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Characterization of Genipin Crosslinked Hydrogels Composed of Chitosan and Partially Hydrolyzed Poly(vinyl alcohol)

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***Abstract*:** Chitosan and 80 % hydrolyzed poly(vinyl alcohol) (PVA) were used to prepare semi-interpenetrating networks of varying ratios of the constituents. The hydrogels were crosslinked using genipin, a naturally occurring nontoxic cross-linking agent. The swelling behavior of these hydrogels was studied in deionized water at 25, 35 and 45 ^{o}C and in media of different pH at 25 ^{o}C. The swelling behavior of the gels was found to be dependent on temperature, pH of the medium and the amount of PVA present in the gel. States of water in the hydrogels swollen at 25 ^{o}C and pH 7 were determined using Differential Scanning Calorimetry (DSC). The amount of freezing water in the swollen hydrogels increased whereas the amount of nonfreezing bound water decreased with the increase in PVA concentration in the gels. The gelation behaviour of the pregel solutions were also studied.

Introduction

Research in the area of hydrogels is becoming increasingly important due to large number of applications of these materials especially in biomedical and pharmaceutical sciences [1]. Hydrogels are macromolecular networks which can absorb large amounts of water while maintaining a distinct three-dimensional structure [2]. The network of the polymer chains is formed due to physical interactions or covalent crosslinking between the chains. These materials are sensitive to environmental parameters such as pH, temperature, solvent concentration, ionic concentration and electric fields. These properties, either singly or in combination, has led to widespread interest in the use of hydrogels for drug delivery [2, 3] and as scaffolds for cell or tissue culture [4].

For applications in the medical field, it is desirable that the hydrogels are prepared from the natural polymers that are known to be biocompatible and biodegradable. Chitosan, a deacetylated derivative of one of the most abundant polysaccharides chitin, falls into this category and has been widely used. It has been found to be non-toxic, biocompatible and enzymatically biodegradable [5]. To improve the hydrophilic character of its hydrogels, chitosan is blended with hydrophilic polymers such as poly(vinyl alcohol) [6] and poly(vinyl pyrrolidone) (PVP) [7, 8].

Although poly(vinyl alcohol) is a synthetic polymer, it is used as a basic material for a variety of biomedical applications due to its inherent nontoxicity, non carcinogenicity and good biocompatibility as well as desirable physical properties such as elastic nature and its ability to form strong films. Its use in contact lenses, skin replacement material, vocal chord reconstruction, artificial cartilage replacement etc. has been reported [9].

Stability to the hydrogel structure is improved by crosslinking the chain of one or all the polymers present in the gel. Many crosslinking agents such as glutaraldehyde, formaldehyde, epoxy compounds, dialdehyde starch etc. have been used. All these compounds are chemically synthesized and are not free from the problems of physiological toxicology. Recently it has been reported that chitosan can be cross-linked with a naturally occurring cross-linking reagent, genipin [10, 11].

Genipin is found in traditional Chinese medicine and is extracted from gardenia fruits [12]. It has been reported that genipin is an effective crosslinking agent and can react spontaneously with amino groups or proteins to form dark blue pigments [10]. Genipin has also been found to be much less cytotoxic than glutaraldehyde [13].

Hydrogels composed of chitosan and PVA have been studied by many researchers. The gels have been crosslinked using different crosslinking agents such as glutaraldehyde [6], formaldehyde [14] and UV radiation [15]. In our previous study [16], we reported the genipin crosslinked hydrogels composed of chitosan and varying amounts of 100% hydrolyzed PVA. The present work is related to the prepartion and characterization of genipin crosslinked hydrogels composed of chitosan and 80% hydrolyzed PVA.

Results and discussion

The swelling kinetics of the hydrogels in deionised water at pH 7 and at 25, 35 and 45 °C are shown in Figs. 1-3.

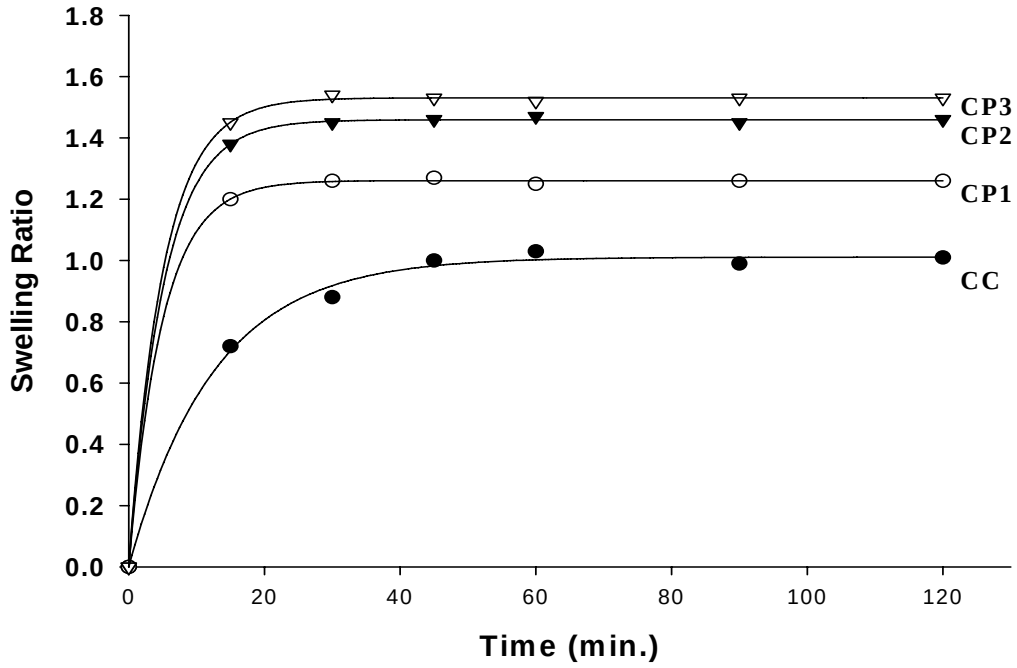

Fig. 1. Swelling kinetics of the hydrogels at 25 °C and pH 7.

All the hydrogels swelled rapidly and reached equilibrium state in less than one hour. Swelling of the hydrogels increased with the increase in PVA content. CP3 showed the maximum while CP1 showed the minimum swelling at all the temperatures.

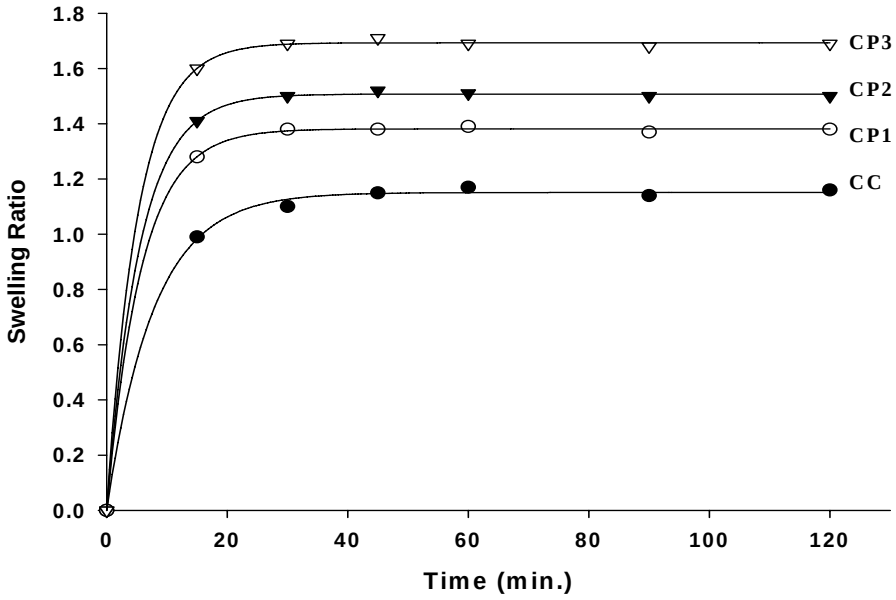

Fig. 2. Swelling kinetics of the hydrogels at 35 °C and pH 7.

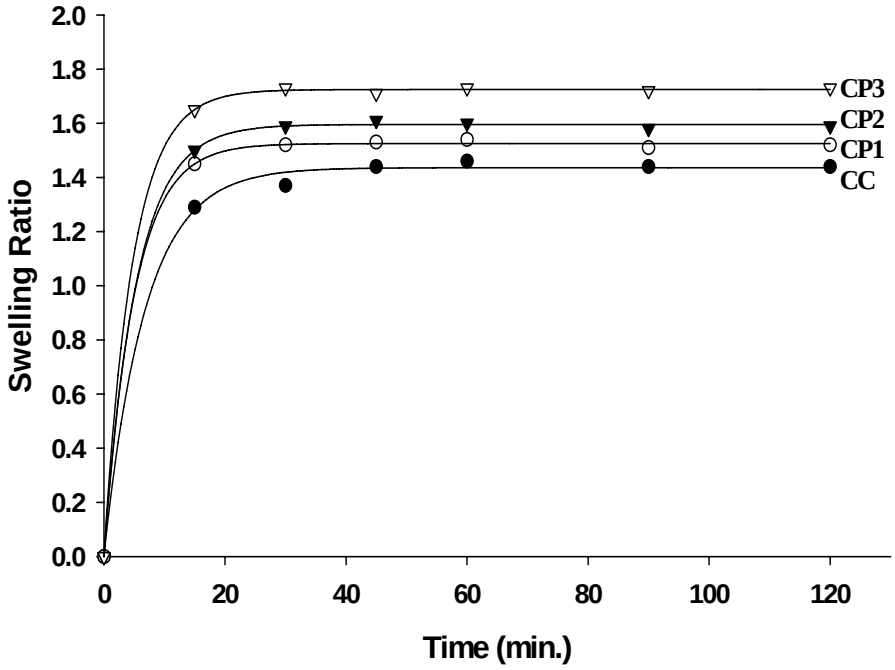

Fig. 3. Swelling kinetics of the hydrogels at 45 °C and pH 7.

The temperature dependent equilibrium swelling behavior of the hydrogels at pH 7 at different temperatures is summarized in Fig. 4. All hydrogels exhibited a temperature responsive behaviour and the swelling of all the hydrogels increased with temperature. Crosslinked chitosan showed the highest temperature dependent swelling. Addition of PVA to the gels decreased the temperature response and swelling in the case of CP3 increased only slightly with temperature.

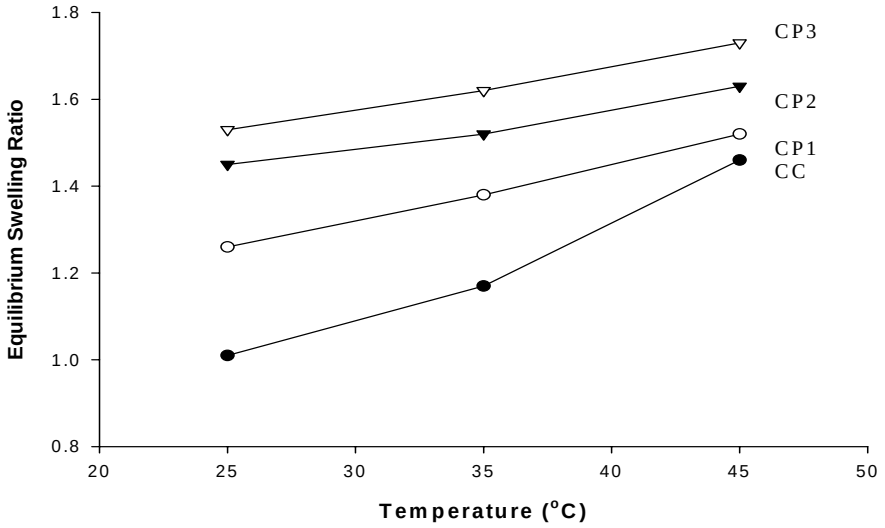

Fig. 4. Equilibrium swelling ratio of the hydrogels at various temperatures at pH 7.

The effect of temperature on swelling is explained on the basis of dissociation of interactions as the temperature is increased which causes relaxation of the polymer chains, thus allowing more water within the hydrogel network. A similar temperature dependent swelling behaviour for chitosan/PVA hydrogels has been reported [15, 16].

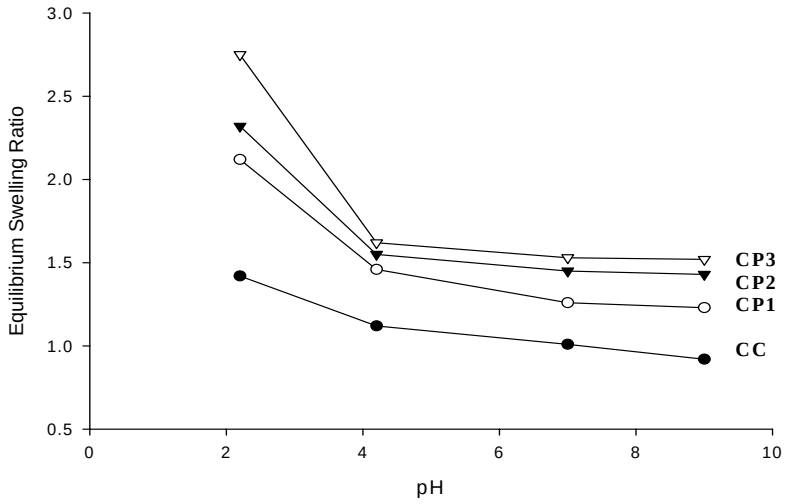

Fig. 5. Equilibrium swelling ratio of the hydrogels at various pHs at 25 °C.

The swelling behaviour of the hydrogels at 25 ^{o}C in buffer solutions of pH 2.2, 4.2, 7.0, and 9.0 are presented in Fig. 5. The hydrogels showed pH-sensitive behavior which is typical of ionic polymer hydrogels. For all the hydrogels, the maximum swelling was observed at low pH and it decreased as the pH of the solution increased. The content of chitosan and PVA in hydrogels affected the swelling ratio. CP3, which contained the highest amount of PVA, showed the maximum swelling at all pH's and the swelling decreased with the decrease in PVA content. Higher

swelling in acidic medium is due to the protonation of the amino groups of chitosan which leads to polymer chain repulsion and dissociation of interactions. Swelling decreases with the increase in pH as the amino groups become deprotonated and repulsion in the polymer chains recedes resulting in the shrinking of the network. In the basic medium, the swellability of the hydrogels remained relatively unchanged.

States of water in the hydrogels were determined from the DSC measurements. Three types of water are known to be present in the hydrogels and are referred to as nonfreezing bound water, intermediate freezing water and free freezing water [17, 18]. Nonfreezing bound water refers to immobilized water molecules which are bound to the hydrophilic sites of the polymer chains and shows no melting peak. Intermediate freezing water molecules preferentially orient around the bound water and have a melting endotherm below 0 ^{o}C. Free freezing water molecules show greater degree of mobility and show a melting endotherm around 0 ^{o}C.

Fig. 6 shows the DSC heating scans for the hydrogels fully swollen in deionized water at 25 ^{o}C. Crosslinked chitosan (CC) shows only one broad endothermic peak whereas others show two well resolved peaks. The peaks below 0 ^{o}C (around –9.0 ^{o}C to –6 ^{o}C) and around 0 ^{o}C are attributed to the intermediate and free freezing water respectively.

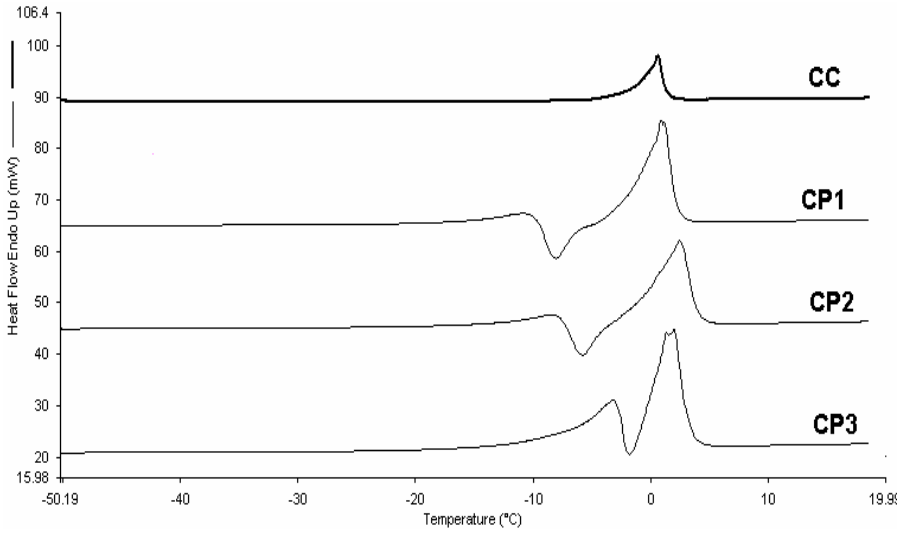

Fig. 6. DSC heating scans of the hydrogels.

The enthalpy values associated with the melting peaks (ΔH_{endo}) for various hydrogels, given in column 3 of Tab. 2, were used to calculate the free and bound water in the hydrogels. EWC represents the percentage of total water present in the hydrogel

$$EWC = W_b + (W_f + W_{if}) \quad (1)$$

where W_b is the amount of nonfreezing bound water, W_f and W_{if} are the amounts of free water and intermediate freezing water respectively. ($W_f + W_{if}$) can be taken as total freezing water in the hydrogel.

The amount of total freezing water can be estimated by taking the ratio of the enthalpy change associated with the endothermic peak (ΔH_{endo}) for intermediate and free water to the enthalpy of fusion (ΔH_{fus}) for pure water (333.7J/g).

$$(W_f + W_{if}) = (\Delta H_{endo}/\Delta H_{fus}) \times 100 \quad (2)$$

Tab. 1. States of water in the hydrogels.

Sample	EWC (%)	ΔH_{endo} (J/g)	Total freezing water (%)	Nonfreezing bound water (%)
CC	49.3	100.5	30.1	19.2
CP1	56.1	108.9	32.6	23.5
CP2	59.5	141.1	42.3	17.2
CP3	60.6	178.0	53.3	7.3

EWC, total freezing and bound water contents of various hydrogels are listed in Tab. 2. It is clear from the Tab. 2 that the equilibrium water content and total freezing water in the hydrogels increased with the addition of PVA whereas the nonfreezing bound water decreased. The results indicate that PVA increased the hydrophilic character of the hydrogels.

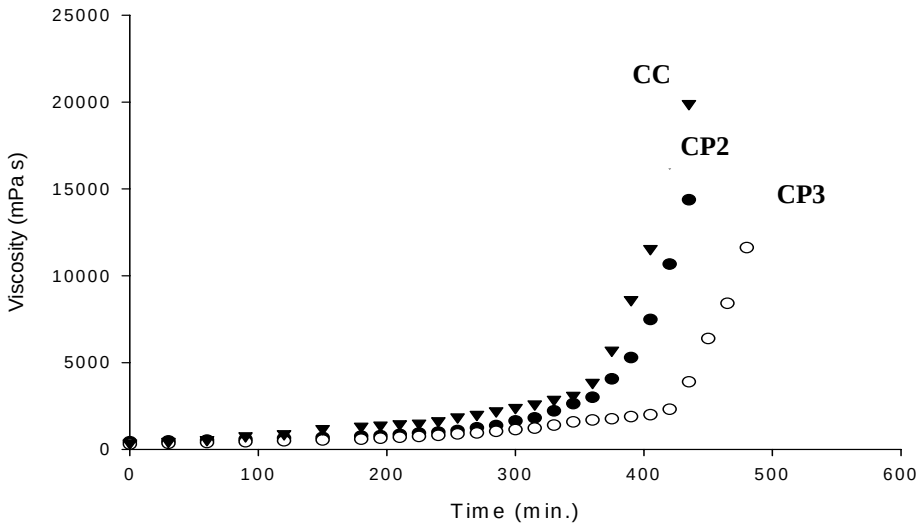

Fig.7. Gelation behaviour of CC, CP2 and CP3 at 25 ºC.

Changes in viscosity of the pregel solutions of CC, CP2 and CP3 with time are shown in Fig. 7. As the pregel turns into a gel, its viscosity starts to rise sharply. Chitosan took about 6 h to form a gel and gelation time increased as PVA was added. CP2 and CP3 formed a gel in approximately 7 h and 8 h respectively. Presence of PVA slowed down the crosslinking reaction between the chitosan and genipin.

Conclusions

Hydrogels of chitosan and 80 % hydrolyzed PVA were prepared and crosslinked using a naturally occurring nontoxic cross-linking agent, genipin. The swelling ratios of the hydrogels were measured in deionized water at different temperatures and in

media of different pH. The hydrogels swelled rapidly and exhibited a swelling response to temperature and pH. The swelling increased with the decrease in the pH and increase in the temperature. PVA rich hydrogels swelled more in both stimuli. The amount of freezing water and nonfreezing bound water was investigated by DSC. The results revealed that increase in the amount of PVA in the hydrogels increased the EWC indicating gels becoming more hydrophilic in nature. The amount of nonfreezing bound water decreased while the amount of freezing water increased with PVA content of the gels.

Experimental part

Materials

Chitosan with 85% degree of deacetylation and buffer tablets and solutions were obtained from Fluka, U.K. 80% hydrolyzed poly(vinyl alcohol) with molecular weight $9.0x10^3$–$1.0x10^4$ and genipin were purchased from Aldrich and Challenge Bioproducts Company respectively. All chemicals were used without further purification.

Preparation of hydrogels

Chitosan was dissolved in 1% aqueous acetic acid solution at room temperature with continuous mechanical stirring for several hours to obtain a 1.5% (w/v) solution. The chitosan solution was filtered through a sieve to remove any undissolved matter. PVA was dissolved in deionised water with mechanical stirring for 1 h to obtain a 5% (w/v) solution.

Varying amounts of 5% PVA solution were added to the chitosan solution to obtain mixtures having PVA:chitosan mass ratios of 6:1, 3:1 and 1:1. Genipin solution of concentration 0.5% (w/v) was slowly added to the mixtures under constant stirring. The detailed composition and designations of the pregels are listed in Tab. 2.
The pregel solutions were poured into polystyrene Petri dishes and allowed to undergo gelation at room temperature for 12 hours. By this time the color of the gels became bluish green. The hydrogels were dried at 55 °C and obtained films of thickness of approximately 0.2 mm. Films were stored in a vacuum until further use.

Tab. 2. Compositions of the hydrogels.

Designation	Vol. of chitosan (cm^3)	Vol. of PVA (cm^3)	Vol. of genipin (cm^3)	Mass ratio Chitosan : PVA
CC	40	0	4	1 : 0
CP1	40	2	4	1 : 0.17
CP2	40	4	4	1 : 0.33
CP3	40	12	4	1 : 1

Swelling measurements

The swelling kinetics of the hydrogels was investigated in deionised water at 25, 35 and 45 °C. Each film sample of surface area approximately 25 mm^2 was weighed and placed in a stainless steel wire mesh. The mesh containing the film sample was then

submerged into a 250 ml beaker containing the swelling medium and covered with parafilm to avoid evaporation. At preset time intervals the films were withdrawn and their wet weights were determined after blotting gently with a filter paper. The above procedure was continued for one more hour after a constant wet weight was observed. Measurements were conducted in triplicate.

To study the effect of pH on swelling behaviour, the dry films of the hydrogels were placed in buffer solutions of pH 2.2, 4.2, 7.0, and 9.0 maintained at 25 ^{o}C and weight of the swollen films were taken after 12 h. The swelling ratio, SR, was calculated from the equation:

$$SR = [(W_s - W_d) / W_d] \quad (3)$$

where W_d and W_s are the weights of the samples in the dry and swollen states respectively. Average of three trials was taken.

The equilibrium water content (EWC) was calculated using the following equation:

$$EWC\ (\%) = [(W_e - W_d) / W_e] \times 100 \quad (4)$$

where W_e represents the weight of the swollen state at equilibrium.

Differential Scanning Calorimetry

Perkin Elmer Pyris 6 DSC was used to investigate the states of water in the hydrogels fully swollen in deionized water at 25 ^{o}C. Samples of mass approximately 1-5 mg were scanned from –50 °C to 20 °C with a heating rate of 5 °C/min under nitrogen flow. The amounts of free and bound water were calculated from the EWC and the enthalpies of melting. Average of five measurements was taken.

Gelation behaviour of the hydrogels

The transition from the pregel solution to the hydrogel was followed by measuring the viscosity of the pregel solution at preset time intervals at 25 °C. 20 ml of the pregel solution were placed in a vial and using the Haake Visco Tester 7 Pt100 fitted with L4 spindle and viscosity was measured at intervals of 30 min in the beginning and of 15 min when the viscosity started to increase.

Acknowledgements

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