ELSEVIER

Polymer 41 (2000) 6055–6059

polymer

Differential scanning calorimetry studies on the mechanism of skin-softening effect of sodium acetylhyaluronate ☆

T. Oka*, N. Ueno, T. Yanaki

New Technology Research Laboratories, Basic Research Center, Shiseido Co. Ltd., 1050 Nippa-cho, Kohoku-ku, Yokohama-shi, Kanagawa 223-8553, Japan

Received 9 August 1999; received in revised form 12 October 1999; accepted 22 November 1999

Abstract

A novel humectant, sodium acetylhyaluronate (AcHA), was found to have an excellent skin-softening effect on the stratum corneum. To clarify the mechanism of the skin-softening effect, the hydration behavior of AcHA was investigated by using differential scanning calorimetry (DSC). The results suggested that the amount of adsorbed water of AcHA in powder form was equal to that of HA. However, the DSC results showed that the bound water content in the stratum corneum treated with AcHA was markedly greater than that of HA-treated stratum corneum. Apparently, AcHA could enhance the intrinsic water holding capacity of stratum corneum. These findings indicate that the excellent skin-softening effect of AcHA is due to the enhancement of the bound water content in the stratum corneum.

Keywords: Differential scanning calorimetry; Skin-softening effect; Bound water

1. Introduction

In the area of research and development for cosmetics, study on moisturizing skin is one of the essential and important research tasks. Numerous studies on moisturizing skin and on the useful effects of humectants have been reported [1–7]. It is well known that (1) water and stratum corneum lipids play an important role in maintaining healthy and fresh skin [8–10] and (2) the water content influences the flexibility of the stratum [11–13]. However, due to aging, surroundings, physical constitution, and other factors, the stratum corneum always has a tendency to lose its water [14]. The reduction of the water content in the stratum corneum results in dry skin and formation of wrinkles. Therefore, to maintain normal water content in the stratum corneum, it is useful to apply humectants to skin [15–17].

There are various humectants, which are formulated in skin care cosmetics. Among these humectants, sodium hyaluronate (HA) is used for humectant or thickener as a cosmetic ingredient, because HA shows useful physical characteristics such as high water retention, high viscoelasticity, high thread-forming ability, and high biocompatibility [18]. In order to enhance these important functions of HA as a novel humectant, we have attempted to introduce hydrophobic groups to HA structure. This introduction of hydrophobic moieties would transform HA into an anchoring humectant. Fig. 1 shows the proposed concept of anchoring of the humectant. If hydrophobic moieties, which play the role as an anchor, are introduced to a polymer humectant, the humectant can adhere to the skin surface that is essentially hydrophobic due to the cover of sebum. The idea of anchoring humectant is based on the fact that the hydrophobic/hydrophobic interaction increases the intrinsic moisturizing effect of the polymer. After numerous investigations for finding HA derivatives, we eventually developed a novel HA derivative, sodium acetylhyaluronate (AcHA). In a previous paper, we reported the synthesis and usefulness of AcHA for cosmetic products [19]. It was shown that AcHA had superb moisturizing and excellent skin-softening effects on the stratum corneum. In particular, the skin-softening effect of AcHA was significantly stronger than those of other humectants and the strength of the effect depended upon degree of substitution (DS) of acetyl groups to HA. However, no detail of the mechanism of skin-softening effect on the stratum corneum has been elucidated.

Differential scanning calorimetry (DSC) is a well-established instrument for evaluating the hydration status of polymer or skin in terms of the change in water [20–23]. Inoue has reported on DSC studies which investigated the melting behavior of water in the stratum corneum [24]. It was shown

☆ Part of this study was presented at the Third Scientific Conference of the Asian Societies of Cosmetic Scientists at Taipei, Taiwan on May 24, 1997.

* Corresponding author. Tel.: + 81-45-542-1331; fax: +81-45-542-8817.

E-mail addresses: takashi.oka@to.shiseido.co.jp (T. Oka), norio.ueno@to.shiseido.co.jp (N. Ueno), toshio.yanaki@to.shiseido.co.jp (T. Yanaki).

0032-3861/00/$ - see front matter
PII: S0032-3861(99)00852-6

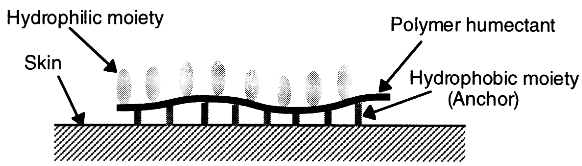

Fig. 1. The proposed concept of an anchoring humectant.

that 20–30% of water in the stratum corneum is bound water which interacts strongly with the protein or lipids in the stratum corneum, and the rest of water (70–80%) is unbound water which solubilizes the water-soluble components such as amino acids in the stratum corneum.

In this paper, in order to elucidate the mechanism of the skin-softening effect on AcHA, the hydration behaviors of AcHA and stratum corneum were investigated by using DSC technique.

2. Materials and methods

2.1. Materials

Chemicals used in this study were cosmetic grade or reagent grade and were used without further purification. The sheets of stratum corneum were prepared by the method reported previously, and they were stored in a vacuum desiccator over silica gel before use [19,25]. Water used in this study was purified by an ion-exchange water purification system Type G-10B (Organo, Tokyo, Japan). Sodium acetylhyaluronate (AcHA) was synthesized by the procedure reported previously [19,26]. In this study, we used AcHA which contained 2.6–3.8 acetyl groups per repeating disaccharide unit (namely, degree of substitution, DS). The weight-average molecular weight of AcHA was approximately 150 kDa (Fig. 2). This polymer could dissolve in 90% (w/w) aqueous ethanol.

2.2. Surface tension measurements at air/water interface

A series of aqueous solutions for HA, and AcHA (the concentrations were 10^{-7}–10^{0} g/dl, respectively) were prepared. The surface tensions at various concentrations were measured after equilibrium at constant temperature of 25°C by SURFACE TENSOMETER ST-1 (Shimadzu, Kyoto).

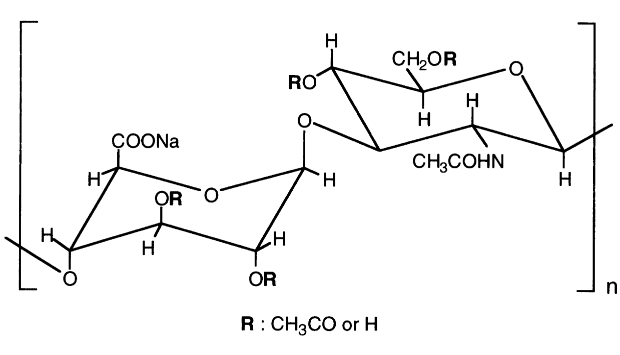

Fig. 2. Structure of sodium acetylhyaluronate (AcHA).

2.3. Measurement of amount of water adsorbed by AcHA

To measure the amount of water adsorbed by HA or AcHA in powder form, samples were equilibrated with constant relative humidities (32.8, 52.3, 75.3, or 93.6%) for 120 h. After being weighed, the samples were dried in a vacuum oven at 105°C for 4 h and weighed again. The amount of adsorbed water of each sample was calculated by the following Eq. (1):

Amount of adsorbed water (%)

$$= \frac{(\text{Wet weight} - \text{Dry weight})}{\text{Dry weight}} \times 100 \qquad (1)$$

2.4. Measurement of bound water content

Bound water content of HA or AcHA was measured by DSC, which was carried out by a differential scanning calorimeter DSC-8230D (Rigaku Electronics, Tokyo) equipped with cooling apparatus [27,28]. The amount of absorbed water in HA or AcHA was determined from the change in weight. Before DSC measurements, aqueous solutions of HA or AcHA (0.2% (w/w), 30 μl) in an aluminum pan (5 mm inner diameter, 2.5 mm deep) were dried in a silica gel desiccator for various periods of time (0–6 h) for preparing samples with various water contents. For the measurement of bound water content of the stratum corneum pre-treated with HA or AcHA, a sheet of stratum corneum (3 × 3 mm) was immersed in 0.2% (w/w) aqueous solution of HA or AcHA for 24 h, and rinsed with water (5 ml) for 10 s. To obtain various degrees of hydration, the samples of the wet stratum corneum were dried in a silica gel desiccator for various periods of time. The samples were folded up and placed in an aluminum pan, which was sealed before the measurement. After being cooled down to −40°C with liquid nitrogen, the pan was heated to 40°C at a rate of 5 K/min to measure the enthalpy of fusion of water (ΔH). Since the total water content at $\Delta H = 0$ indicates no free-water, water content is presented as bound water only. Free water content was calculated from ΔH and the heat of fusion per gram of water using Eq. (2). Although the value of fusion enthalpy of water for calculating free water is 334 J/g in Ref. [27], ΔH of free water measured by the apparatus used in our study was 315.6 J/g. On this account, the calibration was made using the value of 315.6 J/g throughout this experiment. Bound water content was obtained by subtracting the free water content from the

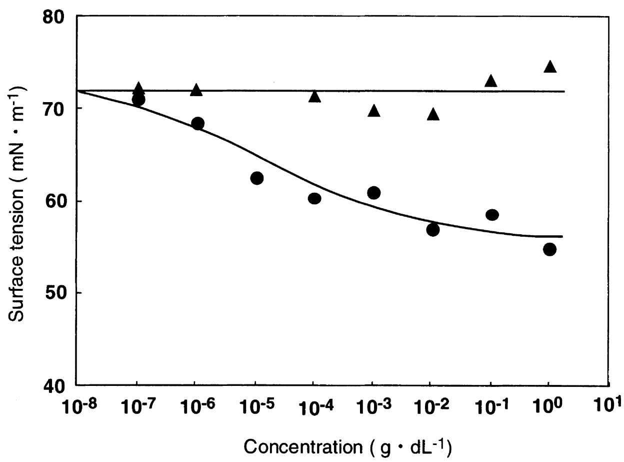

Fig. 3. Surface tension of sodium hyaluronate (HA) and sodium acetylhyaluronate (AcHA). The filled triangles (▲) and the filled circles (●) represent HA and AcHA, respectively.

total amount of water (Eq. (3)).

$$\text{Free water content} = \frac{\Delta H}{315.6} \quad (2)$$

$$\text{Bound water content} = (\text{Total water content}) - (\text{Free water content}) \quad (3)$$

3. Results and discussion

3.1. Surface tension reducing ability of AcHA

To investigate the amphiphilic property of AcHA, surface tensions of various concentration of aqueous solution were measured: Fig. 3 shows the results of the surface tension measurements on AcHA and HA. Since the surface tension of 1.0% (w/w) AcHA was approximately 55.0 mN/m, which was lower than that of 1.0% (w/w) HA (74.0 mN/m), AcHA has the surface tension reducing ability. The result is supported by the amphiphilic property of AcHA.

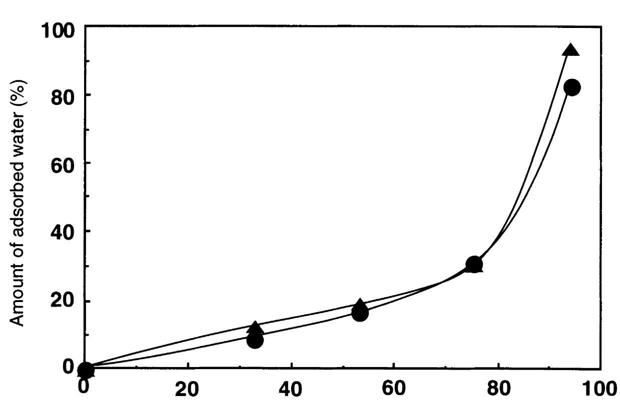

Fig. 4. Water absorbing capacity of sodium hyaluronate (HA) and sodium acetylhyaluronate (AcHA). The filled triangles (▲) and the filled circles (●) represent HA and AcHA, respectively. The *x*-axis shows the relative humidity (RH) value at which the sample was equilibrated.

3.2. Water absorbing capacity of AcHA

It is well known that the flexibility of the stratum corneum depends upon its water content [6,8]. To clarify the mechanism of the skin-softening effect of AcHA, the water-retaining ability of AcHA was compared with that of HA. The hygroscopic nature of AcHA was evaluated using its powder form. Fig. 4 shows that the hygroscopic nature of AcHA is almost identical with that of HA at various relative humidities (RH). Since an excellent humectant such as HA, used in many cosmetic products, should retain a large amount of water to protect skin from dryness at low humidity condition, the hygroscopic nature of AcHA is also an excellent property for protecting skin. The skin-softening effect of humectants depends on their water-holding capacities; hence the higher the water-holding capacity, the more the skin-softening effect [29]. However, strong skin-softening effect of AcHA remains unexplained by its hygroscopic nature.

3.3. Hydration behavior of AcHA powder

To investigate the hydration behavior of AcHA powder, DSC analysis of bound water was performed. Aqueous solutions of AcHA (2.0–100%) were prepared, and the enthalpies of fusion of free water were measured by DSC. Fig. 5 shows the DSC heating curves of the water adsorbed by AcHA. Since the melting of adsorbed water produced a characteristics asymmetric pattern and the melting curves (lines B–D shown in Fig. 5) of adsorbed water appeared earlier than that of pure water (line E), the water in the sample (lines B–D) melted at lower temperature compared with that of pure water. The shape varied according to the water content and the peak area of fusion became smaller with increasing concentration of AcHA. This means that the melting of water was not observed when a very small amount of water was adsorbed on AcHA (line A). That is, with a 67.9% solution of AcHA, the peak completely disappeared indicating that only bound water was present in the sample.

It is well known that water adsorbed on a polymer humectant exists as bound water with a firm attachment and/or as free water which is much more loosely attached. In general, bound water does not freeze even below 0°C, because the bound water attaches firmly to the polymer. Therefore, the enthalpy of fusion (ΔH) of water, obtained by DSC, can show only free water [28]. Free water content was calculated from ΔH value and the heat of fusion per gram of water (315.6 J; Eq. (3)). Bound water content was obtained by subtracting the free water content from the total water content. The changes in content of the free water and the bound water against the total water content in powder of HA

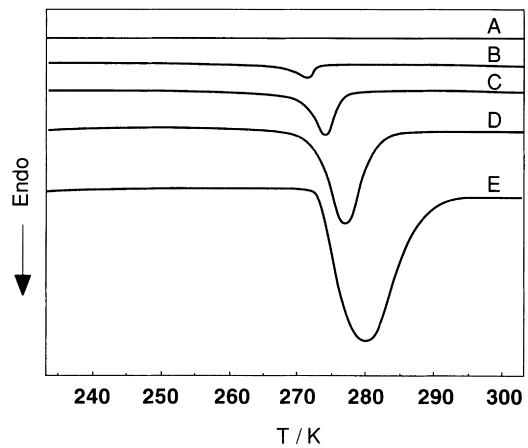

Fig. 5. DSC heating curves of aqueous solutions of acetylhyaluronate (AcHA). The concentration of AcHA solution is 69.9% (line A); 33.6% (line B); 15.2% (line C); 5.8% (line D). Line E represents the DSC curve for pure water.

and that of AcHA were compared (Figs. 6 and 7). The *y*-axis shows the free or bound water content. When completely dry powder adsorbs moisture, water in the powder theoretically exists only as bound water. Free water appeared only following the saturation with bound water. Hydration of AcHA was compared with that of HA by the maximum bound water content without free water. Both showed similar patterns, and the maximum bound water content was 0.89 g per gram of both HA and AcHA. Since AcHA and HA can hold the same amount of pure bound water content, the superiority of the skin-softening effect of AcHA could not be explained by its water holding capacity.

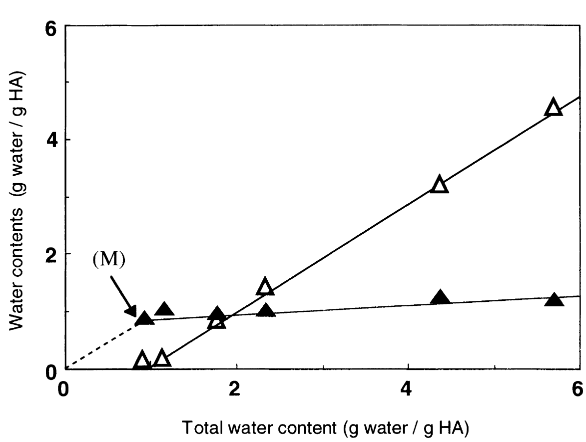

Fig. 6. Changes in contents of bound water and free water of sodium hyaluronate (HA) that has various water contents. The open triangles (△) represent the free water content that was calculated from Δ*H* and the heat of fusion per gram of pure water (315.6 J/g); the filled triangles (▲), the bound water content, which was calculated from the subtraction of the free water content from the total water content. The solid lines are the regression lines of free water and bound water content. The dashed line indicates that the theoretical bound water increases before free water appears. The point M where the regression line of the bound water starts to appear, indicates the maximum bound water content without free water.

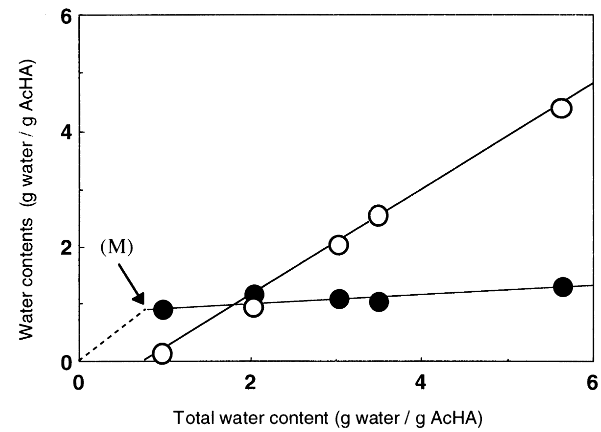

Fig. 7. Changes in the contents of bound water and free water of sodium acetylhyaluronate (AcHA) that has various water contents. The open circles (○) represent the free water content that was calculated from Δ*H* and the heat of fusion per gram of pure water (315.6 J/g); the closed circles (●), the bound water content, which was calculated by the subtraction of the free water content from the total water content. The solid lines are the regression lines of free water and bound water content. The dashed line indicates that the theoretical bound water increases before free water appears. The point M where the regression line of the bound water starts to appear, indicates the maximum bound water content without free water.

3.4. Effect of AcHA on bound water content in the stratum corneum

To investigate the bound water content in the stratum corneum treated with AcHA, DSC measurements were performed. Fig. 8 shows that the maximum content of the bound water per 100 mg of dry stratum corneum increased from 12 to 44 mg by the treatment with AcHA. This increase (32 mg/100 mg dry tissue) was higher than that of HA (24 mg/100 mg dry tissue). The result indicates that AcHA enhanced the bound water capacity of the stratum corneum without the increase of free water. The effect is superior to that of HA suggesting the importance of bound water on the water retention in stratum corneum.

Further, to investigate the effect of the degree of

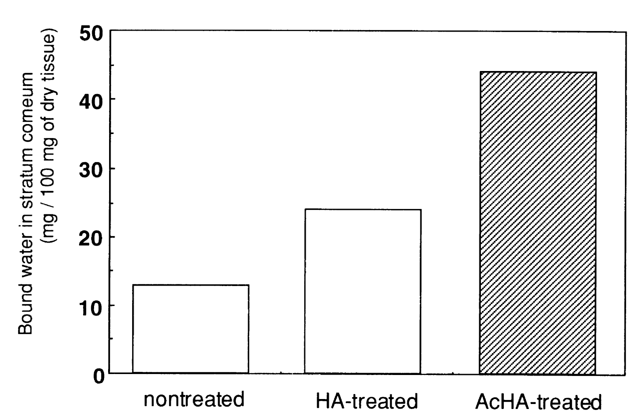

Fig. 8. Bound water contents in the stratum corneum treated with sodium hyaluronate (HA), sodium acetylhyaluronate (AcHA), and non-treated control.

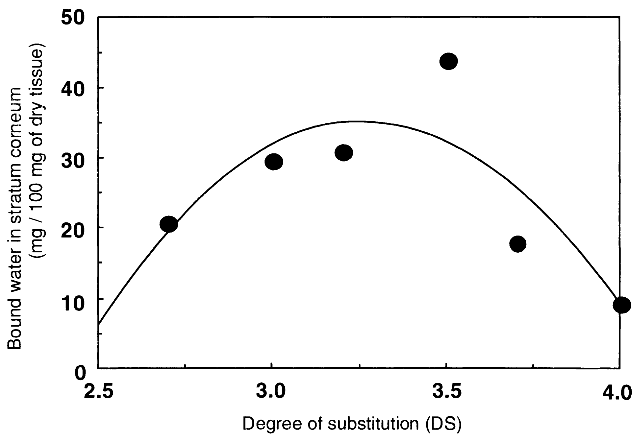

Fig. 9. Relationship between the bound water content in the stratum corneum and sodium acetylhyaluronate (AcHA) which has various degree of substitution (DS) of AcHA. The solid line is the regression line of DS value and the bound water content. There is an optimal DS value around 3.3. Since the DS value of AcHA reflects its hydrophobicity/hydrophilicity balance, a relationship between the bound water content in the stratum corneum and the affinity of AcHA to the stratum corneum may be found.

substitution (DS) of acetyl groups of AcHA, the bound water content in the stratum corneum was treated with AcHA with various DS values. Fig. 9 shows the relationship between the bound water content in the stratum corneum and the DS value of AcHA. The bound water content in the stratum corneum showed the maximum value when DS value is around 3.3. In a previous paper [19], we reported that the degree of the skin-softening effect depends upon the amount of bound water in the stratum corneum and there is an optimal DS value around 3.3. These results suggest that the strength of the skin-softening effect corresponds to the amount of bound water in the stratum corneum. Since the DS value of AcHA reflects its hydrophobicity/hydrophilicity balance, there is also a relationship between water retention in the stratum corneum and the affinity of AcHA to the stratum corneum.

The results indicate that AcHA is compatible with the concept of anchoring humectant (Fig. 1). To explain the strong skin-softening effect of AcHA, the following mechanism is proposed. Humectant sticks to the stratum corneum and prevents the evaporation of water from skin surface, which has a natural tendency to become harder with decreasing hydration. Therefore, AcHA can firmly adhere to the stratum corneum and prevents water-loss from the skin. AcHA helps to sufficiently soften the stratum corneum which is allowed to increase its hydration for a long period of time.

To summarize the results, we can conclude that an amphiphilic polymer, AcHA, was found to show moisturizing effect by enhancing the bound water in the stratum corneum and producing a strong skin-softening effect, compared with HA. Kuroda reported the characterization of the effects of poly(2-methacryloyloxyethyl phosphorylcholine) (MPC) on the stratum corneum functions and its application [30,31]. It was shown that the application of poly(MPC) on normal skin increased the water content in the stratum corneum, reduced the epidermal water loss, and improved the skin surface conductance. These multifunctional characteristics of poly(MPC) are typically found in an amphiphilic polymer.

Further investigation is needed to clarify the mechanism of the skin-softening effect of AcHA. In the near future, we expect to further elucidate this process.

Acknowledgements

The authors sincerely thank Dr T. Hatakeyamma for valuable advice and helpful discussions on the DSC experiments.

References

[1] Chernosky ME. J Soc Cosmet Chem 1976;27:365–76.
[2] Rawlings AV, Scott IR, Bowset PA. J Invest Dermatol 1994;103:731–40.
[3] Imokawa G, Hattori M. J Invest Dermatol 1985;84:282–4.
[4] Yamamura T, Tezuka T. J Invest Dermatol 1989;93:160–4.
[5] Denda M, Kitamura K, Elias PM, Feingold KR. J Invest Dermatol 1997;109:84–90.
[6] Blank IH. J Invest Dermatol 1953;21:259–71.
[7] Foreman MI. Biochim Biophys Acta 1976;437:591–603.
[8] Blank IH. J Invest Dermatol 1952;18:433–40.
[9] Park AC, Baddiel CB. J Soc Cosmet Chem 1972;23:13–21.
[10] Takenouchi M, Suzuki H, Tagami H. J Invest Dermatol 1986;87(5):574–6.
[11] Denda M, Koyamma J, Namba R, Horii I. Arch Dermatol Res 1994;286:41–6.
[12] Andrew WF, Gregory JK. J. Invest. Dermatol 1986;86:598–602.
[13] Elias PM. J Invest Dermatol 983;80:44s–9s.
[14] Cua AB, Whilhelm KP, Maibach HI. Arch Dermatol Res 1990;282:283–8.
[15] Imokawa G, Kuno H, Kawai M. J Invest Dermatol 1991;96:845–51.
[16] Ozawa T, Nishiyama S, Horii I, Kawasaki K, Kumano Y, Nakayama Y. Skin Res 1985;27:276–88.
[17] Kumano Y. J Fragrance 1986;79:64–71.
[18] Tonooka N. Skin Res 1985;27:296–302.
[19] Oka T, Uemura M, Ueno N, Yanaki T. J Soc Cosmet Chem 1999;50:171–84.
[20] Walkley K. J Invest Dermatol 1972;59:225–7.
[21] Jacobi OT. Pro Si Sect Toilet Goods Assoc 1959;31:22–4.
[22] Bulgin JJ, Vinson IJ. Biochim Biophys Acta 1967;136:551–60.
[23] Middleton JD. Br J Dermatol 1968;80:437–50.
[24] Inoue T, Tsujii K, Okamoto K, Toda K. J Invest Dermatol 1986;86(6):689–93.
[25] Kilgman AM, Christphers E. Arch Dermatol 1963;88:702–5.
[26] Tanghe LJ, Genung LB, Mench JW. Acetylation of cellulose. In: Whistler RL, editor. Methods in carbohydrate chemistry, vol. 3. New York: Academic Press, 1963. p. 193–8.
[27] Nakamura K, Hatakeyama T, Hatakeyama H. Polymer 1983;24:871–6.
[28] Hatakeyama T, Yoshida H, Hatakeyama H. Polymer 1987;28:1282–6.
[29] Takahashi M, Yamada M, Machida Y. J Soc Cosmet Chem 1984;35:171–81.
[30] Ishihara K, Ueda T, Nakabayashi N. Polym J 1990;22(5):355–60.
[31] Ishihara K, Nakabayashi N. J Polym Sci Part A 1991;29:831–5.