ACETYLATION OF LINSEED HYDROGEL: SYNTHESIS, CHARACTERIZATION, ISOCONVERSIONAL THERMAL ANALYSIS AND DEGRADATION KINETICS

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Linseed (*Linum usitatissimum* L.) hydrogel (LSH), consisting of polysaccharide rhamnogalacturonans, was acetylated using acetic anhydride and 4-dimethylaminopyridine (DMAP). Acetylated LSH (ALSH) was thoroughly characterized using different spectroscopic techniques. A moderate to high degree of substitution (2.11-2.91) of acetyl groups on LSH was obtained, as calculated by the acid-base titration method. Isoconversional thermal analysis and degradation kinetics of LSH and ALSH were studied. The TG curves of LSH and ALSH exhibited two- and one-step exothermic degradation, respectively. The energy of activation (E_a) values were calculated by the Kissinger and FWO models for the first step of degradation of LSH and ALSH, and were found in the range of 118.32 and 169.18 kJ mol^{-1}, respectively. The comprehensive index of thermal stability (ITS) was found to be 0.41 and 0.49 for LSH and ALSH, respectively. Thermal data showed that thermal stability was imparted to LSH after acetylation.

Keywords: acetylation, degradation kinetics, linseed hydrogel, polysaccharide, thermal analysis

INTRODUCTION

Nowadays, hydrogel-able smart polysaccharide materials are being widely used in sustained/controlled/targeted release formulations of different drugs[1-5] in order to enhance their therapeutic efficiency.[6,7] Among hydrogel forming polysaccharides, naturally occurring polysaccharides mostly offer pH,[8] solvent, salt and saline responsive properties,[9-11] which are of high demand to improve oral formulations for better drug delivery applications.

Keeping in view the wide use of such hydrogel-able polysaccharides of natural origin, there is dire need to search thermally stable polysaccharides for novel formulations to attain higher shelf life, which is an important criterion.[12] Acetylation is a valuable tool to get enhanced thermal stability of polysaccharides.[13,14] This enhanced stability can be accessed from thermogravimetric analysis of materials.[12]

Linseed (*Linum usitatissimum* L.) is a commercial crop usually cultivated to get seeds and fibers due to wide applications in the food industry.[15] The mucilage extruded from linseed is mainly composed of some valuable polysaccharides,[16,17] rhamnogalacturonan along with a minor fraction of arabinoxylan.[16,18-21] Rhamnogalacturonan is a combination of high molecular weight fraction (1510 kDa) and low molecular weight fraction (341 kDa). These fractions are composed of galactose, galacturonic acid, rhamnose and fucose.[17,22] Usually, polysaccharides are considered as biocompatible and biodegradable materials. Therefore, LSH was evaluated as stimuli responsive biomaterial and was used as a release retarding material in oral dosage form and wound healing applications.[9,23,24]

Herein, we report on the acetylation of LSH and its characterization. Also, our objective has

been to perform comparative isoconversional thermal analyses (FWO and Kissinger methods) to determine the thermal stability (ITS), degradation kinetics (E_a, n, Z) and thermodynamic parameters (ΔH, ΔS, ΔG) of the hydrogel-able polysaccharide isolated from linseed and its acetylated derivative. According to the findings of the present investigation, LSH may find applications in pharmaceuticals for improved formulation design.

EXPERIMENTAL

Materials

Linseeds were purchased from a local market, cleaned from superfluous material by passing through different meshes and stored at ambient temperature in an air-tight container. The solvents used were of analytical grade and procured from Sigma-Aldrich, USA. DMAP was obtained from Alfa Aesar, England.

Measurements

Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize LSH and acetylated LSH (ALSH). Potassium bromide (KBr) was used to prepare the pellets of the samples, which were dried at 50 °C for 2 h in a vacuum oven before recording the spectrum on the FTIR instrument (IR Prestige-21, Shimadzu, Japan). ^{1}H NMR spectra of LSH were recorded on a Bruker Avance 600 MHz spectrometer, equipped with a triple-resonance RT probe (Billerica, MA, USA). Deuterated solvents (DMSO-d_6) were used to prepare the LSH and ALSH samples (10 mg/mL). The ^{1}H NMR spectra were processed using TopSpin software. The thermal decomposition temperature of LSH and ALSH was recorded on an SDT Q600 thermal analyzer (TA Instruments, USA) under nitrogen flow (100 mL/min) at heating rates of 5, 10, 15 and 20 °C/min from ambient to 800 °C. Thermal degradation data were processed by Universal Analysis 2000 v 4.2E software.

Isolation of linseed hydrogel (LSH)

LSH was extracted using a hot-water extraction process, as reported by one of the authors.[9] Briefly, cleaned linseeds were soaked in distilled-deionized water for two days and then heated at 80 °C for 30 min. Mucilage was extracted from the seeds by mild pressing through a nylon mesh. The hydrogel obtained was washed with *n*-hexane to remove any fatty/waxy material and then with an excess of distilled-deionized water. LSH portion was separated through centrifugation (3500 rpm, 5 min). LSH was initially air dried and then placed in an oven at 60 °C under vacuum for 24 h. The dried LSH was milled and stored in a vacuum desiccator.

Acetylation of LSH, a typical reaction

LSH (1.0 g) was suspended in DMSO (40 mL), followed by the addition of acetic anhydride (3.5 mL) using DMAP (40 mg) as a catalyst. The suspension was heated at 80 °C with continuous stirring for 6 h under nitrogen. Acetylated LSH (ALSH 1) was dried at 50 °C after precipitation and washed with ethanol. A similar reaction and work procedure were used for ALSH 2 and ALSH 3.

Yield: (1.02 g); DS: 2.11; FTIR: 1749 (CO_{Ester}), 2963 (CH), 1376 (CH_2), 3440 cm^{-1} (OH), 1042 (COC); ^{1}H NMR (DMSO-d_6; 600 MHz; NS 64): 2.05 (CH_3), 3.13-5.62 (Repeating unit – Hs).

Analytical data of ALSH 2

Yield: (1.12 g); DS: 2.53; FTIR: 1752 (CO_{Ester}), 2936 (CH), 1373 (CH_2), 3425 cm^{-1} (OH), 1043 (COC); ^{1}H NMR (DMSO-d_6; 600 MHz; NS 64): 2.05 (CH_3), 3.13-5.62 (Repeating unit – Hs).

Analytical data of ALSH 3

Yield: (1.33 g); DS: 2.91; FTIR: 1738 (CO_{Ester}), 2926 (CH), 1376 (CH_2), 3514 cm^{-1} (OH), 1044 (COC); ^{1}H NMR (DMSO-d_6; 600 MHz; NS 64): 2.06 (CH_3), 3.14-5.62 (Repeating unit – Hs).

Calculation of degree of substitution (DS)

For determining the DS of acetylation onto LSH by the acid-base titration method, ALSH (100 mg) was stirred with 0.1M NaOH aqueous solution (50 mL) for saponification. The solution was then neutralized with 0.01M HCl solution. After that, 1M NaOH solution (known volume) was mixed with this neutral solution. The DS of acetylation was calculated after neutralizing the excess NaOH with 0.1M HCl solution, using the following formula:

$$DS = \frac{n.NaOH \times M(RU)}{Ms - Mr(RCO) \times n.NaOH} \quad (1)$$

where *n.NaOH* is the number of moles of NaOH added after saponification, *M(RU)* is the molar mass of the repeating unit of the polymer, *Ms* is the mass of sample taken and *Mr(RCO)* is the molar mass of ester functionality.

Thermogravimetric analysis and degradation kinetics of LSH and ALSH

Initial (Td_i), maximum (Td_m) and final (Td_f) thermal decomposition temperatures were calculated from the TG curves of LSH and ALSH 3. Thermal kinetics was calculated by the method Flynn-Wall and Ozawa (FWO), *i.e.* a model free and isoconversional method, as described in Equation 2:[25-27]

$$\ln \beta = \ln \frac{AE_a}{Rg(\alpha)} - 5.331 - 1.052 \frac{E_a}{RT} \quad (2)$$

where β is the heating rate, A is the pre-exponential factor, E_a is the activation energy, R is the general gas constant and T is the temperature at the conversion rate (α). The value of α was calculated by Equation 3:

$$\alpha = \frac{(W_o - W_t)}{(W_o - W_f)} \quad (3)$$

where W_o is initial mass, W_f is final mass and W_t is the mass of sample at any temperature T.

To calculate the value of α at different heating rates, a graph was plotted between lnβ and 1000/T results in a straight line. The value of E_a was then calculated from the slope of this graph.

Kissinger proposed a method for the determination of the order of the thermal degradation reaction (n).[28] This method is based on the shape index, which is the ratio of tangential slopes of the DTG curve at right and left inflection points, and is given in Equation 4:

$$S = \left[\frac{\left(\frac{d^2\alpha}{dt^2} \right)_L}{\left(\frac{d^2\alpha}{dt^2} \right)_R} \right] \quad (4)$$

where subscripts R and L are values at right and left inflection points, respectively. The values of n can be calculated as a function of the S value using Equations 5 and 6:

$$n = 1.88S \left(S \geq 0.45 \right) \quad (5)$$

$$n = 1.26S^{0.5} \left(S \leq 0.45 \right) \quad (6)$$

Thermodynamic analysis

Thermodynamic parameters, such as Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS), of the thermal degradation of LSH and ALSH 3 were investigated using the Eyring-Polanyi equation, which is shown below:[29]

$$k = \frac{k_B T}{h} e^{-\frac{\Delta G}{RT}} \quad (7)$$

where ΔG is the Gibbs free energy, k_B is Boltzmann's constant and h is Planck's constant. The linear form of Equation 7 is given as:

$$\ln \frac{k}{T} = \frac{-\Delta H}{R} \times \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S}{R} \quad (8)$$

where k is the rate constant, k_B is Boltzmann's constant, ΔH is the enthalpy of activation, R is the gas constant, T is the absolute temperature, h is Planck's constant and ΔS is the entropy of activation.

A straight line is obtained by plotting ln k/T *vs.* $1/T$. The slope of the straight line gives the ΔH value, while the intercept gives the ΔS value for a given thermal degradation reaction.

The index of thermal stability (ITS) is the main parameter to describe the intrinsic thermal stability of a polymer. Therefore, ITS values were calculated from the area under the TG curves of LSH and ALSH 3 using Doyle's method.[30]

Swelling properties of LSH

Swelling properties of LSH were determined in deionized water at ambient temperature (30 °C) and at 50 °C, using an already reported method.[9] Briefly, LSH (0.1 g) was taken in an empty tea bag, dipped in deionized water and kept at 30 °C for a specific time interval. The tea bag containing swollen LSH was hung for some time to drain excess water. Swelling capacity was determined using Equation 9. Similarly, the swelling of LSH was also determined at 50 °C.

$$\textit{Swelling capacity} = (w_t - w_0 - w_c)/w_0 \quad (9)$$

where w_t is the weight of the wet tea bag containing swollen LSH, w_o is the weight of dry LSH and w_c is the weight of the wet tea bag.

Equilibrium (after 24 h) swelling properties of LSH and ALSH were also observed using the above mentioned procedure.

RESULTS AND DISCUSSION

Synthesis and characterization of LSH acetates

Synthesis

LSH was acetylated with acetic anhydride using 4-dimethylaminopyridine (DMAP) as a catalyst.[14] This efficient reaction yielded a DS of acetylation onto LSH of 2.11-2.91. It was noted that the reaction suspension became soluble after 3 min in each case. The product was obtained by precipitation and purified by washing with ethanol. The acetylated LSH (ALSH) was found soluble in dimethylsulfoxide (DMSO), acetone, *N*,*N*-dimethylacetamide (DMAc) and $CHCl_3$. The results, reaction conditions and DS are shown in Table 1.

FTIR spectroscopic analysis

The FTIR spectroscopic technique was employed for the identification of ester peaks in ALSH (Fig. 1). The FTIR spectra of acetylated LSH show successful esterification due to the appearance of distinct ester peaks at 1749, 1752 and 1738 cm^{-1} in ALSH 1, ALSH 2 and ALSH 3, respectively. Other peaks at 1042, 1043 and 1044 cm^{-1} indicate the presence of COC and CH_2 stretching at 1376, 1373 and 1375 cm^{-1} for ALSH 1, ALSH 2 and ALSH 3, respectively. It was also observed that, as the molar concentration of acetic anhydride increased from 1:6 to 1:18, the DS also rose from 2.11 to 2.91, which is also evident from the FTIR spectra of acetylated derivatives of LSH.

^{1}H NMR spectroscopic analysis

The structure of LSH was characterized by ^{1}H NMR spectroscopy (Fig. 2). Rhamnogalacturonan is a major component of LSH, which is a highly branched structure and is composed of rhamnose, galacturonic acid, galactose, fucose and xylose.[19,31] The presence of these constituents is verified through ^{1}H NMR spectra by comparing the specific signals with already reported results.[16,17,32] The ^{1}H NMR spectrum of ALSH 3 (Fig. 3) shows successful esterification due to

appearance of acetyl methyl protons centred at δ 2.06 (1.90-2.11) ppm and sugar protons absorbed in the range of δ 3.14-5.62 ppm.

Table 1
Reaction parameters and results of the synthesis of acetylated LSH

Sample	Reactants[a]	Yield (g)	DS[b]	Solubility
ALSH 1	1.0 g, 3.5 mL, 40 mg	1.02	2.11	DMSO, acetone, DMAc, $CHCl_3$
ALSH 2	1.0 g, 7.0 mL, 40 mg	1.12	2.53	DMSO, acetone, DMAc, $CHCl_3$
ALSH 3	1.0 g, 10.5 mL, 40 mg	1.33	2.91	DMSO, acetone, DMAc, $CHCl_3$

[a]LSH, acetic anhydride, DMAP; [b]Degree of substitution calculated from acid base titration after saponification

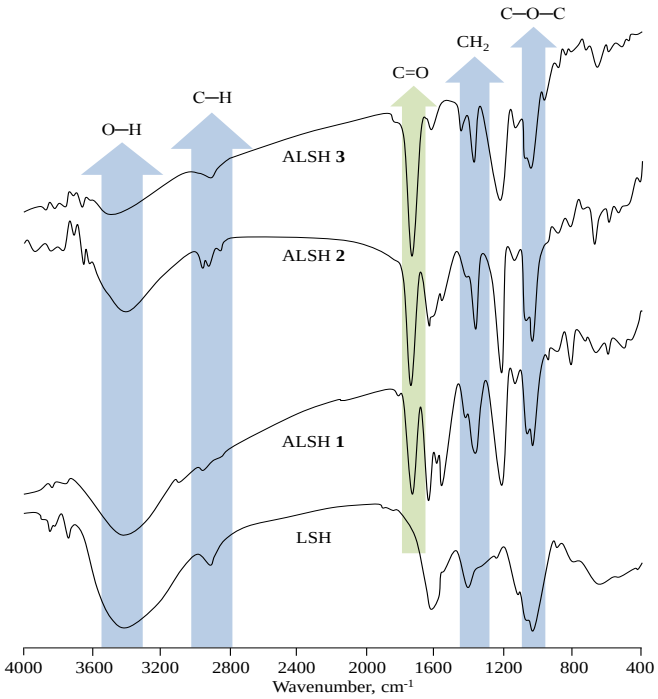

Figure 1: FTIR spectra of LSH, ALSH 1, ALSH 2 and ALSH 3

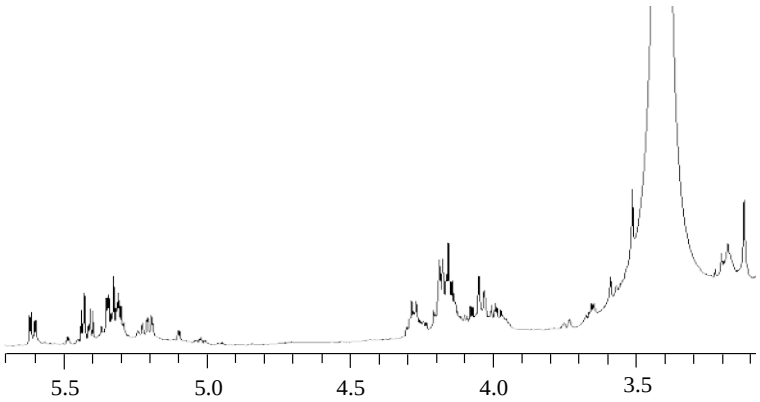

Figure 2: ^{1}H NMR spectrum of LSH (600 MHz, ppm, DMSO-d_6, 40 °C)

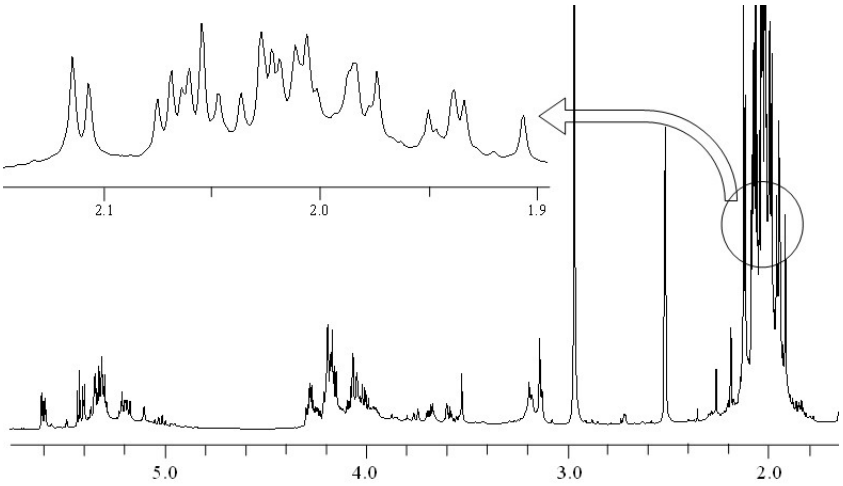

Figure 3: ^{1}H NMR spectrum of ALSH 3 (DS 2.91), (600 MHz, ppm, DMSO-d_6, 40 °C)

Thermal analysis and degradation kinetics

Thermal analysis

Thermal degradation behaviors of LSH and ALSH 3 were evaluated at multiple heating rates from ambient temperature to 800 °C. The TG curves of LSH and ALSH 3 exhibited 8.49% and 4.42% loss of mass in the temperature range of 50 to 130 °C, due to moisture evaporation from the sample.[4] The TGA of LSH revealed that degradation occurred in two steps, where the second step was due to furfural formation.[4] The average Td_i and Td_f values of the first step of LSH degradation were found to be 156 and 354 °C, respectively. The average mass loss at four different temperatures for the first step of LSH degradation was approximately 46.71%, whereas the average Td_m value for this step was found to be 287 °C. The TG and DTG curves of LSH are shown in Figure 4.

Similarly, ALSH 3 was also subjected to multiple heating rates to get its degradation profile, which exhibited single-step degradation (see Fig. 4). The thermal degradation of ALSH 3 showed average Td_i, Td_m and Td_f values at 151, 328 and 419 °C, respectively, with a mass loss of 79.03%. The significantly higher Td_m value (328 °C) of ALSH 3, as compared to that of LSH (287 °C), indicates that thermal stability was imparted to LSH after acetylation. The thermal decomposition temperatures, weight losses and char yields of LSH and ALSH 3 are displayed in Table 2.

Degradation kinetics

Flynn-Wall-Ozawa (FWO), which is an isoconversional method, was employed to evaluate different kinetic parameters, such as energy of activation (E_a) and frequency factor (A). The Kissinger method was used to find the order of thermal degradation reactions (n). The E_a values of the degradation steps for LSH and ALSH 3 were found to be 118.32 and 169.18 kJ mol^{-1}, respectively (Table 3). The higher E_a value for ALSH 3 indicates that the acetylated hydrogel is more stable than the unmodified hydrogel. Figure 5 (a) and (c) show the plots of α *vs*. T curves of thermal degradation for LSH and ALSH 3, respectively, at different heating rates. The FWO plots between logβ and 1000/T(K^{-1}) for thermal degradation at several degrees of conversion for LSH and ALSH 3 are also shown in Figure 5 (b) and (d), respectively. The Kissinger method revealed that the thermal decomposition reactions of LSH and ALSH 3 followed first-order kinetics.

Table 2
Mean thermal decomposition temperatures, weight loss % and char yield % of LSH and ALSH 3 at multiple heating rates

Sample	Step	Td_i (°C)	Td_m (°C)	Td_f (°C)	Weight loss % at Td_f	Char yield (wt%)
LSH	I	156	287	354	46.71	9.11 at 600 °C
ALSH 3	I	151	328	419	79.03	11.77 at 600 °C

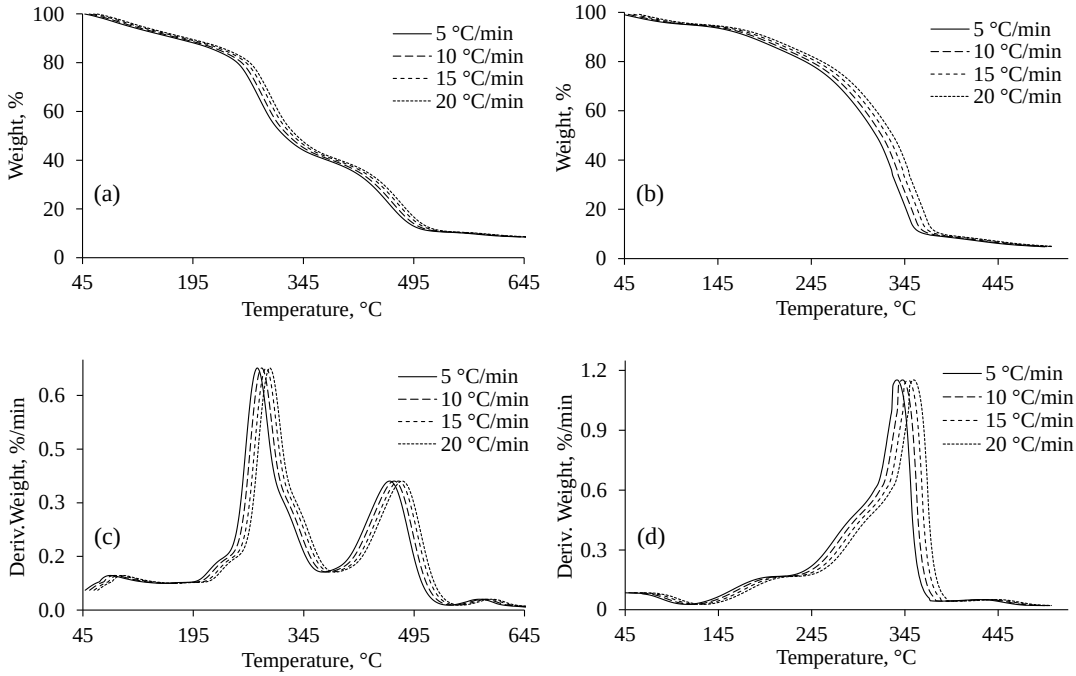

Figure 4: Overlaid TG and DTG curves of LSH (a, c) and ALSH 3 (b, d), respectively, recorded at multiple heating rates

Table 3
Thermal kinetics and thermodynamic parameters of LSH and ALSH 3

Sample	Method	Step	R^2	n	E_a (kJmol^{-1})	ln$A$	ΔH	ΔS	ΔG	IPDT	ITS
LSH	FWO	I	0.979	-	118.32	27.22	113.66	-36.58	134.14	350	0.41
	Kissinger	I	-	0.87	-	-	-	-	-		
ALSH 3	FWO	I	0.966	-	169.18	38.97	164.11	61.97	126.30	295	0.49
	Kissinger	I	-	1.03	-	-	-	-	-		

Thermodynamic analysis and index of thermal stability

The TG data of LSH and ALSH 3 were used to calculate various thermodynamic parameters, such as ΔH, ΔG and ΔS (Table 3). The area under the TG curves was used to calculate the index of thermal stability (ITS) values, which is important to evaluate thermal stability. The mean ITS values for LSH and ALSH 3 were calculated to be 0.41 and 0.49, respectively. The mean ITS value of ALSH 3 is higher than that of LSH, showing that ALSH 3 possesses greater thermal stability. Moreover, ITS values for LSH (0.41) and ALSH 3 (0.49) are higher than those reported for other hydrogels, such as hydrogels from *Astragalus gummifer* (0.38), *Acacia nilotica* (0.40), *Argyreia speciosa* (0.35), *Acacia modesta* (0.42), *Ocimum basilicum* (0.41), *Plantago ovate* (0.39), *Salvia aegyptiaca* (0.33) and *P. ovate husk* (0.41). It means LSH and ALSH 3 are more thermally stable than many reported polysaccharides.[33]

Swelling capacity of LSH

It was observed that LSH has a reasonably high swelling capacity at 50 °C (Fig. 6). It was also observed that by increasing the water temperature (50 °C), the swelling capacity of LSH was also increased. Comparing the swelling of LSH at 30 °C and 50 °C, a significant difference in the rate of swelling was observed up to 12 h at 50 °C.

Equilibrium swelling of LSH and ALSH was calculated to compare the swelling properties before and after acetylation of LSH. After 24 h, the swelling capacity of LSH and ALSH was 42.99 and 12.33 g/g, respectively.

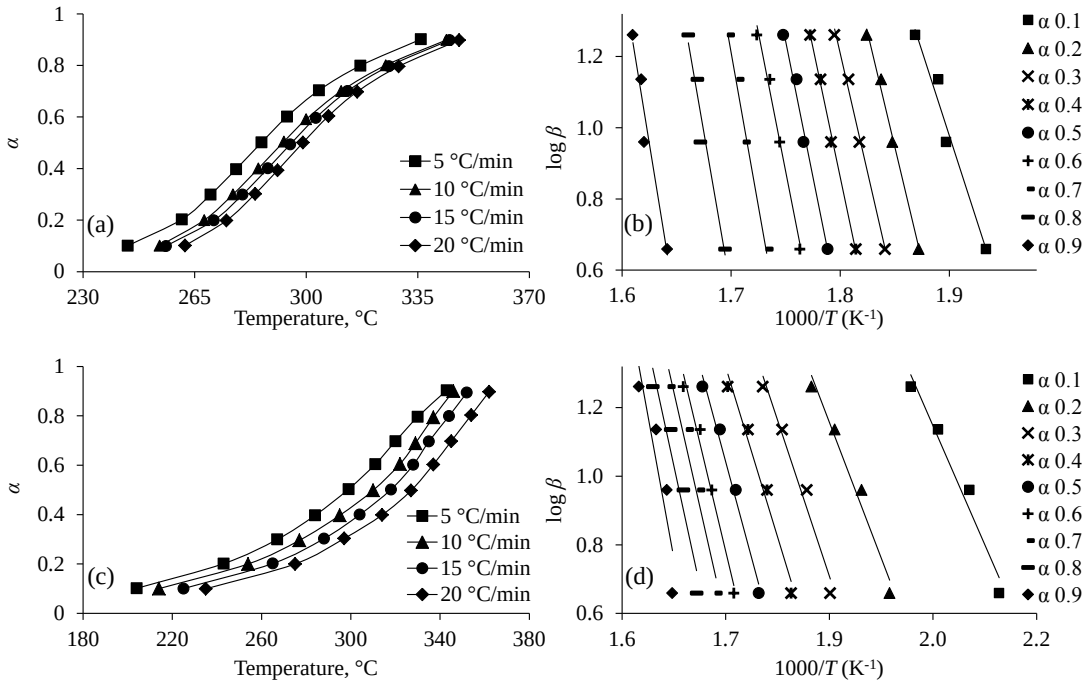

Figure 5: *α vs*. *T* plots of thermal degradation of LSH (a) and ALSH 3 (c) at multiple heating rates and Flynn-Wall-Ozawa (FWO) plots between log *β* and 1000/*T* (K^{-1}) for calculation of E_a of degradation steps at several degrees of conversion for LSH (b) and ALSH 3 (d)

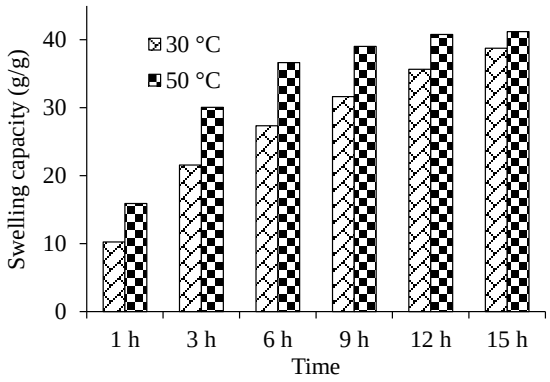

Figure 6: Swelling capacity of LSH at 30 °C and 50 °C

CONCLUSION

Thermal degradation analysis of linseed hydrogel (LSH) demonstrated that it is a thermally stable polysaccharide with Td_m of 287 °C. Therefore, formulation pharmacists/chemists can consider the option of using LSH for achieving higher shelf life of drugs. Secondly, LSH appeared to be a modifiable polysaccharide towards its acetylated derivatives. Acetylated LSH (ALSH) exhibited extra thermal stability (Td_m 328 °C), as compared to unmodified LSH. First-order degradation reaction kinetics was noted for both LSH and ALSH. Several kinetic and thermodynamic parameters were determined and their values were also found higher for ALSH than LSH. The high thermal stability of LSH and ALSH makes them competitive candidates to commercially available polysaccharides for drug tablet formulation.

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