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Cosolubilization of non-polar drugs in polysorbate 80 solutions

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Abstract

This study investigated the cosolubilization phenomenon of three non-polar drugs (hydrocortisone, β-estradiol, and ethynylestradiol) in polysorbate 80 solutions. It was found that the solubility of any drug decreased in the presence of other steroidal compounds. In an attempt to understand the observation, the author proposed a model to describe and to predict the drug solubility in the presence of other non-polar drugs in a non-ionic surfactant. The model indicates that, in a non-ionic surfactant solution that contains both drugs D_a and D_b, the total solubility $[D_a^{tot}]$ is related not only to the physical chemical properties of D_a (micellar equilibrium constant K_a, the intrinsic solubility $[D_a]$), as well as the total surfactant concentration $[S^{tot}]$, it is also related to the physical chemical properties of the D_b. Mathematically, the $[D_a^{tot}]$ decreases as the product of the micellar equilibrium constant K_b and the intrinsic solubility $[D_b]$ increases. The model was also put to the test by comparing the cosolubilization data obtained experimentally with the data calculated from the proposed model. The fact that these two sets of data were in good agreement lent strong support to the newly proposed model.

Keywords: Solubilization; Cosolubilization; Surfactant; Polysorbate 80; Hydrocortisone; β-estradiol; Ethynylestradiol

1. Introduction

Multiple drug lead compounds are often seen to be used/formulated in combination in solution, suspension, or in solid for fast pharmacological and toxicological screening in early drug discovery process. The combined use of multiple drugs, or combination therapy, is also on the rise where more drugs are mixed in a single delivery system, as the therapy has shown the promise to produce synergistic effects. Many examples have been reported recently: tetracycline with flurbiprofen for postmenopausal osteoporosis (Aoyagi et al., 1996), gatifloxacin with other antimicrobial agents against pseudomonas aeruginosa (Gradelski et al., 2001), telmisartan with hydrochlorothiazide for hypertension (Lacourciere et al., 2001), etc. Most of these pharmaceuticals are quite non-polar and poorly water-soluble. Naturally one may wonder if

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the coexistence of multiple drug compounds would have any effect on the solubilization of any particular drug. This is an issue of great importance, as the drug solubility has direct impact on the drug dissolution, absorption, and ultimately, the bioavailability.

This study aims to investigate this phenomenon by using three structurally similar non-polar drug compounds (hydrocortisone, β-estradiol, and ethynylestradiol) in a non-ionic surfactant polysorbate 80 solutions. The drug solubility data will be generated for situations where the drug is solubilized alone, and solubilized in the presence of other drugs. It is of great interest of this research to explore if the solubility of any particular drug would alter as a result of other drug presence in an environment that are void of physical/chemical as well as electronic interactions among drugs, and drug-micelles. It is of equal interest to explore the mechanism of this cosolubilization phenomenon, and how it relates to the physical chemical properties of all components in the system.

2. Materials

All the steroid compounds: hydrocortisone, β-estradiol, ethynylestradiol, were purchased from Sigma and were used without further purification. They were used as with the abbreviation as H, E, EE, respectively. The surfactant polysorbate 80 was also purchased from Sigma.

3. Methods

3.1. Solubility determinations

The phase-solubility method developed by Higuchi and Connors (1965) was used to measure the solubility for H, E, EE, H+E, H+EE, E+EE. Sample vials were prepared in triplicate by adding an excess amount of drugs to vials containing 5 ml of polysorbate 80 solutions at various concentrations (w/v): 0, 0.5, 1, 2.5, 5, 10, 15, and 20%. The vials were then closed with a TEFE-lined rubber-stoppers and were rotated at 40 r.p.m. using an end-over-end mechanical rotator (Vankel, VK750D) at 25 °C for 5 days. Samples with drug crystals present indicate that the drug reached its equilibrium solubility. For samples containing two different drugs, the crystals of both drugs were found (DSC method) in the vial before equilibrium solubility was measured. The crystals/precipitates were removed through 0.45-μm TEFE syringe filters (Millipore). The drug solubility at various polysorbate 80 concentrations was determined using an HPLC assay described in the following section.

3.2. Analytical method

The stability-indicative HPLC assays were used to quantitate the concentrations of H, E, and EE. The drug purity was determined by using diode array detector during the process of method developments. The HPLC system used a Waters 2690 separation module and Waters 2487 dual λ absorbance detector (Waters Corporation). The column was Synergi Max-RP column, 150 cm × 4.6 mm (Phenomenex Corporation); the flow rate was 1 ml/min; the detection was made at 254 nm for samples containing H and 280 nm for E and EE. The injection volume was 50 μl for all the samples. A mobile phase composed 40% acetonitril and 60% water was used to elute H, E, and EE, with a retention time of 3.1, 10.1, and 13.0 min, respectively. Standard solutions for each drug were tested on both inter- and intra-days and were found to be linear at concentrations 0.5, 5, 25, 50, 100 μg/ml with a RSD less than 1.2%. The solubility data are averaged values from triplicate samples with a relative standard deviation (RSD) less than 2.0%. For simplification, only the average data were used in Figures.

4. Results and discussions

Fig. 1 shows that the phase solubility profile for each individual drug. Apparently the drug solubility is a function of polysorbate 80 concentration, where EE is for ethynylestradiol (closed diamonds), H for hydrocortisone (closed squares), and E for β-estradiol (closed triangles). The drug solubility increase follows the order as EE > H >

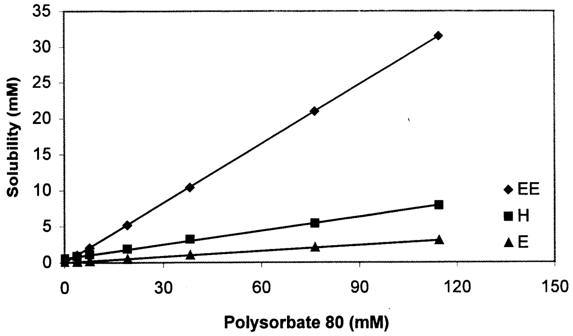

Fig. 1. Total solubilities of ethynylestradiol (EE, ◆), hydrocortisone (H, ■), and β-estradiol (E, ▲) in polysorbate 80 solutions.

E. The solubilization slope was directly obtained from these curves (see Table 1) from which the micellar equilibrium constant *K* (unit: M^{-1}) was calculated. Also listed is the intrinsic solubility of each drug.

Fig. 2a–c present the drug cosolubilization phenomenon: Fig. 2a showed that the H solubility in different situations: solubilized alone (closed diamonds), cosolubilized with saturated E (open squares), and cosolubilized with saturated EE (open triangles). It can be seen that, compared to the situation where H is solubilized alone, the H solubility decreased slightly when E was present and decreased more significantly when EE was present. The solubility decrease trend was observed at all surfactant concentrations. The similar observation was made in Fig. 2b where the E solubility decreased in the presence of H or EE. Fig. 3c shows the variation of the EE solubility when it is cosolubilized with either H or E. Interestingly, the EE did not experience dramatic solubility change.

Table 1
Physico-chemical properties for β-estradiol (E), ethynylestradiol (EE), and hydrocortisone (H)

Drug	[*D*] (mM)	log *P**	Solubilization slope	*K* (M^{-1})
H	0.564	1.43	0.064	123.4
E	0.009	4.01	0.027	3071.5
EE	0.033	4.52	0.275	16450.4

* Calculated log *P* values from ACD/log *P* suite software (2000 version).

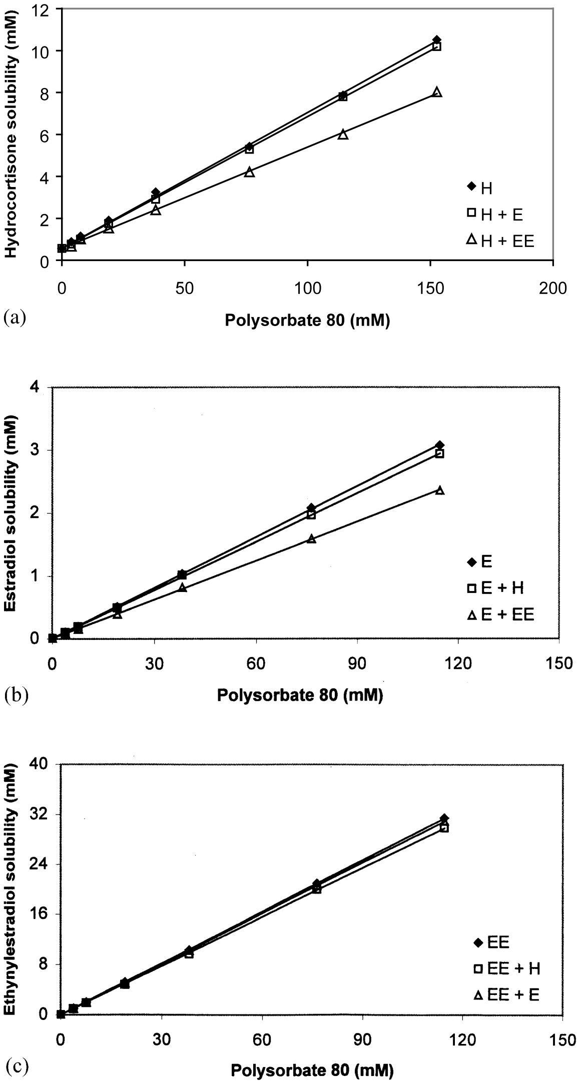

Fig. 2. (a) Total hydrocortisone (H) solubility in polysorbate 80 solutions: H (◆), H+E (□), and H+EE (△). (b) Total β-estradiol (E) solubility in polysorbate 80 solutions: E (◆), E+H (□), and E+EE (△). (c) Total ethynylestradiol (EE) solubility in polysorbate 80 solutions: EE (◆), EE+E (△), and EE+H (□).

This is an interesting observation: the presence of one drug seems to be related to the solubility decrease of the other drug in the same micellar solution. Though there has been a large volume of literature on drug solubilization by surfactants, the cosolubilization phenomenon is rather new and not often discussed. Among limited reports,

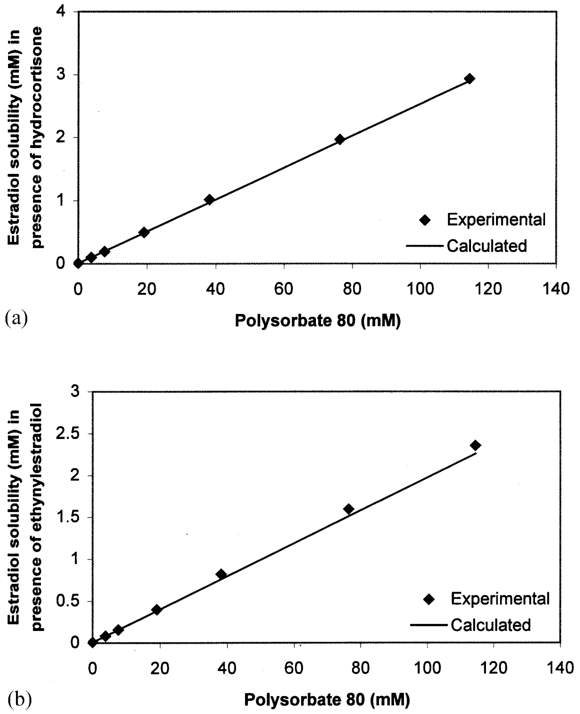

Fig. 3. (a) Calculated (—) vs. experimental (◆) solubilities of β-estradiol (E) in presence of saturated hydrocortisone (H) in polysorbate 80 solutions. (b) Calculated (—) vs. experimental (◆) solubilities of β-estradiol (E) in presence of saturated ethynylestradiol (EE) in polysorbate 80 solutions.

Crooks and Brown (1973) found that the solubility of chloroxylenol was dramatically reduced in a non-ionic surfactant cetomacrogol as the methyl paraben concentration increased. It was hypothesized that when compounds are solubilized within similar micellar regions it is likely that the compounds compete with each other for the solubilization site which leads to a diminished solubility of each. There are also studies conducted on the simultaneous solubilization of some steroids in polysorbate 40, tetradecyltrimethylammonium bromide and sodium dodecyl sulfate (Lõvgren et al., 1978; Lundberg et al., 1979). These studies indicate that when the compounds are located at different sites of micelles, palisade layer versus micellar core, they are solubilized independently of each other; and if in the same region of the micelles, there were alteration in the solubility of both compounds. This in a sense supports the cosolubilization hypothesis put forward by Crooks and Brown (1973).

To better understand this phenomenon, the author proposed a simple mathematical model that is based on the equilibrium theory (see Appendix A). For a non-ionic surfactant solution that contains both drug D_a and drug D_b, the model is constructed as such that both compounds are in an environment where they establish equilibrium, though separately, with the free micellized surfactants, and essentially compete for the free micellized surfactants in a manner that restrained by the established equilibrium. The D_a and D_b are of similar structural category, and should be reside in the close region of the micelles. The proposed model for the total solubility of drug D_a can be described as

$$[D_a^{tot}] = [D_a] + \frac{K_a[D_a]}{1 + K_a[D_a] + K_b[D_b]}[S^{tot}] \qquad (1)$$

where the $[D_a]$ and $[D_b]$ are the drug intrinsic solubility, the K_a and K_b are the drug micellar equilibrium constants, and the $[S^{tot}]$ is the total surfactant concentration. This model shows that $[D_a^{tot}]$ is a function of K_a, $[D_a]$, K_b, $[D_b]$, and $[S^{tot}]$. More importantly, it shows that the $[D_a^{tot}]$ is likely decreased in the presence of D_b. The extent of D_a solubility decrease depends on the physical chemical properties of both drugs D_a and D_b via the product of micellar equilibrium constant and the intrinsic solubility. If the presence of drug D_b is overwhelming, i.e. $K_b[D_b] \gg K_a[D_a]$, Eq. (1) can be written as Eq. (2)

$$[D_a^{tot}] = [D_a] + \frac{K_a[D_a]}{1 + K_b[D_b]}[S^{tot}] \qquad (2)$$

On the other hand, if the presence of D_b is insignificant, i.e. $K_a[D_a] \gg K_b[D_b]$, Eq. (1) can be written as Eq. (3).

$$[D_a^{tot}] = [D_a] + \frac{K_a[D_a]}{1 + K_a[D_a]}[S^{tot}] \qquad (3)$$

The model makes it feasible to explain and understand the phenomenon of drugs when they are cosolubilized in micellar solutions. Close examination of Fig. 2a found that the product of EE ($K_{EE}[D_{EE}] = 0.275$) is far greater than E

($K_E[D_E] = 0.027$), almost 10-fold, which explains why the drug H solubility is lower in the presence of EE than in the E. Similar reasoning can be applied to the Fig. 2b. In the Fig. 2c, as the product for H ($K_H[D_H] = 0.064$) and for E are relatively close, but are both significantly smaller than the product for EE, which explains why the EE solubility decrease is less significant in either H or E, as shown in Eq. (3). The model also suggests that any single physico-chemical parameter of the drug (D_b), be it the intrinsic solubility, micellar equilibrium constant, can not determine the extent of the possible effect on other drug's solubility (D_a). For example, the H has the greatest intrinsic solubility (0.564 mM), meaning that the drug molecules are most available in solution to be incorporated into micelle, while EE has the greatest micellar equilibrium constant (16 450 M^{-1}), indicating that the drug molecules can be most efficiently incorporated into the micelles. Yet it is not up to either one of which but the product of two parameters ($[D_b]$ and K_b), as well as the difference between the magnitude of the product $K_b[D_b]$ and $K_a[D_a]$. To further illustrate, Table 2 listed the drug solubility of H, E, and EE, respectively, in 15% polysorbate 80 concentrations, in the presence of other drugs. It shows that the drug D_a, when cosolubilized with the drug D_b, has various solubility decrease, as the product of K_b and $[D_b]$ varies. An approximate 25% solubility decrease is observed for H in the presence of saturated EE and less than 1% in the presence of saturated E. Again this is because the product of $K_{EE}[D_{EE}]$ is greater than that of $K_E[D_E]$.

Table 2
Solubility $[D_a^{tot}]$ of hydrocortisone (H), β-estradiol (E), and ethynylestradiol (EE) in 15% polysorbate 80 solutions in presence of saturated drug D_b

In the presence of D_b	The total solubility of D_a (mM)		
	H	E	EE
H	7.86 ± 0.12	2.94 ± 0.04	19.97 ± 0.04
E	7.80 ± 0.05	3.08 ± 0.03	20.58 ± 0.05
EE	6.00 ± 0.06	2.36 ± 0.05	20.98 ± 0.03

This study also compared the E solubility obtained directly from the experiment and the solubility calculated by Eq. (1). Fig. 3a,b showed that the predicted $[D_E^{tot}]$ data (solid lines) were very similar to those $[D_E^{tot}]$ from experimental (diamond symbols) in the presence of either H, or EE. The same was observed for other drugs (data not present). This lent support to the effectiveness of the proposed model.

The cosolubilization of non-polar drugs in micellar solutions is an interesting phenomenon. In the absence of drug–drug, and drug–micelles chemical/physical interactions (non-polar steroids in non-ionic surfactant), this model provides a new perspective in drug–micelles thermodynamic (equilibrium) interactions, and how the physical chemical properties of both drugs, intrinsic solubility and micellar equilibrium constant alike, determine the extent of drug cosolubilization phenomenon. Though the proposed model met with success in this study, the model should be treated with caution. The formation and variation of micelles can be far more complex: it is believed that these structurally similar steroids are resided in a very close region of micelles, which leads them to compete with the specific sites. It is possible that compounds of increased structural diversity may induce complex alterations in solubility. For example, one compound might actually induce a reorganization of the micelle structure and allow increased uptake of another compound, or both mechanisms (which makes the micelles increase or decrease the solubilization capacity) are at work (Attwood and Florence, 1983.). In addition, the greatly increased amount of incorporation of drug molecules into the micelles also alters the micelles property (mixed micelles).

Appendix A: Attachment

The proposed model describes a thermodynamics system where two non-polar drugs D_a and D_b are solubilized in a non-ionic surfactant solution. The basic requirement for the model is that all components involved should be stable both chemically and physically, and there should be no drug-drug self-association.

In this system, the model assumes that there exists equilibrium among each drug and the free micellized surfactants in solution. The total drug concentration for drug D_a and D_b can be described in Eqs. (A1) and (A2), where $[D_a]$ and $[D_b]$ are intrinsic solubility, and $[D_aM]$ and $[D_bM]$ are the drug D_a with micellized surfactants and D_b with micellized surfactants, respectively.

$$[D_a^{tot}] = [D_a] + [D_aM] \tag{A1}$$

$$[D_b^{tot}] = [D_b] + [D_bM] \tag{A2}$$

By this assumption, D_a and D_b establish equilibrium separately with free micellized surfactants in solution. This is a simplified version of drug-micelles interactions, which does not consider the species such as $[D_aD_bM]$. The similar approach can be found in a few modeling studies on drug-micelle interactions for weakly electrolytic drug compounds in a pH controlled micellar solutions (Rippie et al., 1964; Lopes et al., 1992; Li et al., 1999; Jinno et al., 2000). In these studies, it is often assumed that unionized and ionized drug species, which are treated as separated drug species, establish equilibrium separately with free micellized surfactants, and there is no species such as $[D_uD_iM]$.

When in equilibrium, the $[D_a]$ is an intrinsic solubility, which relates to the drug partition coefficient ($\log P$) and the melting point. The $[D_aM]$ is related to micellar equilibrium constant for drug D_a, K_a, by Eq. (A3), where $[M]$ is the free micellized surfactant concentration. By similarly reasoning, the $[D_bM]$ can be written as in Eq. (A4). The K_a or K_b, is related to the physical property of that particular drug such as polarity, size, and shape (Attwood and Florence, 1983; Yalkowsky, 1999).

$$[D_aM] = K_a[D_a][M] \tag{A3}$$

$$[D_bM] = K_b[D_b][M] \tag{A4}$$

The total micellized surfactant concentration $[M^{tot}]$ in solution is equal to the sum of free micellized surfactant concentration $[M]$, micellized surfactants that contain drug D_a, and D_b respectively, as in Eq. (A5).

$$[M^{tot}] = [M] + [D_aM] + [D_bM] \tag{A5}$$

The $[M^{tot}]$ equals to the total surfactant concentration $[S^{tot}]$ when the critical micelle concentration (CMC) is small. This is often true for most of the surfactants. Eq. (A5) can be rearranged into Eq. (A6) by combining Eqs. (A3) and (A4).

$$[M] = \frac{[S^{tot}]}{1 + K_a[D_a] + K_b[D_b]} \tag{A6}$$

Taking Eq. (A6) into Eq. (A3) gives the full expression of $[D_aM]$. Incorporating this into Eq. (A1) yields Eq. (A7).

$$[D_a^{tot}] = [D_a] + \frac{K_a[D_a]}{1 + K_a[D_a] + K_b[D_b]}[S^{tot}] \tag{A7}$$

Similarly, combining Eqs. (A6), (A4) and (A2) gives Eq. (A8).

$$[D_b^{tot}] = [D_b] + \frac{K_b[D_b]}{1 + K_a[D_a] + K_b[D_b]}[S^{tot}] \tag{A8}$$

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