Evaluation for Intrinsic Skin Permeation of Unstable Compounds

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An evaluation method is proposed for the intrinsic skin permeation rate of unstable compounds. Vitamin C and vitamin E were used as the model compounds. The degradation of vitamin C and E in the solutions followed first-order kinetics with degradation constants of 0. 26 h^{-1} and 0.014 h^{-1}, respectively. The apparent skin permeation profiles of vitamin C and E *in vitro*, approximated by a nonlinear profile of the polynomial regression method, was corrected for intrinsic permeation rate considering first-order degradation in the receptor solution. The intrinsic profiles evaluated agreed well with the ones determined from radio-labelled compounds, indicating the feasibility of the present analysis.

Key words skin permeation; vitamin C; vitamin E; degradation

In *in vitro* study of skin permeation of a drug for developing a transdermal drug delivery device, we often encounter difficulty in obtaining an intrinsic skin permeation rate due to degradation of the compounds in the receptor solution. Since the drug is continuously degraded in the receptor media after penetration, the apparent rate of permeation is underestimated, more or less, by the presence of the degradation. One possible approach to overcome this problem is to use a stabilizing agent. However, this is not always applicable. In the present study, we have developed a method for determining the intrinsic rate of permeation.

Materials and Methods

Vitamin C was obtained from Sigma Chemical Co. (St. Louis, MO). Radio-labeled vitamin C (1-[1-^{14}C]ascorbic acid, 10.0 mCi/M) was purchased from E. I. Dupont NEN Research (Boston, MA). Vitamin E (α-tocopherol) and radio-labeled vitamin E , [^{3}H]^{2}-1,3-α-tocopherol, 2.0 mCi/mM) were kindly provided by Hoffmann-La-Roche (Nutley, NJ). The purity of the radio labeled compound was tested by HPLC in line with a liquid scintillation counter (Rack Beta 1214-001, LKB Instruments Inc., Gaithersburg, MD). Tween-80 (ICI Americas, Inc., DE) and Silicone Fluid (Dow Corning 360, 20 cp) were used as obtained. Bioflour (E.I. Dupont NEN Research, MA) was used as a liquid scintillation cocktail. All other chemicals were regent grade. The solvent used in the HPLC assay was HPLC grade. Water was purified by a nanopure water purification system (Sybron/Barnstead, Boston, MA). A HPLC method was used to quantitate the amount which permeated through the skin and to identify the metabolites.

Selection of Receptor Solutions To create optimum selection of the receptor solution in which the oxidation of vitamin C and E is minimized, a stability study containing known drug concentrations was performed in various solutions at 37 °C. Water for preparing the solutions was purified by a Nanopure purification system and was bubbled with nitrogen gas for 5 min to exclude oxygen from the solutions. The stability study was conducted using a Valia–Chien diffusion cell; Teflon films were mounted between the cells of the donor and receptor solutions. The diffusion cell was then covered thoroughly with aluminum foil to prevent photo-oxidation. After determining the degradation rate constants of these two vitamins in various solutions, 50% glycerin solution (pH 3.0) and 5 mM Tween-80 solution were selected as the receptor solutions for vitamin C and vitamin E, respectively.

Permeation Study A freshly excised full thickness of abdominal skin of a female hairless mouse (5—7 weeks old, Jackson Lab., HRS/J Strain) was mounted between the half cells of the *in vitro* skin permeation system, Valia–Chien diffusion cell.[2] Then, the selected donor and receptor solutions were charged in each cell compartment. Fifty percentage glycerin as a donor (13 ± 1 mg/ml) and receptor solutions for vitamin C, and silicone fluid (13 ± 1 mg/ml) and 5 mM Tween-80 as a donor and receptor solutions for vitamin E, were charged into the diffusion cell, respectively. At predetermined time intervals, 30 μl of receptor solution was withdrawn and assayed for the drug concentration with HPLC or liquid scintillation counting. The total amount of drug which permeated through the skin was plotted as a function of time. The permeation rate and lag time were determined from the steady state permeation profile and the time intercept of the profile, respectively.

Evaluation Method of Intrinsic Permeation Rate The intrinsic permeation profiles were computed from the apparent time profiles of the concentration by the following mass balance equation in the receptor solution:

$$VdC/dt = S_a J(t) - f(C)V \quad (1)$$

where C = concentration of drug in receptor cell at time t, (μg/ml), $J(t)$ = flux (μg/cm^2·h), $f(C)$ = function of degradation kinetics, S_a = permeation area of skin (0.64 cm^2), t = time (h), V = volume of receptor solution (3.5 ml).

When the degradation kinetics of the tested compound follow first-order kinetics, Eq. 1 can be expressed as follows:

$$VdC/dt = S_a J(t) - KVC \quad (2)$$

where K is a degradation constant for tested compound (h^{-1}).

To correct the effect of degradation, experimental data of $C(t)$ was approximated by a nonlinear profile of the polynomial regression method. 0.05 has been selected as the weight in the regression analysis. The Marquardt method was used to find the best fitting equation to the apparent permeation profile. $J(t)$ was then integrated analytically to calculate the cumulative amount of permeated drug.

Results

Stability of Vitamin C in Donor Solution Vitamin C was stable in the present donor solution (13 ± 1 mg/ml) during 72 h period: 98% of vitamin C remained stable in the donor solution at 72 h. This finding coincides with the work by Bandelin and Tuschoff.[3] They reported that 93% of vitamin C (C_0: 5 mg/ml) remained in 50% glycerin solution after 240 d at room temperature. The initial concentration of vitamin C in solution determines the rate of decomposition.[3,4] In a higher concentration range, vitamin C is relatively stable. Based on our stability study, we assume that the initial concentration of vitamin C (C_0) in the donor solution remains constant during the permeation study.

Stability of Vitamin C in Receptor Solution Bandelin and Tuschoff[3] and Nixon and Chawla[4] have studied the effect of the initial concentration of vitamin C on the stability of itself. They observed that the degradation of vitamin C proceeded more rapidly with a low concentration of vitamin C. A preliminary skin permeation study

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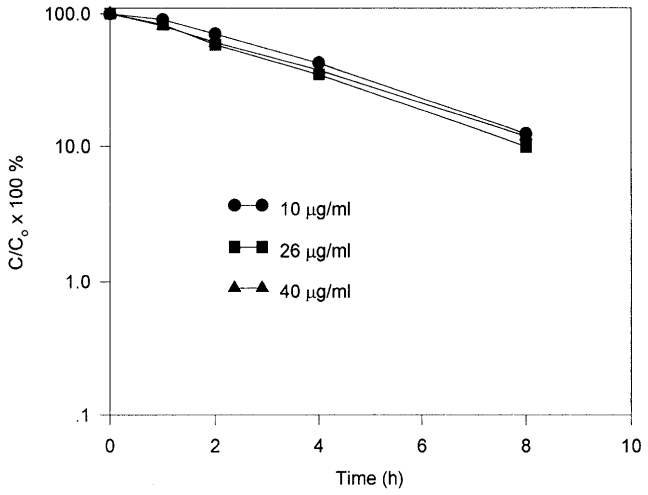

Fig. 1. Stability of Vitamin C (10—40 μg/ml) in Receptor Solution, 50% Glycerin Solution (pH 3.0)

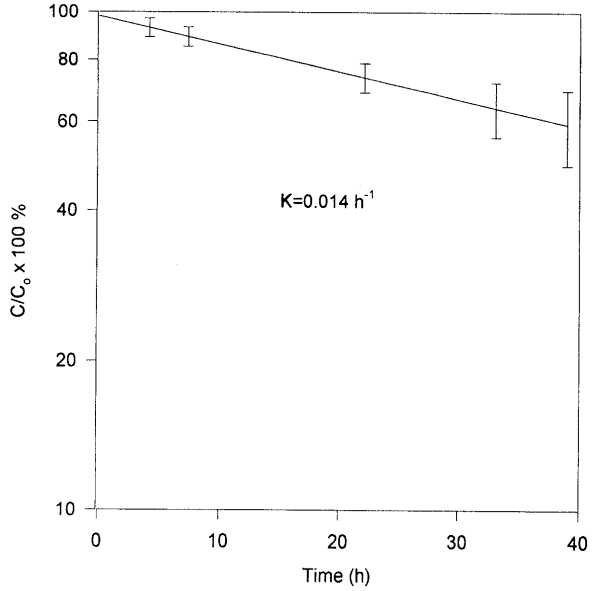

Fig. 2. Stability of Vitamin E (178 μg/ml) in Receptor Solution, 5 mM Tween-80 aq. Solution

of vitamin C was conducted to determine the concentration range in the receptor solution as a function of time. Figure 1 shows the stability of vitamin C in the concentration range used in the skin permeation experiment (10—40 μg/ml), which is almost 1000 times smaller than that in the donor solution. At low concentrations, vitamin C degraded quickly, independently of the concentration, and followed first-order kinetics. Although the initial concentration of vitamin C in solution determines the rate of decomposition in a large scale, based on our stability study, it may be safe to treat the degradation of vitamin C in the receptor according to first-order kinetics. The degradation rate constant obtained for vitamin C was 0.266 h^{-1} and was used for the evaluation of the intrinsic permeation rate of vitamin C.

Stability of Vitamin E in Receptor Solution To maintain a sink condition during the permeation experiment, Tween-80, which can increase the solubility of vitamin E by being incorporated into a micelle, was added to the receptor solution. Imai reported that the solubility of vitamin E in water increased linearly with increasing the concentration of Tween-80.[5] 0.5 mM Tween-80 was chosen after considering the critical micelle concentration of Tween-80 in water (0.4 mM) and the possible skin damage by the surfactant at high concentrations. As shown in Fig. 2, vitamin E approximately follows first-order degradation kinetics with the degradation constant, $K=0.014\,h^{-1}$ which is almost 20 times less than that of vitamin C. The concentration of vitamin E in the donor solution (13 ± 1 mg/ml, in silicone fluid) remains constant (data not shown).[6]

Skin Permeation of Vitamin C Figure 3 shows the concentration–time profiles of vitamin C in the receptor solution. Three individual skin permeation profiles were plotted before correcting for the degradation. A typical bursting was observed initially and thereafter the concentration increased very slowly. For evaluating the intrinsic permeation profile by correcting for the effect of degradation kinetics, the concentration profile of vitamin C was approximated with nonlinear regression analysis using the equation of $Q=b_1t^{b_2}+b_3$ for curve fitting. The experimental profile was then approximated after determining dC/dt and C as a function of time, and the intrinsic permeation rate $J(t)$ was integrated with the Runge–Kutta fourth order method to calculate the cumulative amount of drug which permeated the skin.

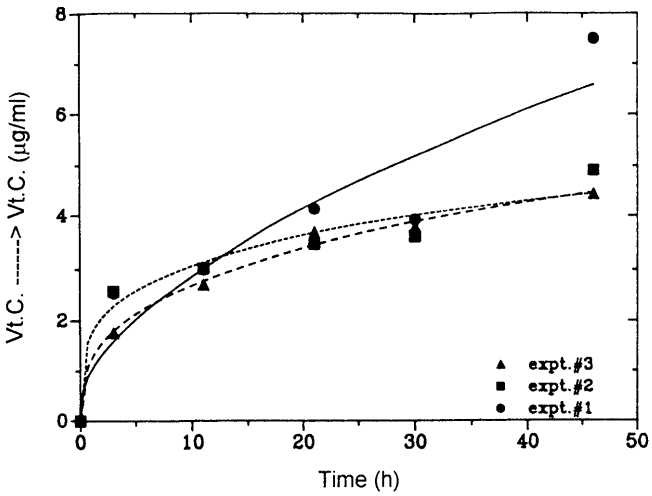

Fig. 3. Curve Fitting of the Bioconversion Data from Vitamin C (Donor) to Vitamin C (Receptor) Using an Arbitrary Function, $b_1t^{b_2}+b_3$

Experiment #1: $b_1=0.609$, $b_2=0.605$, $b_3=0.413$; experiment #2: $b_1=1.717$, $b_2=0.247$, $b_3=0.310$; experiment #3: $b_1=1.255$, $b_2=0.332$, $b_3=-0.902$.

Figure 4 shows the comparison between the intrinsic permeation profile of vitamin C was obtained after correction for the degradation in the receptor solution and the permeation profile of radio-labeled vitamin C. In radio-tracer analysis, the total radioactivity of vitamin C (^{14}C-L-ascorbic acid) which appeared in the receptor solution represents the sum of the degraded and non-degraded vitamin C. The penetration profile for both radio-labeled and corrected profiles of HPLC analysis of vitamin C reached a steady state after 9 h. The rate of steady state penetration under the HPLC assay condition evaluated from the slope of the linear portion of the profile was close to that measured by radio-tracer analysis. There seems to be no lag time, but initial bursting was observed

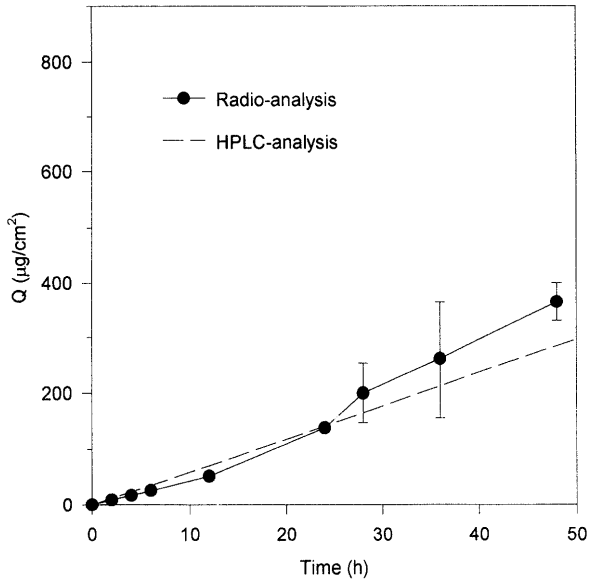

Fig. 4. Comparison of Skin Permeation Profiles of Vitamin C between Radio-Labeled Analysis and HPLC Analysis, Which is the Calculated Intrinsic Permeation Profile of Vitamin C after Considering Degradation Kinetics

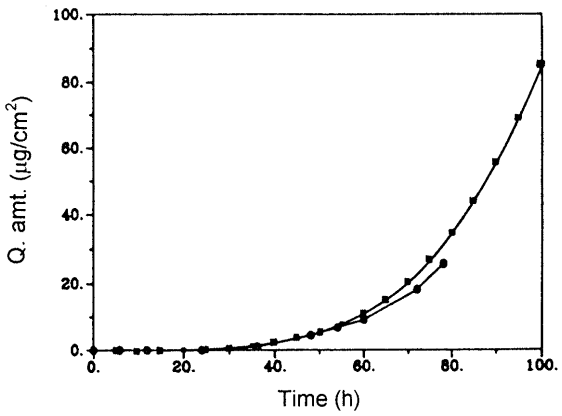

Fig. 5. Comparison of Skin Permeation Profiles of Vitamin E between Apparent and Intrinsic Permeation Profiles after Considering Degradation Kinetics

■, real; ●, apparent.

in HPLC analysis, whereas there is about a 9-h lag time in the radio-labeled vitamin C profile. The difference in skin permeation profiles of vitamin C in the initial period of time between the radio-tracer analysis and the HPLC assay method was due to the endogeneous vitamin C concentration in the skin which was discussed previously.[7] The concentration of endogeneous vitamin C was estimated by solving the mathematical model developed. The concentration was found to be 1.08 μmol/ml.

Skin Permeation Profile of Vitamin E from Vitamin E By employing the same procedure as for vitamin C, the intrinsic permeation profiles of vitamin E were also determined. Figure 5 shows the permeation profiles of the apparent and intrinsic profiles after correcting for degradation. The figure indicates that the degradation of vitamin E is not significant because of its low degradation constant ($K=0.014\,h^{-1}$). The degradation of vitamin E in the receptor solution is virtually insignificant, while that of vitamin C is very significant.

This evaluation method discussed in this report is simple to use and can be applied to second- or third-order degradation kinetics. Even if, in this report, first-order degradation kinetics are discussed, this approach can be applied to any kind of reaction kinetics in general. When we consider only first-order kinetics, this approach can be totally analytical and it becomes very simple to get the solution. The time-dependent concentration profile in the receptor solution can be approximated by an exponential function which can be easily derivatized to obtain dC/dt; therefore, the flux can be calculated analytically. The only one drawback to this approach is the use of the Marquardt method to fit the experimental data. However, this software package is usually available for personal computers. Therefore this approach is simple to use.

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References and Notes

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