Carbohydrate Polymers 156 (2017) 71–76

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Molecular weight kinetics and chain scission models for dextran polymers during ultrasonic degradation

Yuanyuan Pu[a,b], Qingsong Zou[a,b], Dianzhi Hou[a,b], Yiping Zhang[a,b], Shan Chen[a,*]

[a] Center for Sugar Engineering and Technology Research, Guangxi University, Nanning 530004, PR China
[b] College of Light Industry and Food Engineering, Guangxi University, Nanning 530004, PR China

ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 19 August 2016
Accepted 6 September 2016
Available online 7 September 2016

Keywords:
Ultrasound
Dextran
Molecular weight
Kinetics
Chain scission

ABSTRACT

Ultrasonic degradation of six dextran samples with different initial molecular weights (IMW) has been performed to investigate the degradation behavior and chain scission mechanism of dextrans. The weight-average molecular weight (*Mw*) and polydispersity index (*D* value) were monitored by High Performance Gel Permeation Chromatography (HPGPC). Results showed that *Mw* and *D* value decreased with increasing ultrasonic time, resulting in a more homologous dextran solution with lower molecular weight. A significant degradation occurred in dextrans with higher IMW, particularly at the initial stage of the ultrasonic treatment. The Malhotra model was found to well describe the molecular weight kinetics for all dextran samples. Experimental data was fitted into two chain scission models to study dextran chain scission mechanism and the model performance was compared. Results indicated that the midpoint scission model agreed well with experimental results, with a linear regression factor of $R^2 > 0.99$.

1. Introduction

Dextran is a homo-polysaccharide of D-glucose, consisting of more than 50% of α-(1 → 6) linkages in the main chain and other linkages in the branch (Petronijevic, Ristic, Pesic, & Smelcerovic, 2007; Purama, Goswami, Khan, & Goyal, 2009). Dextran is a biopolymer produced via microbial fermentation, particularly from *Leuconostoc mestenteroides* strains (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). The exact structure of dextran depends on specific microbial strains (Robyt, Yoon, & Mukerjea, 2008).

As a polymer, degrees of polymerization of D-glucose determine the molecular weight of dextran. Dextran polymers with different molecular weights have different applications. On the one hand, macromolecular dextran ($Mw > 2 \times 10^7$ Da) can be used as gel filtration media in the fine chemical industry (Majumder, Purama, & Goyal, 2007). Addition of a small amount of high molecular weight dextran improves quality of bakery products such as yeast-raised doughs (Bohn, 1961). On the other, dextran with a certain low molecular weight ($Mw < 8 \times 10^4$ Da) and a narrow molecular weight distribution is often used for preparation of clinical grade dextran which can be used as blood-plasma substitutes in the pharmaceutical industry (Lakshmi Bhavani & Nisha, 2010; Zdolsek et al., 2011). And this clinical product is in a growing demand in recent years. Currently, low molecular weight dextrans were obtained from acid hydrolysis (Guimaraes, Costa, Rodrigues, & Maugeri, 1999) or enzymatic degradation (Kim & Day, 1994) of macromolecular dextrans. However, quality of the degradation products is far from satisfactory, as it has a relatively wide molecular weight distribution which might induce clinical side effects in application. Therefore, a rapid and efficient approach for producing dextrans with a molecular weight range that meets clinical requirements is important.

Ultrasonic treatment, one of the most promising methods of polymers degradation, has received considerable attention for being able to irreversibly lower polymer chain length without causing any chemical change (Suslick & Price, 1999; Taghizadeh & Bahadori, 2009). The use of ultrasonic degradation for producing low molecular weight dextrans has been investigated in the last decades. However, previous studies focused on investigating the effects of several parameters on the reduction of molecular weight. Various factors, including ultrasound parameters (frequency and power), solvent properties (concentration and temperature), and operating parameters (i.e., ultrasonic time, the depth of horn) have been studied (Koda, Taguchi, & Futamura, 2011; Zou et al., 2012), suggesting that the decrease in molecular weight was remarkable at higher power, lower temperature and lower concentration.

The interests of studying polymers degradation kinetics and chain scission mechanism is growing rapidly. Theoretical and

Abbreviations: IMW, initial molecular weights; *Mw*, weight-average molecular weight; *D* value, polydispersity index; HPGPC, High Performance Gel Permeation Chromatography.

* Corresponding author.
E-mail addresses: yuanyuan.pu@ucdconnect.ie (Y. Pu), Qingsong.Zou@ul.ie (Q. Zou), xiaozhihou90@126.com (D. Hou), feyzhang1989@mail.scut.edu.cn (Y. Zhang), chenshan@gxu.edu.cn (S. Chen).

http://dx.doi.org/10.1016/j.carbpol.2016.09.017
0144-8617/

empirical models relating to polymer degradation have been proposed by others (Baramboĭm, Moseley, & Watson, 1964; Malhotra, 1986; Ovenall, Hastings, & Allen, 1958), which described the changes of molecular weight as a function of ultrasonic time. Though the exact mechanism of polymer scission during ultrasonic process is doubtable, it is generally believed that the cavitation effect caused by ultrasound waves is mainly responsible for the chain rupture (Kardos & Luche, 2001). It is assumed that the degradation is a mechanical effect caused by the rapid growth and collapse of microbubbles when the polymer solution was exposed to ultrasound (Basedow & Ebert, 1977; Price, 1990; Price, Daw, Newcombe, & Smith, 1990). Near the collapsing microbubbles, polymer chains are captured in a high-gradient shear field, causing the polymer segments to move at a higher velocity than those farther away from the collapsing cavity in this shear field. It is the stress generated from relative motions between the polymer segments and the solvent that cause chain rupture (Madras & McCoy, 2001). Regarding the location of chain scission, two simplified models have been proposed and summarized in detail by researchers (Aarthi, Shaama, & Madras, 2007; Bose & Git, 2004; Wu, Zivanovic, Hayes, & Weiss, 2008). One is the midpoint scission model, which assumes that the chain rupture occurs at the center of the chain backbone space (Price & Smith, 1991); the other is the random scission model, which assumes that polymers can be degraded randomly and any chain connection has an equal chance of rupture (Jellinek, 1955).

The model of midpoint chain scission ($P(x) \rightarrow 2P(x/2)$) can be expressed as shown in Eq. (1),

$$\ln\left(\frac{M_0 - M_{\lim}}{M_t - M_{\lim}}\right) = k_1 M_{\lim} t \quad (1)$$

where M_0, M_t and M_{lim} represent the initial molecular weight, the molecular weight at a given ultrasonic time t and the limiting molecular weight, respectively; k_1 is the degradation rate constant for the midpoint scission model.

The model of random chain scission ($P(x) \rightarrow P(x-x') + P(x')$) can be expressed as shown in Eq. (2),

$$\frac{M_{\lim}}{M_t} + \ln\left(1 - \frac{M_{\lim}}{M_t}\right) = -\frac{k_2}{c} t \left(\frac{M_{\lim}}{m}\right)^2 + \frac{M_{\lim}}{M_0} + \ln\left(1 - \frac{M_{\lim}}{M_0}\right) \quad (2)$$

where the meaning of M_0, M_t, M_{lim} and t is the same as above; c is the initial concentration of polymer solution; m is for the molecular weight of monomer; and k_2 is the degradation rate constant for the random scission model.

Extensive work has been done on the scission models of different polymers. The midpoint chain scission model was found to be suitable for describing ultrasonic degradation of poly vinyl alcohol (Vijayalakshmi & Madras, 2006), poly ethylene oxide (Vijayalakshmi & Madras, 2005) and polyacrylamide (Vijayalakshmi, 2004). Baxter, Zivanovic, and Weiss (2005) and Wu et al. (2008) have compared the two models by ultrasonic degradation of chitosan, and the results indicated that experimental data showed good agreements with the random chain scission model.

To the best of our knowledge, chain scission model for dextran solution is not available in open literature. In this work, six dextran samples with different IMW (ranging from low molecular weight to high molecular weight) were applied for ultrasonic degradation, aiming to investigate the degradation kinetics (molecular weight changes, polydispersity changes, degradation rates, molecular distributions) during ultrasonic treatment process. Both midpoint scission model and random scission model was used to fit the experimental data for a better understanding of the chain scission mechanism of dextran.

2. Materials and methods

2.1. Dextran samples

Six dextran samples with different initial molecular weights were used in the experiment. Dex-40, Dex-70, Dex-500 and Dex-1000 (with Mw of 4.47×10^4, 6.52×10^4, 5.39×10^5, 11.53×10^5 Da, respectively) were purchased from Pharmacia Fine Chemical Co., Ltd. (New Jersey, USA); Dextran High Fraction (Dex-HF, $Mw = 28.30 \times 10^4$ Da) was obtained from Acros Organics (New Jersey, USA); Dextran Fermentation sample (Dex-F, $Mw = 17.05 \times 10^5$ Da) was produced by *Leuconostoc Mesenteroides* 10074 (China Center of Industrial Culture Collection, CICC) on a sucrose liquid medium in the laboratory.

2.2. Ultrasonic experiment

Ultrasonic degradation of aqueous dextran was conducted by using an ultrasonic generator (Ningbo Scientz Biotechnology Co., Ltd, China) at frequency of 20 kHz and power output of 600 W. Six dextran solutions with an initial concentration of 10 mg/mL were prepared with ultrapure water. A cylindrical glass vessel with a diameter of 40 mm, containing 50 mL of dextran solution, was submerged into an ice bath to keep the solution at a temperature of $10 \pm 1\,^{\circ}C$. The depth of the ultrasonic probe was 15 mm below the solution surface. During the degradation process, 1.5 mL of solution was extracted from the solution at a given ultrasonic time (i.e., 0, 10, 20, 30, 40, 50 and 60 min) for HPGPC analysis. All runs were carried out in triplicate.

2.3. Molecular weight measurement

HPGPC was used to characterize the molecular weight distribution of dextran as described by Zou et al. (2012). Briefly, Shodex Sugar KS-801, KS-805 and KS-G columns were connected in series in the HPGPC system, an Agilent G1362A differential refraction detector was used to capture the refractive index differences between sample and reference cell. The temperature of the columns was maintained at $50 \pm 1\,^{\circ}C$ by a column oven and the ultrapure water was used as the mobile phase with elution flow of 1 mL/min. The standard curve for dextran molecular weight was obtained using a series of standards (Polymer Laboratories Ltd., USA). Samples were collected from dextran solution using disposable aseptic syringes during the ultrasonic degradation process and filtered by 0.45 μm syringe membrane (Membrana, Germany) before HPGPC analysis. The weight-average molecular weight (Mw) and polydispersity index (D value) of dextran solutions at a specific ultrasonic time were recorded for subsequent data analysis.

2.4. Calculation of ultrasonic degradation rate

With ultrasonic treatment, the molecular weight of dextran solvent decreased with increasing ultrasonic time. The degree of the degradation was calculated as shown in Eq. (3),

$$\text{Degradation rate}(\%) = \frac{Mw_0 - Mw_t}{Mw_0} \times 100 \quad (3)$$

where Mw_0 was the initial weight-average molecular weight of dextran, Mw_t was the weight-average molecular weight of dextran at a certain ultrasonic time t.

Table 1
Changes of weight-average molecular weight of each dextran sample during ultrasonic treatment.

t (min)	Dex-40 ($\times 10^4$ Da)	Dex-70 ($\times 10^4$ Da)	Dex-HF ($\times 10^4$ Da)	Dex-500 ($\times 10^5$ Da)	Dex-1000 ($\times 10^5$ Da)	Dex-F ($\times 10^5$ Da)
0	4.47	6.52	28.30	5.39	11.53	17.05
10	4.32	6.25	16.25	2.84	4.51	4.43
20	4.17	6.05	12.62	2.17	3.19	2.78
30	4.03	5.91	11.21	1.80	2.56	2.14
40	3.90	5.73	10.31	1.63	2.17	1.85
50	3.75	5.65	9.65	1.48	1.96	1.61
60	3.69	5.50	8.88	1.37	1.77	1.45

3. Results and discussion

3.1. Changes of Mw in six dextran samples during degradation process

The initial molecular weight, a parameter of considerable importance in ultrasonic degradation, has received much attention in the experiment. Six dextran samples with initial molecular weights ranging from 4.47×10^4 Da to 17.05×10^5 Da were depolymerized for 60 min under current ultrasonic condition. Table 1 shows a summary of the results. It was observed that the molecular weight of dextran decreased as the ultrasonic time increased in all dextran samples. For low IMW dextran samples (Dex-40 and Dex-70), molecular weight decreased gradually with ultrasonic time. However, when the ultrasound was applied to a dextran solution of higher IMW (Dex-HF, Dex-500, Dex-1000, and Dex-F), a sharp decrease in the weight average molecular weight was detected at the beginning of the treatment (10 min), followed by a slight molecular weight decrease. For example, the *Mw* of Dex-HF decreased from 28.3×10^4 Da to 16.25×10^5 Da *Mw* after 10 min ultrasonic treatment, and the *Mw* of Dex-F (a sample with the highest IMW in the study) decreased from 17.05×10^5 Da to 4.43×10^5 Da at the same experimental conditions.

The rate of dextran degradation as a function of ultrasonic time was calculated and is shown in Fig. 1. A linear correlation between the degradation rate and ultrasonic time was observed in the Dex-40 and Dex-70 samples, with degradation rate increasing gradually with time, which indicated a slow ultrasonic degradation progress. However, it was observed that there was a significant degradation in the higher IMW dextran samples and at the early stage (0–20 min). When ultrasonic treatment exceeded 20 min, the molecular weight of dextran gradually reached a certain limit (M_{lim}); a minimum molecular weight that samples can be degraded without changing ultrasonic treatment condition. At this stage, no matter how long the ultrasonic treatments being conducted, the weight-average molecular weight could not be lower than M_{lim}.

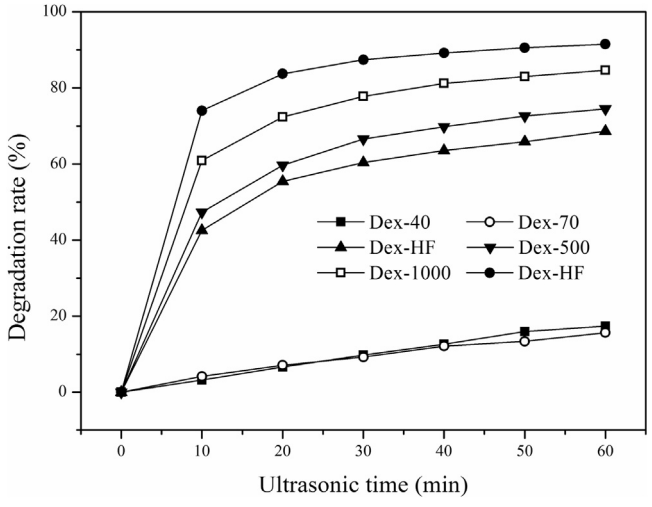

Fig. 1. Changes of degradation rate of six dextran samples with increasing ultrasonic time.

A typical model proposed by Malhotra for describing the molecular weight evolution during polymers degradation is expressed as shown in Eq. (4).

$$\frac{1}{M_t} = \frac{1}{M_0} + Kt \tag{4}$$

Lorimer, Mason, Cuthbert, and Brookfield (1995) used this Malhotra model to describe the dextran ultrasonic process and results provided good linear plots. However, the study was concerned with one dextran with IMW of 9.8×10^5 Da. In the present study, six dextran samples with a wide range of IMW were applied, and the results are shown in Fig. 2. A plot of $1/M_t$ versus ultrasonic time *t* in all samples yields a straight line, where its slope stands for the degradation rate constant *K*, which further proves that the molecular weight evolution of dextran degradation can be accurately expressed by the Malhotra model.

3.2. Changes of D value in six dextran samples during degradation process

Numerous studies have demonstrated that ultrasonic treatment narrows the molecular weight distribution of polymers (Gronroos, Pirkonen, & Ruppert, 2004; Li, Li, Guo, & Li, 2005). Fig. 3 shows the molecular weight distribution of Dex-1000 for a given ultrasonic time. With prolongation of ultrasonic time, the peak area corresponding to macromolecular dextran decreased and shifted gradually toward a lower molecular weight range. Though the peak associated with high molecular weight dextran still presented in the sample that underwent 60 min treatment, a substantial proportion of that has disappeared. The result is consistent with Szu's research (Szu, Zon, Schneerson, & Robbins, 1986), in which Dex-2000 was used as a model to investigate the effect of ultrasonic irradiation on the molecular weight of neutral polysaccharides.

D value (the ratio of weight-average molecular weight and number-average molecular weight, $D = Mw/Mn$) is an important indication in characterizing the polydispersity of dextran. The smaller the *D* value is, the more homologous the dextran solution will be. Fig. 4 shows changes of *D* value as a function of ultrasonic time for all dextran samples. The results showed that the changing tendency of the *D* value is similar to those of molecular weight. In samples of Dex-40 and Dex-70, the reduction in *D* value was slow. In higher IMW samples of Dex-HF, Dex-500 and Dex-1000, the *D* value decreased from 7.4–7.9 at the beginning of treatment (0 min) to 2.7–3.8 at the end (60 min). It is noteworthy that a remarkable drop in *D* value was observed for Dex-F, a sample with a wide molecular weight distribution ($D = 81.51$), when 60 min of ultrasonic treatment was applied ($D = 7.09$), which suggested that ultrasonic treatment is an effective approach to produce a more homologous dextran solution with a narrower molecular weight distribution.

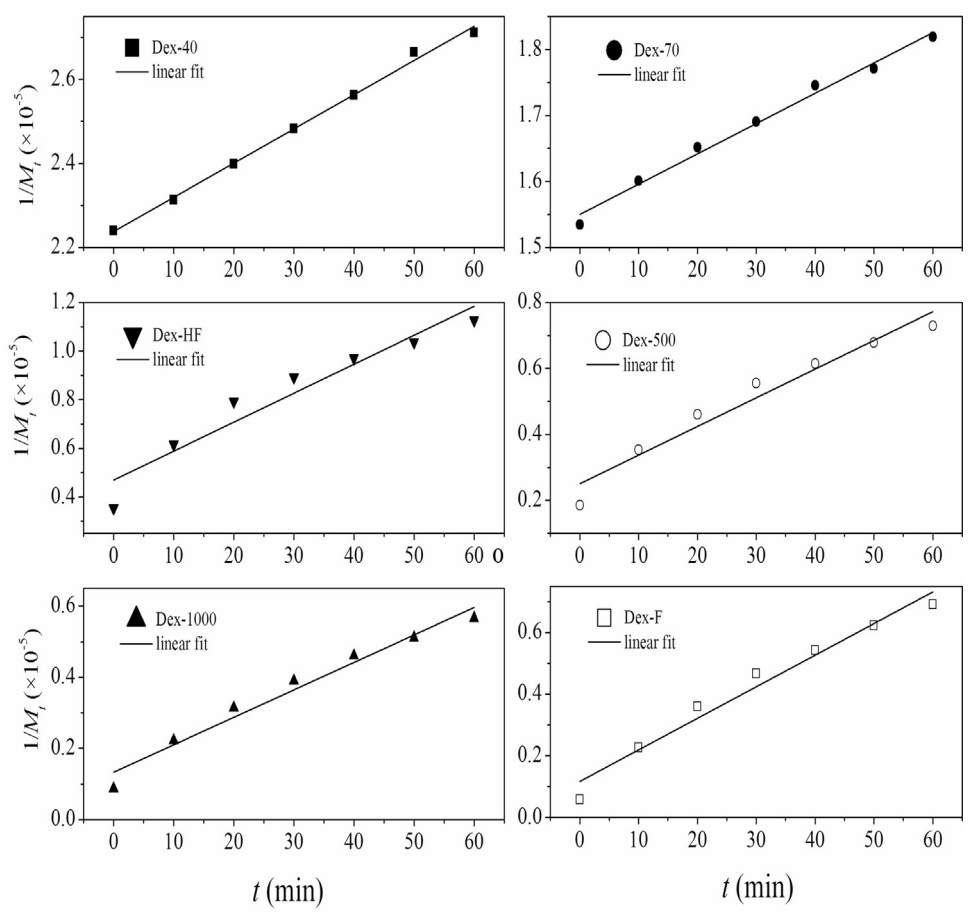

Fig. 2. Plots of $1/M_t$ as a function of ultrasonic time (t) in six dextrans.

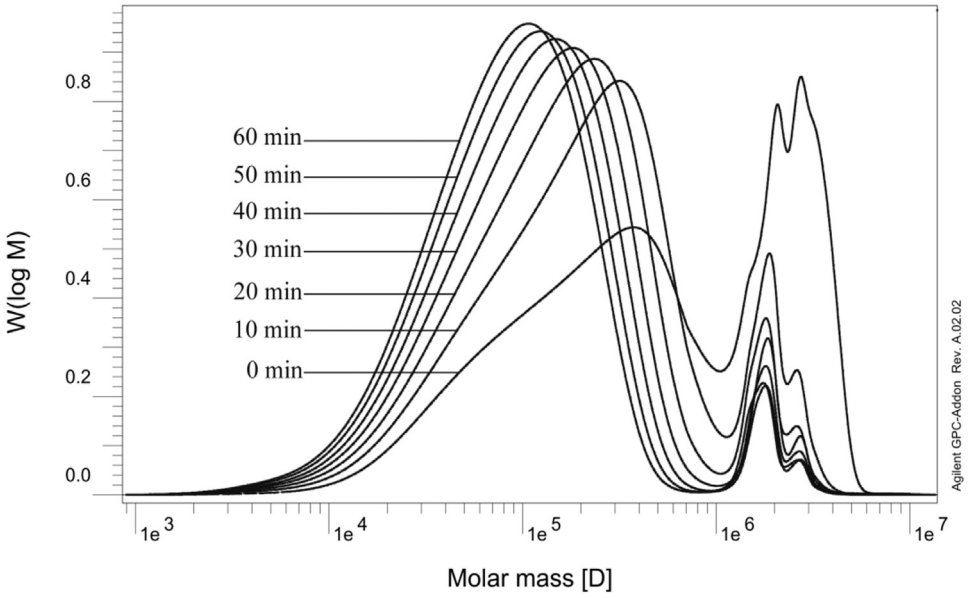

Fig. 3. Molecular weight distribution of Dex-1000 as a function of ultrasonic time.

3.3. Chain scission models for dextran ultrasonic degradation

Several models describing the molecular weight evolution during polymers ultrasonic degradation suggested that the molecular weight decreases rapidly in the early stage and then proceeds slowly to a limiting molecular weight (Akyuz, Catalgil-Giz, & Giz, 2008; Tang & Liu, 2006). The Malhotra model produces a linear relationship between $1/M_t$ and t in our study, but the lacking of a limiting molecular weight is a weak point of this model (Baramboïm et al., 1964). It is merely a phenomenological model exhibiting the changes in molecular weight as a function of time without proposing a scission mechanism (Akyuz et al., 2008).

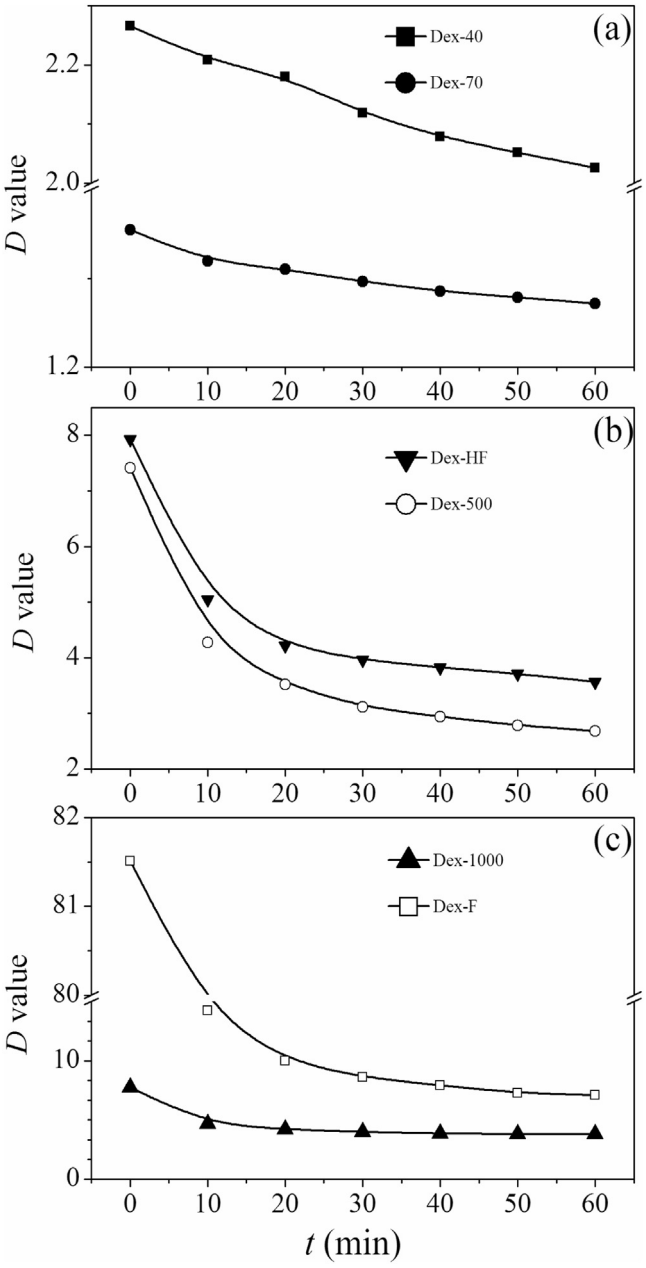

Fig. 4. Changes of *D* value in six dextrans during the degradation process.

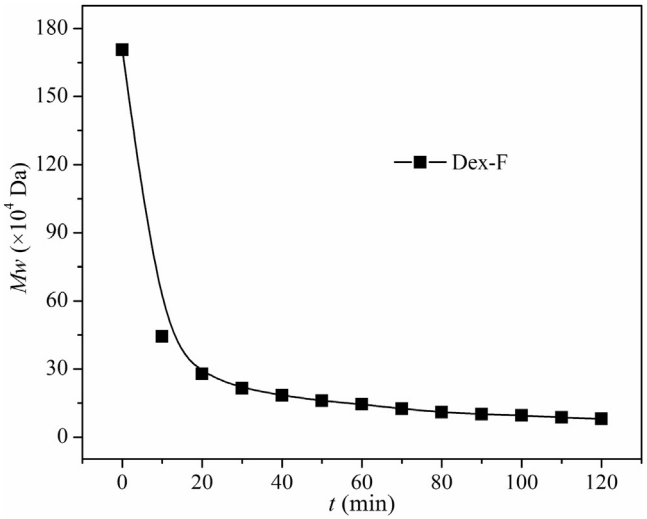

Fig. 5. Molecular weight evolution of Dex-F during 120 min ultrasonic treatment.

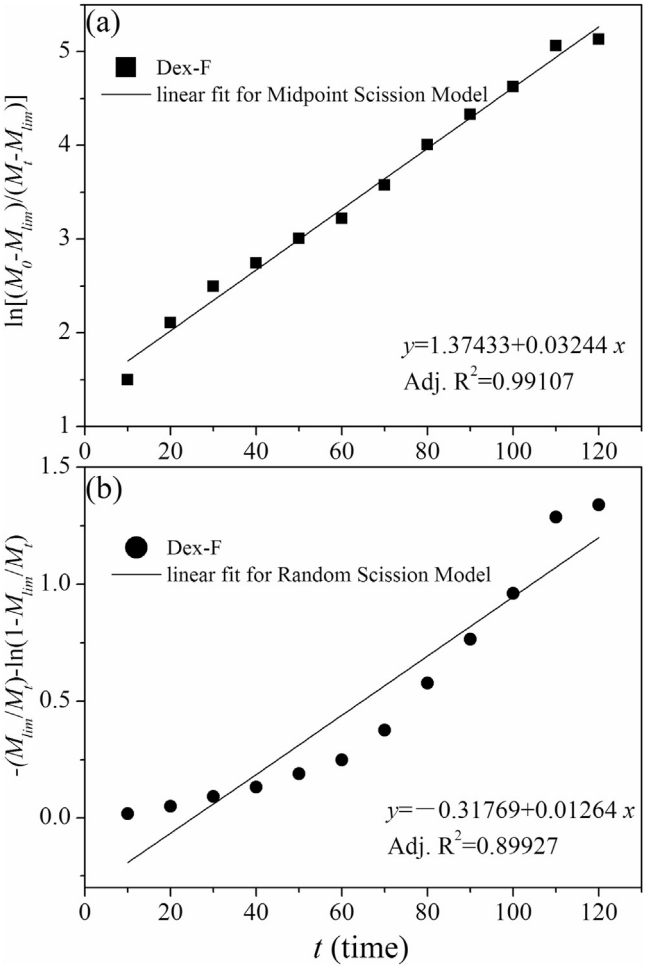

Fig. 6. Comparison of midpoint scission model and random scission model for Dex-F.

The limiting molecular weight (M_{lim}) is an important parameter in understanding the chain scission mechanism of dextran. For this purpose, ultrasonic time for Dex-F was prolonged to 120 min until little change in molecular weight was observed. As shown in Fig. 5, when ultrasonic treatment was carried out for 120 min, the molecular weight eventually tended toward a limiting value of 8.0×10^4 Da, which can be used as the experimental M_{lim} for the dextran chain scission models. A plot of $\ln[(M_{lim} - M_0)/(M_t - M_0)]$ versus ultrasonic time t for the midpoint scission model and $-(M_{lim}/M_t) - \ln(1 - M_{lim}/M_t)$ versus ultrasonic time t for the random scission model are shown in Fig. 6(a) and (b) respectively. A linear curve was observed in the midpoint scission model, while an "S" shape curve was observed in the random scission model. The linear regression factor R^2 for these two models clearly indicates that the midpoint scission model predicts the experimental data reasonably well, with an adjusted R^2 of 0.99107.

Obviously, the linear correlation of the midpoint scission model is much higher than the random scission model, which suggested that chain breaking position was near the middle of the chain. Reasons for that are probably due to the collapsing cavitation bubbles that tends to occur near the center of gravity of the molecule (Poinot, Benyahia, Govin, Jeanmaire, & Grosseau, 2013). The current

experimental data (see Table 1) can be well explained by the midpoint scission model. Due to the fact that dextran chain near the midpoint of the dextran molecules was divided in two in the ultrasonic process, the higher the initial molecular weight of the sample, the faster the molecular weight decreases. Hence, compared to dextran samples with a lower initial molecular weight, effects of ultrasonic degradation on dextran samples with a higher initial molecular weight were more obvious. Another possible explanation for the results could be related to more chances of scission occurs in longer dextran chains. Yanaki, Nishii, Tabata, and Kojima (1983) investigated schizophyllan using midpoint scission model and in his paper the probability of breaking seemed to be higher at middle portions than any other portions of the schizophyllan chain. Similarly, Trzciński and Staszewska (2004) proved in their study that the chain scission of chitosan by ultrasound was not random but occurred at the midpoint of the chain. Our data fits the midpoint random scission model better than the random scission model due to the unique copolymer structure of dextran.

4. Conclusions

The current study showed that ultrasonic treatment reduces molecular weight and polydispersity of six dextran samples, yielding a more homogenous dextran solution with a narrower molecular weight distribution. Significant degradation was observed at the initial degradation stage, particularly for samples with a larger IMW. A kinetic model proposed by Malhotra elucidates the time dependence of molecular weight during the degradation process, with a lack of the limiting molecular weight of dextran. A midpoint scission model, based on a central cleavage assumption, appears to be suitable to describe the results obtained in this study, suggesting that dextran chain scission proceeds in a non-random way.

Ultrasonic treatment is capable of yielding dextran with a lower molecular weight and in the meanwhile improving the homogeneity of molecular weight distribution. As a simple, rapid and controllable method, ultrasonic degradation has a good industrial application prospect for further preparation of lower molecular weight clinical dextran.

Acknowledgements

Financial support from the Project of National Natural Science Foundation of China (21264003) and the Project of Guangxi Natural Science Foundation (2013GXNSFAA019036) are gratefully acknowledged.

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