Sonolytic Degradation of High-Molecular Weight amylose by graphene oxide nanoparticles

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Abstract— **Solution of amylose/GO with different concentrations (5, 10 and 15g/L) and different volumes (25, 50, 75 and 100 ml) were subjected to ultrasonic degradation with power of ultrasound in the range of 30-90W. Ultrasonic treatment of amylose is an alternative, safe method to prepare amylose having different molecular weights, which are more suitable for biomedical and food applications. The changes in viscosity average molecular weight (M_v) were investigated. A method of viscometry was used to study the degradation behaviour and kinetic model was developed to estimate the degradation rate constant. The degradation rate constant was correlated with the power input due to ultrasonic irradiation and reaction volume. It was found that rate constant decreases as the reaction volume and concentration increases. The decrease in rate constant is attributed to the fact that at higher concentration and at higher volume, the intensity of cavitation phenomenon is depressed and therefore the extent of polymer chain breaking decreases. In the case of ultrasound power, the extent of ultrasonic degradation increased with increasing ultrasound power.**

Index Terms—**Amylose, Graphene Oxide, Molecular weight, Ultrasonic, Viscosity**

I. INTRODUCTION

Amylose is the linear component of common starch and characterized by its straight chains which are made up of α-(1→4) bound glucose molecules and it has drawn much attention due to its unique qualities, such as its film-forming ability and strength [1]. Graphene, a kind of two-dimensional nanofiller with a thickness of one atomic layer, is a single layer of sp^2-hybridized carbon atoms. In recent years, much attention and interest have been given to graphene because of its excellent mechanical, electronic and thermal properties [2]. The ability of graphene to enhance the properties of polymers and carbohydrate as a catalyst has also been reported [3].

Application of high-intensity ultrasound to dispersions of carbohydrates can lead to depolymerization because of the intense mechanical and chemical effects associated with cavitation [4]. Cavitational thermolysis may produce hydroxyl radicals and hydrogen atoms that can be followed by formation of hydrogen peroxide [5]. These transient reactive species can subsequently react with carbohydrates. In addition, hydrolysis and cleavage due to the strong

mechanical forces has been reported for a variety of polysaccharides [6]. It is very important to optimize the operating conditions for obtaining maximum benefits in energy efficient manner. This necessitates the identification of the appropriate solution concentration, irradiation power, irradiation time, and reaction volume. Therefore, the specific objective of this study was to determine the influence of ultrasound power, concentration and volume of sonicated solution on the rate constant of degradation process. The goal was to use a kinetics model based on viscometry dada that can be used to predict the rate of degradation of amylose. Finally we provide a comparisonal view that permits us to select an optimum value of parameters to perform a faster process in order to obtain the low-molecular weight amylose.

II. MATERIALS AND METHODS

A. Materials

Amylose $(C_6H_{10}O_5)_n$ was purified from potato starch, with purity of 99% was purchased from Gamay industrial technology Co. Ltd, Shanghai-China. The purchased amylose sample was dissolved in dry DMSO at the concentration of 1mg/mL and centrifuged at 2000×g for 3min to remove any insoluble impurities. Subsequently, Ethanol was added to the supernatant to precipitate the amylose, which was dried under high chromatography (SEC), using monodisperese PEG (M_w=20000 Da) as a calibration standard and water as a mobile phase. SEC results revealed that the amylose has an average molecular weight (M_w= 48600 Da) and polydispersity of 3.78. Graphite was purchased from Aldrich. Other chemicals were supplied from Merck. All chemicals were analytical grade and used as received. Doubly distilled water was prepared using a laboratory distiller device.

B. Methods

1) Experimental setup and procedure

Reactions were carried out in a cylindrical 100ml Pyrex glass vessel. An ultrasound generator (Dr. Hielscher Ultrasonic Processor UP200 H) operating at a fix frequency of 24 kHz and a variable power output up to 100W nominal value, in aqueous media was used for sonication experiments. A titanium-made H3 sonotrode (φ=3mm) immersed in liquid from the open to the atmosphere top of the vessel was used to deliver the ultrasound energy in the reaction mixture. The vessel was fed with a different volume of amylose solution and the reaction temperature, was kept constant at 25±1°C through the use of cooling water circulating through the double-walled compartment, thus acting as cooling jacket.

2) Preparation of the amylose solutions

0.5g of amylose was dispersed in 100mL distilled water, degassed and heated to 100°C before stirred magnetically. The mixture was stirred and heated at 90±5°C for 8h to yield a transparent solution and the cooled to the room temperature prior to use.

3) Preparation of the GO/amylose solution

GO/amylose solution with different amount of GO loadings (0.0, 0.2, 0.4, 0.6 and 0.8 wt %) was prepared. Within the preparing of GO/amylose solution ultrasonic treatment carried out as follow: at the first step the 2g amylose was dispersed into 200mL distilled water and desired amount of GO was subsequently added to the amylose suspension. Then, the obtained suspensions was exposed at ultrasonic radiation at 10, 20, 30, 40 and 50W power of sonication at the aqueous media for 30h and stirred and simultaneously, fixed temperature by means of a thermostat at 30°C. The homogenous solution was obtained and used for experimental purposes.

4) Viscosity measurements

The intrinsic viscosities of the original amylose and its degraded solutions at 25°C were measured using the capillary viscometer (Setavic Kinematic viscometer).
Efflux times were measured for Amylose solutions (ts) and the solvent (t0). Measurement of efflux times were repeated two times and average efflux time was then converted to the ratio of ts/t0, which is proportional to relative viscosity, ηr, of PVP solution.

$$\eta_r = \frac{t}{t_0} \tag{1}$$

$$\eta_{sp} = \eta_r - 1 \tag{2}$$

The intrinsic viscosity [η] values can be related to the specific viscosity, η_{sp} and relative viscosity, η_r by the Huggins and Kramer equations [7].

5) Kinetic model

The rate of degradation is defined as the number of scission that occurs in 1 L in unit time and we must keep in mind that a scission in a chain yields two pieces. Thus, the rate equation of the degradation is as follows [8]:

$$R = \frac{dM}{dt} = kM^n \tag{3}$$

Where, M, is the total molar concentration of the polymer, k, is the rate constant and, n, is the order of reaction with respect to the total molar concentration of the polymer. From the experimental data, it is clear that the degradation rate decreases with increasing solution concentration, so "n" is negative.

Harkal et al. was found that the order of ultrasonic degradation reaction for poly (vinyl alcohol) obtained from this kinetic model is -1[40].

It is noted that solution concentration (g/L) is constant and the total molar concentration (mol/L) increases during the degradation of polymer. The solution of differential Eq. (3) is:

$$M^{1-n} - M_0^{1-n} = (1-n)kt \tag{4}$$

Where M_0 is the initial total molar concentration of polymer. The total molar concentration is related to the number average molecular weight through [41]:

$$M = \frac{C}{M_n} \tag{5}$$

Moreover, viscosity average molecular weight, Mv, is related to the number average molecular weight, Mn, through [42].

$$M_v = [(1+\alpha)\Gamma(1+\alpha)]^{\frac{1}{\alpha}} M_n \tag{6}$$

Mv is related to the intrinsic viscosity, [η], through Marck-Houwink equation:

$$M_v = \left(\frac{[\eta]}{K}\right)^{\frac{1}{\alpha}} \tag{7}$$

Where α and k are the Mark–Houwink constants. Finally, [η] can be related to the specific viscosity, ηsp, and relative viscosity, ηr, by Huggins and Kramer equations:

$$\frac{\eta_{sp}}{C} = [\eta] + K'[\eta]^2 C \tag{8}$$

$$\frac{Ln\eta_r}{C} = [\eta] + (K' - 0.5)[\eta]^2 C \tag{9}$$

From Eq. (8) and (9), intrinsic viscosity is:

$$[\eta] = \frac{\sqrt{2(\eta_{sp} - Ln\eta_r)}}{C} \tag{10}$$

Substitution of Eq. (10) in (7) and Eq. (7) in (6) yields

$$M_n = \left(\frac{[2(\eta_{sp} - \ln \eta_r)]^{0.5}}{Ck[(1+\alpha)\Gamma(1+\alpha)]}\right)^{\frac{1}{\alpha}} \tag{11}$$

Finally, the substitution Eq. (11) in (5) yields:

$$M = \left(\frac{C^{\alpha+1}k[(1+\alpha)\Gamma(1+\alpha)]}{\sqrt{2}}\right)^{\frac{1}{\alpha}} \Delta\eta \tag{12}$$

In addition, substitution Eq. (12) in (4) yields:

$$\Delta\eta^{1-n} - \Delta\eta_0^{1-n} = (1-n)\left(\frac{\sqrt{2}}{C^{\alpha+1}k[(1+\alpha)\Gamma(1+\alpha)]}\right)^{\frac{1-n}{\alpha}} kt \tag{13}$$

Or

$$\Delta\eta^{1-n} - \Delta\eta_0^{1-n} = k't \tag{14}$$

III. RESULTS AND DISCUSSION

1) Effect of concentration on rate of degradation

At the level of interatomic distances within the macromolecules, there is still some debate regarding the place where the bond breakage occurs. By analogy with the chemical degradation, it is expected to take place at the weakest links in the polymer backbone, but some works suggested that it mainly occurs at the midpoint of the polymer chains and the existence of a final limiting molecular weight is predicted, below which ultrasounds have no more effect. Several studies propose a random chain breakage but still consider that some bonds are more resistant, in relation to the decrease in the scission rate constant as lower values of degree of polymerization are reached [9]. Due to the polydisperse nature of most polymers, an accurate analysis of the degradation kinetics is almost impossible without information about the location of chain scission and the dependence of rate coefficients on the molecular weight of the polymer. Two simplified models, based on different assumptions of the location of chain scission, have been proposed to quantitatively describe the degradation process of polymers [10].

Although, a number of different rate models have been proposed for the degradation of polymers, but in this study a simple model was employed via viscometry, Using Eq. (14). A negative order for the dependence of the reaction rate on total molar concentration of amylose solution within the degradation process was suggested. In the initial sonication times, for different concentration of polymer we calculate total molar concentration of polymer. The slopes of plots give the initial rate of degradation using Eq.20, the plot of lnR versus ln[M] are linear. The slope of curve is -0.7, which suggest the order of reactions with respect to total molar concentration of polymer. From substitution of the value of "n" in Eq.14, we obtain the following:

$$\Delta\eta^{1.7} - \Delta\eta_0^{1.7} = k't \tag{15}$$

2) Effect of power of ultrasound on rate of degradation

Fig. 1 shows the effect of increasing changing ultrasound power on relative viscosity (η_r) of amylose solution as a function of the sonication time at 5g/L initial polysaccharide concentration under air. As seen, η_r decreases with increasing the nominal applied power from 30 to 90W.

In aqueous phase sonolysis, there are three potential sites for sonochemical activity, namely: (i) the gaseous region of the cavitation bubble where volatile and hydrophobic species are easily degraded through pyrolytic reactions as well as reactions involving the participation of hydroxyl radicals with the latter being formed through water sonolysis:

$$H_2O \rightarrow H^{\bullet} + OH^{\bullet} \tag{16}$$
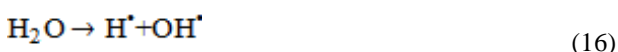
(ii) the bubble–liquid interface where hydroxyl radicals are localized and, therefore, radical reactions predominate although pyrolytic reactions may also, to a lesser extent, occur and (iii) the liquid bulk where secondary sonochemical activity may take place mainly due to free radicals that have escaped from the interface and migrated to the liquid bulk. It should be pointed out that hydroxyl radicals could recombine
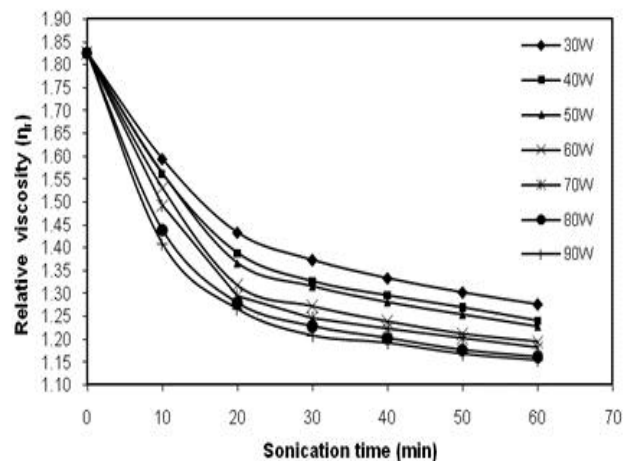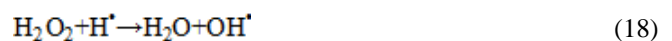

Fig. 1. The relationship between η_r and sonication time in ultrasonic process, for different power of ultrasound at 25°C.

Yielding hydrogen peroxide, which may in turn react with hydrogen to regenerate hydroxyl radicals:

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \tag{17}$$

$$H_2O_2 + H^{\bullet} \rightarrow H_2O + OH^{\bullet} \tag{18}$$

Given that amylose is a non-volatile and soluble polysaccharide, hydroxyl radical-mediated reactions occurring primarily in the liquid bulks as well as at the bubble interface are likely to be the dominant degradation pathway. The plots of $\Delta\eta^{1.7} - \Delta\eta_0^{1.7}$ versus sonication time for different powers of ultrasound are presented in Fig.2. The apparent degradation rate constant, k′, defined in Eq. 14, can be estimated from the slopes of the plots in Fig.2. Based on these results, degradation rate constants, k, were calculated that are shown in Table 1. It can be seen that at the same concentration, the extent of degradation increases with an increase in applied ultrasound power. In quantitative terms, in 60min of irradiation time the extent of degradation at 90W is three times higher as compared to degradation at 30W.
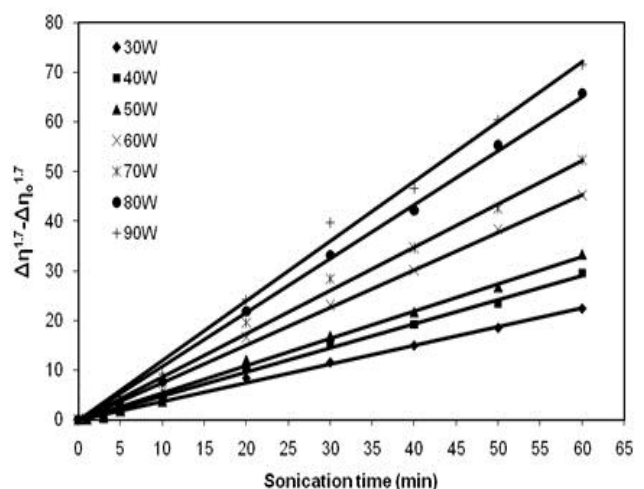

Fig. 2. The plot of $\Delta\eta^{1.7} - \Delta\eta_0^{1.7}$ versus the sonication time in ultrasonic process, for different power of ultrasound at 25°C.

US (W)	C	Volume	$\overline{M}_v$	$k \times 10^9$
30	5	100	88.990	1.538
40	5	100	77.244	1.990
50	5	100	72.539	2.244
60	5	100	61.782	3.098
70	5	100	57.079	3.571
80	5	100	50.372	4.445
90	5	100	48.029	4.922
30	5	75	47.695	5.263
30	5	50	46.692	5.550
30	5	25	43.687	6.027
30	10	100	73.547	2.222
30	10	75	67.160	2.686
30	10	50	62.790	2.977
30	10	25	59.430	3.333
30	15	100	88.990	1.521
30	15	75	84.295	1.746
30	15	50	74.556	2.147
30	15	25	60.101	3.221

Table 1. The effect of power of ultrasound, concentration and volume of solution on the degradation rate constants, and viscosity-average molecular weight, M_v of degraded chitosan samples at 25°C and at the end of 1h.

3) Effect of volume on rate of degradation

Fig. 3 shows the effect of the reaction volume on the extent of degradation (depicted in terms of the change in $\Delta\eta^{1.7}-\Delta\eta_0^{1.7}$ at 15g/L amylose concentration. It can be easily seen from the figure that the extent of degradation decreases with an increase in the reaction volume at same supplied ultrasonic power dissipation. To give a quantitative idea, in 60min of irradiation time the extent of degradation at 25 ml reaction volume is 100% higher as compared to degradation at 100 ml reaction volume. This is attributed to the fact that, increase in the reaction volume decreases the power density of the system (power dissipation per unit volume) resulting in a corresponding decrease in the cavitational activity. Also in the case of ultrasonic horn, the active cavitational volume is restricted very near to the transducer surface (there is only one transducer in the case of ultrasonic horn unlike ultrasonic bath type of systems where multiple transducers can be used; thus in the case of ultrasonic bath, the effect of reaction volume would be different though it is not within the scope of present work) resulting in non-uniform distribution of the cavitational activity. Indeed with an increase in the operating volume, the non-uniformity of the cavitational activity increases (more dead zones where the cavitational activity is minimal) resulting into detrimental effects.

That the trends are exactly identical though the extent of degradation and extent of enhancement obtained with lowering the reaction volume is different. Rate constants for the various experiments are given in Table 1. Also, limiting viscosity average molecular weights in different experimental conditions has presented in this table. The initial viscosity

average molecular weight is 264.196 kDa and as seen, when the volume of solution decreases, with decrease in concentration, the limiting M_v show a decreasing state. This result is in accordance with our findings about the effect of volume changes on rate constants.
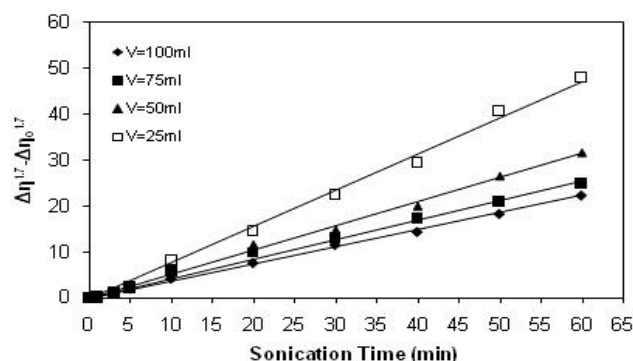

Fig. 3. The plot of $\Delta\eta^{1.7}-\Delta\eta_0^{1.7}$ versus the sonication time for the concentration of 5g/L, for different volumes of solution at constant power of ultrasound (30W) at 25°C.

IV. CONCLUSIONS

The effect of reaction volume and concentration on the ultrasonic degradation of amylose in solution was investigated. The experimental results indicated that the degradation rates reduced with increasing reaction volume. This could be explained by the reduction in energy dissipation per unit volume. It was observed that, at the same reaction volume, increase in solution viscosity decreases the rate of reaction. In addition, the experimental results indicated that the degradation rate reduced with increasing solution concentration and increased with increasing of input power of ultrasound. This is an agreement with earlier studies. Applied kinetic model interpreted the experimental findings in an optimal way and thus, viscosity is a practical approach for monitoring the degradation of polymers in a solution.

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