Investigation of the effects of orientation on freeze/thawed Polyvinyl alcohol hydrogel properties

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Abstract

Although hydrogels produced via freeze-thawing (F/T) technique have been thoroughly studied in literature, stretching hydrogels in between freeze-thawing cycles could change its properties and might be modulated for drug delivery systems. The PVA hydrogels and Caffeine-contained PVA hydrogels were created by freezing for 20 minutes using liquid nitrogen and 4 hours thawing at 4° C. The results revealed that the PVA/CAF hydrogel with two F/T cycles followed by two stretching (S) cycles delivered the best response. Furthermore, this sample had the highest crystallinity degree and Young's modulus of 36 % and 1462 MPa, respectively, which makes this hydrogel very stiff in comparison to all other samples. Moreover, it fits in the Hixson-Crowell drug release model with the fastest drug releasing rate, 15 minutes, and the highest swelling degree of 470 %. Therefore, the study suggests that the number of F/T cycles and stretching cycles influences the properties of the resulting hydrogels and the caffeine release rate.

Keywords: Hydrogel; Freeze-thaw cycle; Polyvinyl alcohol; Polymer orientation; Caffeine; Drug release kinetics

1. Introduction

As a biomaterial, hydrogel plays an important role in biomedical applications. Hydrogels have great potential to produce various type of products for wound care, hygiene products, tissue engineering and drug delivery [1]. Hydrogels proved to have the benefits of improving water retention ability and controlled drug release. PVA is a water-soluble synthetic polymer which can form highly swollen gels [2]. The network of Polyvinyl alcohol (PVA) polymer chains contain a large number of hydroxyl groups (-OH) that are responsible for the polymer's hydrophilicity [3]. These hydrophilic side chains result in a structure capable of trapping water without dissolving in the polymer matrix [2]. Furthermore, the numerous hydroxyl groups present on the backbone of PVA hydrogel offers the possibility of attaching drugs and cell

signalling molecules [4]. Therefore, PVA has been identified as a suitable candidate to be used for pharmaceutical applications in releasing biological and medical materials in a controlled way [5]. Yet, they are not limited to use for long-term biomedical applications as membranes or coatings for implantable devices [6].

Orientation can induce a uniaxial polymer chain alignment while maintaining sufficient porosity and water content (>95 %). The stretched hydrogels are mechanically stronger and easier to handle than typical hydrogels of the same composition and dimensions [7]. In addition, they can induce alignment for cells seeded on or within the polymer matrix to increase cells adhesion, migration, and orientation in the field of tissue regeneration. Wang et al. have investigated that cells which are randomly oriented in non-stretched hydrogels. Under the effect of orientation, the growth of cells are aligned almost perpendicularly to the stretching direction [8]. Furthermore, the highly orientated hydrogels exhibit Hixson-Crowell drug release kinetics. This model is normally used to evaluate the dissolution rate and is proportional to the surface area of spherical particles or tablets. It interprets the drug release rate of immediate-release products [9].

The effect of stretching after the formation of freeze/thawed PVA films have been investigated as a function of the stretching ratio by Fukumori and Nakaoki [10], the stretched PVA after annealing at 130° C shows that the molecular morphology of extended-chain crystals can further improved its tensile strength and Young's modulus. However, we believe that the effect of stretching of PVA hydrogels in between freeze-thawing cycles can exhibit important parameters and physical aspects for modulation of drug delivery as well as mechanical properties. The hypothesis of this is, as the stretching occurs the polymer chains have a tendency to align in the direction of the stretching and, in between the freeze-thawing cycles, physical crosslinking occurs throughout this orientation. In addition, the mechanical and chemical properties of PVA hydrogels are tuneable. The number of F/T cycles, temperature domain of freezing and thawing, stretching ratio and rate, concentration of PVA and drug, as well as molecular weight of PVA can all alter the polymer structure if the aim is to analyse this behaviour [11]–[13].

For these reasons, in this study, the development of PVA hydrogels have acted as a drug carrier, while caffeine is utilised as a model drug. Caffeine is used to prepare fast dissolving drug delivery systems due to its high water solubility [14], [15]. It is widely used as an analgesic adjuvant/mild stimulant in combination with analgesic (e.g. paracetamol, ibuprofen, and

aspirin). This type of medicines can augment their bioavailability and deliver rapid onset of action [16], [17]. The resultant PVA/CAF hydrogel samples have undergone detailed characterisation of chemical, polymer orientation, mechanical and thermal properties. A drug release study and swelling study have also undertaken and compared with the pure PVA hydrogel samples.

2. Experimental

2.1 Materials

PVA (Mw: 195,000 g/mol) with 98–98.8 % hydrolysed was purchased from Sigma-Aldrich. Anhydrous caffeine powder (Mw: 194.19 g/mol), used to produce PVA/CAF hydrogels, was purchased from Amresco. Distilled water was used as the solvent in the study.

2.2 Preparation of pure PVA and PVA/caffeine solutions

10 % w/v pure PVA solution was prepared by mixing 10 g PVA polymer powder with 100 ml distilled water. In addition, 10% w/v PVA/CAF solution was prepared by mixing 10 g PVA polymer powder and 3% caffeine powder (according to 10g of PVA) with 100 ml distilled water.

Dissolution was achieved by heating both the mixtures to 75° C for 90 minutes using electromagnetic stirrer. The mixing process can help to get a homogeneous and crystal clear solution. When the polymer was no longer apparent and the mixture was clear, the solution was cooled for 10 minutes at room temperature to remove any bubbles in the solution.

2.3 Freeze-thawing

A small portion of the polymer solution was cast into the pre-made dumbbell-shaped High Impact Polystyrene (HIPS) mold. They were then subjected to 20 minutes fast freezing using liquid nitrogen and the solidified solutions were thawed for 4 hours at 4° C. Upon thawing, hydrogels were formed. This process is referred to as F/T technique. The samples were produced with the thinnest possible configuration to refrain from obtaining two-strong orientations (x axis and y axis) that would interfere in the results and develop into a complex behaviour.

Table 1. Summary of the PVA hydrogel samples prepared.

Sample Code	Freeze-thaw cycle	Uniaxial orientation cycle (100% stretching strain)
PVA 1FT	1	0
PVA/CAF 1FT	1	0
PVA 2FT	2	0
PVA/CAF 2FT	2	0
PVA 1FT1S	1	1
PVA/CAF 1FT1S	1	1
PVA 2FT2S	2	2
PVA/CAF 2FT2S	2	2

Table 1 exhibits the types of samples prepared in this study. All samples with different F/T cycles and uniaxial orientation cycles (100 % stretching strain per cycle) (Figure 1) were dried at 40° C in Heraeus VT-5042 Vacuum Oven for 3 days. To maintain the geometry of PVA hydrogels, samples were dried using clamps in the extremities. This allowed the water to evaporate and minimizes the effect of forcing the polymer chains to deform. The samples were characterized by dissolution study, swelling behaviour, tensile strength, MDSC, FTIR, DMA and SEM. All the analysis have been performed on the given samples within two months to minimise the effect of aging.

Figure 1. PVA sample with one F/T cycle and one uniaxial orientation cycle.

2.4 Solvent casting

Solvent casting (SC) was performed with the polymer solutions described in Section "Preparation of pure PVA and PVA/caffeine solutions". 1 ml of each solution was pipetted into a Petri dish and homogeneously spread on its glass surface. The Petri dishes were left at room temperature for at least 2 days. The solvents were allowed to evaporate completely before being stored or tested.

2.5 Scanning Electron Microscopy

The hydrogel composite orientations were observed using a scanning electron microscope (SEM) (TESCAN Perfomance in Nanospace) with the back-scattered electron (BSE) mode. Prior to imaging, the samples were sliced to obtain cross-sectional regions and sputter-coated with a gold using Baltec SCD 005 for 110 s at 0.1 mBar vacuum. Images were recorded at an accelerating voltage of 15 kV and a magnification range of 300-1,000.

2.6 Fourier transform infrared spectroscopy

PerkinElmer Spectrum One Fourier transform infrared spectroscopy (FTIR) was used to investigate the existence of chemical interactions between caffeine and PVA. The IR spectra were recorded in the spectral range of 4000–500 cm^{-1} and subsequent analysis were carried out using Spekwin32 spectroscopy software.

2.7 Differential scanning calorimetry

For thermal analysis, differential scanning calorimetry (MDSC) was carried out using DSC2920 Modulated DSC from TA instruments. All the samples were sealed in hermetic aluminium pans (Hermetic Pans, TA Instruments, USA) with the sample weight around 7-10 mg. Each sample test was performed with a heating ramp mode from 20 °C to 280 °C and heating rate of 10 °C/min under the flow of nitrogen gas.

The degree of crystallinity, Xc of the hydrogel samples was determined from the endothermic area using the following equation:

$$X_c = \frac{\Delta Hf}{\Delta Hf_o} \quad (1)$$

where ΔHf is the measured enthalpy of fusion of the PVA hydrogel sample and ΔHf_o is the thermodynamic enthalpy of fusion for 100% crystalline PVA (ΔHfo=150 J/g) [18]–[21].

Results of DSC presented in this manuscript, a five-digit number that was automatically obtained by the equipment provider and should not be taken literally.

2.8 Swelling behaviour

The swelling degree of the hydrogel samples was measured by immersing the dried samples in Petri dishes that were filled with distilled water. The samples were allowed to soak at room temperature. The swollen hydrogels were removed from the water and blotted with filter paper to remove any surface water. The samples were weighted and subsequently placed in water over the 24 hours of soaking. The degree of swelling was calculated using the following equation:

$$Degree\ of\ swelling = \frac{W_t - W_d}{W_d}\ x\ 100 \quad (2)$$

where W_t is the weight of swollen hydrogels at regular intervals and W_d is the weight of dried hydrogels.

The percentage of water content of hydrogel samples was calculated by measuring the weight of swollen hydrogels at regular intervals (W_t) until the hydrogels reached the equilibrium state of swelling (weight= Ws) using the following equation:

$$Water\ content\ (\%)\ = \frac{W_t - W_d}{W_s - W_d}\ x\ 100 \quad (3)$$

2.9 Dynamic mechanical analysis

The viscoelastic behaviour of the dried hydrogel samples was measured using a TA Instrument Q800 DMA with a tension mode. The samples were cut into rectangular shapes (25×10 mm). They were tested at 3 °C/min heating rate with 1 Hz frequency over the temperature sweeps in a range of −80 °C to 220 °C and oscillation amplitude of 10 µm under a nitrogen atmosphere. The loss tangent, also defined as Tan Delta was obtained for each sample. It is equal to the ratio of loss modulus (G") to the storage modulus (G′).

Results of DMA presented in this manuscript, a five-digit number that was automatically obtained by the equipment provider and should not be taken literally.

2.10 Tensile strength

Tensile test was used to evaluate the effect of the F/T cycles with uniaxial orientation on mechanical properties of the dried hydrogel samples. The test was carried out on a table top mechanical testing machine (Lloyd Instruments LRX material tester, AMETEK.Inc.) equipped with a gauge length of 60 mm and a drawing rate of 50 mm/min. The dried hydrogel strips of

160 mm length, a width ranging between 14 and 1.5 mm and a thickness ranging between 1.5 and 0.25 mm were prepared. The dried samples were folded a little bit at the end of both sides to facilitate gripping in the mechanical testing apparatus. During the tests, the samples were stretched to break. All samples must broke in the middle and not at the clamps. The mechanical test was repeated three times for each sample and the average values were used to plot the stress vs strain curves. The Young's modulus (E) of each samples was calculated by finding the maximum slope of the stress vs strain curves as followed:

$$Young's\ modulus = \frac{Stress}{Strain} = \frac{\frac{F}{A}}{\frac{\Delta L}{L_o}} \qquad (4)$$

where F is the force in Newtons (N), A is the cross section area in m^2, ΔL is extension measured in metres and Lo is the original length measured in metres.

2.11 Dissolution studies

Dissolution testing was evaluated using a DISTEK Model 2100B dissolution system. 50 mg of the dried PVA/CAF hydrogel samples were weighted and tested in a phosphate buffer of pH 7.4 at 37 °C. The stir rate was set to 50 rpm with 900 ml of dissolution buffer used per vessel. The test was carried out for 60 minutes. 3 ml of each sample was taken every 5 minutes for the first 30 minutes and every 10 minutes for the next 30 minutes. 3 ml of dissolution buffer was replaced after each time of samples removal to maintain a sink condition. The taken samples were analysed using a UV/vis spectrometer (Shimadzu Spectrophotometer UV-1280) at 273 nm with a 1 cm quartz cuvette. The concentration of caffeine release from the PVA hydrogels into the buffer was determined.

The dissolution profiles were evaluated using model dependent approaches such as zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell model. The five models were all based on different mathematical functions. The first mathematical model is called zero-order model, which describes the drug release rate as independent of its concentration of the dissolved substance. The second model is called first-order model, which describes the drug release rate depends on its concentration of the dissolved substance. The third model is called Higuchi model, which describes the drug release by Fick's law of diffusion [22]. The fourth model is called Korsmeyer–Peppas model, which suggest the drug transport mechanism of the polymeric materials was controlled by more than one process according to the value of release exponent, n. For the case of diffusional release mechanisms from polymeric films, $n \leq 0.45$ corresponds

to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to Super case II transport [23]. The fifth model is called Hixson–Crowell model, which describes the release from systems where there is a change in surface area and diameter of particles or tablets [24]. The determination coefficient (R^2) was used as an indicator of the fitting level of the data to each model [25].

3. Results and discussion

Visual observations

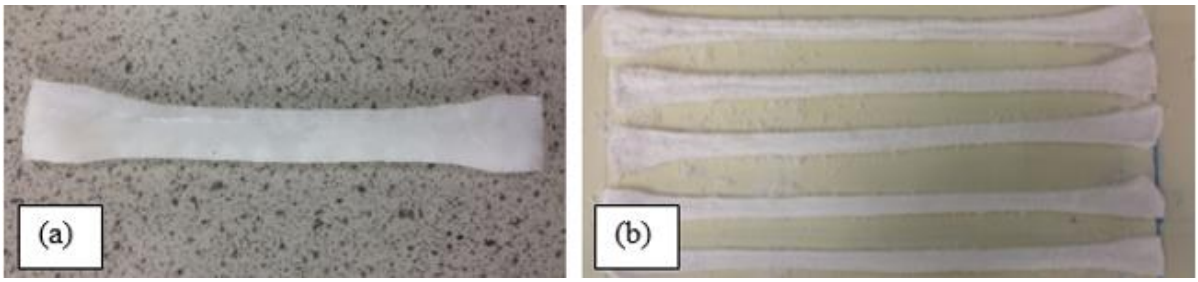

Figure 2. Freeze-thawed PVA hydrogel (a) before and (b) after stretching.

Samples stretched presented a larger surface area due to the stretching mechanism but with a similar appearance to non-stretched samples (Figure 2). The technique used to stretch the hydrogels was effective since no cracking or fractures in between the production (F/T cycles) of the stretched samples was detected.

3.1 Scanning Electron Microscopy

SEM images (Figure 3) were taken to visualize the microstructure morphologies of the interior part of hydrogel samples. In these images, differences can be seen between PVA hydrogels that underwent only F/T cycles and PVA hydrogels that underwent F/T cycles with uniaxial orientations. The non-stretched samples (Figure 3A-B and 3E-F) presented a known morphology typical of PVA hydrogels. Small amounts of orientations were observed due to the drying mechanism to maintain its geometry. For stretched samples, it was possible to identify an increase in orientation in the morphology structure, with more cycles of stretching and F/T. However, when comparing the stretched samples to the non-stretched samples, non-stretched samples seems to indicate a smoother surface. Whereas stretched samples seem rougher, this can be deduced, it is purposes, based on the polymer chains that are forced physically to change their direction which occurs with

the stress applied to the extremities of the stretched; and it actuated on the hydrogel as point of forces and this results in the alignment of the chains in the direction of these points. This concept is further looked at the FTIR scans. The PVA/CAF 1FT1S and PVA/CAF 2FT2S samples have less orientations, being assigned to recrystallized caffeine during drying process, as compared with the PVA 1FT1S and PVA 2FT2S samples. The formation of an amorphous dispersion of the drug in PVA hydrogels was desired for better orientation and drug dissolution. Furthermore, the alignment of these hydrogels appeared to be toward a very specific direction (generally upwards in the SEM images (Figure 3) and this was due to the stretching mechanism produced, since one side was glued and the other side promoted the stretching, the chains were then aligned towards that specific point of force.

Moreover, the images also exhibited differences between pure PVA and caffeine-content PVA hydrogels. During F/T cycles, caffeine tends to form crystals in long needles-shape [26], [27]. When compared with pure PVA hydrogel samples via SEM, the surface of caffeine-content PVA hydrogel sample (Figure 3H) was rougher. This was due to the drug crystallisation and it might have increased the crosslink of the hydrogel system, leading to these chains to react as points of force against the one applied by the clamp in the drying mechanism. With more F/T cycles in PVA/CAF hydrogels exhibited a more aligned direction and it was improved with more stretching cycles.

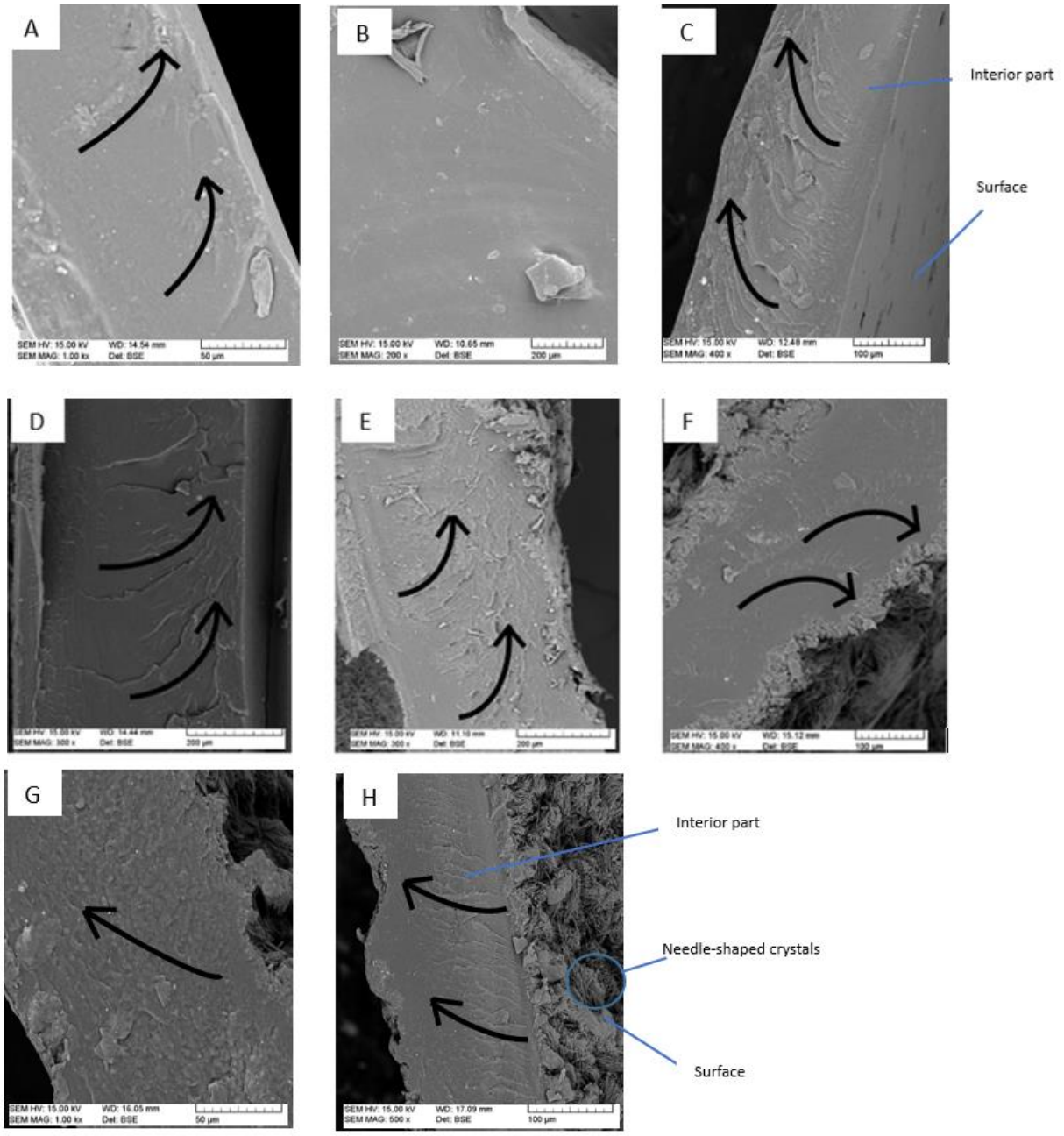

Figure 3. SEM micrographs of the interior part of PVA hydrogel. (A)PVA 1FT, (B) PVA 2FT, (C) PVA 1FT1S, (D) PVA 2FT2S, (E) PVA/CAF 1FT, (F) PVA/CAF 2FT, (G) PVA/CAF 1FT1S, (H) PVA/CAF 2FT2S.

3.2 Fourier transform infrared spectroscopy

The FTIR spectra (Figure 4) have shown the characteristic peaks of the pure PVA and caffeine-content PVA hydrogel samples. Both type of hydrogels exhibited no differences in the characteristic FTIR molecular mode for PVA. All major peaks related to hydroxyl and acetate groups were observed. The large bands observed between 3570 and 3200 cm^{-1} [28] were linked to the stretching O–H from the intermolecular and intramolecular

hydrogen bonds (region (I) in Figure 4A and 4B). The very broad band around 3300 cm^{-1} was attributed to the presence of water that was absorbed by PVA molecular chains as the orientation of chains leaded to a significant storage of water. In addition, the pure PVA hydrogels have broader and higher O-H peaks in comparison with PVA/CAF hydrogels. It is a fact that the peaks of the functional groups belonging to caffeine decreased and disappeared because they have been hidden by the strong and broad bond stretches of PVA hydrogel [22]. The vibrational band observed between 3000 and 2850 cm^{-1} [28] referred to the stretching C–H from alkyl groups (region (II) in Figure 4A and 8B). The peaks between 1750 and 1735 cm^{-1} [1] (region (III) in Figure 4A) were due to the stretching C=O from acetate group remaining from PVA. While, the spectra showed two peaks in the region (III) in Figure 4B for the C=O stretching, which came from both acetate and carbonyl groups [29]. Furthermore, Figure 4B also shown the major peaks of caffeine at 3500-3100 cm^{-1} (N-H stretching), 3100-3000 cm^{-1} (CH3 stretching), 1600-1400 cm^{-1} (C=C stretching from aromatic ring), 1360-1080 cm^{-1} (C-N stretching) and 900-690 cm^{-1} (C-H bending & ring puckering) [29], [30]. The above data suggests that there were no physical or chemical interactions between PVA and caffeine in the hydrogel samples.

The degree of crystallinity of the hydrogels were obtained at 1141 cm^{-1} (region (IV) in Figure 4A and 4B), which was further discussed in DSC section. The presence of caffeine resulted in broader peak at 1141 cm^{-1} due to the increase in crosslinking density caused by drug crystallisation. This was also supported by the work published by Seif and his research team, where it was shown that caffeine was a hydrophilic model drug with high crystallisation tendency. When caffeine combined with PVA (hydrophilic polymer), it was used to form immediate release matrices [31].

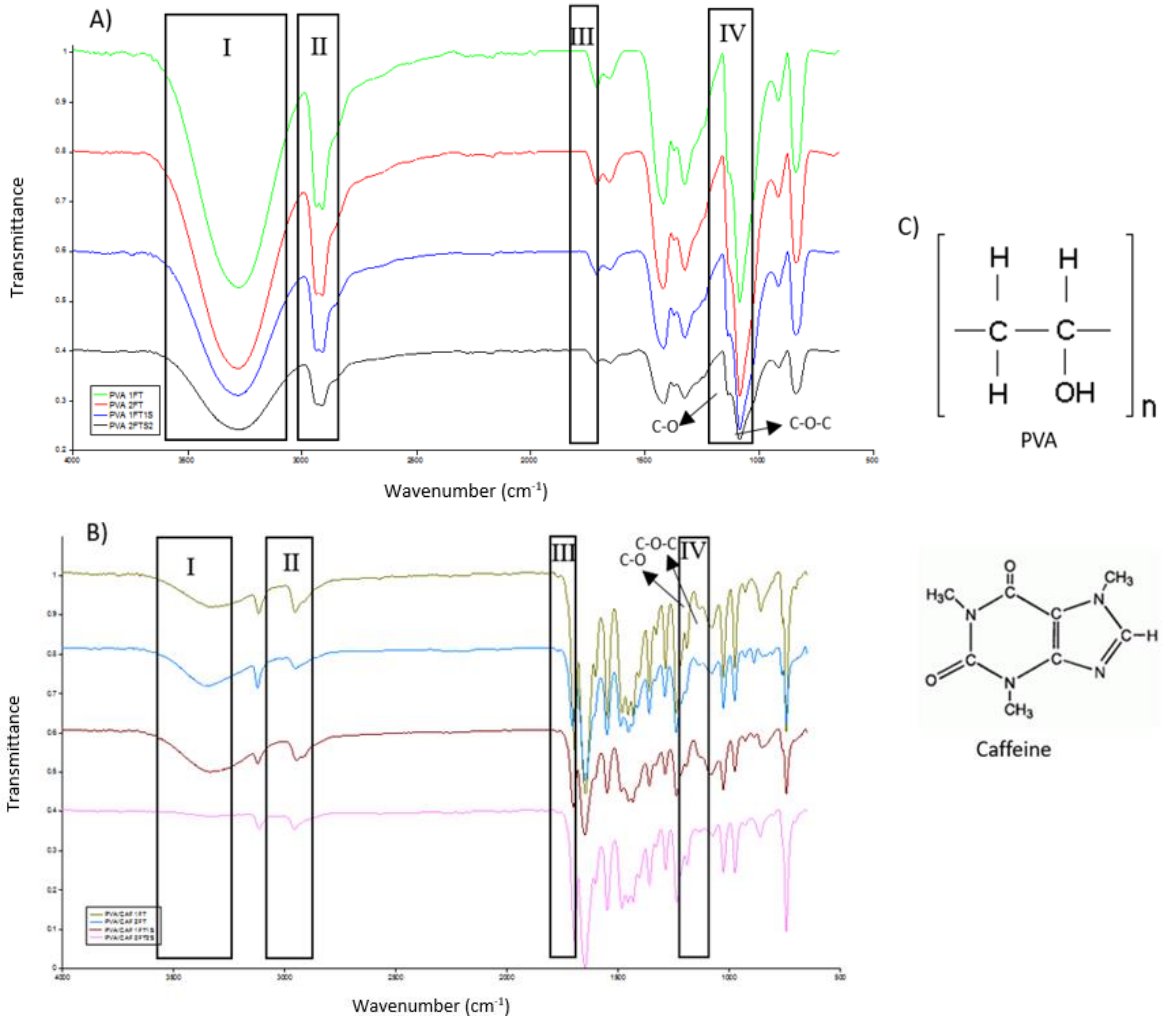

Figure 4. FTIR spectra of the original samples (a), PVA (b), PVA/CAF (c), PVA & CAF chemical structure.

3.3 Differential scanning calorimetry

A typical DSC thermogram for a pure PVA sample exposed to repeated F/T and orientation cycles was shown in Figure 5. The peak at the range of 40-64 °C, designated as the α relaxation, represents the glass transition temperature (Tg) of PVA. The Tg of the solvent casted of pure PVA sample was at 64 °C and the Tg decreased to around 40 °C with the presence of F/T and orientation cycles. This low value was caused by a plasticising effect in the presence of water in the hydrogel samples and, consequently decreases the Tg [32]. While, the broad peak observed at approximately 140 °C represented the evaporation of residual water present in the samples [33], which also designated as the β relaxation, was due to the relaxation in the PVA crystalline domains. The relatively large and sharp peak with a peak around 223 °C, represented the melting temperature (Tm) of the PVA crystalline domains [34]. Upon the increase of F/T

and orientation cycles, the endothermic curve of PVA became sharper and its peak shifted to a higher temperature. In another case, the effect of caffeine on the thermal transitions of PVA is showed on Figure 6. The Tg appeared to increase with the addition of caffeine and the transitions picked up in the region of 45 to 66 °C for the PVA/CAF samples. An increase in Tg implied less chain mobility and therefore more inter- and intra-molecular hydrogen bonding contained in the PVA polymer [32]. However, the Tm was decreased from approximately 224 °C to between 215 °C and 218 °C in the presence of caffeine. The decrease seems counter-intuitive but it is explained by a study with caffeine and PET where caffeine reduces the free volume of the polymer by filling a portion of the idealized Langmuir "microvoids" [35]. This excess free volume where the caffeine is stored, decreases and further disappears when the Tg is reached [36], then any caffeine in the PVA volume will be liberated to enrich the dissolved mode as the temperature increases and this effect produces a reduction in Tg values for the mixture compared to the neat polymer.

The degrees of crystallinity as shown in Figure 7 were calculated by determining the area under the melting peak in Figure 5 and 6. As expected, increasing the F/T cycles leads to an increase in crystallinity. In addition, samples with stretching exhibit higher values of crystallinity compared to non-stretched samples and it further increases with more stretching. The ordered structure presented by the stretching allows a more crosslinking density due to the easy arrangement of the chains; this further improves the Tg of the polymer. Furthermore, the PVA/CAF hydrogel samples have higher degree of crystallinity in comparison to pure PVA hydrogel samples. The increase in crystallinity in PVA/CAF hydrogels is due to the caffeine is a crystalline material [22]. As the caffeine exhibited an endothermic peak around 235 °C (Figure 6), corresponding to crystalline caffeine melting point [37], crystallisation of caffeine did not only occurred in PVA during F/T cycles, but also in the drug itself. Studies have shown that caffeine remain in solid form at the melting point of PVA in hydrogel samples [38]. Furthermore, the increase of axial orientation has also exhibited the growth of the hydrogel crystallinity which caffeine seems to have further improve it. On the other hand, the DSC curve of the solvent casted PVA/CAF hydrogel did not show the characteristic melting peak of crystalline caffeine. This finding suggests that crystalline caffeine was converted into the amorphous state during dissolution into water [39].

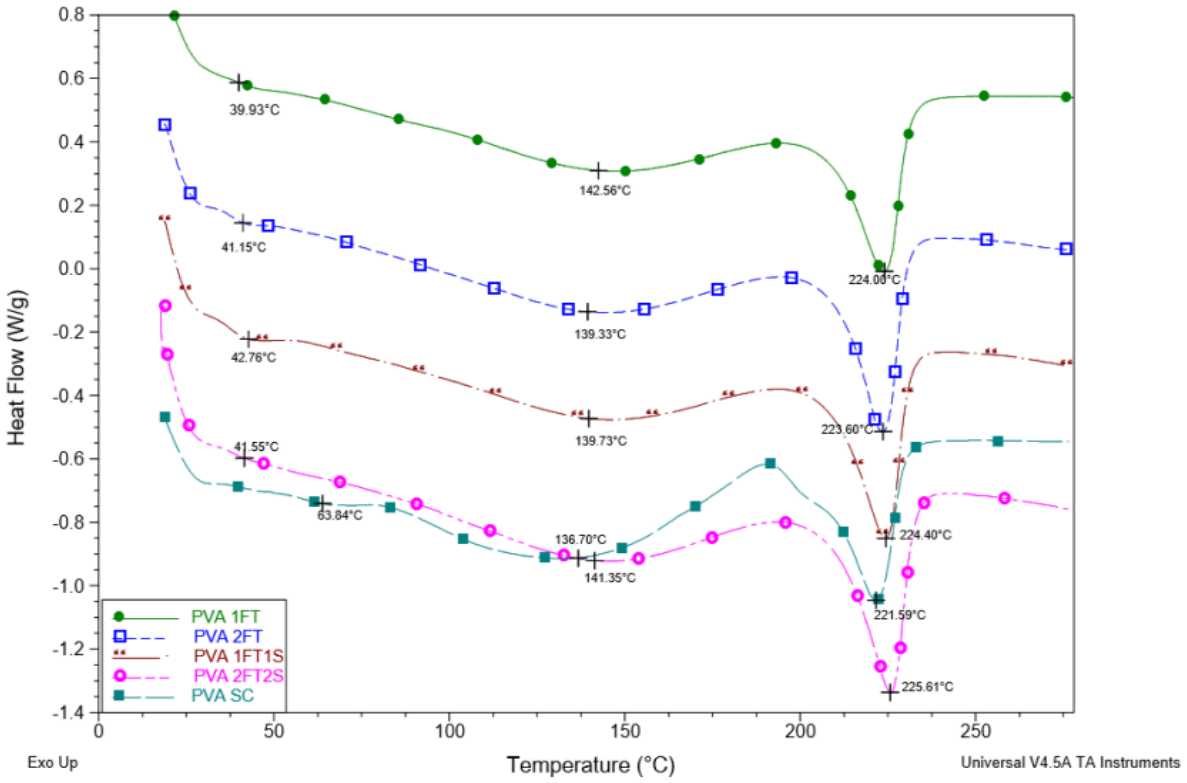

Figure 5. Thermal transitions in PVA samples.

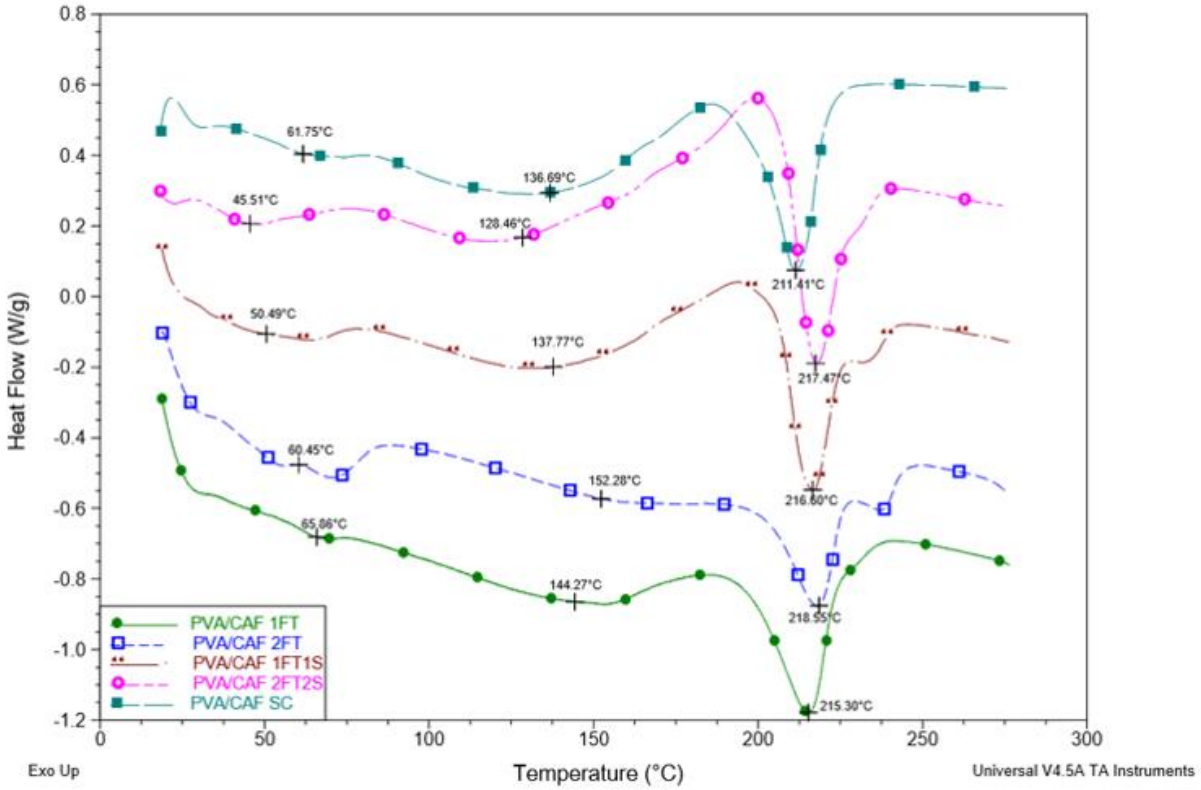

Figure 6. Thermal transitions in PVA/CAF samples.

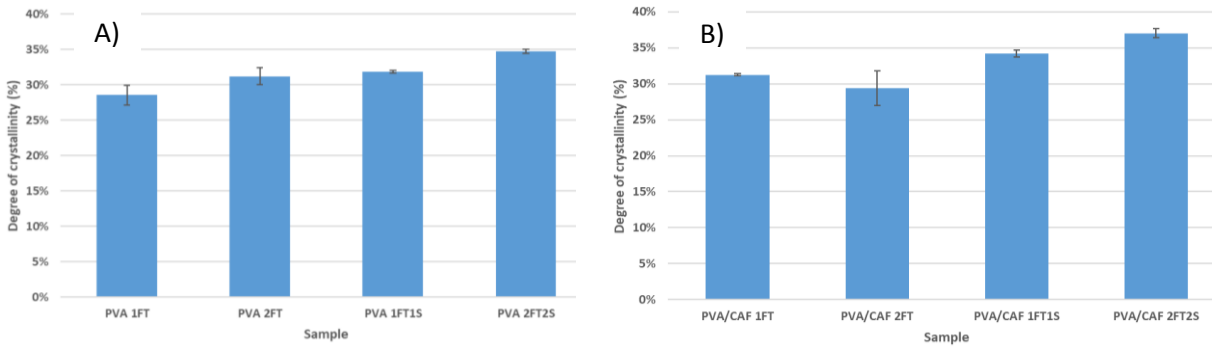

Figure 7. Degree of crystallinity, (A) PVA samples, (B) PVA/CAF samples.

3.4 Swelling behaviour

Swelling characteristics, particularly the swelling degree in rehydrated pure PVA and PVA/CAF hydrogels were investigated, hydrogel samples were considered as swelling controlled-release devices. Before swelling occurs, the drug molecules were entrapped within the PVA polymer matrix and when the hydrogels absorbed water, the molecular weight between cross-links increases and lead to polymer expansion. This essentially means that the water molecules penetrated between the polymer chains and enable the drug molecules to diffuse out of the swollen polymer networks [1], [40]. Figure 8 depicted the degree of swelling and the water content (%) of hydrogel as a function of time for pure PVA and PVA/CAF hydrogel samples. The swelling kinetics of hydrogels were estimated to vary in the number of F/T cycles and orientation cycles.

In Figure 8A, the maximum degree of swelling in 24 hours for PVA 1FT, PVA 2FT, PVA 1FT1S and PVA 2FT2S are 301 %, 295 %, 380 % and 371 %, respectively. The pure PVA samples which have underwent F/T and orientation cycles have the higher swelling degree than the pure freeze-thawed samples without orientation cycles. In addition, PVA 1FT1S and PVA 2FT2S samples reached the equilibrium swelling after 24 hours of soaking. Whereas, PVA 1FT and PVA 2FT samples did not reach equilibrium within 24 hours, both of the samples have only reached the water content of 91 % and 95 % respectively. This result can further justify that the hydrogels with uniaxial orientation need lesser time to reach the EWC value.

In Figure 8B, PVA/CAF 1FT, PVA/CAF 2FT, PVA/CAF 1FT1S and PVA/CAF 2FT2S have the maximum degree of swelling of 287 %, 262 %, 307 % and 470 %, respectively. The PVA + CAF samples have a lower swelling degree when compared to pure PVA samples, except for the PVA/CAF 2FT2S. The low swelling values are attributed to the caffeine being a hydrophilic

model drug with high crystallisation tendencies [26]; this caffeine crystallites within the PVA polymer chains leading to small size pores [41] and a decrease in swelling degree. In addition, PVA/CAF 2FT2S has the highest swelling degree of 470 % in contrast to all the PVA and PVA/CAF samples which might be caused by the crystallinity presence of caffeine and the increased number of orientation cycles applied to the hydrogel.

The samples with one F/T cycle (i.e. PVA 1FT, PVA/CAF 1FT) have higher swelling degree than the samples that underwent two F/T cycles (i.e. PVA 2FT, PVA/CAF 2FT), because the degree of swelling consistently decreased with increasing numbers of F/T cycles [42]. Under the effect of orientation, the polymer chains have formed uniaxial polymer chain alignments while maintaining its structure and water content. Therefore, stretched samples gave higher swelling degree in comparison with hydrogel samples without orientation. It is also verified that the release rate of caffeine increase with degree of hydrogel swelling. Furthermore, all PVA/CAF hydrogel samples have achieved an equilibrium state of swelling within 24 hours. An equilibrium was achieved within 7 hours for PVA/CAF 1FT, 19 hours for PVA/CAF 2FT, 4.5 hours for the PVA/CAF 1FT1S and 4 hours for PVA/CAF 2FT2S. A reduction of time was determined to reach the EWC value within PVA/CAF samples confirmed that the presence of caffeine influences the swelling rate since the water can penetrate easily into the polymer network after the caffeine has dissolved into the water.

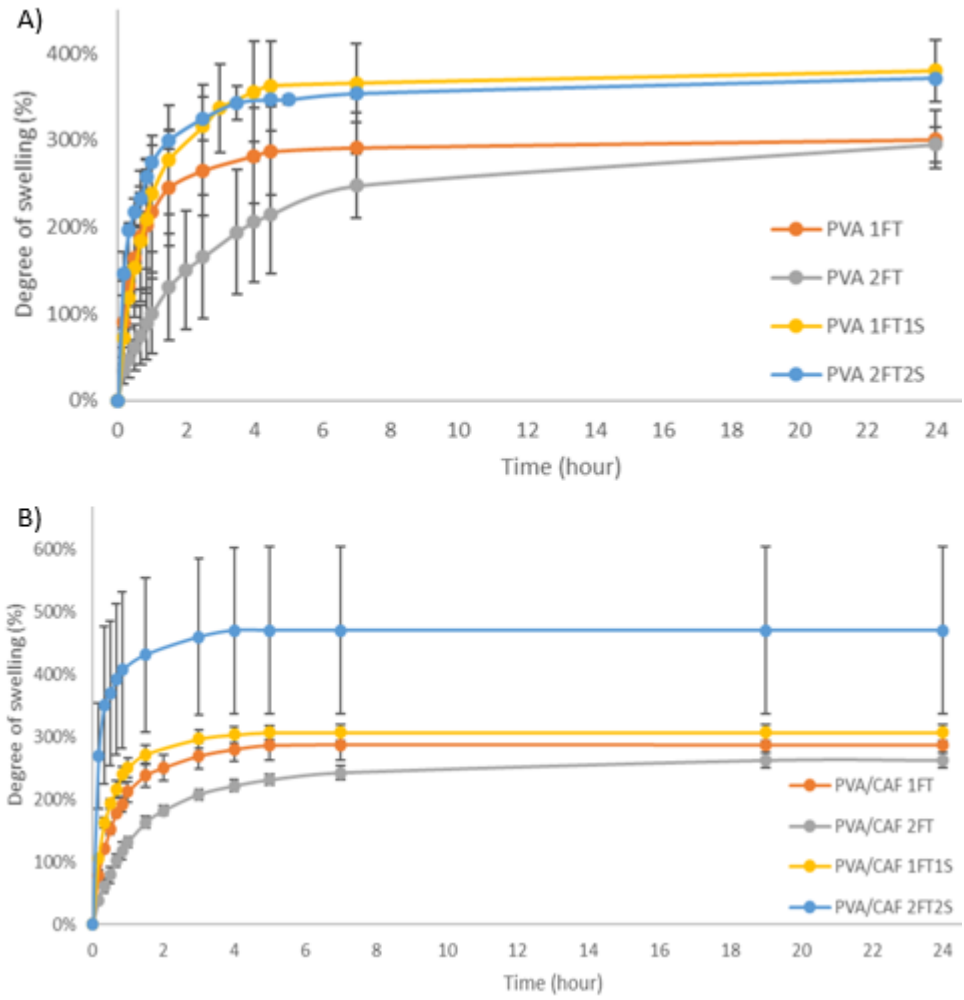

Figure 8. (A) Swelling degree of PVA samples, (B) Swelling degree of PVA/CAF samples

3.5 Dynamic mechanical analysis for PVA/CAF hydrogels

From DMA measurements, the storage and loss modulus as a function of temperature was determined. The presence of a single Tg was observed in the tan δ versus temperature curves as can be seen in Figure 9. The thermogram has shown the Tg of the PVA/CAF 1FT, PVA/CAF 2FT, PVA/CAF 1FT1S and PVA/CAF 2FT2S, being 40 °C, 49 °C, 49 °C, and 52 °C, respectively. As expected, with increasing crosslink density, this transition was shifting toward higher temperatures [43]. This behaviour was due to the hydrogen bonding causing more chain stiffness and rigidity, leading to less flexibility in the PVA [44]. This property was further confirmed by the tensile tests. Moreover, the amount of water attained in the hydrogel was decreased with increasing crosslinking density. In general, as the water content of hydrogel decreases, the storage modulus increases and the Tg also increases [45]. The Tg data obtained from DMA are always

different from that of DSC, and DMA-based Tg is more accurate [46]. While, with increasing uniaxial orientation, the transition was also shifting toward higher temperatures with a corresponding reduction in the value of tan δ. A decrease in Tan δ indicated a decrease in the phase angle δ, which meant the loss (viscous) modulus of the PVA/CAF 1FT1S and PVA/CAF 2FT2S hydrogels were lower than that of the PVA/CAF 1FT and PVA/CAF 2FT [47].

It is known that the mechanical properties of polymer are related not only to its crystallinity (degree of crosslinking) but also to the molecular structure of its amorphous regions [48]. The height of the damping peak in the DMA curve in Figure 9 exhibits the mobility of the polymer molecular chains that leads to damping of energy. The higher the peak, the less restriction there was toward the polymer chain motions [44]. As a result, the damping peak height decreases in the presence of uniaxial orientation, as the polymer chains highly packed together and the restriction for the polymer chain motions increased.

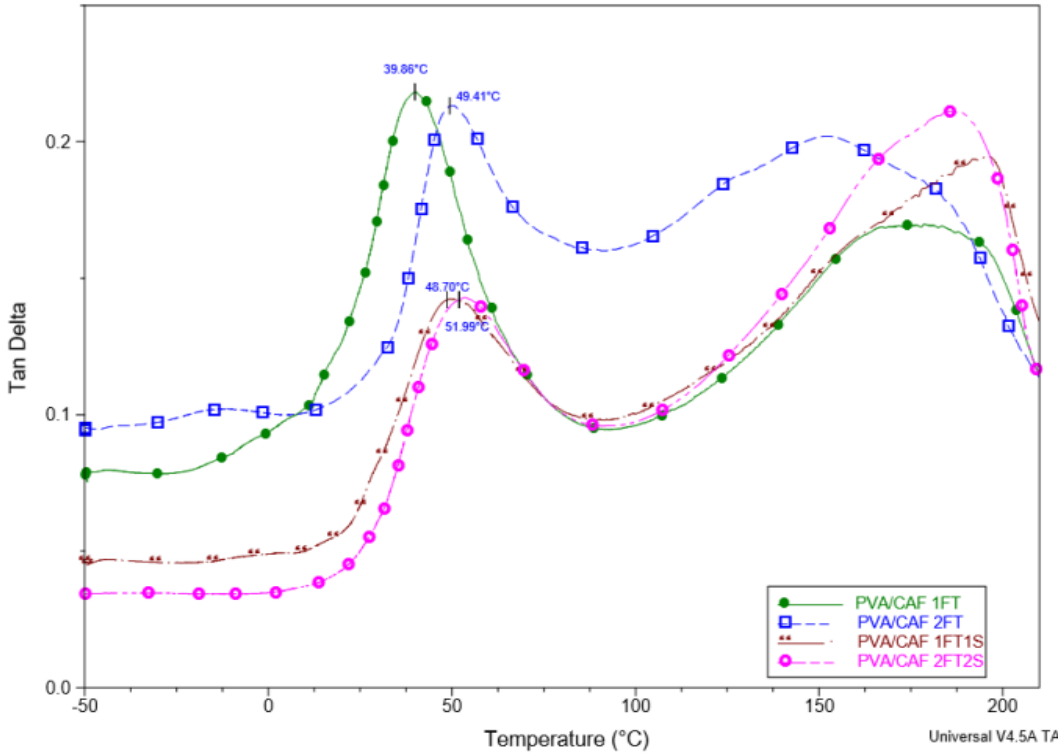

Figure 9. Tan δ of PVA/CAF hydrogels determined by DMA.

3.6 Tensile strength

In the study, the tensile strain at break (ε_B) and the tensile stress at break (σ_B) were analysed. The results in both Figure 10A and 10B showed that PVA and PVA/CAF hydrogel samples

with two F/T cycles have the lowest tensile stress. Hydrogels behave in an elastic manner when they have a lower tensile stress, so, the PVA 2FT and PVA/CAF 2FT samples were highly elastic. In contrast, when there was an addition of orientation cycles applied on the F/T samples, exhibited higher values of tensile stress. As a result, the PVA 2FT2S and PVA/CAF 2FT2S have the highest tensile stress and very rigid which leaded to a decrease in the elasticity. Due to the orientation induced by the stretching it enhanced the crosslinking density via an aligned structure, and consequently improved the mechanical properties, and this was confirmed by the DMA results.

In Figure 10A, there was no remarkable change of tensile strains in the case of the pure PVA samples. It is likely that they have the same ratio of elongation to original length of the PVA, since they all share a similar percentage strain around 219 %. While, there was a significant difference in Figure 10B on tensile strains. PVA/CAF 2FT2S has the lowest tensile strain and PVA/CAF 2FT the highest. This means PVA/CAF 2FT has higher elongation than PVA/CAF 2FT2S. The presence of caffeine and the effect of orientation varied the percentage strain in PVA hydrogels. The reason for the changes of tensile strength was the drug crystallisation [31]. During caffeine crystallisation, caffeine crystallites were being formed within the amorphous region of PVA. This phenomena caused the density, crystallinity, tensile stress and Young's modulus (Figure 11) to increase and elongation at break to decrease. Drug crystallisation can be restricted by fast water evaporation (e.g. electrospinning) which hindered the mobility of caffeine molecules in the hydrogels, thus exhibited the vitrification process [49]. This process gave the incorporated drug limited time to recrystallize favouring the formation of amorphous dispersions or solid solutions state [39], [49].

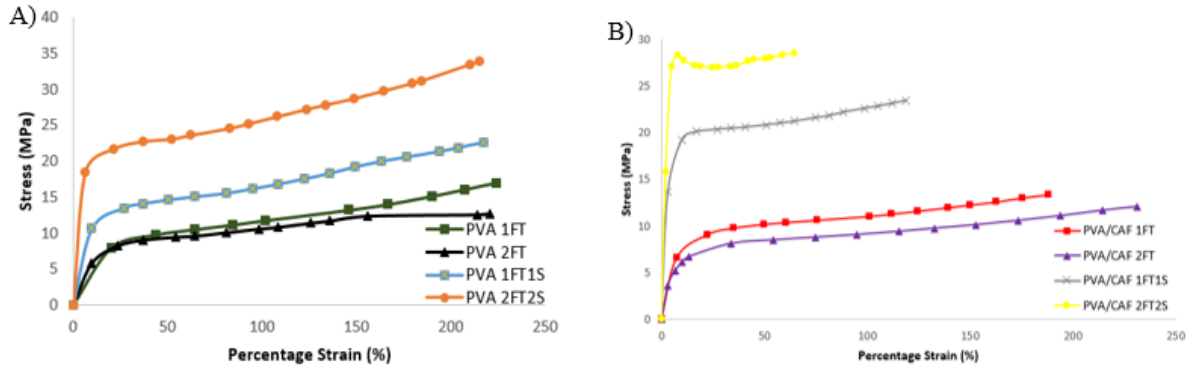

Figure 10. Stress vs strain curve for (A) PVA samples, (B) PVA/CAF samples.

The Young's modulus describes the stiffness of a solid material. The caffeine-content PVA hydrogel samples tend to have higher Young's modulus in comparison with pure PVA samples. In particular, the PVA/CAF hydrogels with orientation have higher Young's modulus than pure

PVA samples with orientation. The PVA/CAF 2FT2S reached the highest Young's modulus of 1462 MPa. It can be deduced that PVA hydrogels tend to increase its solidness and rigidness to undergo stretching when the number of cycles of F/T and orientation increases, as well as drug crystallinity increases [42].

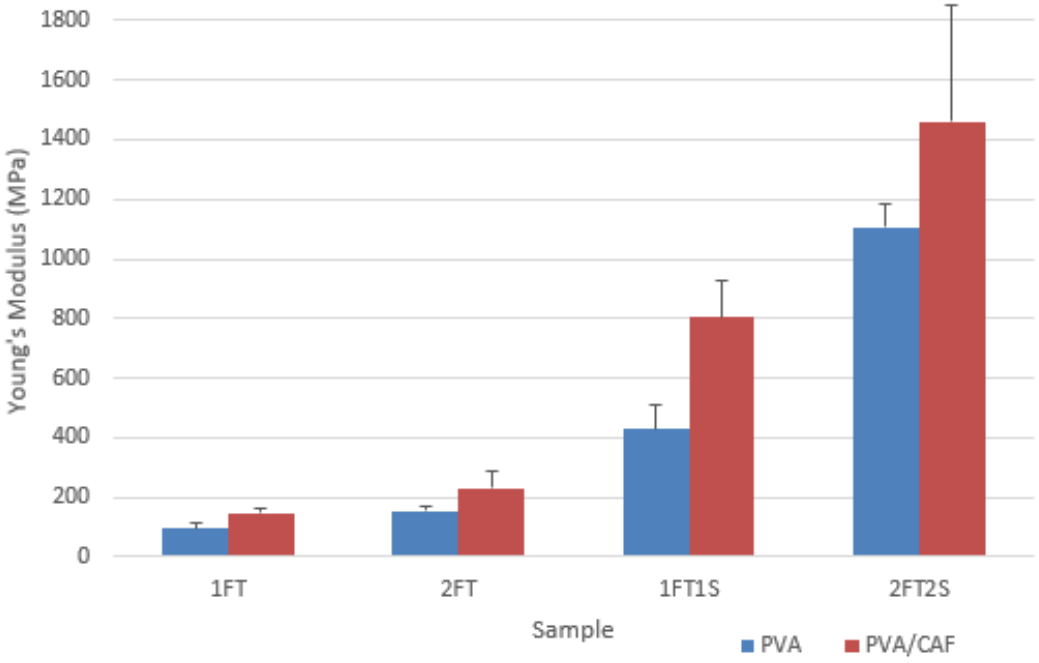

Figure 11 Young's modulus for PVA and PVA/CAF hydrogel samples.

3.7 Caffeine release studies

The release profiles of caffeine-loaded PVA hydrogels was shown in Figure 12. It can be seen that there was a quick drug release at the first 10 minutes which followed by slow rate of drug release. The fully release of caffeine from PVA/CAF 1FT, PVA/CAF 2FT, PVA/CAF 1FT1S and PVA/CAF 2FT2S were obtained at 50 minutes, 60 minutes, 20 minutes and 15 minutes, respectively. This indicates that caffeine-loaded hydrogel samples without stretching have taken much longer to complete the drug release process. Whereas, the hydrogel samples with stretching can completely release the drug between 15 and 20 minutes. 80% of caffeine is released in the rate of 10.0 % release/min and 20.0 % release/min for PVA/CAF 1FT1S and PVA/CAF 2FT2S, respectively. This showed that drug release rate increased with additional uniaxial orientation. In addition, the study showed an inverse correlation between the drug release rate and the degree of crosslinking resulted by F/T cycles. The hydrogel sample that undergoes higher number of F/T cycles (i.e. PVA/CAF 2FT) has a lower caffeine release rate of 8.6 % release/min, when compared to PVA/CAF 1FT which has the drug release rate of

13.3 % release/min at first 10 minutes. It can be explained by the fact that the increase of F/T cycles can cause the polymer network structure become progressively dense and entangled [42]. The increase of crystallinity in the polymer consequently resulted in a decrease of diffusivity of PVA hydrogel and slowed down the drug release rate [50]. Hence, the introduction of uniaxial orientation to the hydrogels can result the polymer chains becoming increasingly aligned. These alignments encourage a rapid penetration of water into the polymer matrices that promote a remarkably fast drug release within an hour.

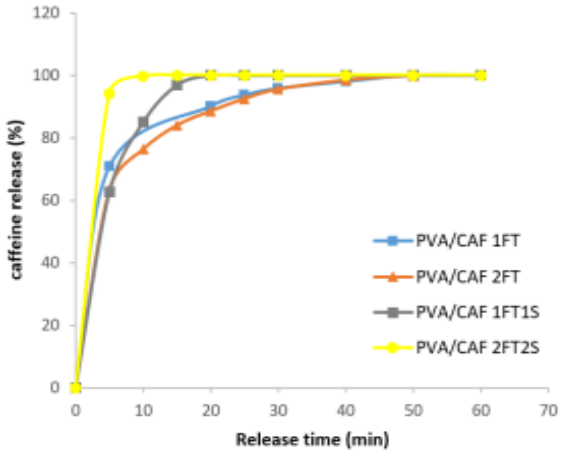

Figure 12. Release behaviour of caffeine from different F/T and uniaxial orientation cycles of PVA hydrogels.

Table 2. Release behaviour of the drug-loaded PVA/caffeine hydrogels.

PVA/CAF	Zero order	First order	Higuchi	Korsmeyer-Peppas		Hixson–Crowell
	R^2	R^2	R^2	R^2	n	R^2
1FT	0.9960	0.9809	0.9808	0.9642	0.1427	0.9871
2FT	0.9800	0.9993	0.9956	0.9992	0.2564	0.9957
1FT1S	0.9696	0.9770	0.9902	0.9941	0.4020	0.9984
2FT2S	0.8033	0.9513	0.8591	0.9062	0.0579	0.9904

In the literature, a number of mathematical models have been used to interpret the drug release data and the associated release mechanism [51]. In this study, the zero-order, first-order, Higuchi Korsmeyer-Peppas and Hixson-Crowell models are used to evaluate the experimental released profile of caffeine obtained at room temperature. The determined fitting parameters (i.e. n and R^2) of all the models are listed in Table 2. In the drug release assays, it was observed that there was an initial burst effect, followed by zero-order kinetics (R^2=0.9960) for PVA/CAF

1FT and first-order kinetics (R^2=0.9993) for PVA/CAF 2FT. The differences in the release behaviour of PVA/CAF 1FT and PVA/CAF 2FT can be explained by the increase of crystallinity and denser network structure of the latter.

The drug release profile of caffeine from PVA hydrogels with orientation (i.e. PVA/CAF 1FT1S and PVA/CAF 2FT2S) have shown best fit with Hixson-Crowell model. The R^2 value for PVA/CAF 1FT1S and PVA/CAF 2FT2S are 0.9984 and 0.9904, respectively. Thus, they were related to the changes in surface area of the hydrogels. The surface area of the orientated samples was directly proportional to the drug release rate. Due to an increase in orientation cycles, the samples surface area/volume ratio increases and it became more active, causing the drug release rate of the samples to increase. It can be deduced that the hydrogel samples with orientation were limited by the caffeine dissolution rate and not by the diffusion that might occur through the PVA polymeric matrix [52].

Although the result obtained in this work demonstrated that the release mechanism of caffeine seems to be proportional to the surface area of the oriented hydrogel, studies performed of PVA and caffeine [22], [53] suggests that the release mechanism is based on the diffusion of the entrapped caffeine to the exterior of the PVA membrane, Fickian diffusion. Although the fitting parameters for a fickian diffusion (Higuchi model) are high for most of the polymers, i.e., they also tend to perform as a diffusion mechanism. These values decreases when the hydrogel is stretched, this is because the preferably method of release changes because of the stress created and the aligned hydrogel produced, which is based on the surface area (Hixson-Crowell).

4. Conclusions

A series of PVA hydrogels with or without caffeine were synthesized by combined freeze-thawing and uniaxial orientation and their properties were investigated in comparison with those without uniaxial orientation. The results revealed that the effect of orientation on these hydrogels modified its structure leads to overall improved properties. Oriented hydrogels increased its mechanical properties, crystallinity and degree of swelling. These positive results were attributed due to the aligned oriented chains that occurred in between the freeze-thawing, with more cycles of F/T a more aligned polymer occurred. The incorporation of caffeine in the aligned hydrogel structure presented a Hixson–Crowell drug release mechanism and this fast caffeine release was justified by the aligned PVA polymer chains that was formed by the physical orientation method. Furthermore, the degree of crystallinity in PVA/CAF hydrogel

samples were higher than pure PVA hydrogels and caffeine crystallisation happened during the preparation of PVA/CAF hydrogels and drying process. Overall, the study believes due to this simple method and easily reproduceable mechanism, this orientated PVA/CAF hydrogel can serve as an ideal biomaterial for drug release applications.

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6. References

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