Aqueous Solubilities of Estrone, 17β-Estradiol, 17α-Ethynylestradiol, and Bisphenol A

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The solubilities of three estrogenic hormones—estrone, 17β-estradiol, and 17α-ethynylestradiol—and the industrial pollutant bisphenol A were measured in water, dilute acid and alkali (pH 4 and 10, respectively), and aqueous KNO_3 (0.01 mol·L^{-1} and 0.1 mol·L^{-1}). The concentrations of saturated solutions, after equilibration at (25.0 ± 0.5) °C with excess solid for 4 days, were determined by HPLC. Six replicate results were obtained for each solute−solvent pair: the coefficient of variation was in most cases < 5 %. The solubilities in pure water with standard deviations were estrone (1.30 ± 0.08) mg·L^{-1}, 17β-estradiol (1.51 ± 0.04) mg·L^{-1}, 17α-ethynylestradiol (9.20 ± 0.09) mg·L^{-1}, and bisphenol A (300 ± 5) mg·L^{-1}. The solubility of each of the hormones was unchanged between pH 4 and pH 7 but was greater at pH 10. At pH 7, the hormones became progressively less soluble as the ionic strength increased from 0.0 to 0.1 mol·L^{-1}. By contrast the solubility of bisphenol A was essentially the same under all of the experimental conditions tested.

Introduction

The aqueous solubilities of hydrophobic organic compounds can influence their distribution, bioavailability, and persistence in the aquatic environment.[1] An important group of environmental pollutants known as endocrine-disrupting chemicals (EDCs) includes a number of steriod hormones, of which the female sex hormone 17β-estradiol has the highest estrogenic activity. One of its major degradation products, estrone, also disrupts endocrine function. These compounds are excreted by humans in a water-soluble conjugated form that is largely not estrogenic. The glucuronide or sulfonide conjugates in domestic wastes are rapidly cleaved and metabolized during transport and treatment, releasing the fully potent hormones with sewage effluents.[1,2] Ethynylestradiol, a highly stable synthetic hormone commonly found in the formulation of oral contraceptives,[3] is a significant environmental contaminant. 17β-Estradiol and its metabolites are also of major concern because estradiol is the principal endogenous steroid estrogen in vertebrates, stimulating the growth and development of the female sex organs. Bisphenol A (BPA), used in the manufacturing of epoxy resins and polycarbonate plastics, is a known EDC of industrial origin.

Although EDCs have been widely recognized as potential environmental pollutants in aquatic and soil environments, solubility data in the literature are highly variable.[1,4−14] For instance, solubilities have been reported over the ranges of (0.8 to 12.4) mg·L^{-1} for estrone [3-hydroxy-1,3,5(10)-estratrien-17-one], (3.1 to 12.96) mg·L^{-1} for 17β-estradiol [3,17β-dihydroxy-1,3,5(10)-estratriene], (3.1 to 19.1) mg·L^{-1} for 17α-ethynylestradiol [17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol], and (109 to 300) mg·L^{-1} for bisphenol A [(2,2-bis(4-hydroxyphenyl)propane)]. In some studies, experimental conditions such as temperature, pH, and ionic strength, which are known to affect the aqueous solubilities of organic compounds,[15] were not reported.

Accurate values of solubilities are important not only for the investigation and modeling of the occurrence and degradation of organic pollutants but also as input data for the estimation of physicochemical parameters such as distribution coefficients (K_{OW} and K_{OC}). The partitioning of estrogens between aqueous and solid phases has been shown to be influenced by their aqueous solubilities.[16] The significance of solubility in environmental modeling may be illustrated by the calculation by Lai and co-workers[5] of sorption coefficients (K_{OC}) from the solubility data of Tabak and co-workers.[1] Had they used the solubility results of Yalkowsky,[7] for example, the calculated sorption coefficients would have been very different.

We are aware of a single report[14] that mentions the effect of pH on the solubility of estradiol but none for the other solutes. Salting out, or decreased solubility in the presence of electrolytes, is a characteristic of a wide range of sparingly soluble organic compounds.[17] It is likely, therefore, that some hydrophobic EDCs are less soluble in saline environments than in pure water, but this does not seem to have been tested.

Because of the paucity of experimental details in the literature, it is difficult to ascertain why there are such large discrepancies between the different solubilities that have been reported. To provide more reliable data, we have measured under well-defined conditions the solubilities of four EDCs: the steroid hormones estrone, estradiol, and ethynylestradiol as well as bisphenol A. The molecular structures and pK_a values of the solutes are shown in Figure 1. Solubilities were measured in water at pH 4, 7, and 10 to cover acidic, neutral, and alkaline conditions and also in (0.01 and 0.1) mol·L^{-1} KNO_3.

Experimental Section

Materials. 17β-Estradiol (E2) [50-28-2] (99.2 % purity), estrone (E1) [53-16-7] (99 % purity), 17α-ethynylestradiol (EE2) [57-63-6] (98 % purity), and bisphenol A (BPA) [80-05-7] (99 % purity) were supplied by Sigma-Aldrich (Melbourne, Australia). The standards were stored in a desiccator in the dark. Analytical reagent grade KNO_3, HCl, KOH, and HNO_3 and HPLC grade methanol and acetonitrile were obtained from Merck (Australia). Milli-Q reagent grade water (Millipore) used for all experimental work including the cleaning of glassware had a conductivity of less than 10 μS m^{-1}.

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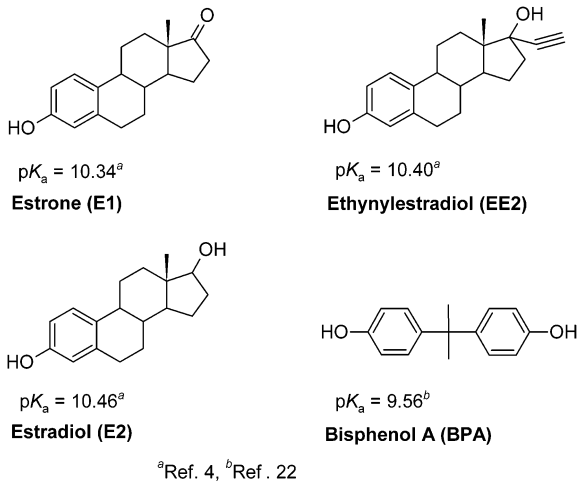

Figure 1. Structures of the steroid hormones and bisphenol A and their pK_a values.

Determination of Solubility. Saturated solutions of each compound under investigation were prepared in 30 mL screw-top borosilicate glass tubes sealed with PTFE-lined caps. Approximately (10 to 20) mg of the solid was weighed into the tubes, and 20 mL of Milli-Q water was added. The quantity of solid was sufficient to be well above the highest reported solubility. The tubes were purged with purified nitrogen, capped, and tumbled end-on-end at approximately 20 rev min^{-1} in a small room maintained at (25 ± 0.5) °C. Initial tests showed that solutions were saturated, giving constant and reproducible solubility data after 4 days. Experiments were conducted at pH 4, 7, and 10, the pH of the suspensions being adjusted as required by the addition of 0.1 mol·L^{-1} KOH or HNO_3. Aqueous 0.01 mol·L^{-1} and 0.1 mol·L^{-1} KNO_3 adjusted to pH 7 were used as solvents in experiments to investigate the effects of ionic strength. After an equilibration period of 4 days, the pH of the samples was measured, and the tubes were left to stand for 1 h. The supernatant solutions were filtered through 0.2 μm glass microfiber filters (Advantec GF-75, 25 mm), with the first 0.5 mL being discarded before analysis by HPLC. Each result presented was obtained from six experiments. Sorption of the compounds to the filters, the walls of the tubes, and the cap liner material was investigated by filtration and analysis of calibration standards and was found to be negligible.

HPLC Analyses. The estrogens and BPA were determined by reverse-phase high-performance liquid chromatography (HPLC) with UV detection at 280 nm (Shimadzu LC-10Ai chromatograph equipped with a Shimadzu SPD-M10AVP photodiode array detector). A Phenomenex Synergy Hydro-RP column (150 mm × 4.6 mm i.d., 4 μm) protected by a guard column with a matching stationary phase was used with a 70:30 acetonitrile/water mobile phase. Six-point linear calibration curves ($r^2 > 0.995$) were obtained from standards prepared by serial dilution of the stock solutions in methanol.

Results and Discussion

Solubility in Water. The solubilities of the four solutes, measured at (25.0 ± 0.5) °C and pH 7, are shown in Table 1, together with selected data from the literature. The wide range of values reported for the aqueous solubilities of these compounds is due, in part, to differences in experimental conditions such as temperature, pH, and ionic strength, which in some studies were not reported. However, even considering these discrepancies, the differences in the relative solubilities of the estrogens are surprising. For example, one study[1] reported that EE2 is the least soluble of the three hormones studied whereas others (including the present one) found it to be the most soluble.

Table 1. Solubilities *S* of Selected Estrogenic Compounds in Water

		S/mg·L^{-1}			
source	t/°C	estrone (E1)	estradiol (E2)	ethynylestradiol (EE2)	bisphenol A (BPA)
this study[a]	25	1.30 ± 0.08	1.51 ± 0.04	9.20 ± 0.09	300 ± 5
ref 10			5	10	
ref 4		0.8	3.9	9.7	
ref 1		12.4	12.96	4.83	
ref 7	25	1.53	3.85	19.1	
ref 11	23	2.1	3.1	3.1	
ref 8	25				120
ref 9	20−25				120−300
ref 12	22−24				253−257
ref 13					109

[a] Standard deviation from six measurements; 25.0 ± 0.5 °C.

Table 2. Variation with pH of the Solubilities S^a of Estrogenic Compounds at (25.0 ± 0.5) °C

	S/mg·L^{-1}			
	estrone (E1)	estradiol (E2)	ethynylestradiol (EE2)	bisphenol A (BPA)
pH 4	1.24 ± 0.06	1.48 ± 0.03	9.10 ± 0.04	298 ± 2
pH 7	1.30 ± 0.08	1.51 ± 0.04	9.20 ± 0.09	300 ± 5
pH 10	2.27 ± 0.08	2.71 ± 0.09	10.80 ± 0.09	319 ± 6

[a] Standard deviation from six measurements.

Preliminary experiments showed that prolonged equilibration of the suspensions was necessary to ensure saturation. After shorter equilibration times (especially < 24 h), the concentrations of the solutes were lower and variable. The reproducibility of the results shown in this and subsequent Tables gives support to the reliability of our experiments.

The order of aqueous solubilities we observed is consistent with the increasing polarities of the estrogens from E1 (one hydroxy group) to E2 (two hydroxy groups) and then EE2 (added ethynyl group at the 17α position on the D ring). BPA, with two phenolic hydroxy groups on a smaller molecule, is expected to be more soluble than the estrogens, confirmed by its much lower K_{OW} reported in the literature.[8,11,18,19]

Effect of pH. In Table 2, we present the solubilities at (25.0 ± 0.5) °C of all four compounds measured at pH 4, 7, and 10. The pK_a values given in Figure 1 are all close to 10. As expected, the solubilities at pH 4 and 7, which are well below the pK_a values, are the same within experimental error, but they are higher at pH 10, when the solutes are significantly ionized.

Schicknuns and Müller-Goymann[14] reported solubilities of E2 (at I = 0.05 mol·L^{-1}) of about (15, 23, and 85) mg·L^{-1} (estimated from the original results given in a bar graph) at pH 7.5, 9, and 11, respectively. Whereas the general trend in solubility is consistent with that of our solubility data, the authors specified neither the time required to form saturated solutions nor the temperature at which the solubility was measured.

The relative increase in solubility at high pH is greatest for the least-soluble compounds, E1 and E2. The relative change in the solubility of BPA is quite small, which suggests that the uncharged diphenolic structure is as readily hydrated as the ionized form.

If the conjugate base A^- of a sparingly soluble weak acid HA is assumed to be completely soluble, then at the pK_a (where the concentrations of HA and A^- are equal) the total solubility should be approximately twice that of the un-ionized form HA, measured at a much lower pH.[20] The observed increases in the

Table 3. Aqueous Solubilities S^a of Estrogenic Compounds in Water and in the Presence of Salt at (25.0 ± 0.5) °C

	S/mg·L^{-1}			
medium	estrone (E1)	estradiol (E2)	ethynylestradiol (EE2)	bisphenol A (BPA)
water	1.30 ± 0.08	1.51 ± 0.04	9.20 ± 0.09	300 ± 5
0.01 mol·L^{-1} KNO_3	0.85 ± 0.02	1.35 ± 0.05	9.10 ± 0.08	301 ± 3
0.1 mol·L^{-1} KNO_3	0.61 ± 0.08	1.10 ± 0.08	8.50 ± 0.08	300 ± 2

[a] Standard deviation from six measurements.

solubilities of E1 and E2 between pH 7 and pH 10 correspond to this prediction, but the corresponding increase is less for EE2. The substantially greater solubility conferred on EE2 by the ethynyl group is probably independent of pH and hence should enhance the solubilities of the ionized and nonionized forms approximately equally. The dominance of this factor over the effect of the ionizable phenolic group probably accounts for the relatively small change in the solubility of EE2 with pH.

Effects of Ionic Strength. To assess whether the solubilities were significantly affected by ionic strength we, compared the solubilities in water with those in neutral solutions of KNO_3 (0.01 mol·L^{-1} and 0.1 mol·L^{-1}). Table 3 shows the results.

There was a pronounced salting out effect with E1, whose solubility was reduced by about 50 % in 0.1 mol·L^{-1} KNO_3, and smaller but significant decreases in the solubilities of the other two estrogens. However, there was very little change in the solubility of BPA with the addition of up to 0.1 mol·L^{-1} KNO_3.

The progressive decrease in the solubilities of the estrogens as the ionic strength increased is consistent with the salting out observed with many organic compounds.[14,17,21] For a 10-fold increase in ionic strength, we observed a (70 to 80) % decrease in the solubility of E2 similar to that reported by Schicksnus and Müller-Goymann.[14] There may be other reasons: for example, Lai and co-workers[5] argued that at higher ionic strength some estrogenic hormones aggregate and flocculate, which would have the effect of reducing their solubility.

The data in Tables 1 to 3 show that the estrogenic hormones and bisphenol A behave quite differently. Not only is BPA much more soluble in pure water, but its solubility is hardly affected by pH or ionic strength, at least within the range of conditions tested in our experiments. By contrast, the hormones follow the trends expected for largely hydrophobic but ionizable organic compounds: their solubilities increase significantly around the pK_a values and decrease as the ionic strength increases.

Finally, we note that the solubilities reported here are for a single temperature, 25 °C. Because temperature has a large influence on the solubilities of most compounds, it is to be hoped that in the future the range of conditions will be extended to both higher and lower temperatures.

Conclusions

A reassessment of the aqueous solubilities at 25 °C of endocrine-disrupting chemicals E1, E2, EE2, and BPA has given a set of data collected under well-defined conditions of temperature, pH, and ionic strength. The aqueous solubilities of estrogens E1, E2, and EE2 were significantly affected by pH and ionic strength.

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