Assessment on the Effects of Liposome-encapsulated Thioglycolic Acid on Hair Perming

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Abstract: In this study, the ethanol injection method was utilized to encapsulate thioglycolic acid (TGA) in liposomes, and 4 lipid formulations were designed to assess the effects of liposome-encapsulated TGA on hair perming. The results showed that the diameter of liposomes without cholesterol was between 112.5 nm and 124 nm, and the diameter expanded to 288 nm to 325 nm after supplementing the liposome particles with cholesterol. The S-shaped waving of hair treated with a pH 9.0 liposome hair perm solution after 4 hair washes was around 19.06 % to 24.73 %, which was higher than the 17.27 % of the control group. Analyses of the cysteine content in hair treated with the first perm solution revealed that the cysteine content in the control group was about 4.40 %, whereas that of the experimental group was 4.51 % to 5.31 %. These results demonstrated that liposomes facilitate the penetration of perm solutions into the hair cortex to cleave the disulfide bonds, thus enhancing the reduction capacity of the first perm solution.

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1. Introduction

With the advancement of the society and the improvement in the quality of life, a wide array of hair products, including hair perm solutions, was developed to meet the needs of various types of consumer. A survey conducted in 2007 revealed that 79.4% (54 cases) of the surveyed perm solutions in the Taiwanese market had incomplete ingredient labels [1]. It is also known that the more the hair was permmed, the greater the keratin loss in the hair [2]. In typical hair perming, thioglycolic acid (TGA) is used as the main ingredient in alkaline perm solutions to cleave the disulfide bonds in the hair, in order to alter the waving of the hair. During the perming process, the first perm solution, a reducing agent, penetrates the hair and cleaves about 45% of the disulfide bonds therein, forming single thiol groups, which are then pressed, reshaped and moved by hair curlers of various shapes, diameters and tensile strengths; the second solution, an oxidizing agent, then brings together the thiol at the new position with another thiol, forming a new disulfide bond, thus resulting in permanent hair modification. In general, hair perm solutions are categorized into three types: acid, cysteine and alkaline.

Liposome is a phosphatidyl choline (PC)-based microparticle; it has a hollow sphere capable of encapsulating molecules, thus making liposome a possible delivery vehicle. Due to its excellent permeability to biological membranes, liposomes have been extensively studied and implemented in various applications. Having both polar and non-polar characteristics, phospholipid liposome may also act as a surfactant; it can bind to most proteins and forms a stable lipoprotein structure, which makes liposome a strong emulsifying agent [3,4]. Previous research results indicated that phospholipid is a good stabilizer in the preparation of emulsions. Currently, liposomes are used in environmentally conscious fabric processing procedures in the textile industry to encapsulate dye molecules, in order to reduce environmental pollution; the related processing techniques have already exhibited promising results [5]. Such liposome-encapsulated dye formulations have demonstrated outstanding coloring effects on coloring protein fibers, such as wool and silk, as liposomes enabled the formulations to dissolve fast, which increased the effectiveness of the coloring processes and also improved the antistatic and anti-shrinkage effects of the fabrics [6,7,8].

Cholesterol (CH) is an important component of biological membranes. Due to its planar structure, cholesterol itself cannot form a bilayer, but may combine with lipids in different concentrations, including a
molecular ratio of cholesterol to lipid of up to 1:1, or even 2:1 [9]. Previous studies reported that the addition of cholesterol had a significant impact on the physical properties of liposome bilayers; upon the addition of cholesterol, the 3-β-hydroxyl group in cholesterol would form a hydrogen bond with the polar head group of phospholipid, resulting in a more compact arrangement of the liposomal lipid membrane and an increased membrane hardness with a reduced fluidity, which may be effective in reducing drug leakage in encapsulating liposomes [10].

The primary objective of this study is to assess the hair perming effect of PC-encapsulated thioglycolic acids. The ethanol injection method was used to prepare the liposomes, and the hair testing was performed under different pH values and with different formulations. This study also investigated the change in hair cysteine content after the first perm solution treatment, aiming to gain insight into the perming effect of liposome perm solutions.

2. Experimental materials and method

2.1 Preparation of Liposome Perm Solutions

As the pH values of perm solutions commonly used in hair salons in Taiwan are between 8.0 and 9.3; pH 8.5, pH 9.0 and pH 9.3 were chosen as the pH values for the 3 first perm solutions to be tested in this study (Table 1). The perm solution creating the best S-shaped waves was then used in the 4 following experiments with liposome perm solutions. The tread hair tress by formula of Ethanol Injection has the best performance.

Liposome perm solutions were prepared by simple instrumentation setup using the ethanol injection method [11]. Phosphatidyl choline (Natterermann, Germany) and cholesterol (Sigma, USA) were dissolved in 10 mL of 99.5% ethanol according to the ratios of 2:0, 2:0.5, 3:0 and 3:0.5 (w/w) to generate 4 lipid solutions. 2 mL of each lipid solution was syringed and added to 8 mL of TGA solution, which was mixed immediately by vigorous shaking for 1 min for liposome formation. The Polidispersity index (The PDI value approaching 0 means the particle sizes approach uniform. When the value falls between 0 and 0.35 it means the range of particle size distribution is narrow. A PDI value of 0.7 or more indicates a boader particle size distribution.) values average particle size, and distribution of particle sizes of the liposomes were analyzed by a Malvern Zetasizer Nano ZS instrument; each liposome sample was scanned 4 times, taking a total of about 3 to 6 min to complete.

2.2 Cysteine Content Analysis

Cysteine (Sigma) was diluted with distilled water to 4 concentrations, 0.98 mg/L, 0.49 mg/L, 0.24 mg/L and 0.12 mg/L, with a final volume of 0.1 mL each. 2.9 mL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman’s Reagent) was added to each sample and the solutions were homogenized and let stand for 3 min. The cysteine contents in the samples were then analyzed by a spectrophotometer (Hitachi, U-1800).

Hair treated with liposome-containing first perm solution was cut into small pieces (about 0.2 mm). 0.01 g of the hair pieces was mixed with 2.9 mL of DTNB and analyzed according to the standard curve of Cysteine concentrations, measured by spectrophotometer to obtain the permeability of liposome perm solutions.

2.3 Hairpiece Fabrication and Perming Procedure

50 strands of real human hair were wrapped in a diameter of 10 mm and a length of 82 mm professional salon plastic curlers with short perm rods. The curler was soaked in 10 mL of first perm solution for 1 min and was saran®-wrapped for 15 min at 50°C. The treated curler was let sit at room temperature for 3 min and rinsed with warm water for 3 min. After wiping off any excess water, the curler was soaked in a second perm solution for 1 min and let sit at room temperature for 15 min, followed by rinsing with warm water. The treated hair strands were removed from the curler and air dried for further evaluation.

2.4 Hair Washing Standards

The perm solutions treated hairpiece was left in a constant-temperature water bath shaker with 1/3 of the shaker volume of clean water, a water temperature of 40 ± 1°C, and a shaking frequency of 200 rpm [12]. The hairpiece was then washed with 400 mL of sodium laureth sulfate (SLES, 60 mg/ml in solution) for 7 min, and rinsed twice with 400 mL of clean water under the same conditions for 3 min.

2.5 S-shaped Waving Evaluation

Evaluation of the persistency of the S-shaped waving in treated hairs was performed as follows: the permed hairpiece was washed 1 to 4 times, and the average length
and the standard deviation of stretched hair were recorded 24 h after the washes. The evaluation was based on the following equation, and the higher the calculated value, the better the waving of the permed hair.

\[
S\text{-shaped waving} = \frac{\text{hair length before the perm} - \text{hair length after the perm}}{\text{hair length before the perm}} \times 100\% \quad (1)
\]

### 2.6 Hair Strength Evaluation

When the tensile strength applied to a hair reaches its intensity limit, the failure strength of the hair would start to decrease until the hair breaks; and the smaller the percentage of failure strength (kgf/mm²) loss while hair breaking, the less damage the hair will derive from the perming. Therefore, the failure strength at hair breaking point was used to determine the elasticity of the hair samples. A thickness meter (Telock SM-1201) was used to measure the diameters of 4 strands of washed hair from each group, and a microcomputer tensile strength tester (GT-7010DI-PC) with a testing velocity of 50 mm/min and a reference distance of 50 mm was employed to evaluate the elasticity of the hair strands.

Percentage of failure strength loss = \[
\frac{\text{breaking point after the 4th wash} - \text{breaking point after the 1st wash}}{\text{breaking point after the 1st wash}} \times 100\% \quad (2)
\]

### 3. Results and discussion

#### 3.1 Analysis of Particle Sizes

Liposome particles aggregate and fuse over time, causing unstable particle sizes and problems with long-term storage; however, this condition can be improved by adding cholesterols to the liposomes. The results shown in Table 2 demonstrate that the addition of 0.5 g of cholesterol resulted in significant difference in the PDI value; although the particle sizes increased in the presence of cholesterols, the occurrence of phase transformation was reduced, which may effectively prevent particle fusion and encapsulation leakage [13].

#### 3.2 Cysteine Content Analysis

The linear regression coefficient R² of the 4 cysteine solutions, with cysteine concentrations of 0.98 mg/L, 0.49 mg/L, 0.24 mg/L and 0.12 mg/L, at 415 nm was 0.9984. As shown in Table 3, the cysteine content of the control group B was 4.40 ± 0.52%, whereas the cysteine contents of groups B1-B4, in which liposome perm solutions were used, were: 4.51%, 4.73%, 5.13% and 5.31%, respectively.

The results suggested that liposome-containing perm solutions exhibited better hair permeability, and that the small particle size of liposomes facilitated the penetration of perming agents into the hair cortex to cleave off the disulfide bonds, thus achieving the desired hair weakening effect. This effect was evident by the higher cysteine contents in groups B1-B4 compared to the 4.40 ± 0.52% of group B.

#### 3.3 Analysis of the S-shaped waving

A, B and C groups of this study were treated with alkaline perm solutions with pH values of 8.5, 9.0 and 9.3, respectively. As shown in Table 4, after 4 hair washes in a month, the S-shaped waving of all three groups were higher than 47% after the perm, and the waving after the 4th hair wash was 17.27% in group B (pH 9.0), indicating the smallest decrease in waving among the 3 groups. Consequently, pH 9.0 was considered the most stable pH value for this study, and the liposome perm solutions were prepared based on the group B solution. Liposome perm solutions for groups B1, B2, B3 and B4 were prepared according to a PC : CH ratio of 2 : 0, 2 : 0.5, 3 : 0 and 3 : 0.5, respectively.

The S-shaped waving of groups B1-B4 after 1 to 4 hair washes were all better than those of the control group B; the 24.73% observed in group B1 after the 4th hair wash was the best S-shaped waving among all of the others. In general, Table 4 shows that when treating hair with perm solutions with liposome-encapsulated TGAs, the S-shaped waving of the treated hair was maintained at higher than 19%, even after 4 hair washes. Therefore, it is evident that liposome perm solutions can improve the S-shaped waving and the persistency of the treated hair.

#### 3.4 Analysis of Hair Strengths

This study on the change in strengths at hair breaking points (kgf/mm²) showed that the strengths of group B decreased from 24.25 after the 1st wash to 20.56 after the 4th wash, and the breaking strengths of groups of perming solutions into the hair cortex to cleave off the disulfide bonds, thus achieving the desired hair weakening effect. This effect was evident by the higher cysteine contents in groups B1-B4 compared to the 4.40 ± 0.52% of group B.

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Table 2  Analysis of liposome particle sizes ; N=4 (Mean±SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>PC (g)</th>
<th>CH (g)</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>2</td>
<td>0</td>
<td>**141.3 ± 4.6</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>B2</td>
<td>2</td>
<td>0.5</td>
<td>**288.8 ± 2.2</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>B3</td>
<td>3</td>
<td>0.5</td>
<td>112.5 ± 2.1</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>B4</td>
<td>3</td>
<td>0.5</td>
<td>192.5 ± 5.0</td>
<td>0.19 ± 0.02</td>
</tr>
</tbody>
</table>

$t$-test  $^*P<0.05$  $^{**}P<0.01$  $^{***}P<0.001$

Table 3  Cysteine contents of permed hair.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cysteine content after the perm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab</td>
<td>g/mL</td>
</tr>
<tr>
<td>B</td>
<td>1.660 ± 0.195</td>
</tr>
<tr>
<td>B1</td>
<td>1.702 ± 0.140</td>
</tr>
<tr>
<td>B2</td>
<td>1.785 ± 0.199</td>
</tr>
<tr>
<td>B3</td>
<td>1.946 ± 0.214</td>
</tr>
<tr>
<td>B4</td>
<td>2.008 ± 0.120</td>
</tr>
</tbody>
</table>
B1-B4 dropped from 27.49, 24.18, 25.00 and 23.92 to 23.84, 21.16, 22.56 and 22.30, respectively (Table 5). The data demonstrated that the percentages of loss in strength at hair breaking points in both the control and experimental groups were significantly reduced by 6-15%, and that the average breaking strength of groups B1-B4 with liposome-encapsulated perming agents was greater than that of the control group B; the average breaking strength of groups B1 and B3 was greater than that of groups B2 and B4. These results indicated that the fiber structure of group B was relatively loose after the perm, and the bonding in the α-keratine intermediate filaments was not strong, causing the hair to be more susceptible to damage by external forces. The hair elasticity of groups B1-B4 was higher than that of the control group, for the following two reasons:

1. The phospholipids supplemented in liposomes may enhance the hair conditioning effect, which was evident by the lower percentages in failure strength loss in groups B3 and B4 compared to those in groups B2 and B1.
2. Liposome-encapsulated perming agents may penetrate the hair cortex more efficiently, to fully reduce and oxidize the disulfide bonds. This was indicated by the higher hair elasticity in groups B1-B4 than that in group B.

### 4. Conclusions

This study assessed the perming effects of liposomes prepared by the ethanol injection method and obtained the following conclusions:

1. The particle sizes of thioglycolic acid-encapsulating liposomes were around 112.5-288.8 nm, and the particles expanded with increased concentrations of supplemented cholesterols.
2. The S-shaped waving of hair treated with liposome perm solutions was higher at pH 9.0 than that of the controls; due to the high lipid contents in phospholipids and cholesterols, the addition of cholesterols to liposome phospholipids significantly improved the strength of treated hairs.
3. The cysteine contents in hair treated with liposome-encapsulated thioglycolic acid were about 4.51-5.31%, higher than the 4.40% of the control group; this observation could be explained by the small particle size of the liposomes, which facilitated the penetration of perming agents into the hair cortex to cleave off the disulfide bonds, thus accomplishing the reduction and oxidation reactions in hair perming. The results described above demonstrated that liposome encapsulation of thioglycolic acid may reduce the occurrence of hair damage from hair perming and may also increase the hair penetration of perming agents, as well as the S-shaped waving and duration of treated hair.

### References