used at each dose level. The effect of first doses were analysed by paired Students's *t*-test. Blood pressure responses to each anorexigenic agent administered repeatedly at the same dose level were subjected to analysis of variance. The compounds were: DITA (3′,4′-dichloro-2-(2-imidazolin-2-yl-thio)-acetophenone hydrobromide, The Dow Chem. Co., Midland, Mich.) and diethylpropion hydrochloride (Tenuate, compliments of the WM. S. Merrel Co. Division of Richardson-Merrel Inc., Cincinnati, Ohio).

Table 1 summarizes the effects of the drugs on pulmonary and systemic arterial blood pressure. It is apparent that the DITA and diethylpropion data do not support linear dose-response relations at the doses used. The pressor response to DITA and diethylpropion were similar in the systemic arterial system. Diethylpropion caused a pressor effect on the pulmonary system a few mm higher than that caused by DITA.

The intravenous administration of diethylpropion caused a transient depressor effect followed by a pressor effect. At 8 mg kg^{-1} it produced an average depressor and pressor response systemically of − 35 and 25 mmHg respectively with a half life duration of 1 and 22 min, respectively (Table 1). With DITA, the first injection of 8 mg kg^{-1} produced only a systemic arterial pressor effect averaging 27 mmHg with a half life duration of 3 min. Subsequent doses of DITA produced biphasic responses similar to diethylpropion.

Tachyphylaxis developed to the systemic and pulmonary arterial pressor effects of the 8 mg kg^{-1} dose of both DITA and diethylpropion (Fig. 1, A and C). Their depressor effects on the systemic arterial blood pressure became more pronounced upon repeated administration (Fig. 1B) but did not cause a significant depressor effect in the pulmonary arterial system (Fig. 1D).

As with (+)-amphetamine, (Abdallah, 1974), tachyphylaxis developed to the pressor effects of DITA and diethylpropion on systemic and pulmonary arterial blood pressure. With aminorex, tachyphylaxis develops only to its pressor effect on systemic arterial pressure but not to its pressor effect on pulmonary arterial pressure (Abdallah, 1974).

The pressor effect of diethylpropion on pulmonary arterial blood pressure was greater overall than that of DITA. This holds true when the compounds are compared to a mg kg^{-1} or μmol kg^{-1} basis. For example, the 2 mg kg^{-1} (10 μ mol kg^{-1}) dose of diethylpropion produced a greater pressor response than the 4 mg kg^{-1} (130 μmol kg^{-1}) dose of DITA.

September 6, 1976

REFERENCES

ABDALLAH, A. H. & WHITE, D. (1974). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **33**, 564.

ABDALLAH, A. H. (1974). *Eur. J. Pharmac.*, **27**, 249–251.

GURTNER, H. P., GERTSCH, M., SALZMANN, C., SCHERRER, M., STUCKI, P. & WYSE, F. (1968). *Schweiz. med. Wschr.*, **98**, 1579–1589; 1695–1707.

PAEPRER, K. (1970). *Arztl. Praxis*, **22**, 1397.

RAU, F. (1969). *Wien. Z. Inn. Med.*, **50**, 506–507.

Adsorption as a possible limitation in solubility determination

S. T. LIU, C. F. CARNEY, A. R. HURWITZ*, *Pharmacy Research and Development, Ayerst Laboratories, Inc., Rouses Point, New York 12979, U.S.A.*

Filtration is an essential step in the determination of solubility. Recently, the most commonly used filters have been cellulosic type membranes. The adsorption of organic compounds by commercial cellulosic (MF–Millipore Filters) filter membranes has been previously reported (Chiou & Smith, 1970; Batra, 1975; Chiou, 1975). The purpose of this study was to determine means to minimize the possible error due to adsorption during solubility determination.

Studies in our laboratory compared adsorption onto two commercial cellulosic filter membranes, I (MF–Millipore) and II (Gelman GA-8) with that onto commercial polycarbonate membrane, III (Nuclepore) or a commercial silver membrane, IV (Flotronics FM 13).

Aqueous suspensions or clear unsaturated solution of drugs had their pH values adjusted with 0·1 N hydrochloric acid or sodium hydroxide. Adsorption onto the filters was assessed after passing successive 5 ml portions of sample through filter membranes held in Pyrex filter holders (Pyrex Microanalysis Filter Holder, Millipore Corp.) or in syringe filter holders (Swinney Filter Holder No. 4310, Gelman Inst.). Filtration time was always kept within 1 min. The percentage adsorption was calcu-

*Correspondence.

lated by dividing the difference between prepared solution concentration and filtrate concentration by the prepared or highest filtrate concentration attained and multiplying by 100. The real contact diameters for 25 and 13 mm filter membranes were 17 and 9 mm respectively. Except where otherwise stated concentrations for all compounds were determined from ultraviolet absorbance values above 240 nm, since above this wavelength water-soluble impurities contained in the filter membranes could be neglected in the analyses. They could be removed when required by prewashing the membrane with 20 ml distilled water.

Table 1 summarizes the results obtained for the adsorption of some acidic, basic and neutral organic compounds onto cellulose acetate membranes. The pH had no significant effect on the extent of adsorption of neutral compounds. The findings of Chiou & Smith (1970) that undissociated acidic compounds were more strongly adsorbed than their ionized forms was confirmed and similarly shown to hold for basic compounds. The relation of compound structure and increased aromatic character to adsorption was demonstrated by comparison of benzocaine to phenothiazine and benzofuran derivatives. Equilibrium adsorption for griseofulvin and warfarin follows Freundlich adsorption (Chiou & Smith, 1970). Results at pH 10 indicated that acetylpromazine gave a Freundlich adsorption isotherm in which the amount adsorbed was directly proportional to the equilibrium concentration. The percentage drug adsorbed onto the filter membrane was 75–82%, essentially independent of the initial concentration over the range 4 to 80 μM.

The degree of adsorption of acetylpromazine onto different types of membrane is shown in Fig. 1. For the same type of membrane, the degree of adsorption increased as the pore size decreased. Filter disc area also had a significant effect on the degree of adsorption. For a particular membrane, significantly less adsorption was observed for the 13 mm disc compared to that for the 25 mm disc. For example, for acetylpromazine at pH 9·65, the first 5 ml filtered through a 25 mm 0·22 μm filter membrane I showed 94% adsorption compared to 52% for a 13 mm disc. Also, saturation of adsorption sites by filtering successive 5 ml portions was more rapidly accomplished for a 13 mm disc than for 25 mm disc.

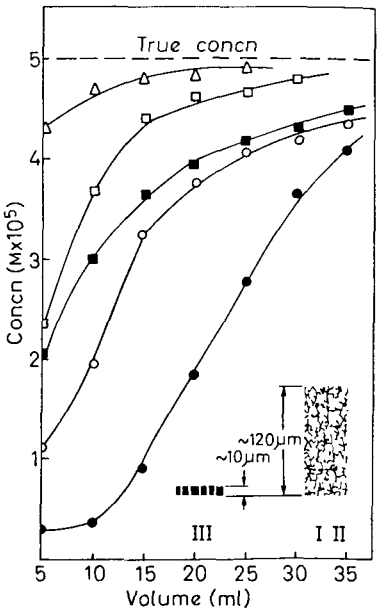

FIG. 1. Comparison of filtration loss of acetylpromazine solution at pH = 9·65; 25 mm 0·22 μm, Filter I ●; 25 mm 0·45 μm, Filter I ○; 13 mm 0·2 μm, Filter II ■; 13 mm 0·22 μm, Filter I □; 13 mm 0·2 μm, Filter III △. Inset shows cross sectional comparison. Ordinate—Concentration of acetylpromazine filtrate. Abscissa—Filtered volume (ml).

Table 1. *Amount (%) adsorbed from 5 ml solution passed through a 25 mm 0·22 μm Millipore cellulose acetate membrane filter (all concentrations 11 to 25 μM).*

Compound	pH	% Adsorbed
Dibenzofuran	—	84
Furobufen (a)	3·70	95
	4·52	77
	6·76	0
Eosin Yellow	3·06	86
	5·16	0
Phenothiazine	—	75
Chlorpromazine (b)	9·20	93
	4·60	9
Acetylpromazine (c)	9·36	94
	6·45	20
	4·52	10
Benzocaine	9·40	8
Griseofulvin	—	26
Medrogestone	—	96

(a) pKa = 4·8 determined by a solubility procedure. (b) pKa = 9·3 (Green, 1967). (c) pKa = 9·3 determined by a solubility procedure.

The data in Fig. 1 compare two cellulosic membranes, I and II, and one polycarbonate membrane, III. Adsorption losses for acetylpromazine at pH 9·65 on the first 5 ml filtration through 13 mm 0·2 μm membranes I, II and III respectively were 52, 59 and 14%. For oestrone the corresponding losses were 20, 49 and 3%. The corresponding losses for medrogestone for membranes I and III were 42 and 21% respectively. Thus significantly less adsorption occurred on the polycarbonate membrane. This was also true for all compounds studied that showed detectable adsorption. The lower adsorption may be due to the basic difference in membrane structure and thickness as shown in Fig. 1. The thinner polycarbonate membrane with straight-through cylindrical pore structure probably offers fewer adsorption sites compared to the cellulose membranes. Adsorption of griseofulvin on the silver

Table 2. *Aqueous solubility for several drugs at 25°.*

Compound	Anal.	Mixing	Solubility in water (μg ml^{-1}) Observed	Lit.
Isosorbide dinitrate	g.c.	Tumbling	550	1089 (a)
Griseofulvin (micronized)	t.l.c.	Stirring	10	8 (b), 10 (c), 142 (d)
Disulfiram	Polarog.	Tumbling	4·09	200 (a)
Diosgenin	g.c.	Tumbling	(0·02)	
Oestrone	g.c.	Stirring	0·80	30 (a)
17-α-Oestradiol	g.c.	Stirring	3·90	3·7 (e), 5 (f), 6·99 (g)
Equilin	g.c.	Stirring	1·41	
Equilenin	g.c.	Stirring	1·52	
Medrogestone (micronized)	u.v.	Stirring	1·82	

(a) Stecher, 1968; (b) Chiou, 1975; (c) Marvel, Schlichting & others, 1964; (d) Sunshine, 1969; (e) Hahnel, 1971; (f) Kabasakalian, Britt & Yudis, 1966; (g) Batra, 1975.

membrane was comparable to that for the cellulose membrane.

Aqueous solubility values were determined for several compounds. Sample suspensions were either tumbled for several days or vigorously stirred magnetically for several hours at 25° to achieve equilibrium. Samples were analysed frequently to ensure equilibration. Reproducibility of the results was within ±5–10%. To minimize adsorption loss and to reduce the volume of suspension required, 13 mm polycarbonate 0·2 μm membranes were used. Successive 5 ml portions of saturated suspension were filtered until the filtrate concentration became constant. The highest constant concentration was recorded as the solubility value. In most cases, the filtrate concentration had attained 95% or more of the final value after filtration of 10 ml. The solubility values obtained are compared with previously reported values in Table 2.

Solubility values higher than those reported in the literature might be expected if adsorption losses had been ignored. However, in general, lower values were observed. An explanation might be that previously reported values were based on determinations made using non-specific analytical procedures. False high solubility values could result from spectrophotometric analysis of materials containing trace amounts of soluble impurities. This was found to be true for griseofulvin and oestrone.

Reliable aqueous solubility values can be assured only when potential adsorption limitations due to membrane filtration are recognized. When sufficient quantities of material are available, several suspension portions can be filtered successively to ensure saturation of adsorption sites in any filter membrane. However, if only limited amounts of materials are available, adsorption can be minimized by employing a small diameter 0·2 μm polycarbonate membrane.

November 12, 1976

REFERENCES

BATRA, S. (1975). *J. Pharm. Pharmac.*, **27**, 777–779.

CHIOU, W. L. & SMITH, L. C. (1970). *J. pharm. Sci.*, **59**, 843–847.

CHIOU, W. L. (1975). *Can. J. pharm. Sci.*, **10**, 112–114.

GREEN, A. L. (1967). *J. Pharm. Pharmac.*, **19**, 10–16.

HAHNEL, R. (1971). *J. Steroid Biochem.*, **2**, 61–65.

KABASAKALIAN, P., BRITT, E. & YUDIS, M. (1966). *J. pharm. Sci.*, **55**, 642.

MARVEL, J. R., SCHLICHTING, D. A., DENTON, C., LEVY, E. J. & CAHN, M. M. (1964). *J. Invest. Derm.*, **42**, 197–204.

STECHER, P. G. (1968). Editor. *Merck Index*, 8th Edition, Rahway, N.J.: Merck and Co.

SUNSHINE, I. (1969). Editor *Handbook of Analytical Toxicology*. Cleveland, Ohio: The Chemical Rubber Co.