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Solubilization of hydrocortisone, dexamethasone, testosterone and progesterone by long-chain polyoxyethylene surfactants

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Solubility and dialysis methods were used to study the solubilization of hydrocortisone, dexamethasone, testosterone and progesterone in aqueous long-chain polyoxyethylene non-ionic surfactant solutions. Partition coefficients, K_m, between micellar and aqueous phases were calculated between 10–50°. K_m decreased with temperature and polyoxyethylene chain length but increased with decrease in steroid polarity. The standard free energy change, ΔG_s^o, for the solubilization of the steroids decreased with decrease in steroid polarity and surfactant hydrophilic chain length but was essentially independent of temperature. The enthalpies and entropies for the process were determined from the variation of K_m with temperature. ΔH_s^o and ΔS_s^o increased with decreasing steroid polarity but were essentially independent of temperature and polyoxyethylene chain length.

Solubilization enhances the solubility of sparingly soluble compounds and indications that surfactants may modify the bioavailability of drugs (Gibaldi & Feldman, 1970) have increased the need for further knowledge. Although solubilization has been extensively investigated (e.g. Mulley, 1964; Swarbrick, 1965; Elworthy, Florence & Macfarlane, 1968), there are few reports on the thermodynamics of the process (Simons & Rhodes, 1971).

In this work, solubilization of hydrocortisone, dexamethasone, testosterone and progesterone in aqueous solutions of long-chain polyoxyethylene non-ionic surfactants was studied and thermodynamic parameters were derived. The aim was to relate the nature of medicinally important solubilizates, expressed by their polarities, to their uptake into micelles formed by pharmaceutical surfactants with various hydrophilic chain lengths, expressed by changes in free energy, enthalpy and entropy. This paper extends work reported at the B.P. Conference 1974 (Barry & El Eini, 1974).

MATERIALS AND METHODS

Surfactants. Well characterized samples of polyoxyethylated cetyl alcohols (Glovers, Ltd., Leeds, U.K.) containing 17, 32, 44 and 63 mol of ethylene oxide (Barry & El Eini, 1976) were used.

Steroids. Hydrocortisone, progesterone and testosterone from E. Merck, Dermstadt, W. Germany; dexamethasone from Sigma Chemicals, St. Louis, U.S.A.

Solubility measurements. Solubilities were determined by stirring excess of solid steroid in solvent and assaying resultant solutions at equilibrium (after 10 days) at a given temperature ($\pm$ 0·1°). Samples, filtered through two washed 0·22 nm Millipore membrane filters, were suitably diluted and assayed spectrophotometrically.

Dialysis studies were made at 25° $\pm$ 0·1° using Perspex cells similar to those of Patel & Foss (1964), with compartment volumes of 24 ml and partition areas of

15·91 cm². A cellulose acetate membrane, 0·091 mm wet thickness, (Scientific Instrument Centre Ltd., London) was used and compartments were stirred with Teflon coated magnetic bars rotated by immersible stirrers. Cellulose acetate is permeable to steroids but not to the surfactants (Short, 1971). Equilibration time varied between steroids but 10 days was sufficient for all systems. At equilibrium samples from each compartment were suitably diluted and assayed spectrophotometrically.

Partitioning studies. Partition coefficients between diethyl ether and water were determined similarly to Flynn (1971).

Thin layer chromatography. R_F values were determined by reverse-phase thin-layer chromatography, with fluorescent silica gel (Kieselgel GF_{254} nach Stahl Type 60, Merck, Dermstadt, Germany) as solid support. Activated plates were impregnated with n-octanol as stationary phase by running a 5% v/v solution in n-heptane to the top and evaporating at 40°. 30% v/v aqueous acetone (mobile phase) achieved the best steroid separation. Spots were located under ultraviolet light (254 nm).

RESULTS AND DISCUSSION

Aqueous solubilities of steroids at different temperatures are in Table 1. Differences in solubility were described in terms of steroid molecular structure (Kabasakalien, Britt & Yudis, 1966). The qualitative polarities of the steroids may be deduced from

Table 1. *Aqueous solubilities of steroids at various temperatures and their R_m values.*

Steroid	R_m	Solubility mol litre^{-1} × 10^4 at					
		10°	20°	25°	30°	40°	50°
Hydrocortisone	0·27	4·78	7·43	8·82	10·34	12·65	15·19
Dexamethasone	0·48	0·82	1·58	2·27	2·52	3·56	4·60
Testosterone	1·04	0·56	0·79	0·81	1·06	1·40	2·10
Progesterone	1·46	0·17	0·22	0·28	0·36	0·38	0·49

the aqueous solubility results indicating a decreasing order of hydrocortisone, dexamethasone, testosterone and progesterone. However, a more useful quantitative expression of a molecule's polarity is its R_m value (Bates-Smith & Westall, 1950):

$$R_m = \log\left(\frac{1}{R_F} - 1\right) \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where R_F is obtained from chromatography. R_m values (Table 1) confirm the relative polarities indicated by aqueous solubility results.

For all surfactant–steroid systems, amount of drug solubilized (mol litre^{-1}) was linearly related to surfactant concentration (% w/w), typically represented by the dexamethasone–surfactant system in Fig. 1. The y-axis intercept equalled the steroid's aqueous solubility indicating that its solubility at the cmc was very close to that in water. This is expected since cmc's of surfactants were very low when compared to surfactant concentrations used.

The linearity between amount of drug solubilized and surfactant concentration indicated that solubilization follows the Distribution Law, according to which solubilizate molecules partition between an aqueous phase and a micellar phase (McBain &

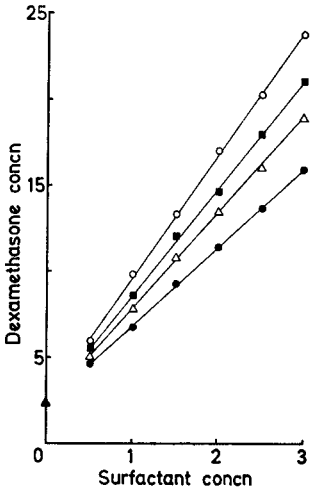

FIG. 1. Solubility of dexamethasone in water (mol litre^{-1} × 10^4), ▲, and as a function of % w/w aqueous concentrations of $C_{16}OE_{17}$, ○, $C_{16}OE_{32}$, ■, $C_{16}OE_{44}$, △, and $C_{16}OE_{68}$, ●.

Hutchinson, 1955). The dialysis investigation in non-saturated systems confirmed that the law was obeyed. D_m and D_w, the micellar and cmc steroid concentrations respectively, were linearly related (for example dexamethasone–$C_{16}OE_{32}$ system, Fig. 2) so that the equilibrium constant for the process or partition coefficient, K_m, is given by

$$K_m = D_m/D_w \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Since the solubilization process is governed by the Distribution Law, K_m may also be expressed as (Humphreys & Rhodes, 1968),

$$K_m = S_m/S_w \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where S_m and S_w are micellar and cmc solubilities of steroid respectively. S_m is estimated by extrapolating the solubility curves to 100% w/w surfactant and S_w may be taken as the aqueous solubility of the steroid because of low cmc's of non-ionic surfactants. Table 2 shows K_m estimated from solubility (K_s) and dialysis (K_d)

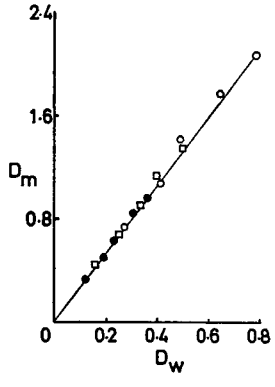

FIG. 2. Equilibrium dialysis of dexamethasone between water and 1, ○, 2 □ and 3, ●, % w/w $C_{16}OE_{32}$ solutions. Units D_w–mol litre^{-1} × 10^4, D_m–mol litre^{-1} × % surfactant^{-1} × 10^4.

data at 25°; the good agreement obtained from solubility and dialysis data indicates the validity of the Distribution Law. This agrees with Simons & Rhodes (1971) who studied the interaction of testosterone with surfactants of the type used here. Partition coefficients between micellar and aqueous phases were progesterone > testosterone > dexamethasone > hydrocortisone, the same rank order as partition coefficients, K, between diethyl ether and water (Table 2).

Table 2. *Partition coefficients of steroids between water and ether, K, and aqueous and micellar phases from solubility, K_s, and dialysis, K_d, at 25°.*

Steroid	$C_{16}OE_{17}$		$C_{16}OE_{32}$		$C_{16}OE_{44}$		$C_{16}OE_{63}$		K
	K_s	K_d	K_s	K_d	K_s	K_d	K_s	K_d	
Hydrocortisone	110	110	101	103	86	87	68	66	1·63
Dexamethasone	314	295	273	269	244	240	199	208	3·89
Testosterone	786	807	661	654	570	588	452	442	56·9
Progesterone	2160	2230	1790	1730	1550	1400	1250	1000	613

The solubilizing efficiency of the surfactants, as indicated by slopes of solubility plots, decreased as polyoxyethylene chains increased, when surfactant concentrations were compared on a weight basis. This was also indicated by decreased K_m with chain length. However, on a molar basis, the order of surfactant solubilizing efficiency was reversed, i.e. the more hydrophilic surfactants appeared more efficient. Similar observations were reported (Nakagawa, 1953; Swarbrick, 1965; Gouda, Ismail & Motawi, 1970) for solubilization of other drugs by polyoxyethylene surfactants; however, no reasonable explanations were offered. Now the number of micelles in equimolar amounts of surfactants may be estimated from partial specific volumes and aggregation numbers so that solubility data of steroids in the micellar phase, S_m, may be used to estimate the number of steroid molecules per surfactant micelle. This estimation assumes that size and hydration of micelles are unaltered when solubilizate is present, which is reasonable as the amount of solubilization is small. Table 3 summarizes micellar solubilization data at 25°. Partial specific volumes, $\bar{V}$, and aggregation numbers, Z, were obtained from hydrodynamic and light scattering studies (El Eini, Barry & Rhodes, 1973, 1976).

Results reveal that what initially seemed inconsistent, in that solubilizing efficiency of surfactants increased with hydrophilic chain length, is valid. Micellar sizes of surfactants decrease while hydration increases as polyoxyethylene chain increases (El Eini & others, 1973, 1976). Therefore, inclusion of non-polar steroids into the increasingly polar micellar environment decreases. This explains decreasing K_m with hydrophilic chain length. However, the number of micelles in equimolar amounts

Table 3. *Micellar solubilization parameters for steroids in n-alkylpolyoxyethylene surfactants at 25°.*

Surfactant	Partial specific volume ml g^{-1}	Aggregation number	Micelles mol^{-1} $\times 10^{-21}$	Steroid molecules per micelle			
				Hydrocortisone	Dexamethasone	Testosterone	Progesterone
$C_{16}OE_{17}$	0·9376	99	6·08	9·1	6·7	6·0	5·6
$C_{16}OE_{32}$	0·9171	56	10·8	7·6	5·3	4·6	4·3
$C_{16}OE_{44}$	0·8972	39	15·4	5·8	4·2	3·6	3·3
$C_{16}OE_{63}$	0·8751	25	24·1	4·0	3·3	2·4	2·3

of surfactants increases as the hydrophilic chain increases and although the number of steroid molecules per micelle is smaller for more hydrophilic surfactants the total amount of steroid per mole of surfactant is greater, hence the observed increase in solubilizing efficiency with increased hydrophilic chain length when molar concentrations are used.

Rectilinear relations were observed between the R_m values and the partition coefficients (K_m) between micellar and aqeous phases (Fig. 3). Such a relation occurred for several types of compounds (Iwasa, Fujita & Hansch, 1965; Hansch, 1971). It indicates that solubilization of steroids by the polyoxyethylene surfactants depends on steroid polarity so that less polar steroids are solubilized to a greater extent. Although K_m increases with decreasing steroid polarity, the number of solubilized

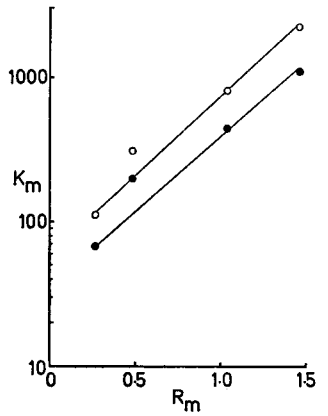

FIG. 3. Relation between the steroid micelle/water partition coefficient, K_m, in $C_{16}OE_{17}$, ○, and $C_{16}OE_{68}$, ●, and their R_m values at 25°. Data for $C_{16}OE_{32}$ and $C_{16}OE_{44}$ were omitted for clarity.

molecules per micelle decreases. Since, from equation 2, the number of solubilized molecules depends on aqueous steroid concentration, it follows that although K_m for a given steroid is high the number of solubilized molecules per micelle is low if aqueous solubility is low.

Unlike ionic surfactants, with relatively small polar head groups, micelles formed by long chain polyoxyethylene non-ionic surfactants, in which the hydrophilic moiety is large, have an alternative locus for solute. Schick (1963) showed that the polyoxyethylene chain is an expanding spiral, i.e., a cone shape, with the narrower end at the surface of the hydrocarbon core. El Eini & others (1973, 1976) showed that micelles of these surfactants were nearly spherical and extensively hydrated. Although there is space for hydrating water in the outer parts of the micelles, there is virtually none close to the hydrocarbon core due to crowding of the polyoxyethylene chains (Macfarlane, 1963). This forms a region which is largely purely polyoxyethylene, rather than polyoxyethylene–water, which may act as a site of solubilization of semi-polar compounds. A cross-section of a polyoxyethylene surfactant micelle thus offers a complete range of polarity, from the non-polar hydrocarbon core, via the semipolar polyoxyethylene to the increasingly polar hydrated polyoxyethylene portion, to pure water on the micellar surface. A likely site for the semipolar steroid drugs would be the unhydrated polyoxyethylene portion close to the hydrocarbon core so that the least polar steroid, testosterone, would be closest to the core and the most polar steroid,

hydrocortisone, furthest from the core and closer to the hydrated polyoxyethylene portion.

The overall steroid solubilities in water (Table 1) and in surfactant solutions increased with temperature but K_m decreased (Table 4). The influence of temperature on solubilization is complex because both aqueous solubility of solubilizate and micellar structure and properties may change (Mulley, 1964) and these changes may or may not be in the same direction. The former factor is simpler and Table 1 indicates that aqueous steroid solubilities increase with temperature. The latter factor is more complex. For non-ionic polyoxyethylene surfactants micellar size increases slightly with temperature until a threshold, about 20° below the cloud point, is reached. Above this temperature size increases exponentially and micelles may become asymmetric (Elworthy & McDonald, 1964). The cloud points of $C_{16}OE_{32}$, $C_{16}OE_{44}$ and $C_{16}OE_{63}$, are above 100° while $C_{16}OE_{17}$ is 94° (El Eini, 1975). These points are well above the highest temperature studied, i.e. 50°. No change in cloud points of surfactants in the presence of steroids was observed, indicating the absence of any observable change in micellar properties (Nakagawa, 1967). This is expected because of the small amounts solubilized, ranging from 2 to 9 molecules per micelle at 25° (Table 3), representing a maximum of 3% of the micellar weight. It is therefore reasonable to assume that micellar structure and properties are only slightly affected by temperature and solubilized steroids. Since steroid solubilities in both aqueous and micellar phases increased with temperature, the decrease in K_m is probably due to the higher temperature coefficient of the aqueous solubility of the steroids.

Knowledge of the thermodynamic parameters controlling the process of solubilization helps to explain the mechanisms involved. The standard free energy change, ΔG_s° (cal mol^{-1}), when one mol of solute transfers from solution to the micelle is given by

$$\Delta G_s^\circ = -RT \ln K_m \quad .. \quad .. \quad .. \quad .. \quad (4)$$

The standard enthalpy change of solubilization, ΔH_s° (cal mol^{-1}), is related to the free energy change by the Gibbs-Helmholtz equation so that at constant pressure P,

$$\frac{-\Delta H_s^\circ}{T} = \left(\frac{\delta \ \Delta G_s^\circ}{\delta T \ \ T}\right)_P \quad .. \quad .. \quad .. \quad .. \quad (5)$$

The standard entropy change, ΔS_s° (cal deg^{-1} mol^{-1}), is

$$\Delta S_s^\circ = \frac{\Delta H_s^\circ - \Delta G_s^\circ}{T} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Thermodynamic parameters are in Table 4.

For all systems ΔG_s° was negative indicating spontaneous solubilization. At constant temperature, $-\Delta G_s^\circ$ decreased with increasing hydrophilic chain length, indicating that solubilizing efficiency was greater for the more hydrophobic surfactants. However, the rate of change of ΔG_s° with percent micellar hydrocarbon content is virtually the same for the four steroids (Fig. 4). If the solubilization was by inclusion into the hydrocarbon core of the micelle, it would be expected that $-\Delta G_s^\circ$ for progesterone, the least polar compound, would be more dependent upon the hydrocarbon content of the micelle than $-\Delta G_s^\circ$ for hydrocortisone, the most polar. This supports the earlier proposal that the steroids are solubilized in the micellar polyoxyethylene layer. The decrease in $-\Delta G_s^\circ$, with hydrophilic chain length, which at first seems to contradict the above proposal, may be explained by considering micellar hydration.

Table 4. *Thermodynamic parameters for the solubilization of steroids by n-alkyl-polyoxyethylene surfactants* (full details in El Eini, 1975).

Surfactant	°C	Hydrocortisone K_m	Dexamethasone K_m	Testosterone K_m	Progesterone K_m
$C_{16}OE_{17}$	10	177	471	1030	2970
	20	120	346	819	2580
	25	110	314	786	2160
	30	99	289	676	2000
	40	83	224	534	1840
	50	55	185	385	1640
$-\Delta G_s^\circ$ kcal mol^{-1}, range		2·57 : 2·91	3·35 : 3·46	3·82 : 3·95	4·50 : 4·75
$-\Delta S_s^\circ$ cal deg^{-1} mol^{-1}, range		7·00 : 7·34	2·57 : 2·68	1·49 : 1·77	−6·05 : −6·70
$-\Delta H_s^\circ$ kcal mol^{-1}		4·94	4·19	4·39	2·74
$C_{16}OE_{32}$	10	149	414	824	2370
	20	105	295	688	2220
	25	101	273	661	1790
	30	85	261	586	1730
	40	71	203	463	1640
	50	46	170	362	1490
$-\Delta G_s^\circ$		2·46 : 2·81	3·30 : 3·39	3·78 : 3·85	4·37 : 4·69
$-\Delta S_s^\circ$		7·40 : 7·76	1·78 : 1·99	−·12 : −·35	−7·60 : −7·89
$-\Delta H_s^\circ$		4·97	3·89	3·74	2·17
$C_{16}OE_{44}$	10	135	364	718	2170
	20	94	274	592	1870
	25	86	244	570	1550
	30	74	239	489	1410
	40	63	185	426	1390
	50	42	159	321	1270
$-\Delta G_s^\circ$		2·40 : 2·76	3·25 : 3·32	3·70 : 3·77	4·32 : 4·59
$-\Delta S_s^\circ$		7·65 : 7·98	1·36 : 1·52	−·50 : −·71	−6·38 : −6·70
$-\Delta H_s^\circ$		4·97	3·71	3·54	2·43
$C_{16}OE_{63}$	10	118	304	573	1670
	20	79	215	475	1420
	25	68	199	452	1250
	30	60	188	405	1200
	40	48	151	355	1120
	50	33	123	268	1070
$-\Delta G_s^\circ$		2·25 : 2·68	3·11 : 3·21	3·57 : 3·65	4·17 : 4·48
$-\Delta S_s^\circ$		9·76 : 9·97	2·11 : 2·34	−·89 : −1·12	−7·35 : −7·57
$-\Delta H_s^\circ$		5·47	3·81	3·30	2·03

It was shown (El Eini & others, 1973, 1976) that the polyoxyethylene surfactants formed heavily hydrated micelles, the hydration varying from 5·2 water molecules per ethylene oxide unit for $C_{16}OE_{17}$ to 10·5 for $C_{16}OE_{63}$. Two of these water molecules are hydrogen bonded to the ether oxygens, the rest being physically trapped by the polyoxyethylene chains mesh (Elworthy, 1960). It therefore seems that trapped water physically hinders incorporation of steroid molecules and since hydration increases with hydrophilic chain length, steroid solubilization efficiency decreases.

The effect of temperature on changes of free energy was slight so that $-\Delta G_s^\circ$ for a given surfactant/steroid system remained essentially constant between 10° and 50°. Increase in micellar volume with temperature, due to unfolding of the polyoxyethylene chains (Kuriyama, 1962; Nakagawa, Kuriyama & Inoue, 1960) would enhance solubilization of the steroids which occurs in the palisade layer. However, this may be counteracted by increased micellar hydration accompanying temperature

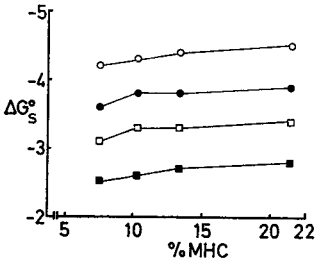

FIG. 4. Variation of the free energy of solubilization, ΔG_s° at 25° C, of hydrocortisone, ■, dexamethasone, □, testosterone, ●, and progesterone, ○, with % micellar hydrocarbon content (MHC).

increase. The essentially constant $-\Delta G_s^\circ$ indicates that the volume increasing effect is similar in magnitude to the opposing hydration effect.

The solubilization efficiency of steroids by a given surfactant, at constant temperature, increased with decreasing steroid polarity. This is illustrated in Fig. 5 where $-\Delta G_s^\circ$ increased as R_m increased. Since the micellar interior is less polar than the aqueous phase it would be expected that steroid molecules would preferentially distribute themselves into micelles and that this preference should increase as steroid polarity decreases.

ΔH_s° was independent of temperature and was negative indicating that solubilization was energetically favoured. It seems that ΔH_s°, for a given steroid, is essentially independent of polyoxyethylene chain length which probably indicates similar interactions upon micellization. However, ΔH_s° becomes more positive as the steroid polarity decreases. This may reflect the degree of disruption of water structure, surrounding the non-polar hydrocarbon groups of the steroid molecules (Nemethy & Scheraga, 1962), which increases with decreasing polarity. Positive enthalpies were obtained for solubilization by polyoxyethylene surfactants of non-polar molecules such as Sudan Red (Schwuger, 1970), Orange OT (Mankowich, 1965) and Yellow OB (Tokiwa & Ohki, 1968).

Hydrocortisone and dexamethasone were solubilized with a negative change in entropy while less polar testosterone and progesterone provided a positive change. Two opposing factors are involved. Insertion of solubilized molecules in micelles restricts molecular movement, i.e. a more ordered state, and provides a negative change in entropy. An opposing effect is that as relatively non-polar molecules leave the aqueous phase for the micelle, the configurational entropy of the water molecules increases due to the breakup of "iceberg" structure surrounding non-polar

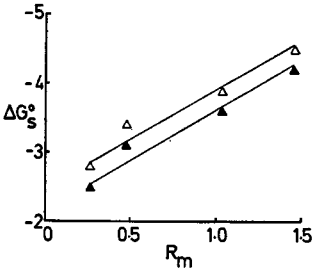

FIG. 5. The free energy of solubilization ΔG_s°, at 25°C, of hydrocortisone, dexamethasone, testosterone and progesterone in $C_{16}OE_{17}$, △, and $C_{16}OE_{33}$, ▲, as a function of their R_m values. Data for $C_{16}OE_{32}$ and $C_{16}OE_{44}$ were omitted for clarity.

groups, i.e. a less ordered state produces a positive change in entropy. For polar steroids, hydrocortisone and dexamethasone, the crowding effect predominates and hence a negative change in entropy occurs on solubilization. It is likely that more polar steroids do not entirely lose their water structuring on entering the micelle, because the region into which they are most likely to be incorporated, i.e. the outer layer of the polyoxyethylene shell, is heavily hydrated by water molecules physically trapped between the chains. Therefore there would be little change in entropy, due to loss of water structure, but a decrease in entropy due to restriction of the solubilized molecules in the micelle.

Less polar steroids, testosterone and progesterone, are solubilized in the less hydrated region of the polyoxyethylene shell, close to the hydrocarbon core, which is largely purely polyoxyethylene. When these steroids leave the aqueous phase, water structure breaks down and is not reformed in the micelle because there is little water at this site. Therefore solubilization of non-polar steroids increases entropy, due to breakup of water structure, which is greater than loss in entropy due to restriction of steroid within the micelle and hence a net positive change in entropy occurs.

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