# Effect of Degree of Deacetylation of Chitosan on the Kinetics of Ultrasonic Degradation of Chitosan

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**ABSTRACT:** The objective of the study was to explore the effect of the degree of deacetylation (DD) of the chitosan used on the degradation rate and rate constant during ultrasonic degradation. Chitin was extracted from red shrimp process waste. Four different DD chitosans were prepared from chitin by alkali deacetylation. Those chitosans were degraded by ultrasonic radiation to different molecular weights. Changes of the molecular weight were determined by light scattering, and data of molecular weight changes were used to calculate the degradation rate and rate constant. The results were as follows: The molecular weight of chitosans decreased with an increasing ultrasonication time. The curves of the molecular weight versus the ultrasonication time were broken at 1-h treatment. The degradation rate and rate constant of sonolysis decreased with an increasing ultrasonication time. This may be because the chances of being attacked by the cavitation energy increased with an increasing molecular weight species and may be because smaller molecular weight species have shorter relaxation times and, thus, can alleviate the sonication stress easier. However, the degradation rate and rate constant of sonolysis increased with an increasing DD of the chitosan used. This may be because the flexibilitier molecules of higher DD chitosans are more susceptible to the shear force of elongation flow generated by the cavitation field or due to the bond energy difference of acetamido and $\beta$-1,4-glucoside linkage or hydrogen bonds. Breakage of the $\beta$-1,4-glucoside linkage will result in lower molecular weight and an increasing reaction rate and rate constant. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 3526–3531, 2003

**Key words:** chitosan; ultrasonic degradation; degree of deacetylation

## INTRODUCTION

Chitin is a high molecular weight polysaccharide linked by $\beta$-1,4-glycoside. It has a structure similar to cellulose. It is composed of *N*-acetylglucosamine and glucosamine.[1] Chitin's most important derivate is chitosan, which is prepared by alkali deacetylation from chitin. Chemical modifications such as carboxymethylation, acetylation, and sulfonation have been used to produce derivates. Chitin, chitosan, and those derivates are called chitinous materials. Chitinous materials are considered to be the most widely distributed biopolymers, having huge resources and are nontoxic and biodegradable. Chitinous materials can be applied in food processing, agriculture, biomedicine, biochemistry, wastewater treatment, membranes, microcapsules, etc.[2]

The physicochemical properties of chitosans depend on intrinsic factors such as the degree of deacetylation (DD), distribution of those acetyl groups, and molecular weight and its distribution.[3] Different molecular weight chitosans are usually prepared by different hydrolysis methods. The ultrasonication method is one of the methods frequently used.[4–11] It has the merits of high efficiency, it can handle a large quantity of a sample, its equipment is easily obtained, it has almost no effect on the DD of treated chitosan,[9,10] has no by-product, etc. High-intensity acoustic radiations cause various changes as they propagate through a medium. These changes occur as a result of several mechanisms such as heating, structural effects, compression and rarefaction, turbulence, and cavitation.

Cavitation is the formation, growth, and violent collapse of small bubbles or voids in liquids as a result of pressure fluctuation[12] and/or a large negative pressure being created.[13] Theoretical calculations indicate that for pure water the negative pressure that is required is about 100 atm for bubbles filled with vapor. Practically, cavitation can be produced at a considerably lower applied acoustic pressure due to the presence of weak spots in the liquid. Weak spots include the presence of gas nuclei in the form of dissolved gases, minute suspended gas bubbles, or tiny sus-

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pended particles. When produced in a sound field at sufficiently high power, the formation of cavitation bubbles will be initiated during the rarefaction cycle.[14] Cavitation may cause enhanced polymerization or depolymerization reactions by temporarily dispersing aggregates or by permanently breaking chemical bonds in polymeric chains.[12] Depolymerization may be due to shock-wave energy, shear stress at the cavitation interphase, local high press and temperature, the relative motion of the polymer segments and solvents, and elongation flow producing stress, etc., during the collapse of the cavity.[15–17] The viscosity of solvent molecules in the vicinity of the collapsing bubble containing monomeric gas was approximately 300 cm $s^{-8}$, which generated a friction force of $3.2 \times 10^{-1}$ N per bond; an order of magnitude larger than that necessary to break the carbon–carbon bond was developed. The shock-wave energy is released as the cavity collapses. The degradation rate should depend upon the acoustic intensity and the size of the macromolecule and it is predicted there would be a minimum chain length below which degradation would not occur.[18]

Ultrasonic treatments have been used to improve the NMR spectrum of ionic high molecular weight polymers such as xanthan, schizophyllan, polyacrylamide, and poly(acrylamide-*co*-sodium acrylate). This is because the resulting lower molecular weight polymers may lessen the problem of air bubbles but this does not affect the tacticity of the comonomer distribution or lead to the formation of a monomer.[19] Liu et al.[20] reported that IR spectra and X-ray graphs showed that the 1-4 linkage is partly broken while the crystalline area remains unchanged. Shyur[10] reported that a chitosan solution treated with ultrasonic radiation at 60°C did not change its DD value as the solution contained no urea; however, the DD of chitosan decreased with an increasing radiation time when the solution contained urea. When the DDs of chitosan were between 47 and 53%, the molecular weights were around 45–639 kDa; those chitosans will be water-soluble. Chen et al.[6] reported that, using a prolonged treatment time and a reaction at lower temperatures, a solution of lower concentration will have a higher degradation rate when ultrasonic radiation is applied to degrade the chitosan solution. Ulanski et al.[7] reported that chitosan in different solvents resulted in different degradation efficiencies.

The above literature focused on the effect of an extrinsic factor such as the chitosan concentration, reaction time, reaction temperature, and solvent used on the degradation rate. Intrinsic factors such as the DD of chitosan on the degradation rate have been rarely discussed. This report explored the effect of the DD of chitosan, the reaction time on the degradation rate, and the rate constant during ultrasonic degradation.

## EXPERIMENTAL

### Preparation of chitin

Chitin was prepared from shrimp (*Solenocera prominentis*) waste by a modified method of Stanley et al.[21] Ground shrimp waste was treated with 0.5*N* NaOH at ambient temperatures to hydrolyze the surface flesh. The alkali-treated waste was washed, then dried and disintegrated to obtain powder. The powder passed through sieves of 40–60 mesh. The flake free powder was soaked in 2*N* HCl for 2 h to remove the minerals until no $CO_2$ evolved. The deminerized powder was soaked in 2*N* NaOH at 80°C to hydrolyze the protein, then washed with water until neutral. The alkali-treated power was soaked in 1% $KMnO_4$ at room temperature for 1 h to oxidize the astaxanthin, then soaked in 1% oxalic acid at 60°C for 1 h to neutralize the $KMnO_4$. This was then washed and dried to obtain a white chitin powder.

### Preparation of chitosan

The chitin powder obtained was suspended in a 50% NaOH solution at a chitin-to-solution ratio of 20 and reacted at 99°C for 1–9 h. A fresh NaOH solution was used to replace old ones after each hour of the reaction. After the reaction, the slurry was washed with water and dried to obtain 60–100% DD chitosan. The reaction was performed under nitrogen to avoid oxidation.[22]

### DD measurement

Infrared spectrometry was used to determine the DD of the chitosans.[23] Chitosan powder was sieved through a 200 mesh, then mixed with KBr (1:100) and pressed into a pellet. The absorbances of amide 1 (1655 $cm^{-1}$) and of the hydroxyl band (3450 $cm^{-1}$) were measured using a Bio-Rad FTS-155 infrared spectrophotometer. The band of the hydroxyl group at 3450 $cm^{-1}$ was used as an internal standard to correct for the disc thickness and for differences in the chitosan concentration in making the KBr disc. The percentage of the amine group's acetylation in a sample is given by $(A_{1655}/A_{3450}) \times 115$. Here, $A_{1655}$ and $A_{3450}$ are the absorbances at 1650 and 3450 $cm^{-1}$, respectively.

### Weight-average molecular weight determination

The static light-scattering method was used to measure the average molecular weight of the chiosan.[24] Chitosan was dissolved in 0.2*M* $CH_3COOH$ + 0.1*M* $CH_3COONa$. Different concentrations (0.001–0.10 g/L) of chitosan solutions were prepared. The solvents and solutions were filtered through 0.02-$\mu$m (Whatman, Anotop 25, USA) and 0.45-$\mu$m (Lida, USA) filters, respectively. The scattered light intensity of the solutions between 30° and 140° was measured by a Malvern light-scattering photometer (Malvern 4700,

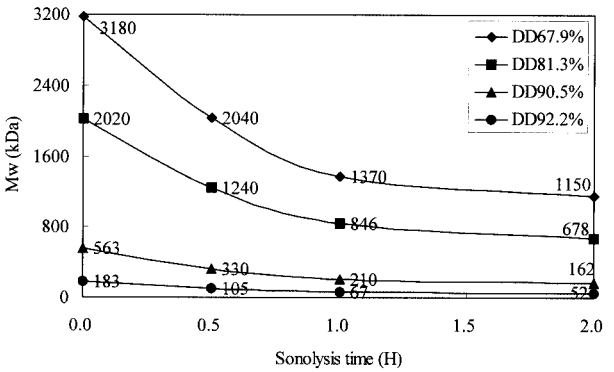


**Figure 1** Changes of weight-average molecular weight of different DD chitosans during ultrasonolysis time.

UK) at 632.8 nm and 30 ± 0.1°C. The molecular weight was calculated from the Zimm plot processed by Malvern software (version 1.26 for Windows). Every sample measurement was repeated five times. Refractive index increments (*dn/dc*) of the chitosan solution reported by Wang et al.[8] were used.

### Ultrasonication degradation reaction

The method of Wang and Qin[9] was followed: A 2% chitosan solution was prepared from a 0.2*M* chitosan–acetic acid solution. Ultrasonic treatments were conducted at 15 W for 0.5, 1.0, and 2.0 h at 98°C with a sonicator (W-380, Heat Systems-Ultrasonic, Inc., USA). Alkali was added to the treated solutions to precipitate the chitosan. The precipitates were collected and washed to neutral, then soaked in methanol twice and lyophilized for later use.

### Degradation rate calculation

The degradation rate ($R$) was defined as the percentage decrease in molecular weight per hour during sonolysis. It can be expressed by eq. (1)[25]:

$$R = [1\text{-}(M_t/M_0)] \times 100\%/t \qquad (1)$$

Here, $R$ is degradation rate of the chitosan molecular weight; $Mw_0$, the molecular weight of chitosan at time zero; and $Mw_t$, the molecular weight of the resulting chitosan after the reaction for $t$ h. To calculate the degradation rate during a certain period of time, eq. (2) was used:

$$R_{n,n+1} = [1 - (M_{n+1}/M_n)] \times 100\%/t \qquad (2)$$

Here, $R_{n,n+1}$ is the degradation rate between $n$th and $n$ + 1th data; and $M_n$ and $M_{n+1}$, the weight-average molecular weight of chitosan subjected to $n$th- to $n$+ 1th-hour sonolysis.

### Calculation rate constant

The degradation reaction by ultrasonic treatment is a first-order reaction; its rate constant ($k$) can be obtained from eq. (3)[26,27]:

$$1/M_t = 1/M_0 + kt/m = 1/M_0 + k't \qquad (3)$$

$k$ is the rate constant ($h^{-1}$) of the molecular weight degradation during sonolysis; $k'$ is measured at mol $g^{-1}$ $h^{-1}$; $t$ is the sonolysis time; $M_0$ and $M_t$, the weight-average molecular weight of chitosan before sonolysis and after reaction for $t$ hours, respectively; and $m$, the molecular weight of the chitosan monomer $m$ = 161 + 42(1 − DD). To calculate rate constant during certain periods of time, eq. (4) was used:

$$1/M_{n+1} = 1/M_n + k_{n,n+1}t/m = 1/M_n + k'_{n,n+1}t \qquad (4)$$

$k_{n,n+1}$ is the rate constant between the $n$th- and $n$ + 1th-data sonolysis.

## RESULTS

### Effect of sonolysis time on molecular weight of chitosan

The results in Figure 1 show the changes of the weight-average molecular weight of different DD chitosans during the ultrasonolysis time. For the same DD chitosan, changes in the molecular weight at an early sonolysis time were more pronounced than were the later ones, which implied that the degradation rate was higher at early reaction periods.

### Effect of sonolysis time on degradation rate

Table I shows the ultrasonic degradation rate ($R$, %/h) and the rate constant ($k$, 1/h, × $10^{-5}$) of different DD chitosans. $R_{0,0.5}$ indicates the degradation rate of chitosan between time 0 and 0.5 h of sonolysis. The results show that degradation rate decreased with an increasing sonolysis time. The degradation rate de-

**TABLE I**
**Ultrasonic Degradation Rate ($R$, %/h) and Rate Constant ($k$, 1/h, × $10^{-5}$) of Different DD Chitosans**

| Ultrasonic degradation rate and rate constant ($k$) | DD (%) | | | |
|---|---|---|---|---|
| | 67.9 | 81.3 | 90.5 | 92.2 |
| $R_{0,0.5}$ | 71.7 | 77.2 | 82.8 | 85.2 |
| $R_{0.5,1}$ | 65.7 | 63.5 | 72.7 | 72.4 |
| $R_{1,2}$ | 16.1 | 19.9 | 22.9 | 22.4 |
| $R_{0,2}$ | 31.9 | 33.2 | 35.6 | 35.8 |
| $k_{0,1}$ (× $10^{-5}$) | 7.0 | 11.8 | 49.5 | 156.1 |
| $k_{1,2}$ (× $10^{-5}$) | 1.8 | 5.1 | 23.1 | 70.6 |
| $k_{0,2}$ (× $10^{-5}$) | 5.2 | 8.4 | 36.3 | 113.4 |

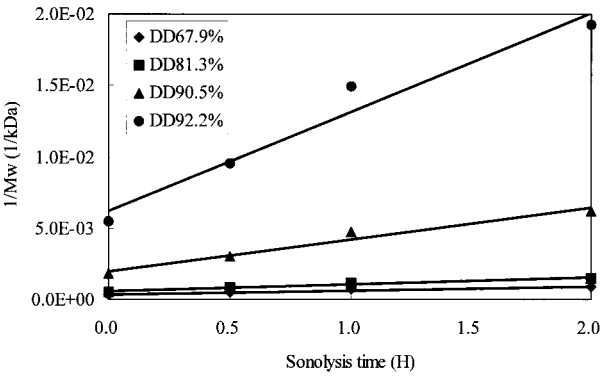


**Figure 2** Reciprocal of weight-average molecular weight of different DD chitosans during ultrasonolysis time.

creased dramatically after 1 h; for example, $R_{0,0.5}$ and $R_{0.5,1}$ are 71.7 and 65.7%/h, respectively, for 67.9% DD chitosan. Both data are significantly larger than that of $R_{1,2}$ of 16.1%. Other DD chitosans showed similar trends.

### Effect of sonolysis time on ultrasonic rate constant

From eq. (3), the slope of the plot of the reciprocal of the molecular weight and sonolysis time (Fig. 2) is $k'$; therefore, the degradation rate constant $k$ can be calculated. Figure 2 shows that the data for sonolysis times longer than 1 h deviated obviously from being linear. Also, from Table I, the rate constants before and after 1 h differed significantly. We plotted of these data separately and used eq. (4) to calculate the rate constant before and after 1 h and they are listed in Table I. The results shows that the rate constants for those both before and after 1 h differed markedly, for example, for DD 67.9% chitosan, $k_{0,1}$ ($7.0 \times 10^{-5}$ 1/h) is larger than $k_{1,2}$ ($1.8 \times 10^{-5}$ 1/h). All other DD chitosans show similar changes.

### Effect of DD of chitosan on degradation rate

The results in Table I show that the degradation rate and the rate constant increased with an increasing DD of the chitosan used. The $R_{0,0.5}$'s are 71.7, 77.2, 82.8, and 85.2 %/h and the $k_{0,1}$'s are $7.0 \times 10^{-5}$, $11.8 \times 10^{-5}$, $49.5 \times 10^{-5}$, and $156.1 \times 10^{-5}$ 1/h for chitosans with a DD of 67.9, 81.3, 90.5, and 92.2%, respectively. For the entire sonolysis reaction, the $R_{0,2}$'s are 31.9, 33.2, 35.6, and 35.8 %/h and the $k_{0,2}$'s are $5.2 \times 10^{-5}$, $8.4 \times 10^{-5}$, $36.3 \times 10^{-5}$, and $113.4 \times 10^{-5}$ 1/h for, respectively, 67.9, 81.3, 90.5, and 92.2% DD chitosans. Those results indicated that the higher the DD the easier it is for molecular degradation to occur during sonolysis.

## DISCUSSION

### Effect of sonolysis time on ultrasonic rate constant

For the same DD chitosan, the molecular weight of chitosan decreased with the sonolysis time and the decrease in molecular weight was more pronounced during earlier reaction times. Chen et al.[6] reported similar results. They reported a molecular weight decrease ratio (MWDR) of 1.4%. Chitosan increased rapidly during the first 15 min of sonolysis; then, the increase in MWDR became slower during later reactions. The MWDR remained nearly constant after 100 min of reaction at 4°C. Similar trends were reported using other polysaccharides, for example, Koda et al.[28] reported that, with ultrasonic degradation carried out at 4°C, 80 W/cm$^2$, on a 0.1%,100 kDa pullulan solution, the molecular weight decreased rapidly during earlier reaction times. The decrease in the molecular weight slowed with the reaction time and reached a constant molecular weight of 5.8 kDa at 36 h. Lii et al.[27] reported that a decrease in the molecular weight of 0.5 % of agarose, κ-carrageenan, and ι-carrageenan

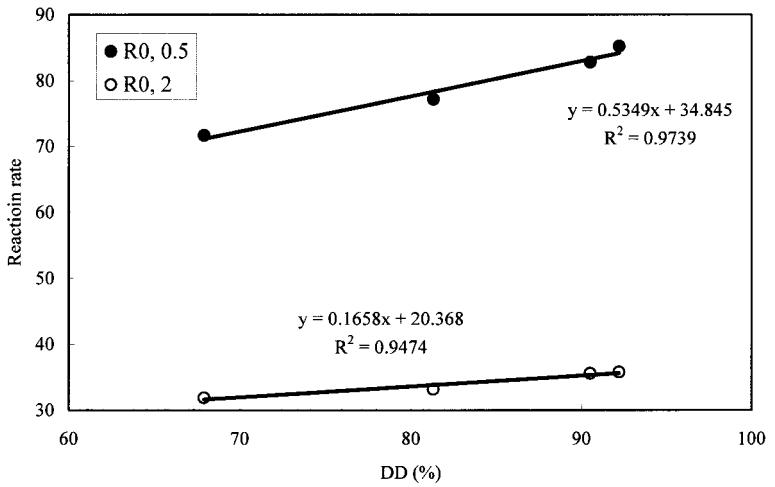


**Figure 3** Ultrasonic degradation rate ($R$, %/h) of different DD chitosans.

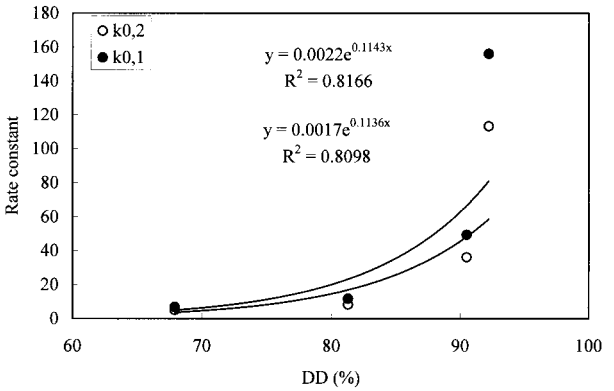


**Figure 4** Ultrasonic degradation rate constants ($k$, 1/h, $\times 10^{-5}$) of different DD chitosans.

**TABLE II**
**Ultrasonic Degradation Rate and Rate Constant of Similar Molecular Weight but Different DD Chitosans**

| Groups | DD (%) | *Mw* (KDa) | Degradation rate (%/h) | Rate constant (1/h, $\times 10^{-5}$) |
|---|---|---|---|---|
| 1 | 67.9 | 2040 | 65.7 | 8.4 |
| | 81.3 | 2020 | 77.2 | 11.8 |
| 2 | 67.9 | 1370 | 16.1 | 1.8 |
| | 81.3 | 1240 | 63.6 | 12.7 |
| 3 | 81.3 | 846 | 19.9 | 5.1 |
| | 90.5 | 563 | 82.8 | 41.4 |
| 4 | 90.5 | 210 | 22.9 | 23.1 |
| | 92.2 | 183 | 85.3 | 133.4 |

solutions by sonolysis at 50°C, 300 W/cm$^2$, showed similar trends.

Decreases in the degradation rate and rate constant (Table I) with an increasing sonolysis time may be due to the chance of being attacked by the cavitation energy, which increased with an increasing molecular weight. Chen et al.[6] reported that the polydispersity of treated chitosan decreased after ultrasonication, which implied that sonolysis occurred at those molecules with a longer chain length. A decrease in the number of those molecules will shift the molecular weight distribution to a lower one. Lorimer et al.[29] also revealed that molecules of a larger molecular weight disappeared gradually with an increasing ultrasonic time and the molecular weight distribution became narrower. Therefore, at earlier periods of sonolysis, degradation occurred with a larger molecular weight species, thus resulting in a rapid decreasing molecular weight and, in turn, an increased reaction rate and rate constant. Another reason may be because smaller molecular weight species have shorter relaxation times and, thus, can alleviate the sonication stress easier.[27]

### Effect of DD of chitosan on reaction rate and rate constant

The results in Figure 3 show a good linear relationship between the sonolysis reaction rate and the DD of treated chitosan. The results in Figure 4 show a good exponential relationship between the sonolysis rate constant and the DD of treated chitosan. The molecular weight of different chitosans used in this study decreased with increasing DD values. In a previous section, larger molecular weight chitosans having a higher reaction rate and rate constant were discussed. However, the results in Figures 3 and 4 show the opposite result, for example, lower molecular weight species (higher DD species) have a higher reaction rate and rate constant. Those results implied that the effects of the DD overwhelmed the effect of the molecular weight on sonolysis. To avoid the effect of the molecular weight of treated chitosan, data of similar molecular weight chitosan were picked up from Figure 1 and their sonolysis reaction rates and rate constants were compared and are listed in Table II. The results show that higher DD chitosans had higher reaction rates and rate constants for all four groups of chitosans, which implied that the DD of chitosan affects the degradation rate prominently. The reasons may be because the flexibilitier molecules of higher DD chitosans[30] are more vulnerable to the shear force of elongation flow generated by the cavitation field or may be because the bond energies of acetamido, $\beta$-1,4-glucoside linkage, and hydrogen bonds are different and because, during sonolysis, the energy of cavitation may preferentially react at the $\beta$-1,4-glycoside linkage (Fig. 5). Breakage of the $\beta$-1,4-glucoside linkage will result in a lower molecular weight and an increased reaction rate and rate constant.

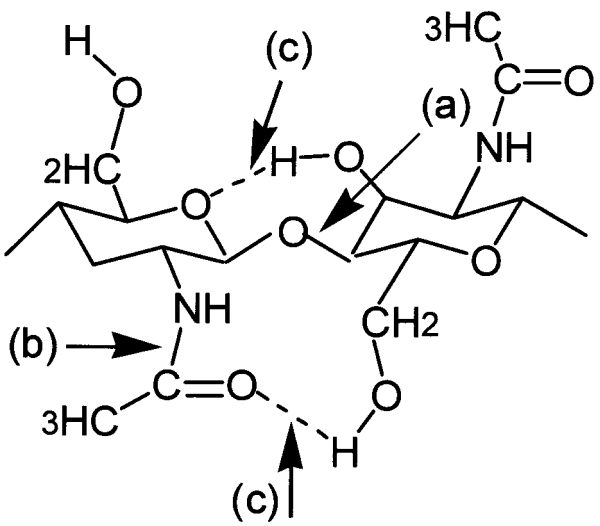


**Figure 5** Possible acting sites: (a) $\beta$-1,3-glucoside linkage, (b) acetamido, and (c) hydrogen bond, by cavitation energy during ultrasonolytic reactions.

## CONCLUSIONS

1. The molecular weight of chitosan decreased with the ultrasonolysis time. The molecular weight–sonolysis time curve for those chitosans broken at 1 h of treatment indicated that the degradation rate changed markedly.
2. The ultrasonic degradation rate and rate constant decreased with an increasing sonolysis time.
3. The ultrasonic degradation rate and rate constant increased with an increasing DD of the chitosans used.

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