

# The Versatility of Polysorbate 80 (Tween 80) as an Ionophore

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**Abstract** □ A number of experiments were performed to illustrate the unusual versatility of Polysorbate 80 (Tween 80) as an ionophore. New ions shown to be transported by it from and to water layers through a model membrane (CH<sub>2</sub>Cl<sub>2</sub>) include H<sub>3</sub>O<sup>+</sup>, Li<sup>+</sup>, Pb<sup>2+</sup>, Co<sup>2+</sup>, piperidinium ion, guanidinium ion, and Paraquat, while two complex ions resisted transport under the conditions used. "Reverse" transport of lipophilic guests (azobenzene, azulene, ferrocene) from and to organic solvents through water was also promoted by Tween 80, but C<sub>60</sub> was not carried. Three water molecules were transported per molecule of KSCN by the Tween.

## Introduction

Previous work has shown that Polysorbate 80 (**1**, Tween 80), a popular surfactant and solubilizing agent used in biochemical, chemical, and pharmacological research and in the food and cosmetic industries, is an effective ionophore,<sup>1</sup> can be used as a phase transfer catalyst,<sup>2</sup> and affects the serum concentrations of calcium ions of rats when included in their diets.<sup>3</sup> It also exhibits, as do poly-(ethylene glycol) 1000 and 18-crown-6, an unusual inverse temperature effect on the rates of transport of potassium thiocyanate through a model membrane.<sup>4</sup>

The present work was undertaken to illustrate the versatility of Polysorbate 80 as an ionophore. It was already known that it is more stable in basic solutions than the tetraalkylammonium salts, and both cheaper and less toxic than the crown ethers.<sup>1</sup> We set out to expand the number and types of ions carried by it, to settle the question of how ions are carried ("naked" or hydrated), and to discover if the "reverse" transport of lipophilic guests through water could be achieved.

Too often, Polysorbate 80 has been used as a surfactant and solubilizing agent in living systems under the assumption that it was totally inert and had no further physiological activity or influence. To test this assumption, we hope to be able to answer the following questions. Is Polysorbate 80 able to transport, not only benign ions, but also potentially dangerous ones (e.g. heavy metal ions), across a membrane? Is it able to transport organic ions as well? Is it able to, not only solubilize into aqueous bodily compartments neutral organic entities, but also to facilitate their movements through membranes into other aqueous compartments, such as cells? How inert and innocuous is Polysorbate 80?

## Experimental Section

**Materials**—Thiocyanate salts, azobenzene, azulene, ferrocene, Paraquat, Methyl Thymol Blue, and Carbon Soot were purchased from Aldrich and, except for the Soot, used without further

purification. Polysorbate 80 was obtained from Fisher Chemical. Spectra were recorded on a Milton Roy Spectronic 301.

**Transport Studies**—The experimental setup has been described elsewhere.<sup>1</sup> A lower layer of 150 mL of approximately 0.8 mM **1** in CH<sub>2</sub>Cl<sub>2</sub> in a 600 mL beaker supports an inner layer of 40 mL of 0.1 M aqueous metal or organic thiocyanate and an outer layer of 0.02 M Fe(NO<sub>3</sub>)<sub>3</sub> in 0.20 M HNO<sub>3</sub>, the latter two layers separated by a glass "cup". The rate of transport is monitored by measuring the increase in the absorbance at 480 nm of the red complex ion Fe(SCN)<sub>2</sub><sup>+</sup> formed in the outer layer with time.

Two exceptions to the above procedure should be noted. Since Pb(SCN)<sub>2</sub> is only very slightly soluble in water, Pb(NO<sub>3</sub>)<sub>2</sub> was used as the salt, and the outer layer changed to a 0.1 mM solution of methyl thymol blue in 0.05 mM succinic acid buffer (pH 5.8).<sup>5</sup>

In the case of the transport of hydronium ions, the inner layer consisted of various concentrations of aqueous HNO<sub>3</sub>, and the outer layer of distilled water. Transport was monitored by measuring the pH of the outer layer.

**Transport of Water Molecules**—These experiments were conducted in a dry bag in an atmosphere of dry N<sub>2</sub>. The inner layer consisted of 40 mL of 0.1 M aqueous KSCN and the outer layer of 25 mL of D<sub>2</sub>O. At regular intervals, including at time zero, 2.0 mL samples of the outer layer were removed and placed in stoppered NMR tubes. At the end, the tubes were removed from the dry bag and the intensity of the absorption peak for H<sub>2</sub>O at 4.65 ppm monitored a minimum of six times. Then, 1.5 mL of each sample was mixed in a UV cuvette with 1.5 mL of 0.02 M Fe(NO<sub>3</sub>)<sub>3</sub> in 0.20 M HNO<sub>3</sub>, and the absorption of the resultant solution followed at 480 nm.

The standard curve used to convert the UV absorptions to concentrations was that used previously.<sup>1</sup> A standard curve to ascertain the transport of H<sub>2</sub>O into the D<sub>2</sub>O of the outer layer was made by adding microliter samples of distilled, deionized H<sub>2</sub>O to 2.0 mL samples of D<sub>2</sub>O and measuring the intensity of the absorption in the NMR at 4.65 ppm; its  $r = 0.9985$ .

Blank experiments were run without KSCN in the inner layer but with Tween in the lower layer, and also without either present.

**Transport of Paraquat**—The colorless Paraquat can be monitored using the intensely colored radical cation which absorbs at 605 nm formed when it is oxidized by alkaline sodium dithionite.<sup>6</sup> A standard curve was prepared by measuring the absorption of a series of solutions made by adding 1.0 mL of aqueous Paraquat solutions to 1.0 mL of freshly prepared 0.5% sodium dithionite in 0.5 M ammonia buffer (pH 9); its coefficient of correlation was 0.9992. Timely 1.0 mL samples of the distilled water in the outer layer were taken during the experiment and, at the end, all added sequentially to 1.0 mL samples of a freshly prepared dithionite solution and the absorption read at 605 nm (dithionite solutions are unstable and must be prepared just before adding to the transported samples).

**"Reverse" Transport of Lipophilic Guests**—In these cases, the lower layer consisted of 150 mL of 0.643 mM aqueous Polysorbate 80; the inner layer of 40 mL of 0.100 M solute (azobenzene, azulene, or ferrocene) in the organic solvent (either hexane or isooctane); and the outer layer of 50 mL of the same pure solvent. Transport was monitored by measuring the absorption of the colored species themselves (azobenzene at 436 nm, azulene at 633 nm, and ferrocene at 442 nm) with time in the outer layer. Standard curves were constructed for each of the solutes: for azobenzene,  $A = 0.00887 + 0.1834[\text{AZB}]$ ,  $r = 0.9945$ ; for azulene,  $A = -0.000963 + 0.3405[\text{Az}]$ ,  $r = 0.9981$ ; for ferrocene,  $A = 0.0113 + 90.65[\text{Fer}]$ ,  $r = 0.9928$ .

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Table 1—Fluxes of Ions via Polysorbate 80<sup>a</sup>

ion	<i>n</i> <sup>b</sup>	10 <sup>8</sup> × flux (mol/s/m <sup>2</sup> ± SD)
K <sup>+</sup>	9	4.94 ± 0.65
Na <sup>+</sup>	7	1.26 ± 0.23
NH <sub>4</sub> <sup>+</sup>	6	2.67 ± 0.31
Li <sup>+</sup>	4	0.40 ± 0.05
H <sub>3</sub> O <sup>+</sup> <sup>c</sup>	4	0.83 ± 0.13
(H <sub>2</sub> N) <sub>2</sub> C=NH <sub>2</sub> <sup>+</sup> (guanidinium)	3	5.44 ± 0.19
C <sub>5</sub> H <sub>10</sub> NH <sup>+</sup> (piperidinium)	5	14.11 ± 0.61
Ca <sup>2+</sup>	6	0.72 ± 0.22
Co <sup>2+</sup>	4	5.43 ± 0.12
Pb <sup>2+</sup> <sup>d</sup>	3	1.81 ± 0.08
Ni(NH <sub>3</sub> ) <sub>6</sub> <sup>2+</sup>	3	0.00
Cr(NH <sub>3</sub> ) <sub>6</sub> <sup>3+</sup>	3	0.00
2 (Paraquat) <sup>e</sup>	3	0.0034 ± 0.0015

<sup>a</sup> Unless otherwise indicated, using 0.8 mM Tween in CH<sub>2</sub>Cl<sub>2</sub> as the lower layer, 0.1 M thiocyanate salt as the inner layer, and 0.02 M Fe(NO<sub>3</sub>)<sub>3</sub> in 0.20 M HNO<sub>3</sub> as the outer layer at 23 °C and 125 rpm. <sup>b</sup> Number of determinations. <sup>c</sup> Using 0.1 M HNO<sub>3</sub> as the inner layer and distilled, deionized water as the outer layer. <sup>d</sup> Using 0.10 M Pb(NO<sub>3</sub>)<sub>2</sub> as the inner layer and 0.10 mM methyl thymol blue in 0.05 M succinic acid buffer (pH 5.8) as the outer layer. <sup>e</sup> Using 0.0778 M Paraquat as the inner layer and distilled, deionized water as the outer layer.

A 0.006 M solution of buckminsterfullerene (C<sub>60</sub>) in toluene was prepared according to the method of Scrivens et al.<sup>7</sup> and 40 mL of it used as the inner layer. Distilled water constituted the outer layer. A standard curve was constructed using the absorption of C<sub>60</sub> at 598 nm.<sup>8</sup> Transport was monitored at the same wavelength.

## Results and Discussion

**Ion Transport**—The number and type of ions shown to be transported by Polysorbate 80 was increased considerably. The original work<sup>1</sup> had studied four biochemically important ions (K<sup>+</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>). In this work, smaller ions were used (Li<sup>+</sup>, H<sub>3</sub>O<sup>+</sup>), as well as, for the first time, heavy metal ions (Co<sup>2+</sup>, Pb<sup>2+</sup>). Some larger organic ions (piperidinium ion, guanidinium ion) are transported, while several complex ions [hexamminecobalt(III)<sup>2+</sup> and hexamminenickel(II)<sup>2+</sup>] are not. Methyl viologen, better known as Paraquat (2), a divalent cation in which the charges are separated, was also transported, but at a considerably lower rate (about 1/150) than any of the other ions. The fluxes of all these ions are summarized in Table 1.

The reluctance of the complex ions to be transported is probably due to the fact that the charges on the metals are so deeply buried within the six ammine groups that no interaction with the oxygen atoms of the Polysorbate 80 is possible, this despite the fact that the ammonium ion itself is easily transported. Chia et al.<sup>9</sup> reported a similar lack of transport of the hexamminecobalt(III)<sup>2+</sup> using the sodium salt of lasalocid as the ionophore; they did, however, effect transport if the receiving layer was spiked with ammonium ion (Lindoy et al.<sup>10</sup> also did this). The much lower rate of transport of the Paraquat may be related to the much greater size and weight that it represents; e.g. the length of the Paraquat molecule is about 10.2 Å compared to a length of only 4.9 Å for piperidinium ion. Apparently, the Tween can completely sequester the latter, but not the former. Also, it may be able to envelope only one of the positively charged nitrogen positions on the Paraquat molecule, leaving the other free to resist removal from the aqueous layer (the positive charges are about 7.1 Å apart).

Leaving Paraquat aside, the ions that are transported move at similar rates. An exception is the piperidinium ion, whose flux is three or more times greater than the other monovalent ions. The reason for this may be that this ion has a lipophilic end that can facilitate solution in the model

Table 2—Fluxes<sup>a</sup> of "Reverse" Transport via Polysorbate 80<sup>b</sup>

guest	concn, M	10 <sup>8</sup> × flux (hexane)	10 <sup>8</sup> × flux (isooctane)
azobenzene	0.0125	3.36 ± 0.72	
	0.025	3.69 ± 1.36	3.77 ± 0.41
	0.050	4.40 ± 1.36	4.45 ± 0.59
	0.075	5.35 ± 0.86	
azulene <sup>d</sup>	0.100	6.32 ± 1.18	6.53 ± 0.21
		( <i>r</i> = 0.9969) <sup>c</sup>	( <i>r</i> = 0.9955) <sup>c</sup>
	0.0125	0.90 ± 0.22	0.89 ± 0.09
	0.025	2.16 ± 0.46	2.06 ± 0.33
ferrocene	0.050	4.33 ± 0.24	4.38 ± 0.60
	0.075	6.63 ± 0.51	6.70 ± 0.59
		( <i>r</i> = 0.9907) <sup>c</sup>	( <i>r</i> = 0.9973) <sup>c</sup>
	0.038		1.53 ± 0.22
buckminsterfullerene (C <sub>60</sub> )	0.077		2.68 ± 0.22
	0.107		3.44 ± 0.71
	0.128		4.03 ± 0.58
			( <i>r</i> = 0.9993) <sup>c</sup>
buckminsterfullerene (C <sub>60</sub> )	0.006	0.00 (toluene)	

<sup>a</sup> Mol/s/m<sup>2</sup> ± SD, with a minimum of three determinations at each concentration. <sup>b</sup> Using 150 mL of 0.8 mM aqueous Polysorbate 80 as the lower layer, 40 mL of the guest at the stated concentrations in the stated solvent as the inner layer, and 50 mL of the same pure solvent as the outer layer, at 23 °C and 150 rpm. <sup>c</sup> Coefficient of correlation of the plot of concentration versus flux. <sup>d</sup> Diederich and Dick<sup>14</sup> report a transport rate for azulene from 0.02 M azulene in hexane through a 0.5 mM cyclophane-type ionophore in water to hexane at 20–22 °C of 8.6 × 10<sup>-6</sup> mol/L/h; they do not reduce this to a flux.

membrane; even the guanidinium ion cannot do this, because the positive charge is distributed throughout the molecule by resonance. Still, this general similarity in fluxes is in contrast to the situation using cyclic ionophores such as the crown ethers, where the correspondence between the size of the cavity and the diameter of the ion is of such importance, so that ions that can fit comfortably within the cavity are transported much more easily than those that are too big or too small for a snug fit. Over a large range of sizes, the poly(ethylene oxide) arms of the Polysorbate 80 are able to envelope many dissimilar ions with similar effectiveness, making it inherently more versatile than the cyclic ionophores.

**"Reverse" Transport of Lipophilic Guests**—One of the major uses of Polysorbate 80 has been to solubilize in water compounds that are otherwise insoluble in it. This led us to believe that lipophilic guests, with very low solubilities, might be able to be transported by Polysorbate 80 from an initial organic layer through an aqueous layer and into a final organic layer. A few examples exist of such "reverse" transport involving specifically constructed hosts (e.g. calixarenes<sup>11,12</sup> and cyclophanes<sup>13,14</sup>) with limited versatility. We chose as our guests strongly colored organic molecules whose absorptions in the visible region could act as their own means of detection.

The results of these experiments are shown in Table 2. Three quite different types of substances (azobenzene, azulene, and ferrocene) were transported with fluxes comparable to those of the ions transported (cf. Table 1). Initially, hexane was used as the organic solvent, but its relatively high volatility led to problems (an adjustment of volume in the upper layers before each reading). These problems were solved by changing to the less volatile solvent isooctane.<sup>15</sup>

With regular ion transport, the flux is first order in the salt. We determined the orders of the transports of our three organic guests in the usual way (using the slope of the line obtained by plotting ln *k* versus ln *c*), with the following results: 0.3–0.4 order for azobenzene, 1.20–1.25 order for azulene, and 0.74 order for ferrocene. This indicates that the rate-determining step of the process, the

Table 3—Transport of Water with Ions through a Model Membrane

components	$n^a$	$10^8 \times \text{flux}^b$ for $\text{H}_2\text{O}^c$	$10^8 \times \text{flux}^b$ for $\text{KSCN}^d$
$\text{H}_2\text{O}$	4	$16.18 \pm 1.13$	
$\text{H}_2\text{O} + \text{Tween}$	3	$18.88 \pm 0.68$	
$\text{H}_2\text{O} + \text{Tween} + \text{KSCN}$	4	$22.29 \pm 2.48$	$1.11 \pm 0.12$

<sup>a</sup> Number of determinations. <sup>b</sup> Mol/s/m<sup>2</sup>  $\pm$  SD. <sup>c</sup> Determined by integration of the NMR peak at 4.65 ppm in the D<sub>2</sub>O outer layer. <sup>d</sup> Determined by the absorption of the  $\text{Fe}(\text{SCN})^{2+}$  complex at 480 nm.

formation of the complex at the initial organic solvent–water interface, is not simple, but complex, depending to some extent on the shape and the size of the guest. Further work is needed to explain these orders.

It is apparent that Polysorbate 80 is as adept at transporting hydrophobic molecules through an aqueous layer as it is in carrying ions through an organic layer. Such versatility is not possible with many artificial (e.g. crown ethers and analogues) or naturally occurring (e.g. valinomycin, monensin, etc.) ionophores, whose cyclic or pseudocyclic structures restrict them to one basic conformation featuring a hydrophilic cavity and a lipophilic exterior. In addition, as is the case with other “octopus” compounds,<sup>12</sup> the size of the guests carried by Polysorbate 80 is not limited by a cavity of a specific size. The relatively free poly(oxyethylene) arms of one or more molecules<sup>11</sup> can envelope and carry guests of widely varying dimensions.

These results should act as a caveat in the use of Polysorbate 80 in living systems. There is always the possibility that the movement of organic molecules that are small enough through membranes can be facilitated by the Tween, causing interference at best and risk at worst. One such example, involving a study of the effect of 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol on the tachycardia of an excised rat heart, has already been documented.<sup>16</sup>

**Are Tween Transported Ions Hydrated or Not**—One of the reasons given for the increased rates of reaction produced by phase transfer catalysis (beyond the fact that the reacting species are in the same phase) is the fact that the ions transported to the organic phase are “naked”, i.e., not hydrated, or at least are less hydrated, than they were in the aqueous phase. Polysorbate 80 has been used as a phase transfer catalyst,<sup>2</sup> and so an experiment was set up to ascertain whether and to what extent water was transported through an organic layer with the cations and their counterions.

The results are shown in Table 3. Two blanks were run. In the first case, no salt was present in the inner layer and no Polysorbate 80 in the lower layer. Despite this fact, water was transported under the usual conditions of the experiment with a significant flux ( $16.18 \times 10^{-8}$  mol/s/m<sup>2</sup>). The second blank involved no salt in the inner layer, but Polysorbate 80 in the lower layer; the flux of water rose to  $18.88 \times 10^{-8}$  mol/s/m<sup>2</sup>, an increase of about 17%.

When KSCN was present in the inner layer and Polysorbate 80 in the lower layer, the flux of the salt was shown to be  $1.11 \times 10^{-8}$  mol/s/m<sup>2</sup> and that of the water  $22.29 \times 10^{-8}$  mol/s/m<sup>2</sup>. Since the flux of the water without the presence of the salt was  $18.88 \times 10^{-8}$  mol/s/m<sup>2</sup>, the KSCN would be responsible for a water flux of the difference,  $3.41 \times 10^{-8}$  mol/s/m<sup>2</sup>. This means that, for each mole of KSCN transported, about three moles of water are transported

$(22.29 \pm 2.48 - 18.88 \pm 0.68) \div 1.11 \pm 0.12 = 3.07 \pm 1.32$ . It is not clear at present whether this water is transported by the cation, the counterion, or a combination of the two. In a similar case, Dang and Kollman<sup>17</sup> postulate on theoretical grounds that three water molecules adhere to the potassium ion in its complex with 18-crown-6. Still, it is clear that the ions in the organic layer, while not truly “naked”, are nonetheless considerably unclothed compared to their situation in water.

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