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Studies on the Reaction of Allyl Glycidyl Ether with Gelatin by Van Slyke Method

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Gelatin was grafted by allyl glycidyl ether (AGE) at 40 ºC under solution pH ranges from 8-13. The Van Slyke method, used for the amino acids analyses quantitatively, was used to determine the conversion rate of the free -NH_2 groups of gelatin. The results showed that the grafting of epoxy compound onto gelatin mainly took place on free -NH_2 groups of gelatin. The highest conversion rate of the free -NH_2 groups was 67.72 %, while the solution pH was 11. The results were consistent with those obtained by colorimetry and gravimetric analysis methods. By comparing with the results obtained from the three analysis methods, we found that the Van Slyke method was the most convenient and reliable method to measure the conversion rate of the free -NH_2 groups. The Van Slyke method is a novel and good method to measure the conversion rate of the free -NH_2 groups.

Key Words: Gelatin, Modification, The Van Slyke method.

INTRODUCTION

Gelatin is one of the proteins obtained from partial hydrolysis of collagen. Due to its good characteristics, such as non-toxic, biocompatibility, *etc*., it has been widely used in producing various pharmaceutical products, film, adhesives, *etc*.[1-5]. In recent years, the modification of gelatin through chemical reaction between amino groups and compounds containing epoxy groups has attracted more attention[6-9]. The modified gelatin can be applied as biomedical materials[10]. Under the selected reaction conditions, grafting reactions mainly take place on the free -NH_2 groups in gelatin[11]. Quantitative conversion rate of the gelatin is the important component of the gelatin modified by epoxy compound.

The gravimetric analysis is considered to be an important method to investigate the content of chemical modification of gelatin[12,13]. Gravimetric analysis shows the increments of grafted gelatin. With the gelatin grafted by allyl glycidyl ether (AGE), the mass of grafted gelatin increases. The absorbance of C=C bond in the organic compound is measured by colorimetry method[14], such as allyl glycidyl ether. The decline in the numbers of the unreacted allyl glycidyl ether reflects the conversion rate of gelatin. However, the amount of free -NH_2 groups with allyl glycidyl ether quantitatively can not be measured by gravimetric analysis and colorimetry methods.

The Van Slyke method is mainly used for the amino acids analyses quantitatively[15,16]. In this study, the Van Slyke method is used to measure the conversion rate of free -NH_2 groups with AGE. The reaction of gelatin grafted by AGE at different pH is investigated. The conversion rates of free -NH_2 groups in gelatin are measured by the Van Slyke, colorimetry and gravimetric analysis. The results obtained from the colorimetry and gravimetric analysis are consistent with those obtained by the Van Slyke method. This study indicates that the Van Slyke method can not only be used for the amino acids analyses quantitatively, but also be used for measuring the conversion rate of free -NH_2 groups in gelatin. The investigation will provide good insight in studying the reaction of free -NH_2 groups in biomacromolecule.

EXPERIMENTAL

Gelatin, which contained over 85 % protein, was used in this work, as supplied by Sinopharm Chemical Reagent Co. (Shanghai, China, AR). The natural moisture content of the gelatin was 7.8 %. Allyl glycidyl ether was purchased from YuDeheng Fine Chemical Co. (Nanjing, China, AR). Distilled water was used as the solvent in all experiments. Acetic acid, sodium nitrite and potassium permanganate, which were used in the Van Slyke testing, were from Bodi Chemical Reagent Co. (Tianjin, China, AR).

Sample preparation: The 10 % aqueous solution of gelatin and the equimolar AGE were employed throughout the experiments. Gelatin and AGE were dissolved separately. The gelatin and AGE solutions were mixed in a three-neck flask after their pH were adjusted to a certain value. The three-neck flask was put inside a water-bath and the temperature

was fixed at 40 ºC. The gelatin aqueous solution was taken out and air cooled after 8 h.

Characterization: ^{1}H NMR spectra of grafted and non-grafted gelatin (D_2O as solvent) were collected by using a Bruker AVENCE 400 spectrometer (Bruker, Switzerland).

Analytical method

Van Slyke method: The Van Slyke method was a method of amino acid detection. The tester was improved[17,18] and used to measure the conversion rate of free -NH_2 groups of gelatin. The procedure for determination of the free -NH_2 groups of gelatin was measuring the N_2 gas evolved by the reaction with nitrous acid.

$$Gel-NH_2 + HNO_2 \longrightarrow Gel-OH + N_2 \uparrow + H_2O$$

Fig. 1. Reaction between gelatin and nitrous acid

The sodium nitrite and acetic acid had to be added first and mixed until the NO gas evolved by spontaneous decomposition of HNO_2 had washed all the air out of the reaction chamber, before the gelatin solution was added. The reaction between the free -NH_2 groups and nitrous acid was maintained at a controlled temperature. The mixture of N_2 and NO (the latter formed by spontaneous decomposition of HNO_2) was transferred to a flask of the type described by Van Slyke, where the NO was absorbed by potassium permanganate. The purified N_2 gas was returned from the flask and driven into a graduated glass tube for measuring volume of N_2 gas. Keeping the water level same within and outside the graduated glass tube, that is, the pressure of N_2 gas within the graduated glass tube was the same as atmosphere pressure.

Recording the volume of N_2 gas (V), atmosphere pressure (P) and room temperature (T), the amount of substance of the N_2 would be calculated. Because of the amount of substance of the N_2 equal that of the free -NH_2 groups in the tested gelatin, the moles of free -NH_2 groups per gram of gelatin was calculated. The conversion rate of the -NH_2 groups was calculated by using eqn. 1.

$$Q(-NH_2) = \frac{(N_0 - N_1)}{N_0} \quad (1)$$

where N_0 was the moles of free -NH_2 groups per gram of gelatin and calculated by the Van Slyke method, N_1 was the moles of free -NH_2 groups per gram of modified gelatin.

Gravimetric analysis method: The conversion rate of gelatin was calculated by using the eqn. 2 after both the gelatin and grafted gelatin were dried at -50 ºC for 24 h in the Alpha 1-2 LD plus freeze dryer (Martin Christ, Germany).

$$Q(W) = \frac{(W_1 - W_0)}{W_0} \quad (2)$$

where W_0 was the mass of the dried gelatin, W_1 was the mass of the dried grafted gelatin. An equivalent amount of gelatin was grafted by AGE and the excess AGE was extracted by the chloroform.

Colorimetry method: The absorbance of C=C bond in AGE was recorded at 239 nm on UV-1700 Ultraviolet Spectrometer (Shimadzu, Japan). The AGE solution was prepared in chloroform. A series of AGE solutions with different concentrations were measured and a standard curve of concentration relative to absorbance was obtained. After the unreacted AGE was extracted from reactive solution, chloroform was recorded at 239 nm on UV-1700 Ultraviolet Spectrometer. The molar mass of the unreacted AGE was calculated through the standard curve. If define n_0 as the molar mass of the initial AGE and n_1 as the molar mass of the unreacted AGE, the conversion rate of gelatin could be calculated by using the eqn. 3.

$$Q(gel) = \frac{(n_0 - n_1)}{n_0} \quad (3)$$

RESULTS AND DISCUSSION

^{1}H NMR characterization: To confirm the reaction between AGE and gelatin, the ^{1}H NMR spectra of AGE, gelatin and AGE-grafted gelatin were performed. The peaks at *ca*. 3.1 ppm were corresponded to -CH_2NH- bonds (a_2) and closed to 3.6 ppm correspond to -CHOH-CH_2O- bonds (b_2), indicating the existence of AGE in gelatin. The peaks appeared near 6.0 ppm corresponded to -CH=CH_2 bonds (e_2) and appeared near 5.2 ppm corresponded to -CH=CH_2 bonds (f_2), they both were characteristic peaks of AGE. The peaks appeared near 4.0 ppm corresponded to -OCH_2- (d_2) bonds. The ^{1}H NMR spectra results indicated the graft of AGE on gelatin.

Conversion rate analysis: Under alkaline conditions, the reaction between the free -NH_2 groups and epoxy groups is an S_{N_2} reaction[19]. With the increase of solution alkalinity, the nucleophilic ability of the amines increases, that is, the grafting reaction becomes easy. The quantity of the free -NH_2 groups is determined by the Van Slyke method. Fig. 2 shows the conversion rate of free -NH_2 groups determined by the Van Slyke method. The conversion rate of free -NH_2 groups increases with the pH from 8.0-10.5. Interestingly, the conversion rate of free -NH_2 groups reaches a maximum (64.19 %) at pH 10.5 and it decreases when pH larger than 10.5. It might be caused by the hydrolysis of gelatin[20].

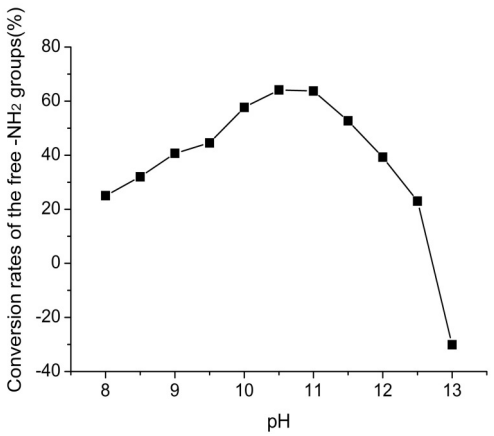

Fig. 2. Conversion rate determined by Van Slyke method

In order to confirm the hydrolysis of gelatin, the hydrolysis rate of gelatin under different pH conditions are studied (Fig. 3). Because of the hydrolysis, the number of the free -NH_2 groups of grafted gelatin increase when pH is more than 10.5. With the quantity of the free -NH_2 groups in grafted gelatin

increasing, it indicates that the conversion rate of free -NH_2 groups in gelatin decreases. Therefore, the revision of the free -NH_2 groups in gelatin is required. The quantity of the free -NH_2 groups increases in virtue of the hydrolysis of gelatin should be deducted from the quantity of the free -NH_2 groups in grafted gelatin. Therefore, the curve c in Fig. 4 embodies the adjusted conversion rate of free -NH_2 groups measured by the Van Slyke method and the conversion rate of free -NH_2 groups reaches a new maximum (67.72 %) at pH 11. However, because of the hydrolysis found at pH 11, the optimal pH for the reaction of AGE with gelatin is 10.5.

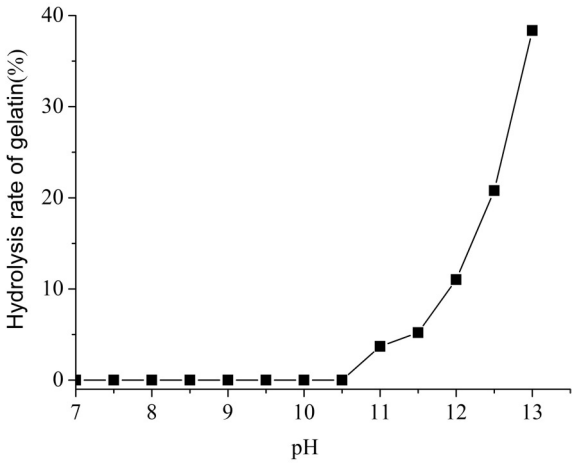

Fig. 3. Hydrolysis rate of gelatin

In order to demonstrate the results obtained from the Van Slyke method, the gravimetric analysis and colorimetry methods are used to measure the conversion rates of free -NH_2 groups of gelatin, respectively (Fig. 4). It can be seen that the shapes of the three curves are the same. However, the conversion rates measured by three different methods are different. The order of the conversion rates is gravimetric analysis (a) > colorimetry (b) > Van Slyke method (c). Gravimetric analysis method shows the increments of grafted gelatin. Colorimetry method shows the absorbance of C=C bond in unreacted AGE. However, the conversion rate of free -NH_2 groups with AGE can not be measured by gravimetric analysis and colorimetry methods. It might be caused by the reaction of the active groups except the free -NH_2 groups.

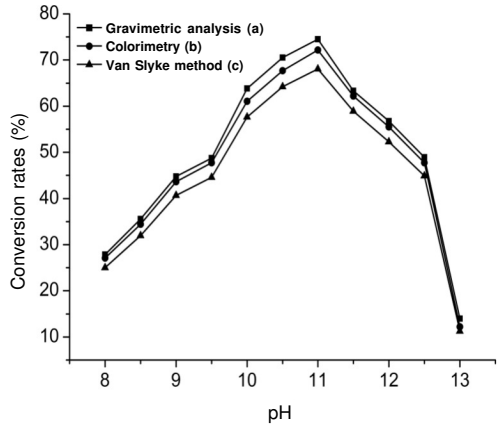

Fig 5. Conversion rates determined by different methods

By the improved Van Slyke method, the -NH_2 groups that reacted with AGE are measured precisely, after deducting the quantity of the free -NH_2 groups which increases in virtue of the hydrolysis of gelatin. The measured data has a good reproducibility[21]. This study indicates that under the selected reaction conditions, the grafting of epoxy compound onto gelatin mainly takes place on the free -NH_2 groups. The difference of the results obtained between gravimetric analysis (a) and colorimetry (b) might be caused by the moisture content of modified product.

Conclusion

Gelatin is grafted by allyl glycidyl ether in the pH range from 8-13 at 40 ºC for 8 h. Under the conditions, the conversion rate of free -NH_2 groups increases from 15.65 % (pH = 8) to 67.72 % (pH = 11), firstly and then decreases to 11.21 % (pH = 13). The decrease of conversion rate for the free -NH_2 groups is caused by the hydrolysis of gelatin. By comparing the results obtained from the Van Slyke, colorimetry and gravimetric analysis methods, it could be concluded that the grafting of epoxy compound onto gelatin mainly takes place on free -NH_2 groups of gelatin. Our studies also illustrates that this is a good way to measure the conversion rate of the free -NH_2 groups by the Van Slyke method.

REFERENCES

1. S.C.G. Leeuwenburgh, J. Jo, H. Wang, M. Yamamoto, J.A. Jansen and Y. Tabata, *Biomacromolecules*, **11**, 2653 (2010).
2. J.J. Han, P. Lazarovici, C. Pomerantz, X.S. Chen, Y. Wei and P.I. Lelkes, *Biomacromolecules*, **12**, 399 (2011).
3. A. Balaji, V.P. Pandey, M.S. Srinath and R. Manavalan, *Asian J. Chem*., **21**, 6277 (2009).
4. S. Jha, S.N. Dikshit and G. Pandey, *Asian J. Chem*., **23**, 4098 (2011).
5. Y.H. Cheng, S.H. Yang and F.H. Lin, *Biomaterials*, **32**, 6953 (2011).
6. L. Kurniawan, G.G. Qiao and X.P. Zhang, *Biomacromolecules*, **8**, 2909 (2007).
7. G. Vargas, J.L. Acevedo and J. López, *Mater. Lett*., **62**, 3656 (2008).
8. R. Zeeman, P.J. Dijkstra, P.B.Van Wachem, M.J. Van Luyn, M. Hendriks, P.T. Cahalan and J. Feijen, *Biomaterials*, **20**, 921 (1998).
9. R. Zeeman, P.J. Dijkstra, P.B. Van Wachem, M.J. Van Luyn, M. Hendriks, P.T. Cahalan and J. Feijen, *J. Biomed. Mater. Res*., **51**, 541 (2000).
10. H.W. Sung and J.S. Shih, *J. Appl. Biomater. Biomech*., **6**, 185 (1995).
11. Y. Di, R.J. Heath and A. Long, *J. Soc. Leath. Technol. Ch*., **90**, 93 (2006).
12. A. Gantar, A. Šebenik and M. Kavcic, *Polymer*, **28**, 1403 (1987).
13. H. Saito, T. Taguchi and H. Kobayashi, *Mater. Sci. Eng. C*, **24**, 781 (2004).
14. R. Wilson, *Anal. Lett*., **24**, 1983 (1991).
15. D.D. van Slyke, *J. Biol. Chem*., **83**, 425 (1929).
16. C. Fraenkel and L. Heinz, *J. Biol. Chem*., **148**, 453 (1943).
17. T.D. Li, X.L. Tang, Q.W. Jiang and J. Xu, Chinese Patent 2011-10249480.1 (2011).
18. T.D. Li, X.L. Tang, Q.W. Jiang and J. Xu, Chinese Patent 201120316998.8 (2011).
19. H.W. Sung, C.S. Hsu and Y.S. Lee, *J. Biomed. Mater. Res*., **31**, 511 (1996).
20. R. Zeeman, P.J. Dijkstra and P.B. Wachem, *Biomed. Mater. Res*., **46**, 424 (1999).
21. H.S.M. Zahangir, M. Ohnishi, S. Kanoh and E. Niwa, *Fisheries Sci*., **2**, 327 (1999).