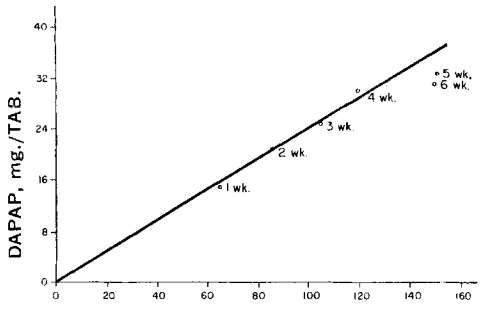

Fig. 3—Product A stored at 50°.

tions (11, 12). Magnesium stearate also has been shown to accelerate the formation of acetylphenylephrine in aspirin–phenylephrine combinations (1). Consequently, the relative effects of water, stearic acid, and magnesium stearate on the rate of formation of SA and DAPAP in aspirin–APAP mixtures were studied. Powder mixtures containing (*a*) 3.25 Gm. aspirin, 1.75 Gm. APAP, and 0.05 Gm. water; (*b*) mixture (*a*) plus 0.05 Gm. stearic acid; and (*c*) mixture (*a*) plus 0.05 Gm. magnesium stearate were sealed in small bottles and stored for 4 weeks at 50°. Subsequent DAPAP analysis of these mixtures indicated 67 mcg./Gm. in (*a*), 50 mcg./Gm. in (*b*), and 48.4 mg./Gm. in (*c*). Thus, magnesium stearate had a marked accelerating effect on the rate of DAPAP formation. Consequently, this lubricant is contraindicated in such formulations.

The pharmacological significance of the presence of DAPAP in these products is not known since information in the literature concerning this compound is scanty. Stockelbach (13) disclosed in a U. S. patent that DAPAP has valuable medicinal properties particularly as an antipyretic, antineuralgic, and sedative remedy. Charlier *et al.* (14) list DAPAP as one of the compounds studied for influence on bile secretion and toxicity, but did not give any data for DAPAP.

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Micellar Solubilization of Testosterone I

In Aqueous Solutions of Polysorbates

By ARVIND L. THAKKAR and NATHAN A. HALL

The solubility of testosterone in aqueous solutions of partially purified polysorbate 20, 40, and 60 was determined by measuring the area under the ultraviolet spectral peaks. There was solubilization below the critical micelle concentration. A linear relationship was observed between the amount of testosterone solubilized and the per cent polysorbate concentration. Polysorbate 60 exhibited the greatest solubilizing capacity and polysorbate 20 the least. The *Z*-value method for determining solvent polarity was employed to investigate the polarity of the environment in which testosterone was solubilized. At low polysorbate concentrations the *Z*-value was similar to that in water. As the polysorbate concentration increased, the *Z*-value decreased. This decrease in *Z*-value paralleled the increase in solubility of testosterone.

RECENT REVIEWS (1, 2) in pharmaceutical literature indicate that the phenomenon of solubilization continues to be of interest. In 1949 Ekwall and Sjöblom (3) published the first study on the solubilization of steroid hormones. They reported the solubility of testosterone in a 10% sodium oleate, 20% sodium myristyl sulfate, and 20% sodium cholate solutions. Nakagawa (4) has measured the solubility of testosterone in 20% aqueous solution of polyoxyethylene sorbitan monolaurate containing about 12 ethyleneoxide groups. Sjöblom and co-workers (5–7), in their

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work with estrogens and corticosteroids, have made extensive investigations of the effect upon solubilization of modifications in the structure of the steroid solubilizate.

Although previous reports on micellar solubilization of steroids have presented information about how the nature of the solubilizate may affect solubilization, little detailed data are available on the effect of the nature of the solubilizer. As part of a study of the effect of different surfactants upon the solubilization of a single steroid, this paper deals with the study of testosterone solubilized in some nonionic surfactants of the polyoxyethylene sorbitan ester series.

It is well known that the electronic absorption spectrum of a substance is appreciably altered when the substance is in the micellarly solubilized form. Riegelman *et al.* (8) have drawn certain qualitative conclusions about the location of molecules solubilized in micellar solutions based upon the alterations in their absorption spectra. Bjaastad and Hall (9) have recently demonstrated the use of the *Z*-value method, an empirical means of solvent polarity developed by Kosower (10), for investigating the polarity of the environment of some solubilized ketones. This method is based upon the assumption that the polarity of the layers of solvent immediately surrounding a molecule or ion will differ appreciably from that of the bulk solution. Kosower has defined *Z*-value as the energy of electronic transition of 1-ethyl-4-carbomethoxy pyridinium iodide, a substance with an ultraviolet spectrum remarkably sensitive to solvent polarity. Application of this method involves determining the spectral characteristics of the solubilizate in a number of solvents of known *Z*-values and establishing a relationship between *Z*-value and the energy of transition which is readily obtained from the wavelength of maximum absorbance. From this relationship the *Z*-value of a solvent or a medium such as a surfactant solution may be derived once the wavelength of maximum absorbance of the solubilizate in the particular solvent or medium is known. Bjaastad and Hall have pointed out the assumptions which must be made in order to apply the *Z*-value method to solubilized systems.

Testosterone is a steroid of relatively simple structure. The ultraviolet absorption spectrum of this α,β-unsaturated ketone is solvent sensitive. The polarity of the environment of testosterone solubilized in aqueous solutions of polysorbates was investigated by the *Z*-value method and is reported in this paper.

EXPERIMENTAL

Materials—Three nonionic surfactants, having the same hydrophilic portion in their molecules but differing in the length of the carbon atom chain of their lipophilic portion, were selected. These were polysorbate 20, polyoxyethylene-20-sorbitan monolaurate; polysorbate 40, polyoxyethylene-20-sorbitan monopalmitate; and polysorbate 60, polyoxyethylene-20-sorbitan monostearate.[1] The commercial samples of the three nonionic agents were partially purified by partitioning between 5 *N* sodium chloride solution and ethyl acetate by the method of Weibull (11). Samples of partially purified polysorbates were dried over anhydrous sodium sulfate, placed in a 60° oven for 24 hr. to remove traces of any organic solvent, and stored in a desiccator until used. Removal of polyethylene and polyoxyethylene glycols was followed by a thin-layer chromatographic procedure (12). Solutions of polysorbates were prepared using water redistilled in the presence of alkaline permanganate from an all-glass distilling apparatus. The critical micelle concentrations of these partially purified surfactants, determined in this laboratory and elsewhere, with benzopurpurin 4B (13), have been reported as 1.0×10^{-2}, 5.5×10^{-3}, and 4.6×10^{-3} Gm./100 ml., respectively (14, 15).

Testosterone N.F.[2] was used as received. Solvents used where of reagent grade.

Solubility Determinations—To a series of screw cap vials containing 10 ml. of polysorbate solutions of known concentration, an excess of testosterone was added. The caps (lined with aluminum foil) were placed on the vials which were then rotated in a water bath[3] at 25.0° ± 0.1° for 96 hr. Although the solutions showed an initial supersaturation, all were found to attain solution equilibrium within the 96-hr. period. The contents of the vials were filtered through a Swinney filter adapter containing a 0.45 μ Millipore filter disk.[4] The ultraviolet absorption spectra were recorded with a Cary model 14 automatic recording spectrophotometer. Matched cylindrical silica absorption cells, 1 and 10 mm. light path, were used. For the determination of the concentration, ultraviolet spectra were recorded at 1 mμ/sec., from 350 mμ to 210 mμ, and the area under the spectral peak determined by multiplying the height of the peak by its width at one-half the height. The area under the peak was found to give a direct relationship to the concentration of testosterone. The observed solubility in each solution (S) was divided by the solubility in water (S_0) to give the relative solubility in the surfactant concentration.

Z-Value Determinations—In order to relate the ultraviolet absorbance of testosterone to the polarity of the solvent, the wavelength of maximum absorbance ($\lambda_{max.}$) of testosterone was determined in a number of solvents of known *Z*-values. The $\lambda_{max.}$ was first estimated from spectra scanned at 1 mμ/sec., then accurate determinations of $\lambda_{max.}$ were made by recording the spectra at 0.25 mμ/sec. for 20 mμ in the vicinity of the maxima. The reported

[1] Marketed as Tween 20, Tween 40, and Tween 60, respectively, by Atlas Chemical Industries, Inc., Wilmington, Del.

[2] Generously supplied by Schering Corp., Bloomfield, N. J.

[3] Gyrotory water bath shaker, model G76, New Brunswick Scientific Co., Inc., New Brunswick, N. J.

[4] While this work was in progress, Cahn (16) reported the presence of surfactants in Millipore filters. The experimental procedures used in this work were checked to assess the effect of the surfactant contributed by the filter. Neither the quantity of testosterone solubilized nor its ultraviolet absorption spectrum was affected by the quantity of surfactant leached from the filter disk.

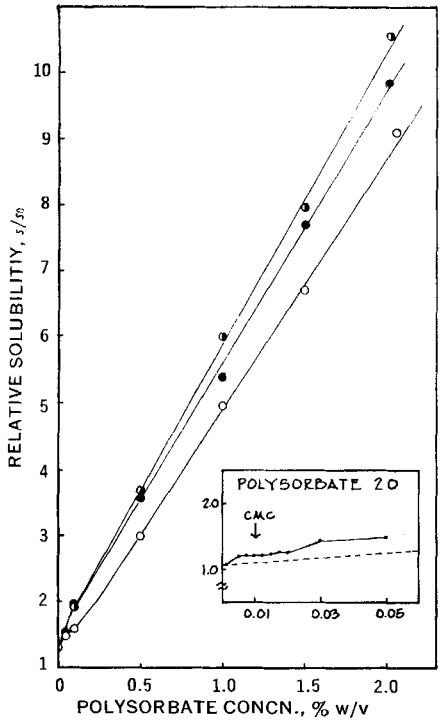

Fig. 1—Relative solubility of testosterone versus *concentration of polysorbate 20* (○), *polysorbate 40* (●), *and polysorbate 60* (◑). *Inset: polysorbate 20 solutions at low surfactant concentrations. Dashed line indicates an extrapolation of the linear portion of the larger graph.*

values of $\lambda_{max.}$ were obtained from an average of three scans and were precise to ± 0.5 mμ.

Spectral measurements for determination of $\lambda_{max.}$ of solubilized testosterone were made using undiluted solutions. In some cases, however, the saturated solutions were too concentrated to permit spectral measurements even with the cells of the shorter light path length of 1 mm. It was necessary in these cases to dilute the saturated solutions with the appropriate surfactant solution. Dilution was kept to a minimum, however. The effect of testosterone concentration upon $\lambda_{max.}$ and Z-value was investigated in some detail for solutions containing polysorbate 20.

RESULTS AND DISCUSSION

Solubility Determinations—The relationship between relative solubility of testosterone and polysorbate concentrations is shown in Fig. 1. After the initial stages of solubilization, the plots are linear, indicating that the amount of testosterone solubilized is directly proportional to polysorbate concentration. Similar observations for other steroids have been made by Sjöblom (5–7) and by Guttman *et al.* (17). The values of the slopes of the linear portions of the curves multiplied by the aqueous solubility of testosterone, in mg./100 ml., gave the solubilizing capacities of polysorbates in terms of mg. of testosterone per Gm. of polysorbate. Table I lists the solubilizing capacities on both a weight and molar basis. Since polysorbates are heterogeneous, the limitation of the significance of the molar values should be borne in mind. The results (Table I and Fig. 1) show that polysorbate 60 has the greatest capacity and polysorbate 20 the least.

The order of solubilizing capacities of the three polysorbates can be directly related to the length of their alkyl portions. In an idealized picture of spherical micelles these alkyl portions may be visualized as being directed inward. An increase in their length would result in micelles of larger size; thus polysorbate 60 micelles would have the largest relative size. It is interesting to note that the solubilizing capacities of the three polysorbates are of similar order of magnitude.

TABLE I—SOLUBILIZING CAPACITY OF POLYSORBATES FOR TESTOSTERONE AT 25°

Surfactant	Solubility: mg. Testosterone/ Gm. Surfactant	Solubility: mole Testosterone/ mole Surfactant[a]
Polysorbate 20	8.29	3.529×10^{-2}
Polysorbate 40	8.95	3.984×10^{-2}
Polysorbate 60	9.64	4.385×10^{-2}

[a] Calculated from the theoretical molecular weights of 1228, 1284, and 1312 for polysorbate 20, 40, and 60, respectively.

TABLE II—SPECTRAL PROPERTIES OF TESTOSTERONE IN SOLVENTS OF VARYING POLARITY

Solvent	Z (9)	$\lambda_{max.}$, mμ ± 0.5 mμ
Water, distilled	94.6	2490
80% Methanol (v/v)	87.1	2448
70% Ethanol	84.5	2445
90% Methanol	85.5	2435
80% Ethanol	84.8	2435
95% Methanol	84.5	2428
90% Ethanol	82.5	2425
95% Ethanol	81.2	2420
Methanol	83.6	2415
Ethanol	79.6	2410
Isopropanol	76.6	2408
Acetonitrile	71.3	2383
Diethylether, water saturated	66.1	2338
n-Hexane	60.4	2313

TABLE III—EFFECT OF TESTOSTERONE CONCENTRATION UPON $\lambda_{max.}$ AND Z-VALUES IN SOLUTIONS OF POLYSORBATE 20

Polysorbate 20 Concn., %	Testosterone % Saturation	Testosterone Concn., mg./100 ml.	$\lambda_{max.}$, mμ	Z
0.01	40	1.15	2490	95.17
	20	0.57	2490	95.17
0.05	40	1.27	2489	94.97
	20	0.64	2486	94.45
0.10	100	3.41	2476	92.57
	20	0.68	2482	93.65
	10	0.34	2483	93.89
0.50	100	6.45	2442	86.13
	50	3.23	2437	85.17
	20	1.29	2437	85.17
	10	0.65	2437	85.17
1.00	100	10.75	2429	83.65
	50	5.38	2426	83.05
	20	2.15	2422	82.29
	10	1.98	2427	83.25

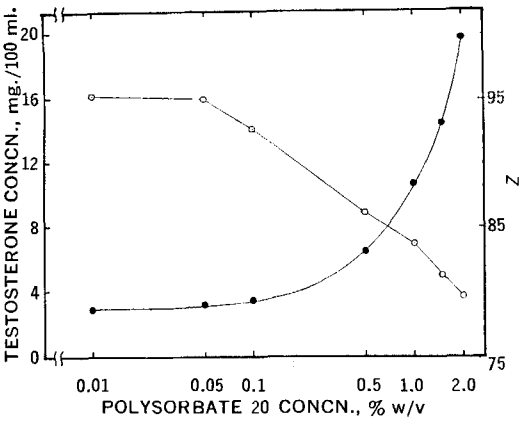

Fig. 2—The relationship between concentration of polysorbate 20 (logarithm), solubility of testosterone (●), and Z-value (○).

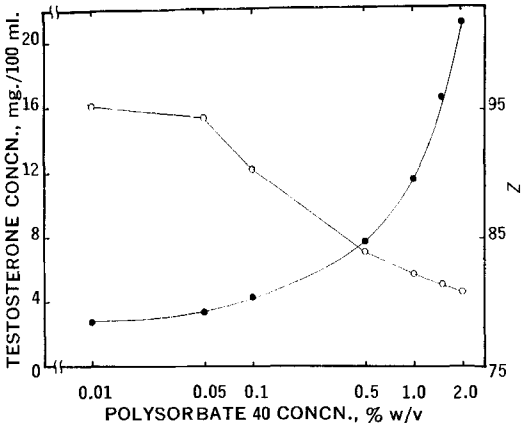

Fig. 3—The relationship between concentration of polysorbate 40 (logarithm), solubility of testosterone (●), and Z-value (○).

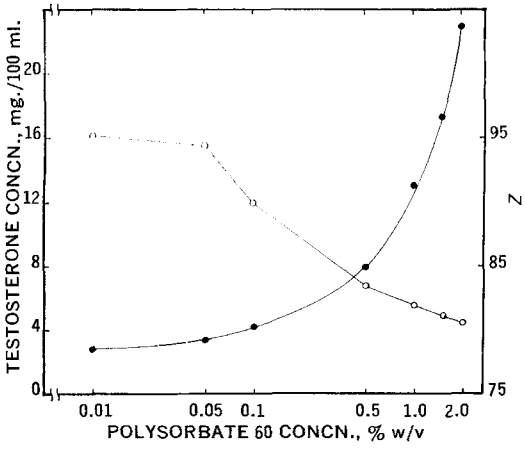

Fig. 4—The relationship between concentration of polysorbate 60 (logarithm), solubility of testosterone (●), and Z-value (○).

In general, solubilization is associated with the presence of micelles, there being little or no solubilization below the CMC. In the present study it was found that testosterone was solubilized below the CMC. This aspect was investigated in some detail with solutions of polysorbate 20 and is illustrated in the inset of Fig. 1. Moilliet *et al.* (18) have suggested that solubilization below the CMC may be explained by the formation of mixed micelles below the usual CMC due to an affinity between the surfactant and the solubilizate. In case of polysorbate 20 and testosterone such an explanation seems plausible.

Z-Value Determinations—The wavelengths of maximum absorbance of testosterone in a wide range of solvents are given in Table II.

The energy of electronic transition for testosterone was calculated from the relationship (19):

$$E_t = 2.859 \times 10^4/\lambda_{max.} \text{ in m}\mu \text{ (Kcal./mole)}$$

When a plot of Z *versus* E_t was made, a straight line with a negative slope was obtained. A negative slope indicates a $\pi \rightarrow \pi^*$ electronic transition (20). By the method of least squares, the slope and intercept were calculated and the following equation obtained for the straight line:

$$Z = -4.00\, E_t + 554.45$$

The Z-values of surfactant solutions were calculated from this equation. Since it was necessary to dilute some of the surfactant solutions saturated with testosterone, the effect of testosterone concentration upon $\lambda_{max.}$ and Z-values was investigated for solutions of polysorbate 20. This is shown in Table III.

It may be seen that any changes in $\lambda_{max.}$ due to dilution are within ± 0.5 mμ, the precision of the method. The corresponding changes in Z should not be significant. This is in agreement with previous observations made with solubilized ketones (9, 14).

Figures 2, 3, and 4 show the relationships among the concentration of polysorbate, solubility of testosterone, and Z-value. At 0.01% polysorbate concentration the Z-value is the same as that of water, and the solubility of testosterone is not appreciably greater than that in water. This concentration is approximately the CMC for polysorbate 20, and for polysorbate 40 and 60 it is higher than the CMC. With increasing polysorbate concentrations and consequent increase in testosterone solubility, the Z-value decreases, indicating an environment of decreasing polarity. The behavior is similar for the three polysorbates.

As Bjaastad and Hall (9) have pointed out, decreasing polarity might be explained by an increase in concentration and organization of micelles as the concentration of surfactant is increased. It may also be due to the solubilized testosterone encountering less polar regions of micellar interior. Guttman *et al.* (17) have suggested that the steroids, prednisolone, methylprednisolone, and fluoromethylone, are associated, by hydrogen bonding, with the polyethylene chain of the nonionic surfactant oxyethylated tertiary octylphenol formaldehyde polymer.[5] Testosterone with its hydroxyl group at C-17 should be capable of association with the ethylene oxide portion on the surface of polysorbate micelles. This would account for the high

[5] Triton WR-1339, Rohm and Haas, Philadelphia, Pa.

Z-value of low concentration polysorbate solutions in which one may expect testosterone molecules to be oriented in the hydrated polyoxyethylene surface of the micelles. The fact that the ethylene oxide chain lengths of the three polysorbates are identical and the observation that the values of testosterone solubility are nearly the same in low concentrations of all the three polysorbates support this view. The decrease in Z-value possibly results from multilayer adsorption. This proposal is substantiated by similar order of testosterone solubilization in higher polysorbate concentrations. If solubilization involved incorporation of testosterone within micellar interior, one would expect a more pronounced difference in the solubilities with a change in lipophilic moiety.

The magnitude of Z-values in 2% polysorbate solutions are comparable to those of ethanol and isopropanol, which also indicates that the environment of solubilized testosterone is quite polar. Although the hydrocarbon interior of micelles is believed to contain some water (21), its polarity might be expected to be lower than those of the alcohols.

Further studies utilizing ionic surfactants with comparable alkyl chain lengths are being conducted and will be reported in a future communication.

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Steric Aspects of Adrenergic Drugs VII

Certain Pharmacological Actions of D(−)-Pseudoephedrine

By J. B. LAPIDUS, ARTHUR TYE, and P. N. PATIL

The ability of D(−)-pseudoephedrine to influence the actions of selected adrenergic drugs was examined in both *in vivo* and *in vitro* experiments. In anesthetized dogs, pretreatment with D(−)-pseudoephedrine can reduce or block the pressor effects of the other ephedrine isomers and (+)-amphetamine. Delayed potentiation was observed with norepinephrine and epinephrine. D(−)-Pseudoephedrine did not affect the pressor effects resulting from bilateral carotid occlusion, and exhibited no unique properties on the rabbit aortic strip. D(−)-Pseudoephedrine had no intrinsic activity on the isolated rat vas deferens but could block the contractions caused by tyramine or D(−)-ephedrine, and potentiate the effects of norepinephrine. D(−)-Pseudoephedrine and D(−)-ephedrine compete for α-adrenergic sites in the reserpine pretreated rat vas deferens and can protect these receptors from dibenamine blockade. It appears that D(−)-pseudoephedrine acts at both catecholamine uptake sites and at α-receptors, but apparently lacks intrinsic effects at the latter site.

In previous papers (1, 2), the authors have presented evidence that D(−)-pseudoephedrine has a unique vascular activity when compared with other isomers of ephedrine. It has virtually no pressor activity (2), yet is a diastereoisomer of D-(−)-ephedrine, the most active pressor member of the four ephedrine isomers. Certain stereochemical consequences of this relationship led to the belief that D(−)-pseudoephedrine should, in fact, be able to antagonize the pressor response to D(−)-ephedrine (1). D(−)-Ephedrine and D(−)-pseudoephedrine have the same absolute configuration at the β-carbon atom (that bearing the hydroxyl group) (Fig. 1). If the assumption is made that the amino group, the hydroxyl group, and the phenyl ring are all involved in the drug–receptor interaction (3), then these two diastereoisomers represent a

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