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The stabilization of L-ascorbic acid in aqueous solution and water-in-oil-in-water double emulsion by controlling pH and electrolyte concentration

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Synopsis

This study presents a new approach that can stabilize effectively L-ascorbic acid in water-in-oil-in-water (w/o/w) double emulsions. Basically, the behavior of L-ascorbic acid in the aqueous phase was observed, considering its molecular deformation. Then, it was found that the stability determined in the aqueous phase by high-performance liquid chromatography (HPLC) showed that the collapse of ionization of L-ascorbic acid played a crucial role in protecting the molecular deformation. Then, the stable aqueous system was incorporated into the internal aqueous phase of the double emulsions. From the HPLC analysis, it was observed that the L-ascorbic acid in an appropriate system showed high molecular stability for a long time. Moreover, in the measurement of *in vitro* skin permeation, the L-ascorbic acid stabilized in this study showed considerable skin permeation ability, indicating its potential applicability in pharmaceutics and cosmetics.

INTRODUCTION

L-ascorbic acid has many biological functions, such as the inducement of collagen synthesis, the strengthening of skin tissues against external attacking factors, reduction in the loss of pigmentation, and anti-free-radical activity (1–6). Unfortunately, however, L-ascorbic acid is very sensitive to light, to the action of oxidizing agents and metal ions, and even to slight heating. Therefore, L-ascorbic acid degrades unavoidably in aqueous solution (7). In order to overcome those problems, a number of methods have been proposed, including the chemical modification of L-ascorbic acid, microencapsulation, and complexation with other moieties (8–12). However, to the best of our knowledge,

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the ultimate stabilization of L-ascorbic acid in the aqueous phase has not been reported in the literature.

The degradation processes of L-ascorbic acid are very complex and contain a number of oxidation/reduction and intermolecular rearrangement reactions (13). Scheme 1 shows well the degradation pathway of L-ascorbic acid in the aqueous solution. Dehydro-L-ascorbic acid, an oxidation form of L-ascorbic acid, is highly unstable in an aqueous solution, which may convert to a variety of species, such as 2,3-diketo-L-gulonic acid, L-xylosome, etc. (14,15). An important clue in the degradation process is that the degradation is initiated by the ionization of the hydroxy groups in the L-ascorbic acid molecule. The control of ionization may provide us a potential route that can protect fundamentally the degradation of L-ascorbic acid in the aqueous system.

In our previous studies, we developed stable w/o/w double emulsions by two-step emulsification method (16). In those studies it was shown that the stable w/o/w double emulsions produced can be used as a useful tool for the stabilization of water-soluble, unstable L-ascorbic acid. In the present contribution, L-ascorbic acid located in the internal aqueous phase of w/o/w double emulsions was stabilized, controlling the pH and ionization property. The effectiveness was then evaluated according to the experimental parameters.

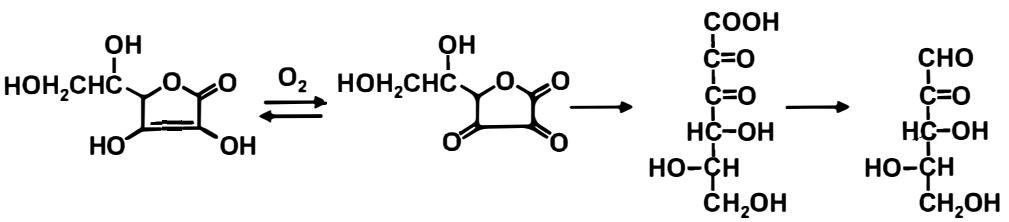
EXPERIMENTAL

MATERIALS

L-ascorbic acid (99.9% assay, Shinyo Pure Chemicals, Japan), magnesium sulfate ($MgSO_4$, Aldrich Chemical Co., USA), and sodium hydroxide (NaOH, Aldrich) were all of reagent grade and used without any further purification. In the preparation of w/o/w double emulsions, the lipophilic primary surfactant was Arlacel P135, a polyethylene glycol (30) dipolyhydroxystearate (Uniquema Americas). The hydrophilic secondary surfactant was Synperonic PE/F 127, an ethoxylated propylene oxide copolymer (ICI, France). Puresyn 4, a hydrogenated polydecene (ExxonMobil Chemical) and xanthan gum (Kelco Biopolymers) were used as oil and emulsion stabilizers, respectively, in the preparation of w/o/w double emulsions.

PREPARATION OF w/●/w DOUBLE EMULSIONS

The double emulsions were produced by two-step process (17–19). In the first step, L-ascorbic acid was dissolved in water together with $MgSO_4$. The adjustment of pH was



Scheme 1. Schematic representation of the degradation of L-ascorbic acid in aqueous solution.

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carried out by adding 10 wt% NaOH solution to the L-ascorbic acid/$MgSO_4$ aqueous solution. The total amount of internal aqueous phase was compensated by adding water. Then the primary water-in-oil (w/o) emulsions were prepared by adding slowly the L-ascorbic acid/$MgSO_4$ aqueous solution to the oil phase composed of Puresyn 4 and Arlacel P135 at a high temperature (70° ± 5°C) while emulsifying at 7000 rpm for 5 min with an MX-5 homogenizer (Nihonseiki Co., Japan). The primary w/o emulsions produced were continuously cooled to room temperature. In the second step, the primary w/o emulsions produced in the first step were reemulsified at 4000 rpm in the aqueous phase containing Synperonic PE/F 127. About 20 g/min was a suitable speed for the primary w/o emulsions. After another 5 min of homogenization, the w/o/w double emulsions produced were stabilized sterically with the aid of a xanthan gum. The w/o/w double emulsions containing L-ascorbic acid in the internal aqueous phase were then sealed in polyethylene plastic tubes and stored at 40°C. The composition for the production of w/o/w double emulsions is summarized in Table I.

OM OBSERVATION

Microscopic analysis was carried out to visualize the morphology of w/o/w double emulsions with an optical microscope (OM) (Nikon Microphot FXA). After 1/10 (w/w) dilution of the double emulsions in DDI water, the droplet morphology was captured with a digital camera at room temperature.

HPLC MEASUREMENT

To determine the concentration of L-ascorbic acid in the w/o/w double emulsions, HPLC measurements were carried out. After dissolving completely 1 g of w/o/w double emulsions containing L-ascorbic acid in 10 ml of methanol, the total volume was adjusted to 100 ml by adding DDI water. Ultrasound was applied to the diluted emulsions for 20 min to break up the droplets perfectly. The HPLC sample solutions

Table I
Standard Composition for w/o/w Double Emulsions

Emulsification step	Ingredient	Concentration (wt%)
Primary w/o emulsion[a]	Puresyn 4	15.5
	Arlacel P135	2
	DDI water	29 (variable)
	$MgSO_4$	0.5
	L-ascorbic acid	3 (variable)
	NaOH[c]	Variable
w/o/w double emulsion[b]	DDI water	39
	Synperonic PE/F 127	1
	Primary emulsion	50
	Xanthan gum[d]	10

[a] 75• ± 5•C; 7,000 rpm; 5 min.
[b] 25• ± 5•C; 4,000 rpm; 5 min.
[c] Sodium hydroxide aqueous solution was added to adjust pH of internal aqueous phase.
[d] 1 g/dl aqueous solution.

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were then prepared by filtering the diluted solutions with a sintered-glass filter. The liquid chromatographic system used was an HP1100 series equipped with a solvent delivery module, a UV detector, and an autosampler. The chromatographic separation was achieved using an YMC-Pack column (YMC-Pack NH_2, A-603, 4.6 × 250 mm I.D. S-5 μm, 12 nm, Japan). The carrier solvent for HPLC analysis was the mixture of 50 mM of $NH_4H_2PO_4$ solution and acetonitrile (50/50 v/v). The flow rate was 1 ml/min. The detection wavelength was set at 264 nm for L-ascorbic acid. The sample injection volume was 20 μl. In this HPLC condition, L-ascorbic acid was detected at the retention time of 6.7 min. The stability of L-ascorbic acid was defined by the ratio of measured concentration to initial concentration, $[AA]_m/[AA]_0$, where $[AA]_m$ is the measured concentration of ascorbic acid in the w/o/w double emulsion and $[AA]_0$ is the initial concentration of ascorbic acid in the w/o/w double emulsion.

IN VITRO SKIN PERMEATION TEST

Female hairless guinea pigs (strain IAF/HA-hrBR) were sacrificed for the *in vitro* skin permeation test. They were all eight weeks old. Abdominal skin was excised, divided, and mounted on Franz diffusion cells (Lab Fine Instruments, Korea). The diameter of each diffusion cell was 0.9 cm and the compartment volume was 5 ml. The receptor compartment was filled with 10 wt% glycerin aqueous solution. The receptor compartment was kept at 32°C by circulating water through an external jacket and stirring constantly with a magnetic bar. In this study, three samples were measured: 5 wt% L-ascorbic acid (pH = 2), 5 wt% L-ascorbic acid (pH = 7), and 5 wt% L-ascorbic acid (pH = 7)/0.5 wt% $MgSO_4$. HPLC analysis was carried out by taking 5 ml of receptor solution at a predetermined time (20–22).

RESULTS AND DISCUSSION

DEGRADATION OF L-ASCORBIC ACID IN AQUEOUS SOLUTION

In many cases, the degradation of L-ascorbic acid has been a reason for the declining quality in the final applications. As summarized in Scheme 1, L-ascorbic acid degrades to various species in the aqueous solution (13). In the aqueous solution, L-ascorbic acid is oxidized readily to dehydro-L-ascorbic acid. The oxidation takes place especially at the most reactive enediol group (10). There it looks clear that the degradation of L-ascorbic acid is started from the enediol group by pro-oxidants such as hydroxy peroxide, hydroxy radicals, and hydroperoxy radicals, as well as by metal ions. Important information that we can obtain is that if the enediol group is protected effectively, it is possible to stabilize L-ascorbic acid in the aqueous solution because one of the key destabilization factors is fundamentally excluded.

PROPOSAL OF L-ASCORBIC ACID STABILIZATION SYSTEM

When L-ascorbic acid is dissolved in water, the pH of the solution decreases sharply due to the dissociation of the hydrogen ion from the enediol group. Considering the pK values for L-ascorbic acid (pK_1 = 4.17, pK_2 = 11.57), the main contribution to the lowering of pH is the hydroxy group located on the number 3 carbon (C3). In order to

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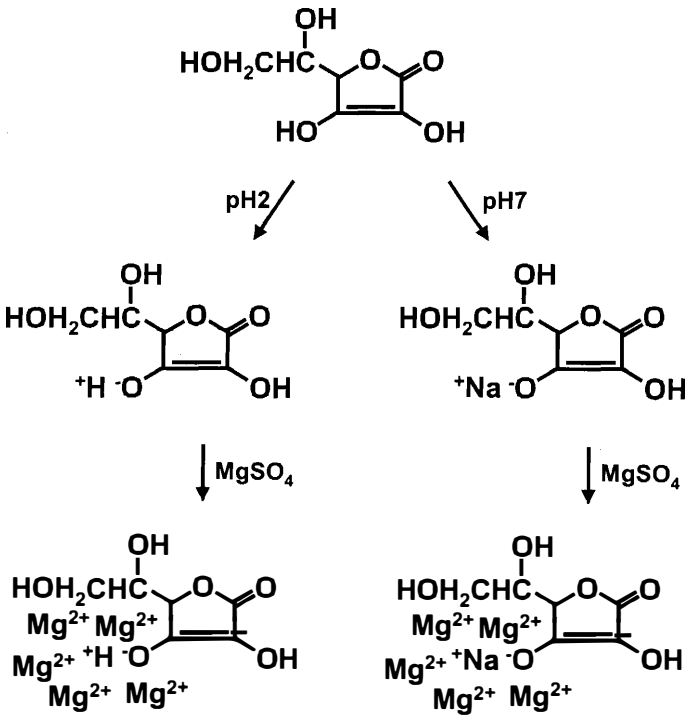

Scheme 2. Schematic representation of the ionic shielding process of L-ascorbic acid in aqueous solution.

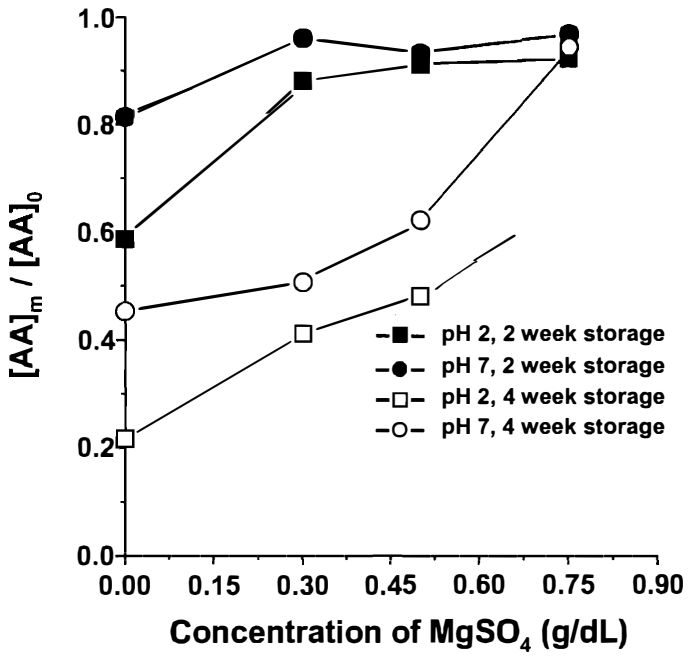

Figure 1. Stability of L-ascorbic acid solutions with the ionic strength at different pH and storage times after 2-week storage (-■-, pH 2; -●-, pH 7) and 4-week storage (-□-, pH 2; -○-, pH 7) at 40°C. In this observation, the concentration of L-ascorbic acid was 3 wt%.

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control the characteristics of the enediol group, especially the hydroxy group attached to C3 in the L-ascorbic acid molecule, we introduce the concept of ionic shielding, which is applied generally to the swelling behavior of hydrogels in an ionic medium condition (23–25). In Scheme 2, the ionic shielding process of L-ascorbic acid is represented schematically. When the ionic strength of the solution is increased, the counterions have a tendency toward binding to the species charged oppositely, which is responsible for the ion–ion interaction. The counterions include charged ionic species such as, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, and Al^{3+}. (In this study, we selected Mg^{2+} as a counterion.) The ionic strength in the system can be controlled by varying the ionic species and their concentration. It can then be said that the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid is possibly hindered.

In this study, the effectiveness of ionic shielding on the stability of L-ascorbic acid was examined by considering the medium's pH. Figure 1 shows the long-term stability of simple L-ascorbic acid aqueous solutions with the ionic strength at a high temperature,

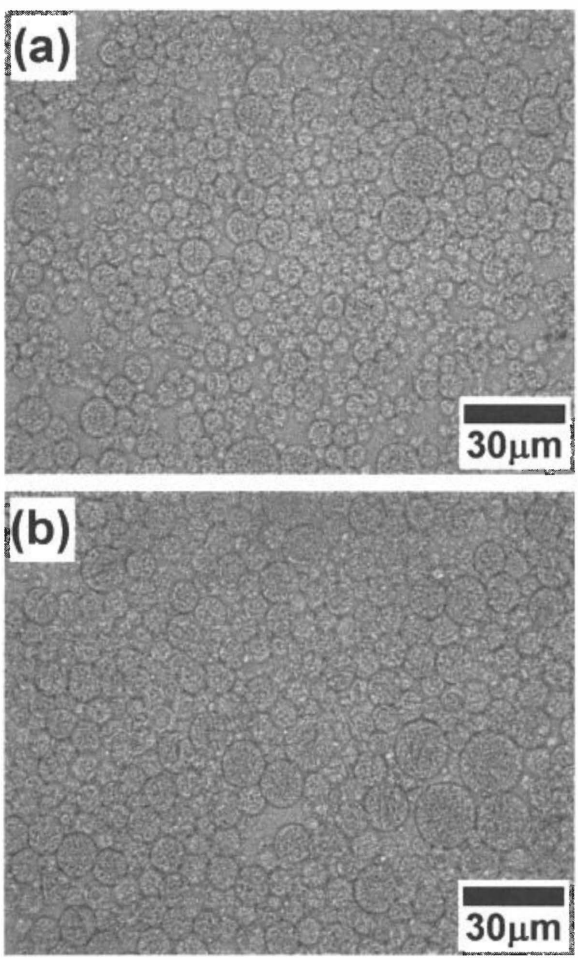

Figure 2. OM photographs for w/o/w double emulsions: (a) w/o/w double emulsions not containing L-ascorbic acid; (b) w/o/w double emulsions containing 3 wt% L-ascorbic acid in the internal aqueous phase.

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40°C. In Figure 1, it is verified that the increase in the ionic strength contributed significantly to the improvement of L-ascorbic acid's stability in the aqueous solution. When the pH of the solution was adjusted to 7, L-ascorbic acid could retain its initial molecular characteristics for a long time, compared with the case of a low pH. It is quite different from the conventional results (9,26). In most instances, L-ascorbic acid is more stable at a low pH. Therefore, it is recommended that for the stabilization of L-ascorbic acid, the system should have a low pH. However, judging from our concept and experimental result, a large amount of hydrogen ion generated at low pH simply shields the ionized L-ascorbic acid (L-ascorbate ion). In our system, L-ascorbic acid showed a better stability at pH 7. Moreover, when the ionic strength was strengthened sufficiently, only a little deformation of L-ascorbic acid was observed. It appears that the ionic shielding generated by the ion–ion interaction was fortified because the dissociation of the sodium counterion takes place readily, compared with that of the hydrogen counterion.

STABILIZATION OF L-ASCORBIC ACID IN w/o/w DOUBLE EMULSIONS

Characterization of w/o/w double emulsions. In this study, a system for the stabilization of L-ascorbic acid was constructed by considering the ionic shielding effect in w/o/w double emulsions. Figure 2 shows an OM image of w/o/w double emulsions containing 3 wt% L-ascorbic acid in the internal aqueous phase. In this OM image, it is reasonable to say that a stable w/o/w double emulsion was formed in the size range of 10–20 μm, with the

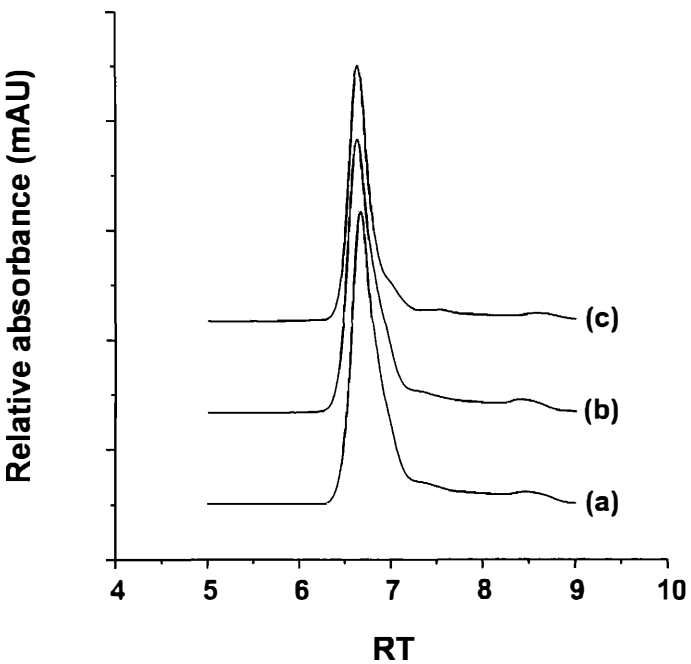

Figure 3. HPLC measurements for the L-ascorbic acid-loading w/o/w double emulsions for different systems: (a) right after the preparation of L-ascorbic acid-loading double emulsion; (b) L-ascorbic acid-loading double emulsion (pH 7) after 10-week storage at 40°C; (c) L-ascorbic acid-loading double emulsion (pH 2) after 10-week storage at 40°C. In this case, the concentration of L-ascorbic acid in the w/o/w double emulsions was fixed at 3 wt%.

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morphology of very fine internal water droplets. Moreover, it is evident that the presence of L-ascorbic acid in the internal aqueous phase didn't have any influence on the morphological change.

Stability of L-ascorbic acid in w/o/w double emulsions. The stability of L-ascorbic acid in the w/o/w double emulsions was evaluated by HPLC measurements. Figure 3 shows examples of HPLC measurements. In the HPLC measurement, L-ascorbic acid was detected at 6.7 min of retention time. From the linear regression of peak area to the concentration, the stability of L-ascorbic acid in the w/o/w double emulsions could be determined precisely. The long-term stability of L-ascorbic acid in the w/o/w double emulsions was measured at time intervals and shown in Figure 4. At low pH, L-ascorbic acid could not avoid degradation, even in the w/o/w double emulsions. However, by merely applying ionic shielding, the stability could be fairly improved, which shows a good accordance with the solutions shown in Figure 1. For a certain time, the stability was good. In the long run, however, the stability started to drop. In contrast, a dramatic improvement in stability could be achieved at pH 7. When ionic shielding was applied appropriately to the internal aqueous phase of w/o/w double emulsions, the initial stability of L-ascorbic acid could be maintained for a long time, even at a high temperature. This reveals that ionic shielding plays a crucial role in stabilizing L-ascorbic acid. The effect of loading amount on the stability of L-ascorbic acid was also observed. Figure 5 shows the stability of L-ascorbic acid with storage time at different loading amounts. It could be found that the stability was not dependent on the loading amount of L-ascorbic acid. From those results, it can be said that once a proper system

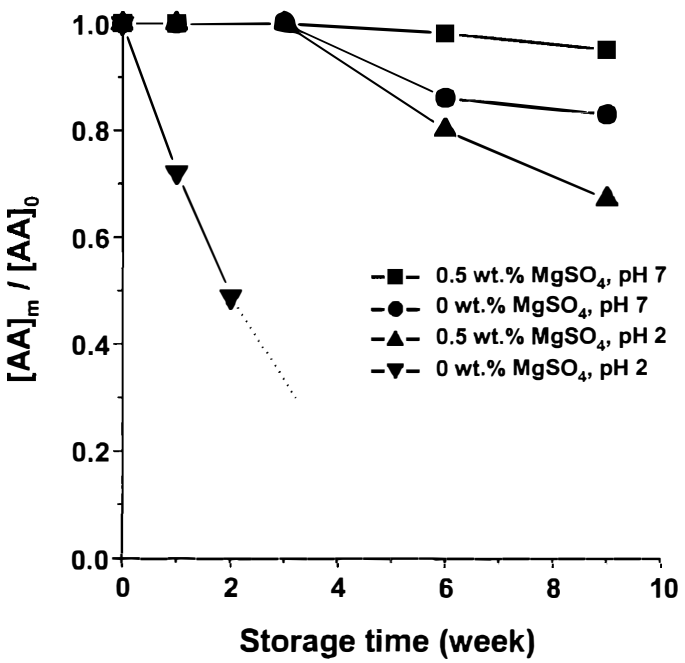

Figure 4. Stability of L-ascorbic acid in w/o/w double emulsions with the storage time (40°C) for different systems: -■-, 0.5 wt% $MgSO_4$/pH 7; -●-, without $MgSO_4$/pH 7; -▲-, 0.5 wt% $MgSO_4$/pH 2; -▼-, without $MgSO_4$/pH 2.

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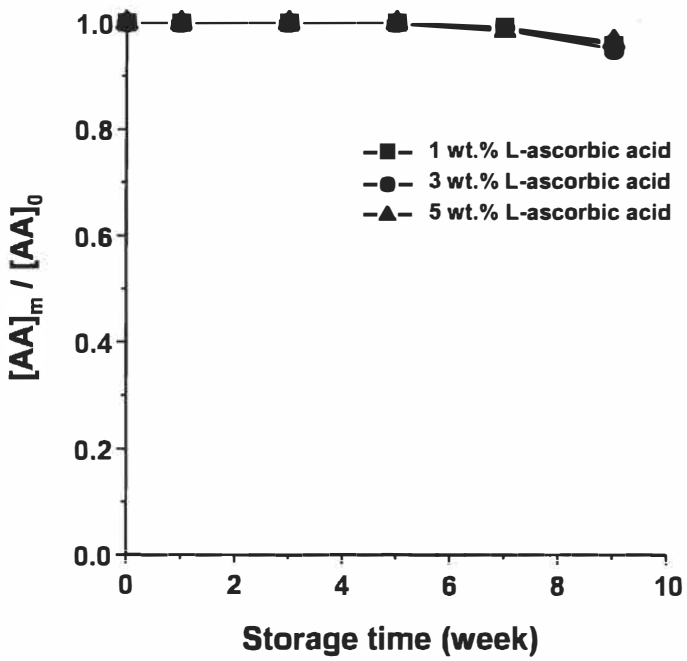

Figure 5. Stability of L-ascorbic acid in w/o/w double emulsions with the storage time (40°C) at different concentrations: -■-, 1 wt% L-ascorbic acid; -●-, 3 wt% L-ascorbic acid; -▲-, 5 wt% L-ascorbic acid. All the samples were shielded with 0.5 wt% $MgSO_4$. The pH of the internal aqueous phase was adjusted to 7.

is established, L-ascorbic acid can be stabilized to a high concentration in the w/o/w double emulsions.

A more attractive result in our study was that the L-ascorbic acid stabilized showed no browning phenomenon. Figure 6 shows a photograph for the color change of L-ascorbic acid-loading w/o/w double emulsions. Due to the deformation of chemical structure, the color of emulsion formulations changes inevitably from a white to a dark brown, which is accelerated at elevated temperatures. Such a phenomenon is a fatal defect in the final applications. In order to confirm the browning effect of w/o/w double emulsions, the

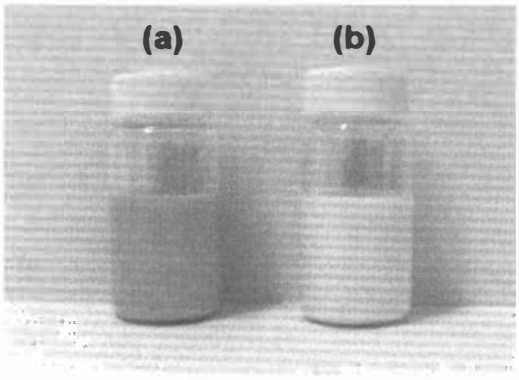

Figure 6. Color change for L-ascorbic acid-loading w/o/w double emulsions after 10-week storage at 40°C: (a) pH 2, (b) pH 7. The concentration of L-ascorbic acid in the emulsions was fixed at 3 wt%.

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color change was observed at an elevated temperature, 40°C. In the case of a low pH, the color of w/o/w double emulsions changed eventually to dark brown (Figure 6a). The browning started to appear within several days. However, when the pH of the internal aqueous phase was adjusted to neutral, we could not observe any change in color (Figure 6b). This result is also direct evidence for the successful stabilization of L-ascorbic acid by means of ionic shielding in the w/o/w emulsion system.

SKIN PERMEATION CHARACTERISTICS OF L-ASCORBIC ACID

Skin permeation of L-ascorbic acid is very important for dermatological applications. In order to show its functions properly, L-ascorbic acid must permeate the stratum corneum and reach viable epidermal and dermal layers or viable subcutaneous layers. However, the barrier property of skin acts as a major obstacle to transdermal drug delivery. Figure 7 shows the *in vitro* skin permeation of L-ascorbic acids. It was observed that the skin permeation efficacy of L-ascorbic acid in the neutral condition is significantly lowered compared with that of the acidic condition. This is because at the neutral condition, the skin, especially stratum corneum, is not injured, as compared to the acidic condition. Also, the lower skin permeation at the neutral condition stemmed possibly from higher ionization, that is, the higher polarity of L-ascorbate ion at the neutral condition. Nevertheless, even though the efficacy was more or less low, it is significant that the skin permeation of L-ascorbic acid still took place successfully. Ionic shielding seems to have negligible influence on skin permeation.

CONCLUSIONS

In this study, we tried to stabilize L-ascorbic acid in w/o/w double emulsions. From the fundamental understanding of the behavior of L-ascorbic acid in the aqueous solution,

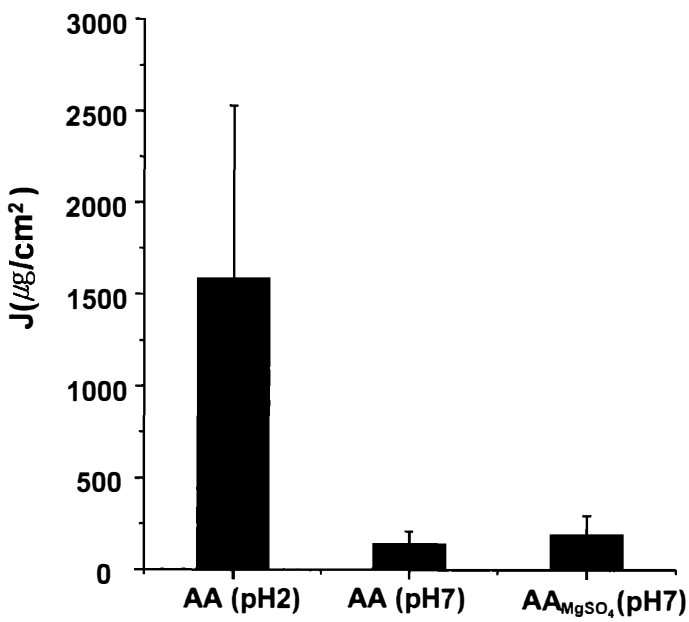

Figure 7. Evaluation of L-ascorbic acid *in vitro* skin permeation with Franz diffusion cell measurements.

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a stabilization system was constructed, considering the ionic shielding and pH adjustment in the stable w/o/w double emulsions. In a proper condition, the L-ascorbic acid could maintain its initial molecular characteristics for a long time (we are still testing). It is notable that the emulsion formulations containing L-ascorbic acid did not show any browning phenomenon, even after long storage at high temperature, verifying directly that the stabilization of L-ascorbic acid is achieved in w/o/w double emulsions. Moreover, the successful skin permeation of L-ascorbic acid stabilized in this study is expected to maintain its functions in the final applications.

ACKNOWLEDGMENT

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