smart dissolution system from Carl Stuart Ltd. The standard solution contained 0.02 g of TH in 600 ml. The hydrogels were cut into discs and tested in a phosphate buffer of pH 7.2 at 37 °C. The stir rate was set to 50 rpm with 900 ml of dissolution media used per vessel. Samples were automatically taken every 15 min and analysed by ultraviolet (UV) light at 276 nm using a 1 cm quartz cuvette on a Perkin–Elmer lambda 2 spectrometer. The dissolution profile was observed from a plot of time versus absorbance.

2.5. Micro-thermal analysis

Micro-thermal analysis was conducted using a Topometrix A 2990 micro-thermal analyzer, which combines an atomic force microscope with a Wollaston type temperature controlled thermal probe. Characterisation was carried out in two modes: micro-modulated thermal analysis (µMDTA), in which thermal transitions are measured, and micro-thermo mechanical analysis (µTMA), in which expansion, softening, melting and glass transitions are measured.

All measurements were performed in air. Topography and conductivity images were obtained by scanning the probe over the surface while maintaining it at a constant temperature. As the probe scanned across the sample surface two images were obtained; (1) surface topography and (2) thermal conductivity. Local thermal analysis (LTA) was performed by positioning the tip at a selected location and subsequently heating, resulting in a sensor (µTMA) and a power signal (µDSC).

The calibration of the sensor was verified using PET of known melting point. A performance check was carried out on a semi-conductor silicon grid, which consisted of raised silicon squares with a 3 µm pitch. This determined whether the system was fully operational. Micro-thermal analysis of the samples was performed on samples of 4×4 mm^2 and fixed onto metallic sample stubs using double sided sticky tape. These samples were cut from the hydrogel and exposed to atmospheric conditions for 7 days to ensure that minimum moisture was present. Images of 50×50 µm^2 were recorded at a scan speed of 20 µm/s.

In each sample, three locations were selected for analysis by LTA. The probe was heated from 0 °C to 350 °C at a heating rate of 20 °C/s using a contact force corresponding to 10 nA (1 nA corresponds to 3–4 nN). One hundred and fifty points per second were recorded using a frequency of 2.2 kHz and a heating amplitude of 3 °C. Analysis was carried out on twenty different locations and representative results are displayed.

3. Results and discussion

3.1. Optical microscopy

The attempts to produce a composite, using conventional PVA freeze thaw techniques, were not successful. The hydrogel created was not cohesive and became easily detached from the film. A viable composite with sufficient integrity was formed only upon the addition of NaOH to PVA. Adhesion may be due to interdiffusion of polymer chains across the interface. This interdiffusion leads to entanglements and physical bonds between the PVA/PAA and the PVA/NaOH/H$_2$O. The level of penetration of the polymer chains is a function of the polymers and the contact time between the two substances [24]. By using a PVA/NaOH/H$_2$O gel and the correct thickness of substrate a viable composite with biomedical potential

was produced. Fig. 3 shows a detailed photograph of the interface of the film and gel. The hydrogel and the film have formed a cohesive structure. The PVA/PAA film maintained its integrity, even though it has become imbibed with water. The film gives the composite structural integrity while the hydrogel provides a reservoir for drugs.

To achieve sustained release, which is independent of the drug molecular weight, compounds may be entrapped in a second phase, which is subsequently incorporated into the drug delivery system [25]. In the system described by the authors, only the hydrogel acts as the drug reservoir, however it may be possible to incorporate an API into the PVA/PAA film. The principal challenge with a two-phase system is in achieving high drug loading in the second phase. Two-phase systems have been investigated to control API release profiles. These systems include the entrapment of liposomes [26].

3.2. Modulated differential scanning calorimetry

PVA exhibits transitions at 85 °C, 143 °C and a large peak above 210 °C. The peak at 85 °C, known as the α relaxation, represents the glass transition temperature of PVA. The relaxation observed at 143 °C, designated as the β relaxation, is due to the relaxation in the PVA crystalline domains. The third relaxation, which occurs at a temperature between 200 and 260 °C, is caused by the melting of the crystalline domains of PVA [27]. As discussed previously by the authors, crystallinity increases with the addition of certain concentrations of NaOH for PVA hydrogels [23].

The melting temperature of TH is 270 °C [28]. When placed in contact with water at ambient temperature, anhydrous TH is known to transform to TH monohydrate [29].

Upon drying of TH, Phadnis and Suryanarayanan postulated that a transition in the region of 145° could be attributed to the solid to solid transition of a metastable anhydrous TH [29].

Fig. 4 shows the thermograms of the samples without any TH present. The melting point of PVA is at 205 °C. The addition of NaOH results in an increase in the melting point of PVA to 228 °C and is consistent with previous work [23]. The endotherm present in the region of 92 °C for the samples is due to the evaporation of residual water [27]. Upon cooling there is only one endotherm present for PVA at 121 °C, which may be due to the β relaxation [27].

Fig. 5 shows the thermograms of PVA/NaOH/TH and PVA/TH samples and TH on its own. The melting point of TH is 274 °C and it shows no other transitions, upon cooling there is a crystallisation endotherm at 255 °C. For the PVA/NaOH/TH sample, as the temperature is ramped up, a transition is shown at 152 °C and upon cooling another transition is present at 166 °C. These transitions may be due to the solid to solid transition of a metastable anhydrous TH [29] or the crystalline β relaxation of PVA [27]. It is interesting that this transition is not present in the PVA/NaOH samples devoid of TH and would indicate that TH is the primary cause. The addition of TH to PVA/NaOH results in a reduced melting point of 203 °C, suggesting that TH interferes with the crystalline structure. The melting point of TH, in the PVA/NaOH/TH samples, is masked and is not clearly visible. The melting point of the PVA/TH samples is essentially the same at 210 °C. A transition is present upon cooling at 127 °C and is similar to the transition present in Fig. 4 and may be due to the crystalline relaxation of PVA or the transition of a metastable anhydrous [27,29].
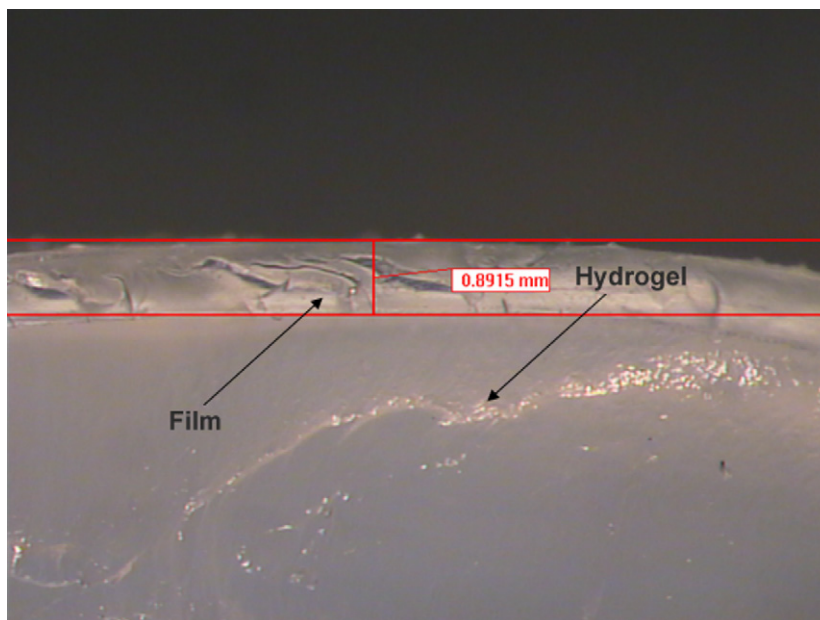

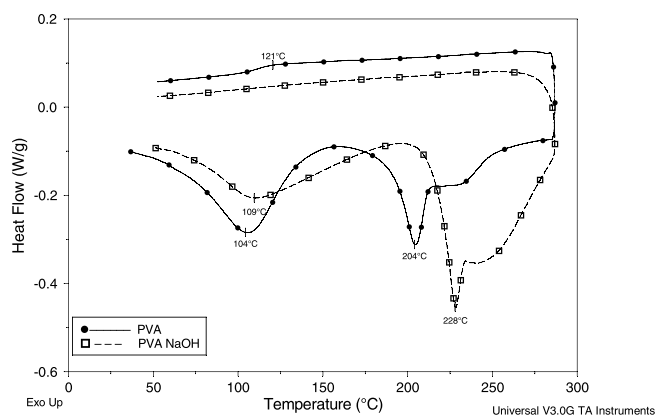Fig. 3. Composite interface between film and gel.

Fig. 4. Differential scanning thermograms of dried samples of PVA/NaOH and PVA.
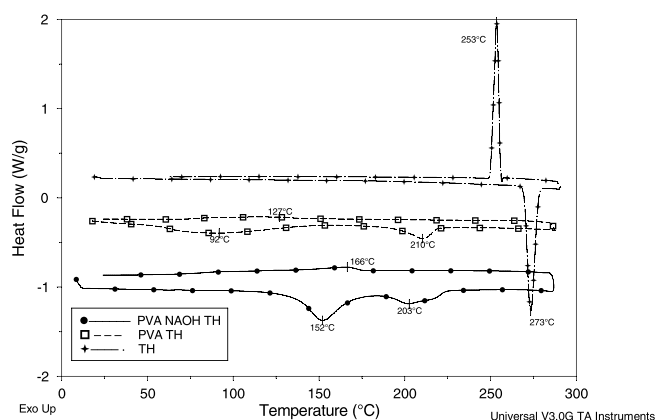

Fig. 5. Differential scanning thermograms of dried samples of PVA/NaOH/TH and PVA/TH and a sample of TH.

PVA gels are believed to consist of crystalline regions consisting of junction zones and amorphous regions consisting of long flexible chains [30]. The extent of crystallinity in a PVA hydrogel has an important effect on the mechanical properties of the gels. Gels with a high crystallinity have reduced elasticity and are fragile, whereas if the crystallinity is too low, gels are poorly coherent [31]. In addition crystallinity has an effect on drug dissolution for TH, resulting in inconsistent drug release [32]. Moji Adeyeye et al. investigated TH drug delivery systems under varying humidity conditions and found that crystal changes occurred whereby the anhydrous TH changed to the monohydrate [32]. The possibility of crystalline hydrate formation in TH and the PVA crystallisation process complicates the design of a consistent reproducible formulation process.

3.3. Micro-thermal analysis

Micro-thermal analysis combines the imaging capabilities of atomic force microscopy with the ability to characterise the thermal behaviour of materials. It is an extension of scanning thermal microscopy which is part of the family

of scanning probe microscopy techniques [33]. The method of local thermal analysis measurements using a micro-thermal analyser is accurate, robust and fast. It is used to study a variety of applications, including drug formulations, polymer blends, interface behaviour in injection moulded components and thickness analysis of polymer films [34–36].

3.3.1. Analysis of relative thermal conductivity

In μTA, the surface can be visualised, according to its response to the input of heat [22]. The thermal images obtained from the μTA are affected by the topography of the samples. As the probe travels over the sample, the current changes to maintain the probe at constant temperature. When the probe is at a higher peak, it is surrounded by less sample and more air. Air has a lower thermal conductivity than the sample and the apparent conductivity appears reduced [34]. The thermal image approach depends on the thermal conductivity between components being sufficiently high, to allow differentiation. It should be stated that differentiation between components using thermal conductivity is not completely reliable, with systems such as HydroxyPropylMethylCellulose (HPMC) Ibuprofen having no clear distinction between different particles [37].

In order to explore the possibility of differentiating between the PVA and TH phases using thermal conductivity, 2 D topography and thermal conductivity images with corresponding pixel intensity histograms of PVA/TH, PVA, and PVA/NaOH were considered and are presented in Figs. 6–8.

Typical variations in thermal conductivity studies are between 0.01 and 0.04 mW [38]. All samples show a relatively uniform conductivity, with slight variations due to the topography of the samples. Since in most cases the variations are superimposed on topographical features, it is difficult to resolve images definitively.

Makovic et al. characterised chiral omeprazole sodium salts and used images and intensity histograms of topography and thermal conductivity to differentiate between amorphous and microcrystalline phases and proposed that conductivity is not a simple reflection of topography [39]. Using this histogram approach, all the images for thermal conductivity show a monomodal distribution, while the topography images all show a multimodal distribution. This suggests that there may be a slight systematic topographic difference in the height due to different phases present. The phases present in the PVA/TH sample may be due to TH, amorphous PVA and crystalline PVA. The phases present in the PVA/NaOH and PVA samples may be due to amorphous and crystalline PVA. However the differences between Figs. 6–8 are small enough to suggest that there is a uniform distribution of the components, specifically the distribution of TH in PVA.

3.3.2. Localised thermal analysis

Localised thermal analysis (LTA) is the process whereby, a thermal probe is placed at a selected point on
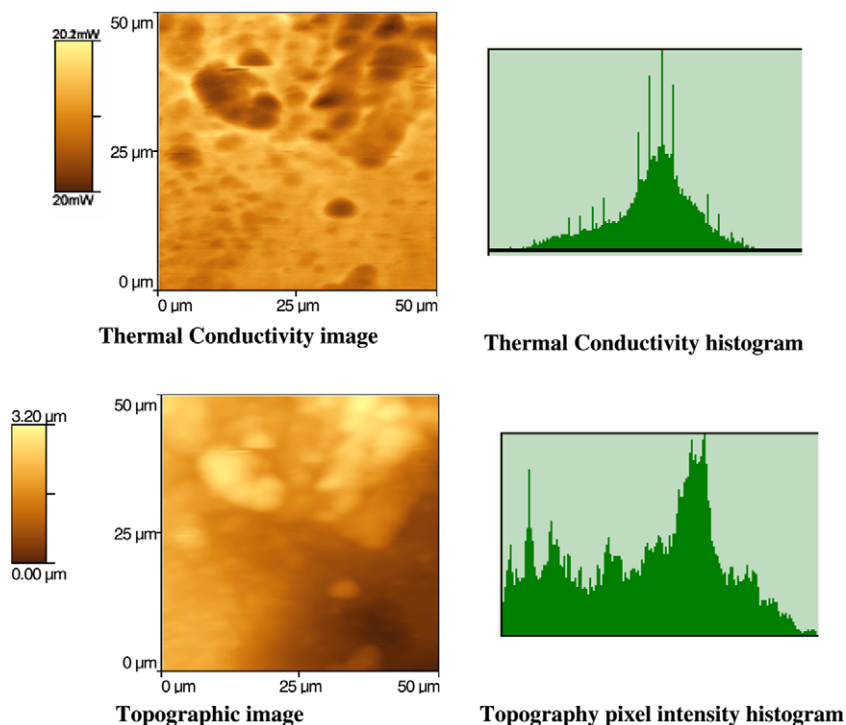
Fig. 6. Dried sample of PVA/theophylline: topography pixel image and thermal conductivity image with corresponding intensity histograms.
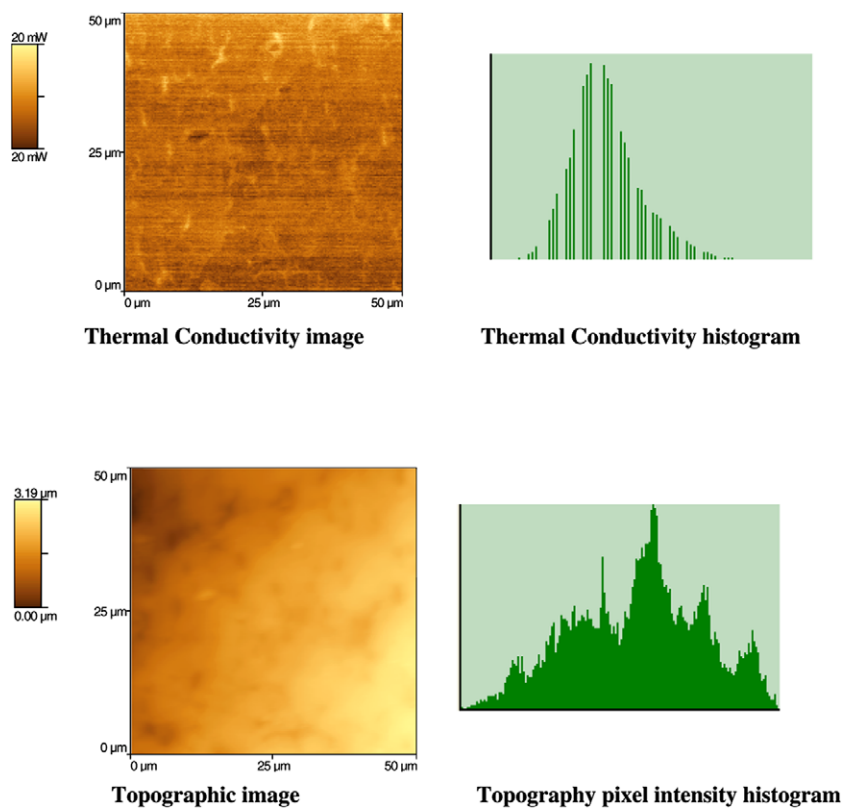

Fig. 7. Dried sample of PVA: topography pixel image and thermal conductivity image with corresponding intensity histograms.

the surface and the temperature ramped linearly with time. The power required to raise the temperature gives a calorimetry signal [33]. In addition to calorimetry, the position of the cantilever is measured. When the probe is placed on the surface, the cantilever is bent to a predetermined extent ensuring a controlled force on the tip. As the temperature
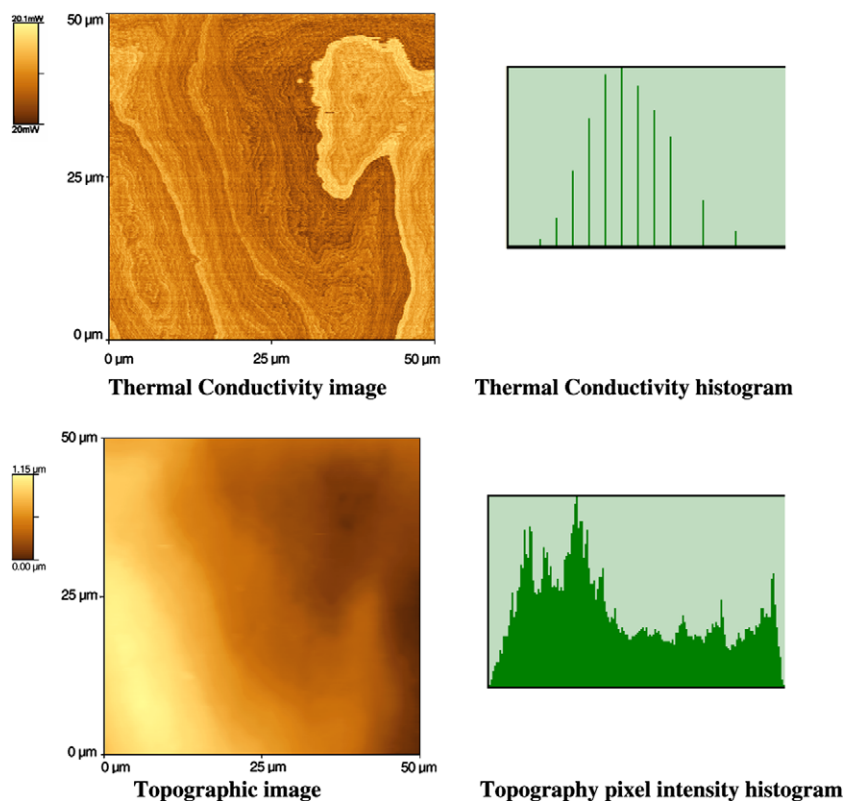
Thermal Conductivity image

Thermal Conductivity histogram

Topographic image

Topography pixel intensity histogram

Fig. 8. Dried sample of PVA/NaOH: topography pixel image and thermal conductivity image with corresponding intensity histograms.

rises the sample will soften and tip deflection is measured. The change in deflection of the cantilever is measured concurrently with the localised calorimetry and allows a micro-thermomechanical analysis of the sample [33]. Conventional DSC yields a specimen average response, as a relatively small sample is placed into a large heating container. Consequently the melting of a sample using μTA may not be directly comparable to DSC [40].

The results of a LTA measurement of frozen PVA in the presence and absence of TH are shown in Figs. 9 and 10. The results of a LTA measurement of frozen PVA with NaOH are displayed in Fig. 11. Examining Fig. 9, the
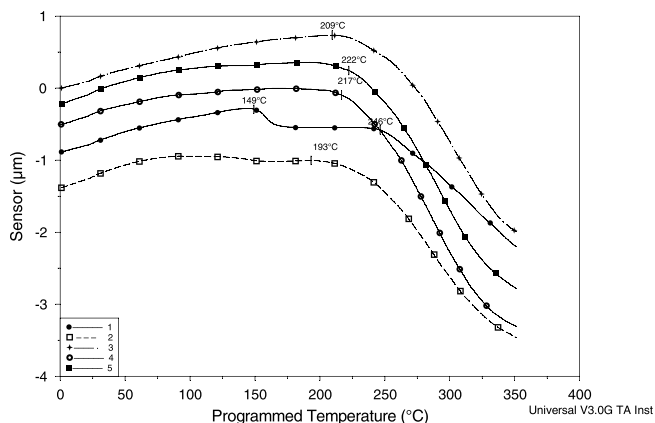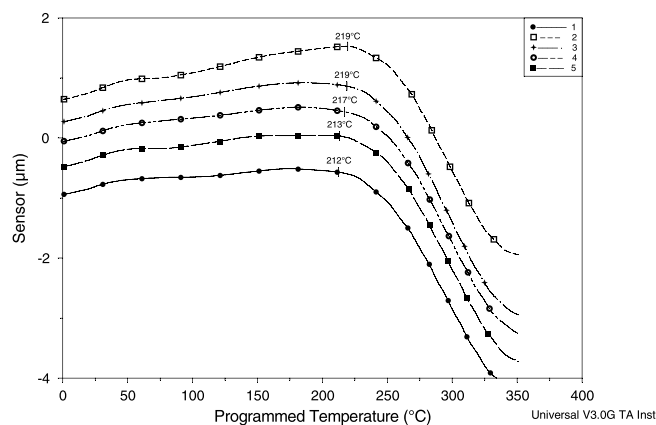

Fig. 10. Dried sample of PVA without TH: micro-MDTA endotherms.

traces for PVA and TH (3,5,4 and 2) show a penetration at 209, 222, 217 and 193 °C, respectively. The penetration of the probe into the material can be expected to occur, once most of the crystals are molten. This melting range agrees with the literature and the DSC results [41]. Trace 1 shows transitions at 149 and 246 °C. The lower transition is probably caused by the β relaxation, which is a relaxation in the PVA crystalline domains observed at 143 °C [27] alternatively, it may be due to the solid to solid transition of a metastable anhydrous TH [29].

The higher temperature transition of 246 °C could suggest the recrystallisation of TH; a similar transition was

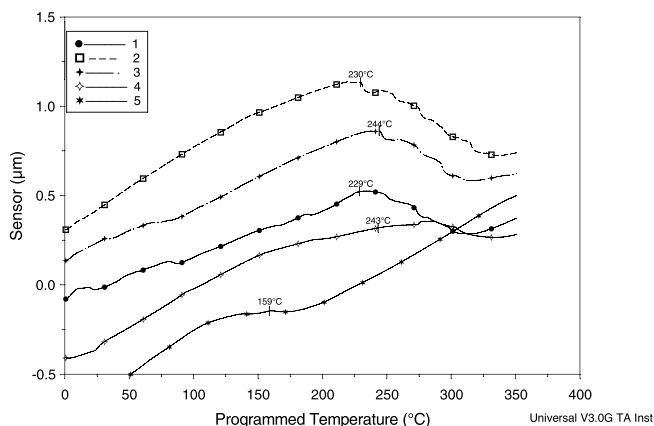Fig. 9. Dried sample of PVA with TH: micro-MDTA endotherms.

Fig. 11. Dried sample of PVA/NaOH without TH: micro-MDTA endotherms.

observed in the MDSC thermograms of TH on its own. For PVA in the absence of TH, as shown in Fig. 10, the melting of the crystalline regions occurs between 212 and 219 °C which is expected.

Examining Fig. 11, the traces for the PVA/NaOH show that the penetration of the probe occurs with greater difficulty and at a higher temperature. The lowest softening point is 229 °C while the highest is 244 °C. This indicates that the addition of NaOH has increased crystallinity and this will result in a stronger resistance to thermally or mechanically induced sliding motion of the chains [23]. This would explain the high tip deflection of the probe [32]. Trace 1 shows a transition at 159 °C which is probably due to the β relaxation [41].

3.4. Theophylline release studies

TH release was studied for a total of 840 min under specified conditions. When % release was plotted versus the square root of time, it showed a linear relationship between 0% and 60% indicating that TH release followed the Higuchi Matrix dissolution model [18]. Release rates
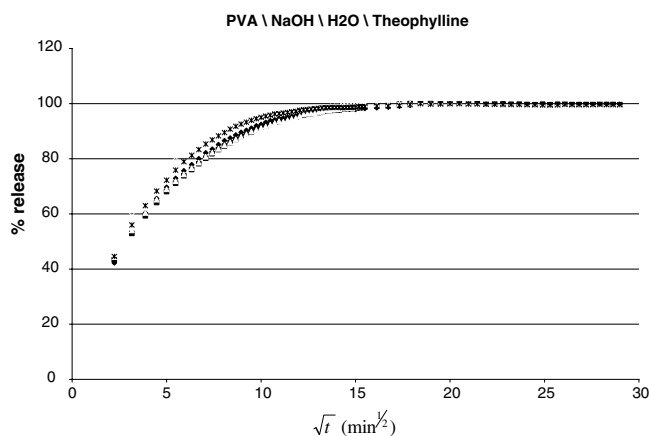
were calculated from Figs. 12 and 13. These were 4.6 (% release/$\sqrt{}$min) for PVA/NaOH and 5.6 (% release/$\sqrt{}$min) for PVA on its own. The slower rate from PVA/NaOH is probably due to the increased crystallinity of PVA/NaOH sample, which retards the release of the drug. In crystalline materials such as TH, hydrogen bonding is relatively weak, though it has been reported that there was a decrease in drug release from TH microcrystalline cellulose pellets prepared by wet granulation due to the formation of additional binding of TH and the microcrystalline cellulose [32].

3.4.1. Diffusion exponent

Peppas presented a simple semi-empirical equation which can be used to analyse data of controlled release of water soluble drugs from polymers [42]. The general form of the equation is:

$$\frac{M_t}{M_\infty} = kt^n \tag{1}$$

where M_t/M_∞ is the fractional release, k is the kinetic constant and n is the diffusion exponent. When $\log M_t/M_\infty$ is plotted against $\log t$, the value of the diffusion exponent was obtained. These were 0.29 and 0.33 for PVA/NaOH/H_2O and PVA/H_2O, respectively. These values are near the Fickian exponent value of $n = 0.45$ discussed by Peppas [15]. Thus TH release was controlled by a pure diffusion mechanism. Shaheen et al. discussed the use of PVA/NaCl/H_2O systems for the delivery of TH [18,19]. The drug release behaviour showed an irregular Fickian diffusion which broadly corresponds to our results.

Hickey and Peppas in the investigation of drug release from PVA found that repeated freezing/thawing cycles led to a denser crystalline structure with the changes in crytallinity having little effect on the mechanism of release [43]. However the formation of salts or crystalline ionic complexes is well established as altering the physicochemical properties of an API, with the inherent drawback of reduced control [44].
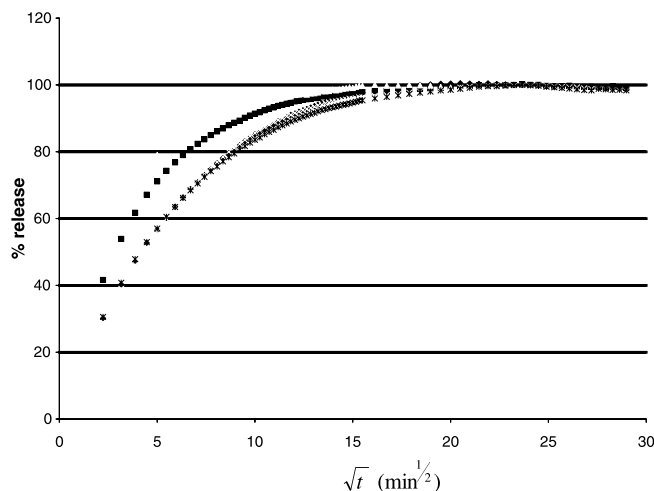

Fig. 12. Drug release profiles from PVA/NaOH/H_2O hydrogels in a phosphate buffer of pH 7.2 at 37 °C.

Fig. 13. Drug release profiles from PVA/H_2O hydrogels in a phosphate buffer of pH 7.2 at 37 °C.

TH forms a monohydrate in the presence of water and additional interactions may occur between TH and the PVA. The relatively fast release may be attributed to the high water content of the hydrogel (>90%). To achieve sustained release which is independent of the drug molecular weight, compounds may be entrapped in a second phase which is incorporated into the hydrogel [25].

4. Conclusion

The results of this study permitted the conclusion that this novel composite technique is suitable for the formulation of porous dosage forms containing TH as an API. The potential applications of PVA hydrogels are limited by weak strength and previous studies, by the authors, showed that the physical stability of freeze thawed PVA hydrogels was improved using NaOH. TH was incorporated into such a PVA hydrogel and its stability was further modified by the combination of the hydrogel and film into a novel composite.

The thermal properties of the various components were examined, as variations in the degree of crystallinity in a pharmaceutical substance may exhibit physiochemical differences that impact at therapeutic, manufacturing, commercial and legal levels.

This effect is further complicated when physically cross-linked hydrogels are used to incorporate an API as these hydrogels rely on hydrogen bonding and/or crystalline effects.

The thermal analysis shows distinct endotherms relating to the crystalline behaviour of PVA and the polymorphic nature of TH. This was shown in the MDSC thermograms where the melting of TH and PVA was examined. Other effects that may be present are related to TH changing to a monohydrate and the relaxation of the crystalline regions of the PVA polymer. The μTA investigation of conductivity and topography suggested that TH was uniformly distributed. The localised thermal analysis showed an endotherm similar to the recrystallisation of TH shown in the MDSC results. This has implications for the analysis of hydrogels where thermal behaviour of drugs is masked by excipients. Micro-thermal analysis provides a greater resolution and an increased confidence in analysis.

The drug release behaviour of the PVA/H_2O/NaOH and PVA/H_2O hydrogels was Fickian and though not greatly affected by the crystallinity, an effect is clearly present. A high release rate was also noted; however there may be scope to modify the drug release behaviour, by the judicious control of the film component of the composite substrate.

Our work suggests that the creation of a composite is a useful technique for producing improved properties and varying drug release behaviour. By varying such parameters as the initial aqueous concentration of PVA, molecular weight of PVA, freezing and thawing conditions, the composition of the substrate and by the use of different APIs, we can likely further modify and enhance the properties of the composite. Such composites with enhanced swelling and physical properties show promise for a variety of applications in the biomedical and pharmaceutical areas.

Acknowledgements

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