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High-Performance Liquid Chromatographic Assay of Methadone, Phencyclidine, and Metabolites by Postcolumn Ion-Pair Extraction and On-Line Fluorescent Detection of the Counterion with Applications

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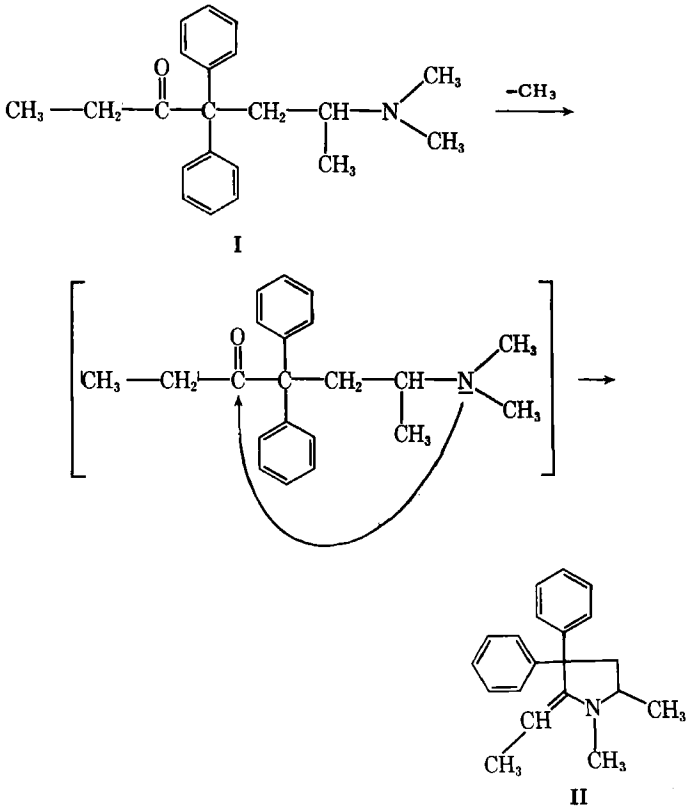
Abstract □ Methadone, phencyclidine, and their metabolites were extracted from plasma and separated on a high-performance liquid chromatographic (HPLC) column using the fluorescent 9,10-dimethoxyanthracene-2-sulfonic acid as a counterion. The chromatographed mobile phase was subsequently extracted on-line with chloroform. The separated organic phase, containing the fluorescent ion-pairs of the investigated amines, was analyzed in the flow cell of a fluorometer (excitation 380 nm, emission 445 nm). The phase separator volume was as small as possible to avoid dead volume. The method was also applied to the bioassay of cocaine with a sensitivity of 1–6 ng/ml of plasma. Application of these assays gave a red blood cell–plasma water partition coefficient for methadone of 3.39 ± 0.26 (*SD*) in a concentration range up to 20 μg/ml, and demonstrated a time-dependent partition with a diffusion half-life of 1.44 min ± 0.26 min (*SD*). The protein binding of methadone determined by ultracentrifugation was concentration dependent and varied between 75–62% at the highest concentration studied (9 μg/ml). The presence of the major metabolite did not have any influence on the protein binding. The results were confirmed by using the red blood cell-partitioning method to determine the protein binding.

Keyphrases □ Phencyclidine—high-performance liquid chromatographic assay of methadone and metabolites by postcolumn ion-pair extraction, on-line fluorescent detection of counterion with applications □ Methadone—high-performance liquid chromatographic assay of phencyclidine and metabolites by postcolumn ion-pair extraction, on-line fluorescent detection of counterion with applications □ High-performance liquid chromatography—assay of methadone, phencyclidine ion-pair extraction of counterion with applications

Classical high-performance liquid chromatography (HPLC) detection of drugs and their metabolites in biological fluids is by spectrophotometry and fluorescence. These methods were expanded by application of electrochemical detectors (1) and derivatization reactions (2) to get high sensitivities. Unfortunately, these direct detection methods are inadequate for some pharmacokinetic studies of drugs that are given in low doses and show low levels in biological fluids. Sensitive HPLC methods have not yet been developed (3) for methadone (I) and its major metabolite, 2-ethylidene-3,3-diphenyl-1,5-dimethylpyrrolidine (II) (Scheme I), nor for phencyclidine (III) and its hydroxylated metabolites (V, VI). These drugs have insufficient UV-absorbance, no fluorescence, and cannot be readily derivatized. Classical carbonyl reactions are not possible with methadone due to the steric effects of the two phenyl rings. Although cocaine (IV) can be spectrophotometrically detected in HPLC, greater sensitivity than the 15 ng/ml reported (4) would be advantageous in forensic and pharmacokinetic studies.



Scheme I—Major pathway of methadone metabolism.

All of these substances are tertiary amines which are readily protonated. They can be extracted into organic

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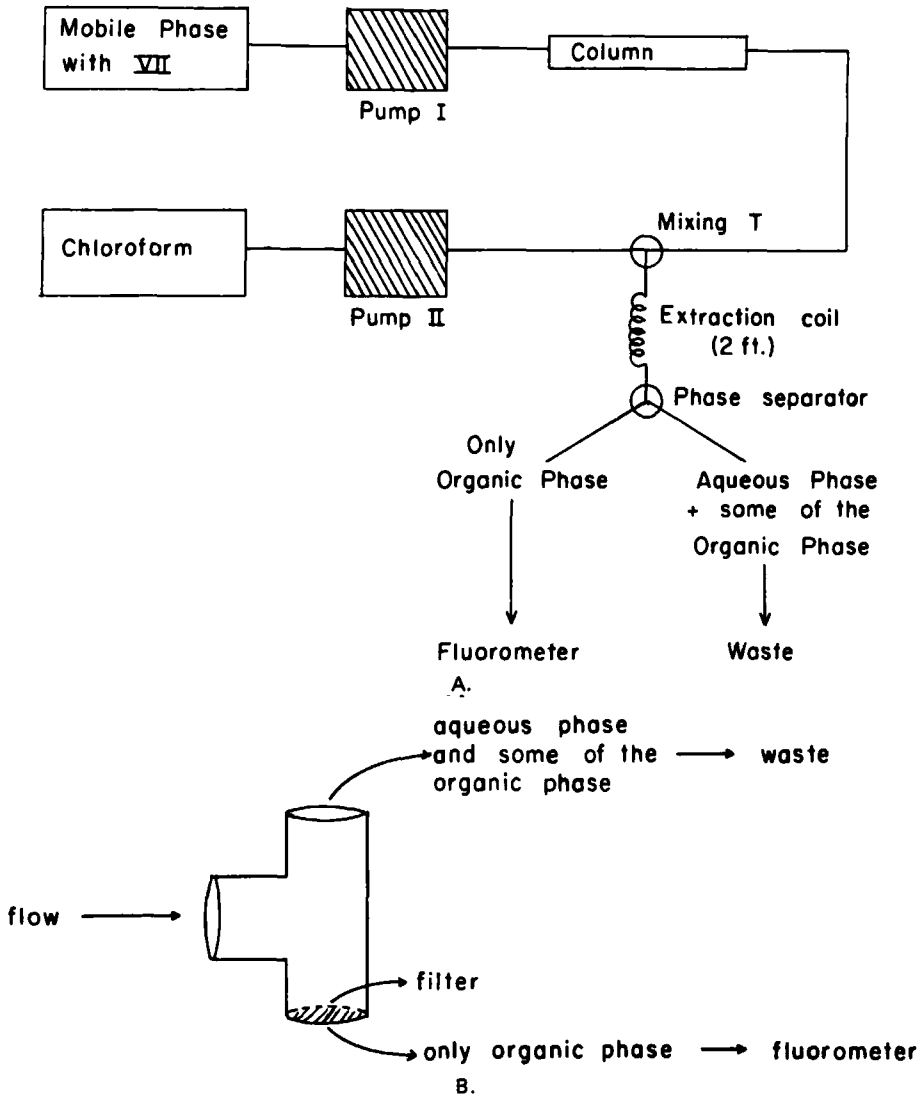
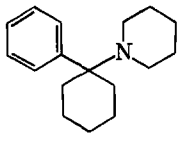


Figure 1—*(A) Schematic representation of HPLC system with postcolumn extraction; (B) phase separator.*

solvents as ion-pairs with aromatic sulfonic acids such as 9,10-dimethoxyanthracene-2-sulfonic acid, (VII). This counterion is highly effective in ion-pair extraction and has a strong fluorescence which can be used for indirect detection of amines with high sensitivity (5–8).



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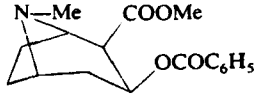

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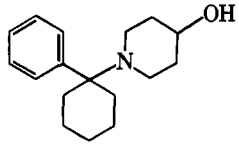

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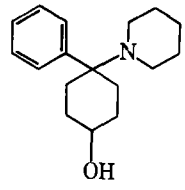
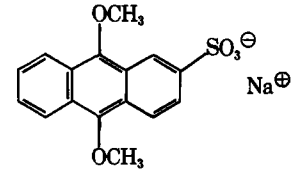


VI

These properties can be utilized after HPLC separation by postcolumn ion-pair extraction and fluorescent detection of the ion-pair. This was described previously (5, 8) where a system was applied to both normal-phase and reverse-phase chromatography. However, a proper phase separator is critical in such systems to provide only one phase for the detector. Commercially available phase separators frequently show significant peak broadening and lessened specificity and sensitivity due to relatively large dead volumes (7). Thus a new phase separator was constructed with a polytef filter and the developed assay systems applied to the determination of the protein binding and red blood cell partitioning of methadone. The plasma protein binding of radiolabeled methadone, reported in the literature, varies widely between 40 and 90% (9–12) with a possible concentration dependence (12).



VII

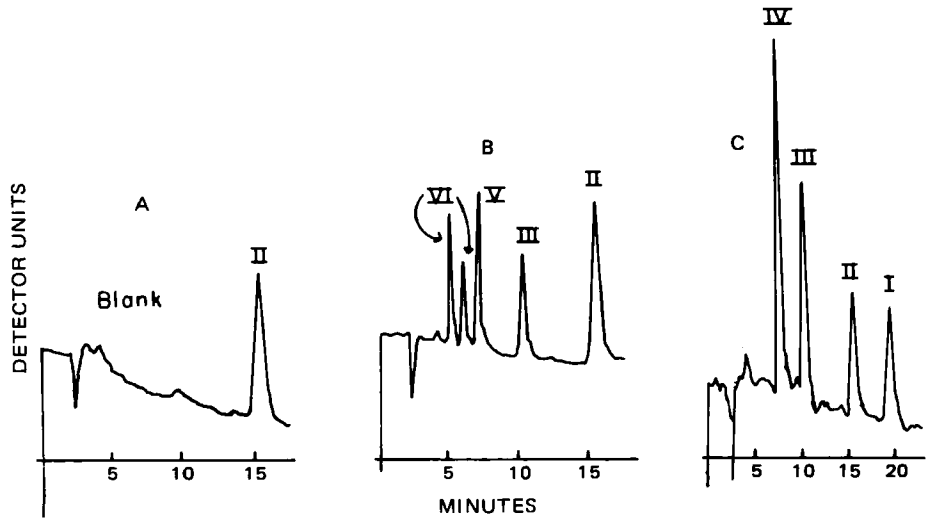


Figure 2—*Chromatograms of 1 ml of plasma alkalinized, extracted with hexane, evaporated to dryness under nitrogen, and reconstituted in 250 μl of 0.025* M *acetate buffer, pH 3.6 with 100 μl injected into the chromatograph. (A) The initial plasma contained 100 ng/ml of internal standard II. (B) The initial plasma contained 50 ng/ml phencyclidine (III), phencyclidine metabolite (V), the* cis- *and* trans-*isomers of phencyclidine metabolite VI, and 100 ng/ml of II as internal standard. (C) The initial plasma contained 60 ng/ml of cocaine (IV), II, methadone (I), and 100 ng/ml of phencyclidine (III) as internal standard.*

EXPERIMENTAL

Materials—The following analytical grade materials were used: sodium acetate[1], acetic acid[1], dibasic and monobasic sodium phosphates[1], sodium chloride[1], and volumetric concentrates of sodium hydroxide[2]. Acetonitrile[3] and chloroform[3] were HPLC-grade; hexane[4] was UV-grade. Methadone[5] (I), 2-ethylidene-3,3-diphenyl-1,5-dimethylpyrrolidine[5] (II), phencyclidine[5] (III), 1-(1-phencyclohexyl)-4-hydroxypiperidine[5] (V) 4-phenyl-4-piperidinocyclohexanol[5] (VI), cocaine[5] (IV), and the sodium salt of 9,10-dimethoxyanthracene-2-sulfonic acid[6] (VII) were used as received. Sodium chloride injection USP[7], sodium heparin injection USP[8], and disposable syringes[9] were used in the preparation of red blood cell suspensions.

Apparatus—For the HPLC assay, the following were used: two pumps[10], an automatic injector[11], a cyano column[12], a fluorescence detector[13], a phase separator constructed from a purchased mixing tee[14] and equipped with a filter made of polytef[15], and a data station[16]. Plasma protein binding was determined with an ultracentrifuge[17]. A laboratory centrifuge[18] was used in the separation of organic extract from plasma and aqueous phases.

HPLC Systems—A postcolumn extraction procedure was used in all HPLC assays (Fig. 1). The mobile phase was a mixture of 0.025 *M* acetate buffer, adjusted to a measured pH of 3.6, and acetonitrile (80:20). It contained 30 mg/liters of 9,10-dimethoxyanthracene-2-sulfonic acid. The flow rate was 2 ml/min with a back pressure of 10 MPa. Immediately after passing the 50°-heated column, the chromatographed mobile phase was mixed with chloroform from another pump at a flow rate of 2 ml/min. The extraction of the ion-pairs into the chloroform was performed in a 0.61-m extraction coil, followed by separation in the constructed phase separator (Fig. 1B).

The phase separator used a mixing tee[14] with upper and lower outlets. It was maintained at a perpendicular angle. Its lower outlet was fitted with a hydrophobic filter made of polytef to increase the efficiency of the phase separation by repelling droplets of the aqueous phase. The back pressures of the two outlets were adjusted so that a constant fraction of the organic phase ran through the lower outlet of the phase separator into the fluorometer with 90% of the extraction mixture going to waste. The excitation wavelength was 380 nm (slit 10 nm), emission 445 nm (slit 5 nm).

Extraction Procedure from Plasma—Freshly prepared dog plasma (1.00 ml) samples were spiked with an appropriate amount of drug and an internal standard. Cocaine, phencyclidine, or its metabolite 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (V) were used as internal standards in the methadone assay. The methadone metabolite (II) was used as an internal standard for the assay of phencyclidine and its metabolites. The plasma was alkalinized with 200 μl of 1 *N* sodium hydroxide and extracted with 6 ml of hexane for 10 min with slow shaking.

The tubes were centrifuged at 2000 rpm for 10 min, and 5 ml of the organic phase was evaporated to dryness under a nitrogen stream. The residue was reconstituted in 250 μl of 0.025 *M* acetate buffer at pH 3.6, and 100 μl was injected into the HPLC system.

Plasma Protein Binding by Ultracentrifugation—Fresh heparinized dog blood was centrifuged for 15 min at 3000 rpm. Plasma aliquots (5 ml) were spiked with different amounts of methadone, and 1 ml was taken for analysis. After ultracentrifugation at 35,000 rpm for 18 hr, 1 ml of the supernatant plasma water was analyzed for methadone.

The influence of the presence of the major metabolite of methadone (II) on the protein binding of methadone was studied in ratios of 1:10, 1:2, 2:1, and 10:1 of II:I.

Red Blood Cell–Buffer Partition Studies of Methadone and its Metabolite (II)—Fresh heparinized dog blood was centrifuged for 15 min at 3000 rpm. The plasma was removed and isoosmotic phosphate buffer pH 7.4, was added to the erythrocytes. Red blood cells were gently suspended and centrifuged for 10 min at 2000 rpm. This washing procedure was repeated three times. Red blood cell suspensions in phosphate buffer were spiked with methadone and its metabolite (II) to yield total drug concentrations in the range of 1–20 μg/ml. The hemocrit was determined routinely using a microcentrifuge with capillary tubes. The spiked suspensions were allowed to equilibrate for 60 min and then centrifuged for 10 min at 2000 rpm. An aliquot of the supernatant solution was analyzed and the red blood cell–buffer partition coefficient was calculated.

Determination of the Protein Binding by Red Blood Cell Partition Method (13)—Fresh heparinized dog blood was spiked with different amounts of methadone, shaken carefully, and centrifuged for 30 min. The plasma concentrations were determined as described previously.

[1] Mallinckrodt Inc., Paris, KY 40361.
[2] Ricca Chemical Co., Arlington, TX 76012.
[3] Fisher Scientific Co., Fair Lawn, NJ 07410.
[4] Burdick & Jackson Laboratories Inc., Muskegon, MI 49442.
[5] National Institute on Drug Abuse, Research Technology Branch, Rockville, MD 20852.
[6] Fluka AG, CH-9470, Buchs, Switzerland.
[7] McGaw Laboratories, Irvine, CA 92714.
[8] The Upjohn Co., Kalamazoo, MI 49001.
[9] Monoject, Division of Sherwood Medical, A. Brunswick Co., St. Louis, MO 63103.
[10] Series 3B Microcomputer Controlled Pump Module, Perkin-Elmer, Norwalk, CT 06856.
[11] Model 420B Auto Sampler, Perkin-Elmer, Norwalk, CT 06856.
[12] CN-μBondapak column, Waters Associates, Milford, MA 01751.
[13] Model 650 Fluorescence Spectrophotometer, Perkin-Elmer, Norwalk, CT 06856.
[14] Tee for 0.8-mm bore tubing, Rainin Instrument Co. Inc., Woburn, MA 01801.
[15] 75-XF pure TFE filter membrane, Laboratory Supplies Co., Chemware Inc., Hicksville, NY 11801.
[16] Model Sigma 15 Data Station, Perkin-Elmer, Norwalk, CT 06856.
[17] Beckman Ultracentrifuge Model LS-50 with rotor Ti 50, Beckman Instruments, Norcross, GA 30092.
[18] Lab centrifuge, International Centrifuge Equipment Co., Needham Hts., MA 02194.

Table I—Statistics of Calibration Curves[a] After Extraction from Plasma (1.00 ml)

Compound	Internal Standard	c_{IS}[b]	m[c]	b[d]	$s_{x,y}$[e]
Methadone	phencyclidine	50.0	131.8	−3.2	3.0
Methadone metabolite (II)	phencyclidine	50.0	109.1	−3.2	3.6
Cocaine	phencyclidine	50.0	36.8	−0.3	0.6
Phencyclidine	II	100.0	83.1	−0.4	3.3
Phencyclidine metabolite (V)	II	100.0	54.6	−2.3	1.8
Phencyclidine metabolite (VIa)	II	100.0	70.7	−7.0	2.9
Phencyclidine metabolite (VIb)	II	100.0	97.3	−1.1	1.1

[a] Concentrations in ng/ml of plasma *versus* peak height ratio relative to internal standard. [b] Concentration of the internal standard in ng/ml. [c] Slope of the calibration curve. [d] Intercept of the calibration curve. [e] Standard error of estimate y on x, concentration in ng/ml plasma on peak height ratio.

Table II—Red Blood Cell–Plasma Partition Coefficients for Methadone at Different Concentrations of Drug

A_{tot}, μg[a]	c_{PW}[b]	V_B[c]	V_{PW}[d]	H[e]	D[f]
45	1.20	18.0	10.80	0.40	3.71
10	1.67	3.5	2.415	0.31	3.29
90	2.66	18.0	11.88	0.34	3.59
20	3.14	3.5	2.415	0.31	3.64
100	4.48	14.0	9.66	0.31	2.92
30	5.01	3.5	2.415	0.31	3.29
180	5.69	18.0	12.06	0.33	3.30
40	6.22	3.5	2.415	0.31	3.70
300	12.71	14.0	9.66	0.31	3.21
500	20.37	14.0	9.66	0.31	3.28
				mean	3.39
				SD	0.26

[a] Total amount of drug added to a red blood cell suspension. [b] Drug concentration (μg/ml) in plasma water. [c] Volume (ml) of the red blood cell suspension. [d] Volume (ml) of the plasma water. [e] Hematocrit. [f] Red blood cell–plasma water partition coefficient.

RESULTS AND DISCUSSION

HPLC System with a Postcolumn Extraction—This HPLC system (Fig. 1) can determine amine concentrations indirectly. Substances are chromatographed as ion-pairs on the column with the ion-pairing VII in the mobile phase used for the detection after coextraction into chloroform in stoichiometric ratios with an organic amine. Stoichiometric highly fluorescent dimethoxyanthracene sulfonic acid of the ion-pairs can be detected with high sensitivity in the flow cell of a fluorometer.

A general and highly sensitive method was developed with the system to analyze methadone, phencyclidine and its metabolites, and cocaine. The designed phase separator (Fig. 1B) has a small inner volume and a hydrophobic filter made of polytef to repel water droplets in the separated organic layer. Such droplets would contain the counterion (VII), which would give irregular and interfering background in the fluorescent detection.

Mobile phase was limited to <20% acetonitrile to maintain a reasonable extractability into the chloroform. However, this lengthened the retention times for methadone and its metabolite (II), on the cyano column, the most polar reverse-phase column available. Column heating to 50° lessened the retention times. The described system and conditions were applicable for all the studied compounds with retention times: methadone (I), 17 min; methadone metabolite (II), 14 min; phencyclidine (III), 10 min; phencyclidine metabolite (V), 7 min; phencyclidine metabolite (VI), 5 and 6 min (*cis* and *trans* forms); cocaine (IV), 6 min.

In the chromatogram the background from biological fluids was satisfactory when hexane was used for extraction (Fig. 2A); however, it was unsatisfactory when chloroform was used for extraction. Typical chromatograms of substances at 50–60 ng/ml in plasma are shown in Figs. 2B and 2C. Calibration curves were set up to determine the sensitivity of the method. Statistics for calibration curves of the substances are given in Table I. The limit of sensitivity for all compounds was in the range 1.0–6.0 ng/ml of plasma ($2 \times s_{xy}$).

Determination of the Red Blood Cell–Plasma Water Partition Coefficient—The red blood cell partition coefficient of a drug can be defined as:

$$D = \frac{C_{RBC}}{C_{PW}} = \frac{A_{RBC}}{A_{PW}} \times \frac{V_{PW}}{(V_B - V_{PW})} = \frac{(A_{tot} - A_{PW})V_{PW}}{A_{PW}(V_B - V_{PW})} = \frac{A_{tot} - A_{PW}}{C_{PW}(V_B - V_{PW})} = \frac{A_{tot} - C_{PW}V_{PW}}{C_{PW}(V_B - V_{PW})} \quad \text{(Eq. 1)}$$

where D is the red blood cell partition coefficient, C_{RBC} is the concentration of the drug in the erythrocytes, C_{PW} is the concentration in plasma water, A_{RBC} is the amount of drug in the erythrocytes, A_{PW} is the amount in plasma water, A_{tot} is the total amount of drug added to the red blood cell suspension, V_B is the volume of the red blood cell suspension, and V_{PW} is the volume of plasma water.

Red blood cell–plasma water partition coefficients were determined for 10 different concentrations of methadone (Table II) and averaged D = 3.39 ± 0.26 (*SD*). There was no concentration dependence for partitioning in the studied concentration range up to 20 μg/ml.

The partitioning was time dependent. Diffusion half-life into the red blood cells was determined for three different concentrations and the average was 1.44 ± 0.26 (*SD*) min (Fig. 3).

Protein Binding of Methadone by Ultracentrifugation—The ultrafiltration method for the determination of the protein binding of

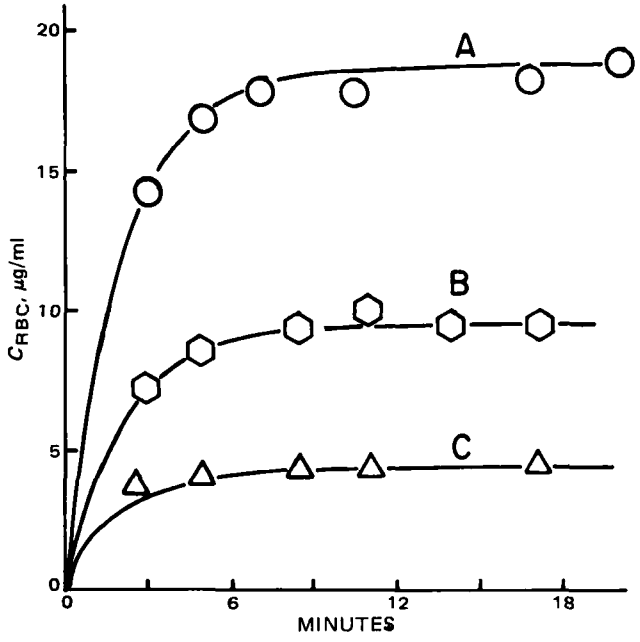


Figure 3—*Time dependence of the red blood cell partitioning in three different concentrations of methadone. The curves through the experimental values, concentrations of drug in red blood cells (C_{RBC}) over time, were fitted for a diffusion half-life of 1.44 min. The concentrations of methadone in plasma water were: (A) 5.7 μg/ml; (B) 2.7 μg/ml; (C) 1.2 μg/ml.*

Table III—Percent Protein Binding of Methadone by the Ultracentrifugation Method

c_{pre}[a]	c_{post}[b]	r[c]	V_{dil}[d]	m[e]	$f_b \times 100$, %[f]
99.9	27.2	0.272	0.005	1.001	72.8
199.6	59.1	0.296	0.010	1.002	70.4
497.5	124.1	0.249	0.025	1.005	75.2
990.1	342.6	0.346	0.050	1.010	65.6
1960.0	677.5	0.346	0.100	1.020	65.8
4760.0	1870.0	0.393	0.250	1.050	61.9
9090.0	3636.0	0.400	0.500	1.100	62.3

[a] Concentration of methadone in plasma before ultracentrifugation in ng/ml. [b] Concentration of methadone in plasma water after ultracentrifugation in ng/ml. [c] Ratio between the concentrations after and before ultracentrifugation. [d] Volume in ml of spiked solution that was added to 5 ml of plasma. [e] Dilution factor $(5 + V_{dil})/5$. [f] Percentage of methadone bound to plasma proteins.

Table IV—Protein Binding of Methadone in Presence of its Major Metabolite (II) at Different Drug–Metabolite Ratios

R[a]	c_{pre}[b]	c_{post}[c]	r[d]	V_{dil}[e]	m[f]	$f_b \times 100$, %[g]
10.5	1.996	0.679	0.340	0.11	1.022	66.9
10.5	1.996	0.717	0.359	0.11	1.022	65.0
2.0	1.942	0.743	0.383	0.15	1.030	62.9
2.0	1.942	0.623	0.321	0.15	1.030	69.0
0.5	1.887	0.511	0.271	0.30	1.060	74.4
0.5	1.887	0.684	0.362	0.30	1.060	65.5
0.1	1.640	0.656	0.400	1.10	1.220	64.7
0.1	1.640	0.622	0.379	0.10	1.220	66.7

[a] Drug–metabolite ratio for 2 μg of methadone/ml of plasma. [b] Concentration of methadone in plasma before ultracentrifugation in μg/ml. [c] Concentration of methadone in plasma water after ultracentrifugation in μg/ml. [d] Ratio between the concentrations after and before ultracentrifugation. [e] Volume of ml of spiked solution that was added to 5 ml of plasma. [f] Dilution factor $(5 + V_{dil})/5$. [g] Percentage of methadone bound to plasma proteins.

methadone was not useful because of high binding to the ultrafilters. Therefore, the protein binding was determined by the ultracentrifugation method, where methadone-spiked plasma was separated into plasma proteins and plasma water and the plasma water was assayed. The calculation of the fraction of drug bound to the plasma proteins was based on the following principles.

The equilibrium constant between protein-bound methadone and methadone in the plasma water can be defined as:

$$K = \frac{c_b}{c_f} = \frac{A_b}{A_f} \times \frac{V_{PW}}{A_{Pr}} = \frac{A_b}{A_f} \times \frac{V_{PW}}{c_{Pr} V_{PW}} = \frac{A_b}{A_f c_{Pr}} \qquad \text{(Eq. 2)}$$

where c_b is the concentration of protein-bound methadone, c_f is the concentration of the free methadone in the plasma water, c_{Pr} is the protein concentration of the plasma, A_b is the amount of methadone bound to the protein, A_f is the amount of methadone free in the plasma water, A_{Pr} is the amount of protein in the plasma water, and V_{PW} the volume of the plasma water. If the protein concentration in plasma is constant:

$$K_s = K c_{Pr} \qquad \text{(Eq. 3)}$$

then,

$$K_s = \frac{A_b}{A_f} \qquad \text{(Eq. 4)}$$

Since the plasma is slightly diluted by a volume V_{dil} of the spiking solution, the dilution factor is:

$$m = \frac{V_{PW} + V_{dil}}{V_{PW}} \qquad \text{(Eq. 5)}$$

and the protein concentration after spiking is:

$$c'_{Pr} = \frac{C_{Pr}}{m} \qquad \text{(Eq. 6)}$$

Thus, the equilibrium in the diluted plasma is:

$$K = \frac{c'_b}{c'_f} = \frac{A'_b}{A'_f c'_{Pr}} = \frac{A'_b m}{A'_f C_{Pr}} \qquad \text{(Eq. 7)}$$

Table V—Plasma Protein Binding of Methadone by the Red Blood Cell–Plasma Partition Method[a]

A_{tot}[b]	V_B[c]	H[d]	c_{Pl}[e]	$f_b \times 100$, %[f]
10.0	4.5	0.49	1.90	63.1
20.0	4.5	0.49	4.05	67.2
30.0	4.5	0.49	5.79	64.1

[a] The red blood cell–plasma water partition coefficient used was 3.39. [b] Total amount of methadone added to the blood in mg. [c] Volume of blood in ml. [d] Hematocrit. [e] Concentration of methadone in plasma in μg/ml. [f] Percentage of methadone bound to plasma proteins.

and

$$K_s = m \frac{A'_b}{A'_f} \qquad \text{(Eq. 8)}$$

where the primed values are the amounts in the diluted sample studies.

Methadone concentrations were assayed before (c_{pre} in plasma) and after ultracentrifugation (c_{post} in plasma water). The ratios of these two concentrations are:

$$r = \frac{c_{post}}{c_{pre}} = \frac{A'_f}{A'_f + A'_b} \qquad \text{(Eq. 9)}$$

Thus, from Eqs. 8 and 9:

$$K_s = m \frac{1 - r}{r} \qquad \text{(Eq. 10)}$$

The fraction of methadone bound to the plasma proteins, f_b, is

$$f_b = \frac{A_b}{A_b + A_f} \text{ or } \frac{1}{f_b} = 1 + \frac{A_f}{A_b} \qquad \text{(Eq. 11)}$$

so that on realization of Eqs. 4, 10, and 11, the fraction bound is

$$f_b = \frac{m(1 - r)}{r + m(1 - r)} \qquad \text{(Eq. 12)}$$

and can be calculated from the ratio of the methadone concentrations before and after ultracentrifugation. The results are summarized in Table III. Protein binding of methadone was concentration dependent in agreement with the previous studies by equilibrium dialysis (12) of 100–10,000 ng/ml of plasma. Protein binding varied 75–62% to give the low value at the highest 9 μg/ml of plasma studied.

The determination of the protein binding of methadone was repeated in the presence of different amounts of its major metabolite (II). Ratios, R, between parent drug and metabolite were 10:1, 2:1, 1:2, and 1:10 for a methadone concentration of 2 μg/ml of plasma. The results in Table IV indicate no significant effect of a 10-fold greater concentration of metabolite on the protein binding of methadone.

Protein Binding of Methadone by Red Blood Cell–Plasma Partitioning—Protein binding of a drug can be calculated from the known or assayed concentrations in blood and plasma after equilibration on the premise that only free drug can partition into the erythrocytes. The fraction of the drug bound to the plasma proteins can be calculated (13) from:

$$f_b = 1 - \frac{A_{tot} - c_{Pl} V_b (1 - H)}{D c_{Pl} V_b (1 - H)} \left(\frac{1}{H} - 1 \right) \qquad \text{(Eq. 13)}$$

where A_{tot} is the total amount of drug added to the volume V_b of the blood, D is the red blood cell–plasma water partition coefficient, H the hematocrit, and C_{pl} the concentration of the drug in the plasma. The

results in Table V agree with the results that were obtained by the ultracentrifugation method. Significant amounts of methadone are bound to the plasma proteins in the therapeutic range. The results confirm previous data (12), but disagree markedly from the results of other investigators (9, 10).

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Physical Chemistry of Freeze-drying: Measurement of Sublimation Rates for Frozen Aqueous Solutions by a Microbalance Technique

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Abstract □ The sublimation rate of frozen solutions was studied as a function of freezing rate, thickness of dried product (l), temperature, residual air pressure, and solute concentration. Data are presented for pure water, aqueous potassium chloride, aqueous povidone, and aqueous dobutamine hydrochloride–mannitol (System I). The resistance of the dried product to water vapor flow ($\hat{R}_p$) was evaluated from the sublimation rate and the sample temperature. The primary experimental technique was based on freeze-drying a cylindrical microsample isothermally, with the sample suspended from one arm of a vacuum microbalance. Methodology to evaluate resistance data from vial freeze-drying experiments is also described. In separate experiments, samples in the form of a thin (15-μm) film were visually observed through a microscope during freeze-drying. Freeze-drying of most samples appeared to occur by water vapor escaping through open channels created by prior sublimation of ice. Contrary to the usual theoretical model, $\hat{R}_p$ is neither independent of temperature nor directly proportional to l. Rather, $\hat{R}_p$ decreases with increasing temperature and the l dependence is normally of the form $\hat{R}_p = (A_0 + A_1 l)/(1 + A_2 l)$, where A_i ($i = 0, 1, 2$) are constants. In several cases, $\hat{R}_p$ is very large near $l = 0$, decreases sharply at $l \simeq 0.1$ cm, and obeys the above equation where $l > 0.2$ cm, a result suggesting an amorphous surface skin which cracks on desorption of water. The temperature dependence of $\hat{R}_p$ suggests that, as the sample temperature approaches the eutectic (or collapse) temperature, hydrodynamic surface flow of adsorbed water is an important flow mechanism.

Keyphrases □ Sublimation rate—measurement for frozen aqueous solutions, microbalance technique, as a function of process variables □ Freeze-drying—methodology of rate measurements for aqueous solutions, influence of process variables, mechanisms of mass transfer in the dried solid □ Dosage forms—freeze-drying of aqueous solutions, effect of process variables on sublimation rate, mechanisms for mass transfer through the dried solid

As sublimation of ice proceeds during freeze-drying, a dried product layer above the ice is produced which acts as a barrier, or resistance, for transport of water vapor. The dried product resistance is generally regarded as the most important factor in determining the drying rate at fixed sample temperature and therefore has a major impact on the process economics. However, published experimental data are confined to food products or biological tissue samples, and the effects of major process or formulation variables on the dried product resistance have not been studied.

This report describes the direct experimental determination of the resistance of the dried product as a function of freezing rate, thickness of dried product, temperature, residual air pressure, and solute concentration. Data are presented for aqueous potassium chloride, aqueous povidone, and aqueous dobutamine hydrochloride–mannitol in a 1.12:1 weight ratio (System I)[1]. Sublimation rate data for pure water are also presented. To aid in the interpretation of the resistance data, the freeze-drying process was also observed microscopically using thin (~15-μm) samples confined between two glass coverslips. Procedures for determining the dried product resistance from vial freeze-drying studies are also described. Comparison of resistance data determined from vial freeze-drying with corresponding data determined by the microbalance procedure confirms that microbalance data are indeed predictive of the dried product resistance encountered in vial freeze-drying.

BACKGROUND

Freeze-drying, or lyophilization, is a process where a solvent (normally water) is removed from a frozen solution by sublimation. Freeze-drying has several advantages over competing processes for production of pharmaceuticals. First, as freeze-drying is a low temperature process, chemical decomposition is minimized. Second, since the solution may be sterile filtered immediately before introduction into a vial and no powder-handling steps are involved in the subsequent processing of a parenteral product, particulate levels may be reduced to a minimum. Due to the high capital equipment costs and typically long processing times, freeze-drying is frequently regarded as an expensive process. However, due to a lack of fundamental understanding of freeze-drying, a given process is often not optimized for the maximum production rate consistent with product quality. Consequently, freeze-drying, in practice, often is much more expensive than necessary.

[1] DOBUTREX, Eli Lilly and Co., Indianapolis, Ind.

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