Diffusion Behavior of Poly(ethylene imine) into Keratin Fibers Using Microspectrophotometry

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ABSTRACT: In order to investigate the diffusion behavior of poly(ethylene imine) (PEI) into keratin fibers, cross-sectional samples of bleached white human hair treated with PEI were prepared. We were successful in developing a method for analyzing the diffusion behavior of PEI into human hair, which to our knowledge is a first. The diffusion pattern of PEI into human hair, which cannot be determined by optical microscopy, can be determined by our method. After the treatment, the cross-sectioned hair samples were dyed with Orange II and the cross-sectional intensity scans were measured at a wavelength of 487 nm (λ_{max} of Orange II) with a microspectrophotometer. In our method, the diffusion pattern of PEI at pH 11.1 showed Fickian type characteristics. This suggests that the diffusion coefficient of PEI is essentially independent of the PEI concentration. By calculating the diffusion coefficient from the PEI concentration profile, the diffusion coefficient of PEI [number-average molecular weight (M_n) = 300 and 600] into the bleached human hair was found to be on the order of 10^{-10} cm^2/s. In addition, the diffusion coefficient of PEI (M_n = 600) with urea added increased twofold in comparison with that of PEI without urea added. This experiment demonstrated that urea acts as a penetration accelerator for PEI. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 65–71, 2005

Key words: fibers; diffusion; dyes and pigments; poly(ethylene imine); microspectrophotometry

INTRODUCTION

Poly(ethylene imine) (PEI) can be irreversibly adsorbed into hair, and it will completely remain in the hair even after washing in distilled water. Because PEI can be strongly adsorbed into damaged hair, PEI has been used in shampoo and rinses to improve the effects of hair conditioning and hair styling.[1] In contrast, urea has been known and used as a dyeing assistant for low temperature wool dyeing. Numerous articles have been published on the interaction of urea with dyes and keratin fibers.[2–9] The function of urea in low temperature dyeing is considered to be disaggregation of anionic dyes,[2] swelling of wool,[3,4] and removal of a part of the hydrophobic material from the surface of the cuticle layer.[5,6] However, the actual function of urea cannot be clarified because of the complexity of its mechanisms. In particular, measuring the swelling of wool in aqueous urea solutions has given variable results because of differences between the methods of measurement and the history of the wool prior to the swelling experiments.[7] Moreover, the addition of urea into a bleaching agent has been demonstrated to improve the whiteness of wool,[8] and its addition into a reducing agent has been known to improve the reduction effect on human hair.[9]

We developed a novel low temperature coloring method using PEI as a counterion reagent; the human hair was treated beforehand with a PEI solution and then colored with an acid dye (Orange II).[10] As a result of this new method, the coloring and color fastness to shampooing was clearly improved compared to that treated with the usual method. Moreover, the coloring and color fastness to shampooing of the hair pretreated with a PEI solution containing urea was clearly improved compared to that pretreated with a PEI solution not containing urea.[11] The reason for the improvement in the coloring ability is that PEI, which penetrates into the cortex region, exerts counterionization on the acid dye, which in turn improves the penetration of the acid dye. Therefore, it is important to study the diffusion behavior of PEI into keratin fibers.

Chow reported that PEI irreversibly penetrated into human hair as ascertained by the radioactive-tracer technique.[12] However, this method cannot obtain information about the diffusion behavior of PEI into human hair.

In order to study the diffusion behavior of PEI into keratin fibers, cross-sectional samples of bleached white human hair treated with PEI were prepared.

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The PEI parts in the cross-sectional samples were dyed with Orange II, and the influence of the PEI molecular weight and urea on the penetration of PEI into bleached human hair was investigated with optical microscopy. The diffusion behavior of PEI into bleached human hair was analyzed by measuring the diffusion profile of PEI using a microspectrophotometer.

EXPERIMENTAL

Materials

Virgin Chinese white hair bundles (average diameter = 70.6 μm) as keratin fibers were purchased from Staffs Co. (Tokyo). The hair was cleaned with a 0.5 wt % sodium lauryl sulfate solution. After washing in distilled water, the hair was dried at room temperature. PEIs with number-average molecular weights (M_n) of 300, 600, 1200, or 20,000 were supplied by Nippon Shokubai Co., Ltd. (Osaka, Japan). Tissue-Tek O.C.T.4583 compound (Sakura Finetechnical Co., Tokyo) was used as an embedding resin to make up the fiber cross sections. Urea, Orange II acid dye, 1-methyl-2-pyrrolidone (MP), 25 wt % ammonia solution, and 35 wt % hydrogen peroxide solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of bleached human hair

Bleached human hair was prepared according to Tate et al.'s method.[13] Eleven virgin white hair bundles were immersed in a solution of 6.0 wt % hydrogen peroxide (pH adjusted to 10.2 with ammonia water) at a hair/solution ratio of 1:50. The hair bundles were soaked for 1 h at room temperature. After being sufficiently washed in distilled water, the bleached hair bundles were dried at room temperature.

Preparation of hair treated with PEI

As a control, one bleached human hair bundle (sample 1) was treated with MP according to the following procedure. The bleached human hair sample was immersed in a solution of 5.0 wt % MP at a hair/solution ratio of 1:15. The hair sample was soaked at 50°C for 15 min (MP treatment procedure). After being washed in distilled water for 1 min, the hair sample was dried at room temperature.

In addition, four bleached human hair bundles (samples 2, 3, 4, and 5) were immersed in a solution of 10 wt % PEI with varying M_n values (300, 600, 1200, or 20,000) and 5.0 wt % MP at a hair/solution ratio of 1:15. The hair samples were soaked at 50°C and pH 11.1 for 15 min (PEI treatment procedure). One bleached human hair bundle (sample 6) was prepared according to the same procedures as sample 3 except that the sample was soaked for 60 min. One bleached human hair bundle (sample 7) was immersed in a solution of 10 wt % PEI (M_n = 600) and 5.0 wt % MP at 50°C and pH 7.9 for 60 min. All samples were washed in distilled water for 1 min, and then dried at room temperature.

Preparation of hair treated with PEI containing urea

As a control, one bleached human hair bundle (sample 8) was treated with urea according to the following procedure. The bleached human hair was immersed in a solution of 20 wt % urea and 5.0 wt % MP at a hair/solution ratio of 1:15. The hair sample was soaked at 50°C for 60 min (urea treatment procedure). After being washed in distilled water for 1 min, the hair sample was dried at room temperature.

Three bleached human hair bundles (samples 9, 10, and 11) were immersed in a solution of 20 wt % urea and 5.0 wt % MP with a hair/solution ratio of 1:15. The hair samples were soaked at 50°C for 1 h. After being washed in distilled water for 1 min, the hair samples were immersed in a solution of 10 wt % PEI with varying molecular weights (M_n = 600, 1200, or 20,000), 20 wt % urea, and 5.0 wt % MP at a hair/solution ratio of 1:15. The hair samples were soaked at 50°C and pH 11.1 for 15 min (PEI–urea treatment procedure). After being washed in distilled water for 1 min, the hair samples were dried at room temperature.

Evaluation of penetration of PEI into human hair

White human hair fibers treated with PEI as described in the previous section were embedded in a resin (Tissue-Tek O.C.T.4583) and frozen. The frozen blocks were microtomed on a Leica CM1800 (Leica Instruments GmbH, Heidelberger, Germany) to 10-μm thickness and mounted on a slide glass. Next, the PEI penetrated parts of the cross-sectional samples were dyed with a solution of 0.1 wt % Orange II at room temperature with a syringe. Finally, the penetration of PEI into human hair was observed by optical microscopy.

Microspectrophotometry

The cross-sectional intensity scans of the cross-sectioned hair samples were measured using a microspectrophotometer (DMSP-II, Olympus Optical Co. Ltd., Tokyo). This instrument is a microscopic photometer equipped with an interference filter for spectroscopic measurements.

The cross-sectional samples mounted on slide glasses were infused dropwise with a solution of 0.1

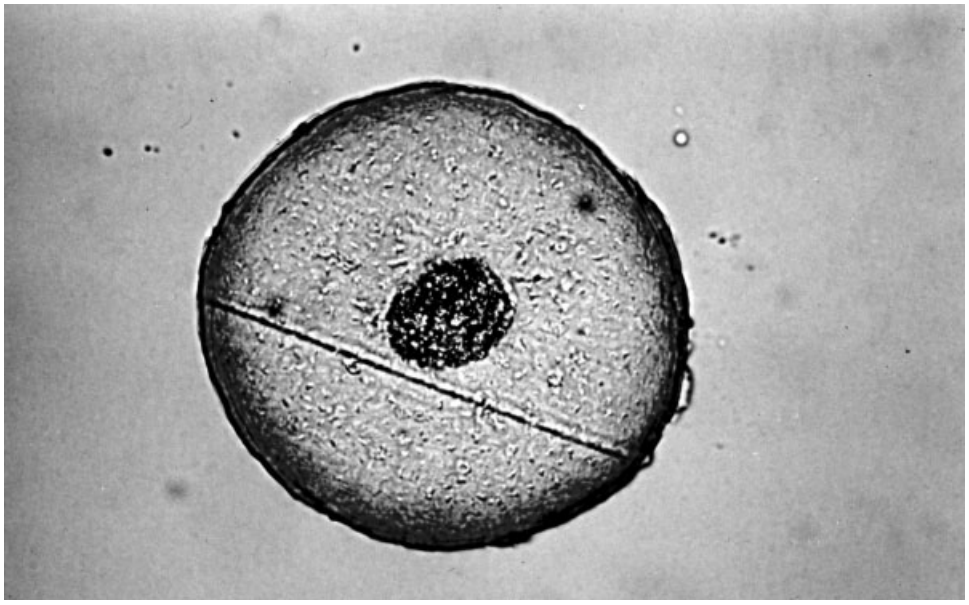

Figure 1 Photomicrograph of the bleached white human hair (Sample 6) treated with PEI (M_n = 600) at 50°C and pH 11.1 for 60 min, then cross-sectioned and finally dyed with Orange II.

wt % Orange II at room temperature with a syringe. The PEI penetrated cross-sectional samples were dyed at room temperature for 1 min. Next, cover glasses were placed on the cross-sectional samples, and then the slide glass mounted cross-sectional samples were set on a specimen stage. Finally, the diffusion pattern of the PEI that had penetrated into the human hair was examined at a wavelength of 487 nm (λ_{max} of Orange II). The cross-sectional intensity scan was measured with the following light source conditions: tungsten filament lamp, 5.3-μm spot diameter, 10 mm/min scanning speed, and 20 mm/min recording speed. In addition, as a blank, the cross-sectional intensity scan of the same cross-sectional sample was measured at a wavelength of 650 nm.

RESULTS AND DISCUSSION

Penetration of PEI into human hair using optical microscopy

PEI is a branched polymer, which has many cationic charges because of the large amount of nitrogen in the molecule.[1] Therefore, the penetration of PEI into the human hair can be observed by dyeing PEI penetrated parts with acid dye (Orange II, etc.).[10,11] Thus, we prepared samples of bleached human hair that were treated with PEI, cross sectioned, and then dyed with Orange II. Finally, the penetration of PEI into the human hair was estimated by optical microscopy. The photomicrograph of the bleached white human hair (sample 6) treated with PEI (M_n = 600) at 50°C and pH 11.1 for 60 min, then cross sectioned, and finally dyed with Orange II is shown in Figure 1. Next, we prepared a sample of bleached human hair that was cross sectioned and then dyed with Orange II. The photomicrograph of this sample (sample 1) is shown in Figure 2. The untreated hair (sample 1) adsorbed the Orange II into the surface of the cuticle only slightly, and it did not adsorb the Orange II into the cortex at all. In contrast, the bleached white human hair (sample 6) treated with PEI (M_n = 600) at 50°C for 60 min adsorbed the Orange II from the cuticle to the cortex. The influence of the PEI molecular weight on the penetration of PEI into the bleached human hair was investigated using the method above (Table I). The penetration of PEI into the bleached human hair was clearly improved by decreasing the M_w of PEI. However, the penetration of PEI into the bleached human hair fell dramatically when the PEI molecular weight was over 1200.

Next, the influence of urea on the penetration of PEI into bleached human hair was investigated by using the above method. In order to be able to correctly estimate the diffusion behavior of PEI, urea needed to be sufficiently penetrated into the bleached human hair. To achieve this, we treated the bleached human hair beforehand with 20 wt % urea at 50°C for 60 min. In the next step, we prepared the cross-sectional samples of human hair treated with 10 wt % PEI containing 20 wt % urea. Finally, the penetration of PEI in the cross-sectional samples dyed with Orange II was estimated by optical microscopy. The photomicrograph of the bleached white human hair (sample 8) treated with 20 wt % urea at 50°C for 60 min, cross-sectioned, and dyed with Orange II is shown in Figure 3. The bleached human hair (sample 8) treated with 20 wt %

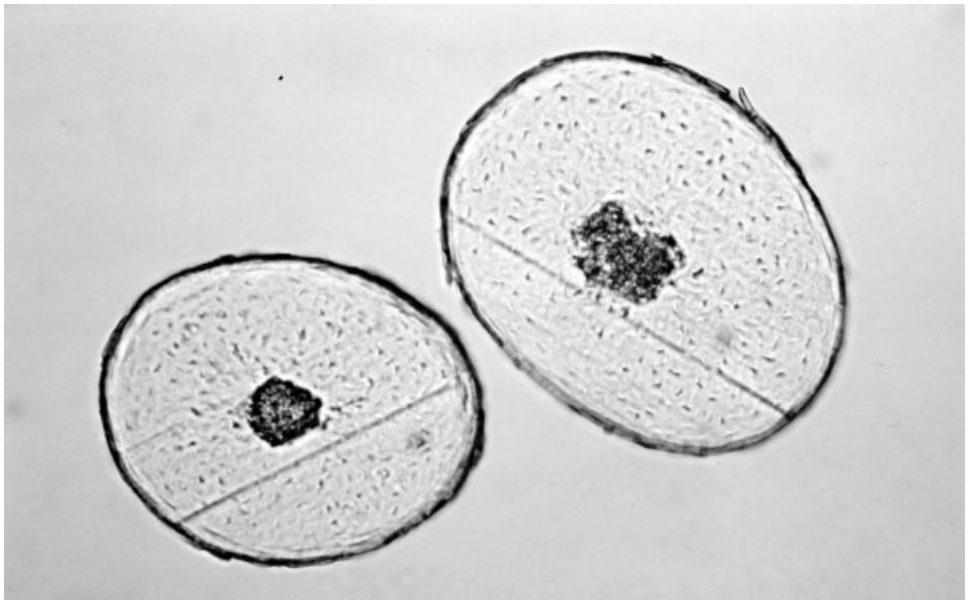

Figure 2 Photomicrograph of the bleached white human hair (Sample 1), which was cross-sectioned and finally dyed with Orange II.

urea adsorbed Orange II into the surface of the cuticle only slightly, but it did not adsorb Orange II into the cortex at all. This suggests that the addition of urea has no effect on the ability to observe the penetration of PEI into the human hair using this method. The penetration of PEI into bleached human hair was clearly increased by adding 20 wt % urea in all cases, despite varying PEI molecular weights (600, 1200, 20,000; Table I).

Diffusion behavior of PEI into human hair

Microspectrophotometry has been used to analyze the diffusion behavior of disperse dyes into various fibers.[14,15] Han et al. have reported that the diffusion coefficient of HC Red 3 (a semipermanent hair dye) into human hair was on the order of 10^{-10} cm^2/s using the same method.[16]

TABLE I
Penetration of PEI Estimated by Optical Microscopy for Cross-Sectional Samples Dyed with Orange II

	Penetration (μm) in sample treated with	
M_n of PEI	PEI	PEI and urea
300	8.35	ND
600	6.02	10.2
1,200	3.25	6.94
20,000	2.78	4.63

The treatment conditions were 50°C at pH 11.1 for 15 min. Here, samples treated with 10 wt % PEI and 20 wt % urea were treated beforehand with 20 wt % urea at 50°C for 60 min. ND, not done.

In this study, the diffusion behavior of PEI into human hair was analyzed by combining microspectrophotometry and dyeing techniques. The diffusion patterns of PEI into human hair could not be determined by microspectrophotometry only, because the PEI does not have a visible spectrum characteristic. However, the diffusion patterns of PEI into human hair could be determined by combining microspectrophotometry and dyeing techniques.

The cross-sectional intensity scans at 487 and 650 nm (blank) of the bleached white human hair (sample 6) treated with PEI (M_n = 600) at 50°C and pH 11.1 for 60 min, cross sectioned, and dyed with Orange II are shown in Figure 4. The Orange II concentration, namely, the PEI concentration, decreased from the fiber surface to the cortex as shown by scanning. In addition, the pseudopeak in the vicinity of the fiber surface arises because of the difference in the refractive index of water and the refractive index of hair fiber (Veckerain phenomenon). Thus, the diffusion profile of PEI was drawn by subtracting the cross-sectional intensity scan at 650 nm (blank) from the cross-sectional intensity scan at 487 nm.

The relative concentration (c/c_0) profiles of PEI (M_n = 600) for different processing times (15 and 60 min) at 50°C and pH 11.1 are shown in Figure 5. Here, c_0 is the PEI concentration at the fiber surface and c is the PEI concentration when the distance from the fiber surface is x. The diffusion pattern of PEI showed Fickian type characteristics. PEI penetrated deeper into the bleached hair when increasing the processing time (from 15 to 60 min). In addition, the c/c_0 profiles of PEI having different M_n values (300 and 600) at 50°C and pH 11.1 for 15 min are shown in Figure 6. PEI pene-

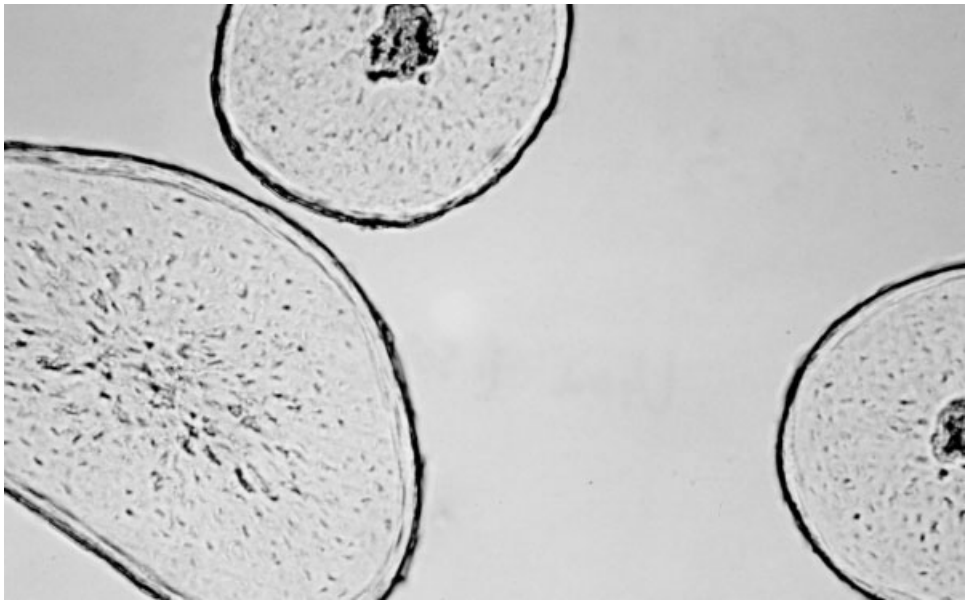

Figure 3 Photomicrograph of the bleached white human hair (Sample 8) treated with 20 wt % urea at 50°C for 60 min, then cross-sectioned and finally dyed with Orange II.

trated deeper into the bleached hair when decreasing the M_n (from 600 to 300).

Moreover, the influence of urea on the diffusion behavior of PEI into bleached human hair was investigated by using the above method. PEI clearly penetrated further into the bleached hair when urea was added (Fig. 7). In addition, the diffusion pattern of PEI at pH 11.1 showed Fickian type characteristics, regardless of whether urea was added. This suggests that the diffusion coefficient of PEI is essentially independent of the PEI concentration.

Determination of diffusion coefficient of PEI

The diffusion pattern of PEI at pH 11.1 showed Fickian type characteristics. In the case of this Fickian type pattern, the diffusion coefficients (D) at each concentration can be determined from the PEI relative concentration profile using Equation (1), which was developed by Matano[17] and Itou et al.,[18] and Equation (2), which was developed by Itou et al.[18] and Karasawa et al.[19] In this case, $D_{c=c1}$ is the diffusion coefficient at concentration $c1$, x is the distance from the fiber surface, c_0 is the PEI concentration at the fiber surface ($x = 0$), c is the PEI concentration when the distance from the fiber surface is x, C is the relative concentration, and β and γ are variable parameters.

$$D_{c=c1} = -\frac{1}{2t}\frac{dx}{dc}\int_0^{c1} x\,dc \tag{1}$$

$$C = \frac{c}{c_0} = \exp(\beta x^{\gamma}) \tag{2}$$

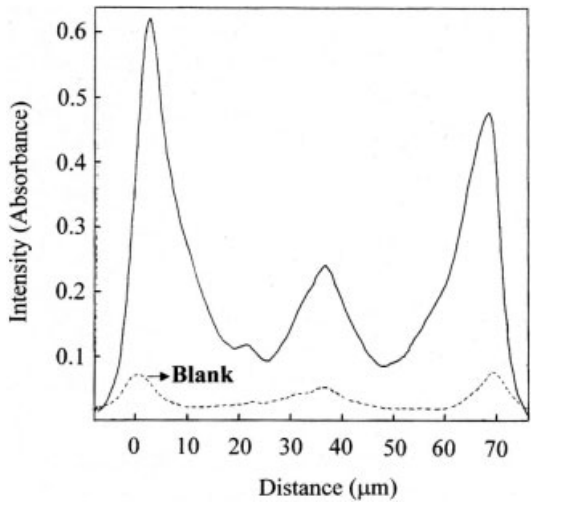

Figure 4 Cross-sectional intensity scans at 487 nm and 650 nm (blank) of the bleached white human hair (Sample 6) treated with PEI (M_n = 600) at 50°C and pH 11.1 for 60 min, then cross-sectioned and finally dyed with Orange II.

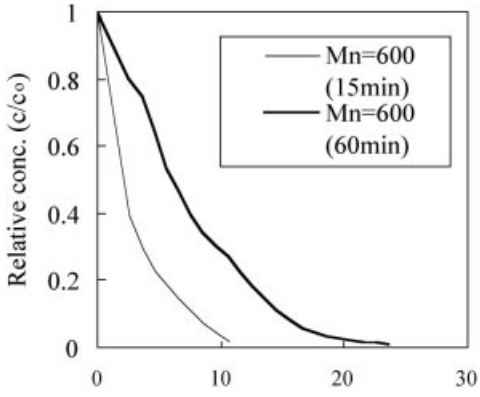

Figure 5 Relative concentration (c/c_0) profiles of PEI (M_n = 600) for different processing times (15 and 60 min) at 50°C and pH 11.1.

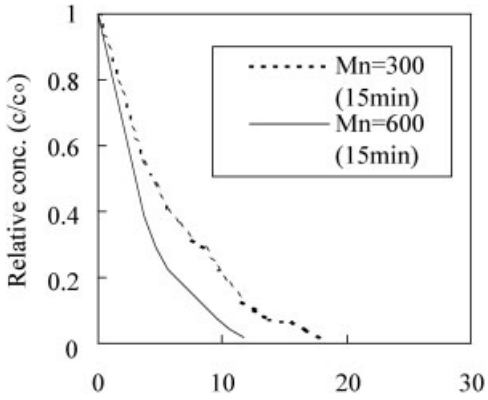

Figure 6 Relative concentration (c/c_0) profiles of PEI having different number-average molecular weights (M_n = 300 and 600) at 50°C and pH 11.1 for 15 min.

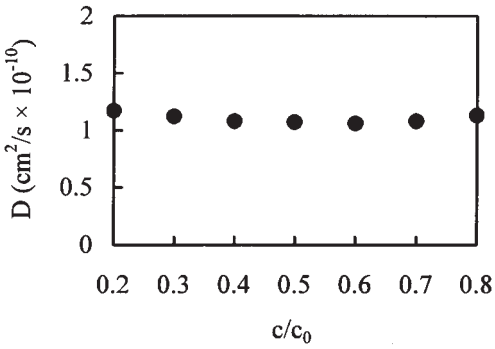

Figure 8 Diffusion coefficients (D) of PEI (M_n = 600) into human hair as a function of the relative concentration (c/c_0) calculated from the PEI relative concentration profile (Figure 5, treatment time: 60 min).

The diffusion coefficients of PEI (M_n = 600) into human hair as a function of the c/c_0 calculated from the PEI relative concentration profile (treatment time = 60 min, Fig. 5) are shown in Figure 8. The diffusion coefficients of PEI as a function of the PEI c/c_0 were constant. Because the diffusion coefficient of PEI for the most part did not depend on the concentration, the diffusion coefficients were determined by calculating the average value from the diffusion coefficient at PEI relative concentrations of 20, 30, 40, 50, 60, 70, and 80%.

The diffusion coefficients determined from the PEI relative concentration profiles are shown in Table II. The diffusion coefficients of PEI (M_n = 600) determined from the PEI relative concentration profiles for 15 and 60 min were in good agreement, indicating that the result obtained by our method was reasonable. In the case of assuming that the diffusion coefficient of PEI (M_n = 600) is 1.34×10^{-10} cm^2/s [the average value calculated from the diffusion coefficients of PEI (M_n = 600) for 15 and 60 min], the diffusion coefficient of PEI with M_n = 300 increased about threefold in comparison to that of PEI with M_n = 600. In addition, the diffusion coefficient of PEI (M_n = 600) with urea added increased about twofold in comparison with that of PEI without urea added.

From this experiment, the diffusion coefficients of PEI (M_n = 300 and 600) into bleached human hair at pH 11.1 are found to be on the order of 10^{-10} cm^2/s.

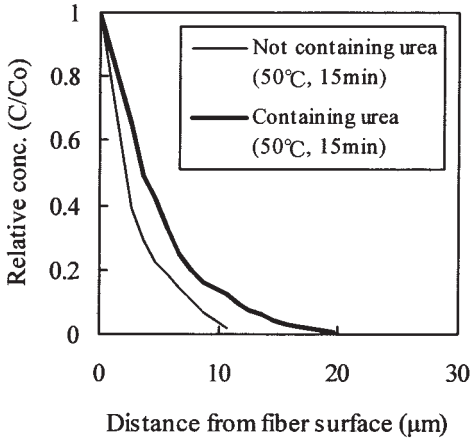

Figure 7 Relative concentration (c/c_0) profiles of PEI (M_n = 600) containing urea or not, at 50°C and pH 11.1 for 15 min.

Influence of pH on penetration of PEI into bleached human hair

The influence of the pH on the penetration of PEI into bleached human hair was investigated. When PEI comes in contact with water, it forms an alkaline solution because of the extraction of a hydrogen proton from the water molecule. Woodward reported that the positive charge ratio of PEI was increased by lowering the pH.[1] Namely, at pH 10.5, 4% of the total nitrogen atoms have a positive charge; at pH 8.0, 6.0, and 4.0, the percentages are 25, 33, and 50% respectively.

The diffusion pattern of PEI at pH 11.1 showed Fickian type characteristics (Figs. 5–7). The isoelectric point of the untreated hair[20] was pH 3.67, and the hair surface had a negative charge when the pH of the hair surface exceeded this isoelectric point. However, the positive charge ratio of PEI at pH 11.1 was so small that interaction between the hair surface and PEI did not occur.

By contrast, the photomicrograph of the bleached white human hair (sample 7) treated with PEI (M_n = 600) at 50°C and pH 7.9 for 60 min, then cross-sectioned, and finally dyed with Orange II is shown in Figure 9.

TABLE II
Diffusion Coefficients Determined from PEI Relative Concentration Profiles (n = 4)

M_n of PEI	Contains urea	Treatment time (min)	$D \times 10^{10}$ (cm^2/s)
300	No	15	4.34 ± 0.62
600	No	15	1.61 ± 0.17
600	No	60	1.07 ± 0.15
600	Yes	15	3.36 ± 0.80

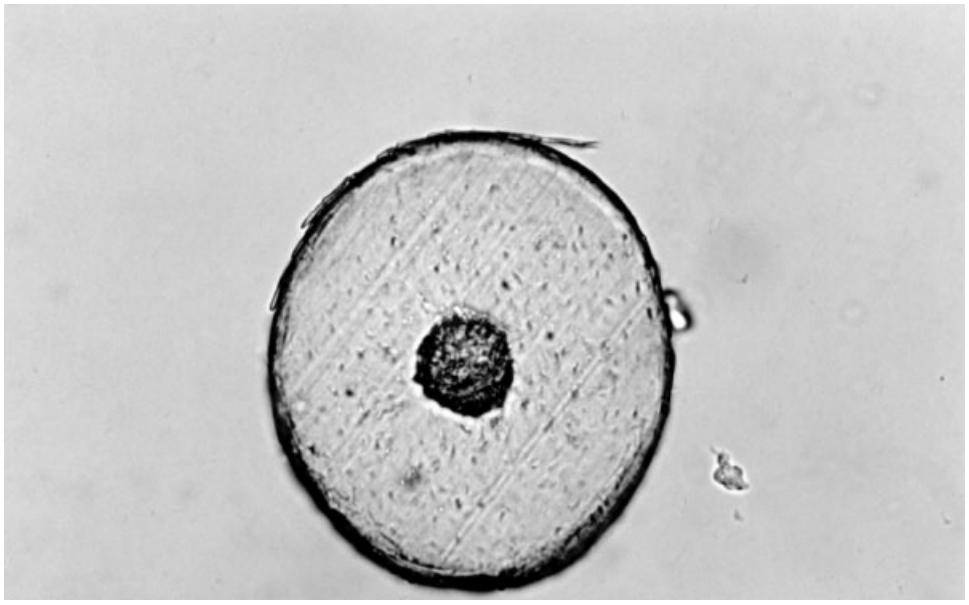

Figure 9 Photomicrograph of the bleached white human hair (Sample 7) treated with PEI (M_n = 600) at 50°C and pH 7.9 for 60 min, then cross-sectioned and finally dyed with Orange II.

The penetration of PEI into the bleached human hair was decreased by changing the pH from 11.1 to 7.9 (compared to Fig. 2). This suggests that the cationic ions of PEI interacted electrostatically with the anionic ions of the human hair, and some PEI was captured in the cuticle, because PEI has a positive charge at pH 7.9.

This experiment showed that the penetration of PEI into bleached human hair clearly depended on the pH of the PEI solution.

CONCLUSIONS

We developed a method for analyzing the diffusion behavior of PEI into human hair. The diffusion pattern of PEI at pH 11.1 showed Fickian type characteristics. This suggests that the diffusion coefficient of PEI is essentially independent of the PEI concentration. By calculating the diffusion coefficient from the PEI concentration profile, the diffusion coefficient of PEI (M_n = 300 and 600) into bleached human hair at pH 11.1 was found to be on the order of 10^{-10} cm^2/s. In addition, the penetration of PEI into bleached human hair depended on the pH of the PEI solution. This suggests that the penetration of PEI into bleached human hair depended on the electrostatic interaction between the anionic ions of human hair and the cationic ions of PEI.

Moreover, the diffusion coefficient of PEI (M_n = 600) with urea added increased twofold in comparison with that of PEI without urea added. This experiment demonstrated that urea acts as a penetration accelerator for PEI.

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