International Journal of Pharmaceutics, 25 (1985) 265–274
Elsevier

IJP 00857

Membrane permeation kinetics of nortestosterone: effect of methyl groups on thermodynamics

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(Received December 27th, 1984)
(Modified version received March 11th, 1985)
(Accepted March 27th, 1985)

Key words: nortestosterone – permeation – silicone membrane – structural conformation – diffusivity – partition – temperature dependency

Summary

The kinetics and thermodynamics of nortestosterone and its methyl derivatives in permeation through silicone membrane were investigated. The permeation parameters involved, such as diffusivity, solubility and permeability, as a function of temperature ranging from 10 to 50°C, were carefully studied as well. An unusual deviation was observed in the Arrhenius plot of the temperature dependence of diffusivity and solubility, which could be explained as the result of a conformation change at temperature beyond 30°C. It was postulated that the removal of 19-methyl group would increase the flexibility of steroidal structure and susceptibility to temperature, leading to the increase in diffusivity and exothermic reactions in solution processes as temperature is raised above 30°C. Hence, an enhancement in the overall rate of membrane permeation was observed.

Introduction

The permeation characteristics of steroids has been extensively studied (Kincl et al., 1968; Sundaram et al., 1978; Kratochvil et al., 1970; Garrett et al., 1968; Most et al., 1972; Roseman et al., 1972; Lacey et al., 1974). The modification of polymer-

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backbone in the silicone elastomers has been explored as well (Friedman et al., 1970). It was shown that the effect of different substituents along the polydimethylsiloxane-polymer backbone upon the permeation of specific steroids is substantial. The marked effect of the variation in molecular structure on the diffusivity of steroids in silicone elastomers stimulated the development of theories and methods.

However, a literature search indicated that no research has been systematically conducted to study the temperature effect on various permeation parameters, i.e. diffusivity and partitioning, of nortestosterone and its methyl derivatives through polydimethylsiloxane membrane. In this study, the effect of temperature on the transport characteristics of nortestosterone and its methyl derivatives, testosterone and methyltestosterone, was studied. The steroids used in the study were reported to be thermally stable (Görög and Szász, 1978). Nortestosterone, with the removal of the methyl group on position 19 in the testosterone structure, has been stated to have a stronger anabolic activity with less virilizing action than testosterone (Applezweig, 1963). Nevertheless, the flexibility of the ring conformation was also found to be enhanced (Carey and Sundberg, 1977). On the other hand, methyltestosterone, in which the oxidation of 17-hydroxy group is prevented by the incorporation of a methyl group at position 17, has been known to be orally effective and is most lipophilic among the steroids in this investigation (Görög and Szász, 1978).

Experimental

Materials

Nortestosterone, testosterone, methyltestosterone (all Sigma Chemicals, St. Louis, MO), polyethylene glycol 400 (Fisher Scientific, Fairlawn, NJ), silicone membranes and silicone fluid 360 (both Silastic Sheetings (HH113930), Silicone Fluid 360 (HH064645), Dow Corning, Midland, MI) were used as received.

Determination of solubility

The solubility of nortestosterone and its methyl derivatives in various aqueous PEG 400 solutions and in silicone fluid was determined by equilibrating an excess amount of steroids in each medium at a specific temperature with constant shaking for 24 h. The solution was withdrawn from each medium using a pre-heated syringe equipped with a 0.45 μm filter. The steroid concentration in the filtrate was determined by UV spectrophotometry (Perkin Elmer, 559A UV/vis Spectrophotometer, Perkin-Elmer, Elmwood Park, NJ) from the peak absorbance at 245 nm.

Permeation through silicone membrane

For studying the permeation of nortestosterone and its methyl derivatives, the well-calibrated Ghannam-Chien membrane permeation system was used (Tojo et al., 1984). A suspension of steroid in 40% aqueous PEG 400 solution was added into donor compartment while the same aqueous medium without the steroid was filled into the receptor compartment. At each pre-determined interval, 10 ml of receptor solution was sampled and replaced with the same volume of fresh, steroid-free PEG

solution. The steroid concentration was analyzed by UV spectrophotometry from the peak absorbance at 245 nm.

Diffusivity and time lag

The diffusivity of steroid molecules (D) through silicone membrane with a thickness of h_m can be determined by:

$$D = h_m^2/6t_1$$

where t_1 is the time lag required to reach steady-state permeation.

Results and Discussion

Cosolvency and interfacial partitioning

Cosolvency is one of the approaches commonly used in pharmaceutical area to enhance the solubility of lipophilic drugs in aqueous medium. Use of a cosolvent system to improve the solubility of slightly soluble, relatively non-polar drugs has become increasingly popular for in vitro drug release and in membrane permeation studies to maintain sink conditions (Chien, 1982). It has been proposed that the increase in solubility of a relatively non-polar drug in a cosolvent system is attributed to the reduction of hydrophobic interaction between drug molecules and water (Yalkowsky et al., 1972). Therefore, by using cosolvents to increase the entropy of solution, it is possible to enhance the solubility of non-polar drug molecules exponentially.

The relationship has been found that the total solubility (C_T) is dependent on the volume fraction of a cosolvent (f_x) added in the aqueous solution (Gould et al., 1984)

$$\log C_T = \log C_w + f_x(\log K_{o/w} - \log K_{o/x}) \quad (1)$$

where C_w represents drug solubility in pure water; $K_{o/w}$ and $K_{o/x}$ are the corresponding partition coefficients for the interfacial partitioning between oil and water and between oil and cosolvent, respectively.

If silicone elastomer or silicone fluid is used as the oil phase, Eqn. 1 becomes:

$$\log C_T = \log C_w + f_x(\log K_{s/w} - \log K_{s/x}) \quad (2)$$

where the subscript s denotes the silicone elastomer or silicone fluid.

From Eqn. 2, it becomes clear that the slope (σ) of $\log C_T$ versus f_x plots should be equivalent to: $(\log K_{s/w} - \log K_{s/x})$. The results in Fig. 1 demonstrate that, as expected from Eqn. 2, the aqueous solubility of nortestosterone and its methyl derivatives increases exponentially as the volume fraction of cosolvent increases. It was observed that the slope (σ) values increase in the sequence of nortestosterone < testosterone < methyltestosterone. The physicochemical properties of these anabolic

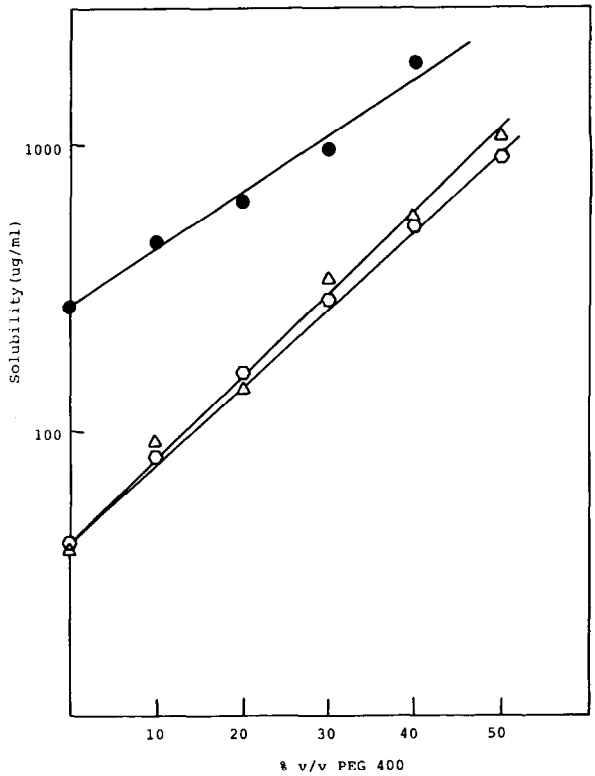


Fig. 1. Dependency of the aqueous solubility of nortestosterone (●), testosterone (○) and methyltestosterone (△) on the volume fraction of polyethylene glycol (PEG) 400, as the cosolvent, at 37°C.

steroids are illustrated in Table 1. The slope values calculated from the relationship of ($\log K_{s/w} - \log K_{s/x}$) are found to be consistent with the results measured from the plot (Table 2). Apparently, the slope of the semi-logarithmic relationship between drug solubility and volume fraction of cosolvent is simply a balance of

TABLE 1

PHYSICOCHEMICAL PROPERTIES OF NORTESTOSTERONE, TESTOSTERONE AND METHYLTESTOSTERONE

Physicochemical properties	Nortestosterone	Testosterone	Methyltestosterone
Solubility (μg/ml) *			
water	288.0	42.8	37.0
silicon fluid	156.0	156.0	238.0
PEG 400	8.18×10^4	1.96×10^4	2.55×10^4
Melting point (°C)	120	165	164

* Measured at 37°C.

TABLE 2

COMPARISON IN SLOPE VALUES BETWEEN EXPERIMENTAL MEASUREMENT AND CALCULATION

	Nortestosterone	Testosterone	Methyltestosterone
log $K_{s/w}$ [a]	−0.266	0.562	0.763
log $K^{1}_{s/x}$	−2.720	−2.099	−2.030
Slope(σ) [b]			
calculated	2.454	2.661	2.793
measured	2.054	2.686	2.858

[a] Calculated from the data in Table 1.
[b] Calculated by Eqn. 4.

distribution between two solvents with different polarities, as measured by log $K_{s/w}$ and log $K_{s/x}$.

The partition coefficient between silicone fluid and water, $K_{s/w}$, is also one of the important parameters in membrane permeation studies, in which the permeability coefficient (P) is directly proportional to the magnitude of $K_{s/w}$. The low σ value (2.054) and negative log $K_{s/w}$ value (−0.266) for nortestosterone at 37°C, in contrast to other analogs, suggests its unusual characteristics. The drastic reduction in the cosolvent effect on the enhancement of nortestosterone solubility, as shown by the low σ value and the high water solubility (288 μg/ml), implies that the nortestosterone molecule has a greater interaction with water than its methyl derivatives. Further, the low melting point of nortestosterone presumes that the removal of the 19-methyl group from the basic steroidal skeleton gives the molecules more freedom to change their conformation, due to the reduction in intermolecular forces.

Thermodynamics of solubility

The temperature dependence of solubility over a temperature range is defined by the following thermodynamic relationship:

$$\log C = \log C_0 + (-\Delta H/2.303RT) \quad (3)$$

where C is the saturation solubility in equilibrium at absolute temperature T; ΔH is the heat of solution; C_0 and R are constants. The equation above indicates an exponential relationship between equilibrium solubility and the reciprocal of absolute temperature.

As can been seen from Fig. 2, the solution process of testosterone and methyltestosterone in either 40% PEG 400 solution or silicone fluid is an endothermic reaction, which implies little solute–solvent interaction. However, the solution process of nortestosterone is composed of two stages: endothermic reaction in the low temperature range (10–30°C) and exothermic reaction at higher temperature (37–50°C). This difference in solution behavior as a function of temperature can be

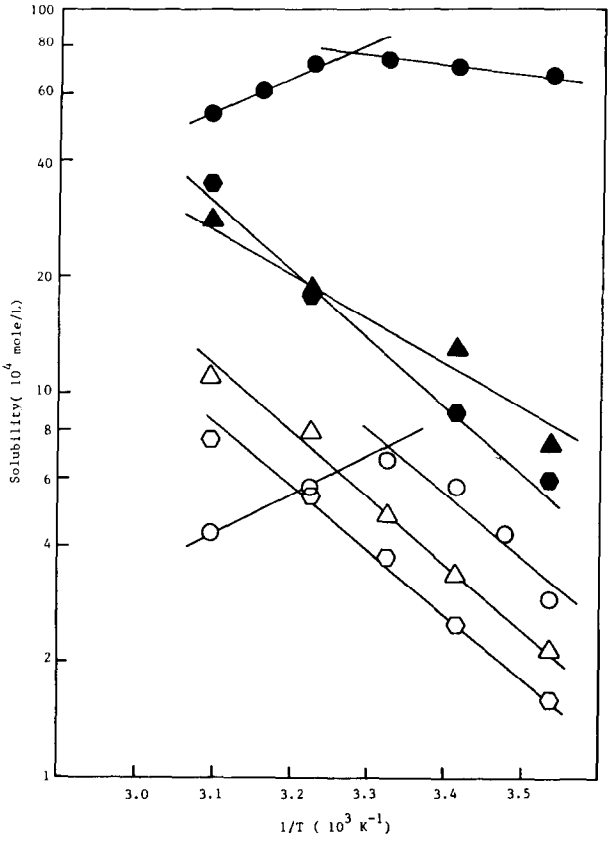


Fig. 2. Semi-logarithmic relationship between saturation solubility in 40% aqueous PEG 400 solution or in silicone fluid and the reciprocal of the absolute temperature as expected from Eqn. 5. Key: (1) 40% PEG 400-nortestosterone (●), testosterone (⬢) and methyltestosterone (▲); (2) silicone fluid-nortestosterone (○), testosterone (⬡) and methyltestosterone (△).

considered as a result of the conformation effect. It is speculated that the structure of nortestosterone becomes more flexible as the temperature rises, thus the interaction between solute and solvent increases. Hence, when sufficient interactions occur between solute and solvent molecules, a quantity of heat, which is in excess of that required to overcome the molecular forces for the solute–solute and solvent–solvent interactions, will be released, i.e. an exothermic process. The absence of the 19-methyl group in the nortestosterone molecule permits the molecular conformation to change in response to the variation in temperature, giving rise to unusual properties. The change in molecular conformation with temperature will be discussed in more detail in the next section.

Thermodynamics of diffusion and permeation

The diffusivity (D) of nortestosterone and its methyl derivatives through the silicone membrane was measured at various temperatures and the relationship was

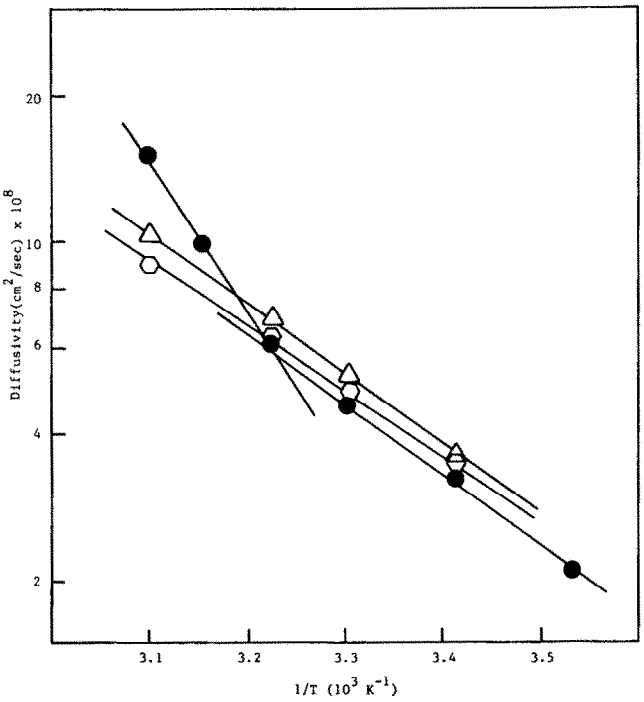


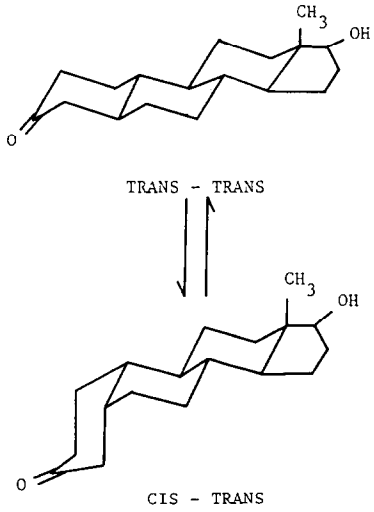
Fig. 3. Arrhenius plot for the diffusivity of nortestosterone (●), testosterone (○) and methyltestosterone (△).

then plotted from the Arrhenius equation in Fig. 3. A linear relationship was observed except for nortestosterone. It was found that the temperature dependency of nortestosterone diffusivity shows a shift at around 30°C, i.e. the diffusivity is more sensitive to temperature variation in the range above 30°C. The breakpoint temperature is very close to the inflection temperature for the solubility profile for nortestosterone in both silicone fluid and 40% aqueous PEG 400 solution (Fig. 2). The change in the energy of activation required for the diffusion of nortestosterone molecules through the polymer membrane, as indicated by the change in the slope values, could imply an alternation of molecular conformation. It was postulated that the absence of the 19-methyl group increases the flexibility of the trans–trans conformation, and, as the temperature rises to a level high enough, it shifts to the cis–trans conformation (Scheme I), which is a more temperature-sensitive dimension (Carey and Sundberg, 1977). Thus, the diffusivity value increases faster as temperature rises.

On the other hand, the permeability (P), which is defined as:

$$P = D \cdot K \tag{4}$$

where K is the partition coefficient for the interfacial partitioning between silicone membrane and aqueous PEG solution, does not show a break in the Arrhenius plot



Scheme I.

for nortestosterone (Fig. 4). A constant temperature dependency observed for the membrane permeability of nortestosterone in 10–50°C range is rather different from that obtained in the diffusivity plot (Fig. 3) as well as the solution process in either silicone fluid or aqueous PEG medium for nortestosterone in the same temperature range. This difference in temperature dependence can be explained as follows.

From Eqn. 4,

$$P = D \cdot C_p/C_s \tag{5}$$

where C_p and C_s represent drug solubility in polymer membrane and elution medium. Thus,

$$\log P = \log D + \log C_p - \log C_s \tag{6}$$

The energy relationship is obtained by using the Arrhenius equation (Chien, 1982) and is expressed as:

$$\Delta H_p = \Delta E_D + \Delta H_{C_p} - \Delta H_{C_s} \tag{7}$$

where ΔH_p, ΔE_D, ΔH_{Cp}, ΔH_{Cs} are the energy required for permeation, diffusion, solution in silicone membrane and aqueous medium, respectively.

If the solubility in silicone membrane (C_p) is substituted by the solubility in silicone fluid (C_p'), which is polydimethylsiloxane of low molecular weight, Eqn. 7 becomes:

$$\Delta H_p = \Delta E_D + \Delta H_{C_p'} - \Delta H_{C_s} - A \tag{8}$$

in which $\Delta H_{C_p'}$ is heat of solution in silicone fluid and A is a constant accounting

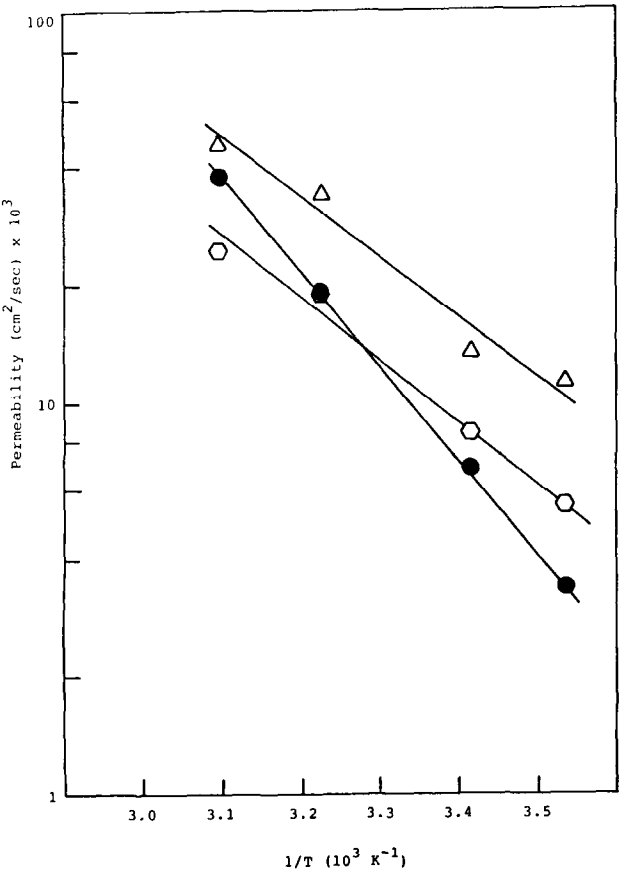


Fig. 4. Arrhenius plot for the permeability of nortestosterone (●), testosterone (○) and methyltestosterone (△).

for the possible difference in the energy requirement between solution in polymer membrane and in silicone fluid.

The result (Table 3) indicates that the energy required for diffusion is almost the same for these steroidal compounds in the temperature range of 10–30°C whereas the diffusion energy (ΔE_D) of nortestosterone is much higher in the temperature range of 30–50°C. Furthermore, the energy for permeation(ΔH_p) of these steroids calculated from the energy requirements for diffusion(ΔE_D) and solution(ΔH_{C_p}, ΔH_{C_s}) is consistent with the value determined directly from the permeability plot. Apparently, the exothermic reaction from the dissolution of nortestosterone in the temperature range greater than around 30°C enhances the diffusion process of nortestosterone in the silicone elastomers.

The data in Fig. 4 compare the temperature dependence for the membrane permeation of nortestosterone with its methyl derivatives in the temperature range of 10–50°C. From the slope values, it appears that the membrane permeation of

TABLE 3

ENERGY REQUIREMENT FOR MEMBRANE PERMEATION OF NORTESTOSTERONE AND ITS METHYL DERIVATIVES

Energy required (kcal/mol)	Nortestosterone			Testosterone	Methyltestosterone
	10–30°C	30–50°C	10–50°C	10–50°C	10–50°C
ΔE_D	6.06	14.41	–	6.08	6.00
ΔH_{C_p}	10.67	−3.85	–	7.28	7.64
ΔH_{C_s}	1.66	−4.56	–	7.05	6.73
A *	4.15	4.15	–	–	–
ΔH_p calculated	10.92	10.97	–	6.31	6.91
measured	10.95	10.95	10.95	6.80	6.70

* Calculated from Eqn. 8.

nortestosterone is more sensitive to temperature than its methyl derivatives. The energy requirement for membrane permeation listed in Table 3 demonstrates that both testosterone and methyltestosterone have a similar energy requirement for permeation (6.8, 6.6 kcal); however, nortestosterone demands more energy (10.95 kcal) for permeation across the silicone membrane.

References

Applezweig, N., Steroid Drugs, McGraw-Hill, New York, 1963, p. 93.

Carey, F.A. and Sundberg, R.J., Advanced Organic Chemistry, Plenum, New York, 1977, p. 91.

Chien, Y.W., Novel Drug Delivery Systems, Marcel Dekker, New York, 1982, Ch. 9.

Friedman, S., Koide, S.S. and Kincl, F.A., Sustained release hormonal preparations. VII. Permeability of three types of silicone rubber to steroids. Steroids, 15 (1970) 679.

Garrett, E.R. and Chemburkar, P.B., Evaluation, control and prediction of drug diffusion through polymeric membranes. I. Methods and reproducibility of steady state diffusion studies. II. Diffusion of aminophenones through Silastic membranes. J. Pharm. Sci., 57 (1968) 944.

Görög, S. and Szász, G.Y., Analysis of Steroid Hormone Drugs, Elsevier, New York, 1978, p. 49.

Gould P.L., Goodman,, M. and Hanson, P.A., Investigation of the solubility relationships of polar, semi-polar and non-polar drugs in mixed co-solvent systems. Int. J. Pharm., 19 (1984) 149.

Kincl, F.A., Benagiano, G. and Angree, I., Sustained release hormonal preparations. I. Diffusion of various steroids through polymer membranes. Steroids, 11 (1968) 673.

Kratochvil, P., Benagiano, G. and Kincl, F.A., Sustained release hormonal preparations. VI. Permeability constants of various steroids. Steroids, 15 (1970) 505.

Lacey, R.E. and Cowsar, D.R., Factors affecting the release of steroids from silicones. In Controlled Release of Biologically Active Agents, Plenum Press, New York, 1974, Ch. 5.

Most, C.F., Jr., Copermeant enhancement of drug transmission rates through silicone rubber. J. Biomed. Mater. Res., 6 (1972) 3.

Roseman, T.J., Release of steroids from a silicon polymer. J. Pharm. Sci., 61 (1972) 46.

Sundaram, K. and Kincl, F.A., Sustained release hormonal preparations. II. Factors controlling the diffusion of steroids through dimethyl polysiloxane membranes. Steroids, 12 (1968) 517.

Tojo, K., Sun, Y., Ghannam, M. and Chien, Y.W., Characterization of a membrane permeation system for controlled-drug delivery studies, AIChE, May, 1984, in press.

Yalkowsky, S.H., Flynn, G.L. and Amidon, G.L., Solubility of nonelectrolytes in polar solvent. J. Pharm. Sci., 61 (1972) 983.

Yalkowsky, S.H. and Roseman, T.J., In Yalkowsky, S.H. (Ed.), Techniques of solubilization of Drugs, Marcel Dekker, New York, 1981, p. 91.