Comparison of Plasma Concentrations of Warfarin Measured by Both Simple Extraction and TLC Methods

P. G. WELLING, K. P. LEE, URMILLA KHANNA*, and J. G. WAGNER

Abstract □ Human volunteers received 25-mg. single oral doses of sodium warfarin. Ninety-three plasma samples were independently assayed by: (*a*) a simple extraction method, and (*b*) a TLC method using a UV instead of a fluorescent end-point. Both assays employed 7.5-cm. pathlength cells and the Cary 14 UV spectrophotometer. The assays were concluded to be equivalent and to measure only unchanged warfarin after single doses, since: (*a*) the least-squares line forced through the origin when assays by the extraction method were plotted against assays by the TLC method had a slope of 1.00; (*b*) of the differences (TLC − extraction), 44 were positive, 2 were zero, and 47 were negative; and (*c*) average elimination half-lives of warfarin estimated from terminal plasma concentrations measured by the two methods were not significantly different. The absolute value of the differences averaged 0.17 mcg./ml. with a *SD* of 0.14 mcg./ml. One may assume that each assay had an average deviation of 0.085 mcg./ml. from the "true" warfarin concentration and that the deviation was independent of the plasma concentration in the range studied (0.1–4.5 mcg./ml.).

Keyphrases □ Warfarin, plasma determination—method comparison □ Extraction procedure—warfarin determination in plasma □ TLC—warfarin determination in plasma □ UV spectrophotometry—analysis

An analytical method, developed by O'Reilly *et al.* (1), for the measurement of unchanged warfarin in plasma has been used by several investigators (2–6) to study the absorption, distribution, and metabolism of this compound and to correlate prothrombin times with plasma concentrations of the drug. Although other analytical techniques have been used (4, 7), the O'Reilly assay, involving a series of extractions and UV determination, has proved to be the simplest and most reliable procedure.

Recently, Lewis and Ilnicki (8) published, in abstract form, data suggesting that the O'Reilly assay was not specific for unchanged warfarin in plasma. TLC evidence was mentioned, indicating that two other compounds, presumably warfarin metabolites, could be detected at a stage in the assay which necessitated their being included in the final estimation. The implication of this report is clear and throws some doubt on the validity of all data using the original assay.

Interest in the pharmacokinetics of warfarin made it necessary to investigate this problem further. The data reported here are part of a more detailed study, the results of which will be published later. The objects of this particular investigation were: (*a*) to increase the sensitivity of the existing O'Reilly assay by variation of plasma and solvent volumes and measuring techniques in order to measure lower plasma drug concentrations, and (*b*) to develop a new assay, based on the abstract of Lewis and Ilnicki (8), specific for unchanged warfarin and equally as sensitive as the modified O'Reilly assay. Thus, the second assay would assess the validity of the first.

EXPERIMENTAL

Assay Methods—*Modified O'Reilly Assay*—After some experimentation, the following procedure was found to give reproducible linear absorbance-to-concentration ratios over the required plasma concentration range (0.1–4.5 mcg./ml.).

To 1,2-ethylenedichloride[1] (EDC, reagent grade, 20 ml.) in a square-sided 50-ml. bottle, fitted with a polyethylene-lined screw cap, were added plasma (4 ml.), distilled water (2 ml.), and 3 *N* hydrochloric acid (1 ml.). The bottle was tightly closed and horizontally agitated in a Kahn shaker for 10 min. The contents were then poured into a 125-ml. separator fitted with a Teflon tap. The bottle was allowed to drain into the funnel for 30 min., which was sufficient time for separation of the organic phase from the emulsion. The EDC layer was drained into a second separator, to which was then added 0.1 *M* phosphate buffer, pH 7.2 (5 ml.). After shaking for 4 min. and allowing 4 min. for phase separation, the EDC layer was run off. Exactly 15 ml. of this washed EDC extract was added to a third separator, shaken for 4 min. with 2.5 *N* sodium hydroxide (5 ml.), and allowed to stand for 4 min.

The EDC layer was discarded, and the alkaline aqueous phase containing sodium warfarin was poured into a 15-ml. centrifuge tube. This was centrifuged at 1500 r.p.m. for 5 min. to separate residual EDC; the absorbance of the clear aqueous solution was read by scanning in a 7.5-cm. pathlength cell from 380 to 280 mμ in a Cary 14 spectrophotometer.

The subject's zero-hour plasma was carried through each assay batch and was used as the reference solution in the spectrophotometer. Pooled plasma, from the hospital blood bank, was spiked with warfarin (0.5 ml. of a 40 mcg./ml. aqueous solution per 9.5 ml. of plasma) to a concentration of 2.0 mcg./ml. This was included in each assay batch and measured against unspiked blood bank plasma to determine assay reproducibility.

In this and in the TLC assay, a baseline drift was observed when scanning from 360 to 308 mμ, giving an average net absorbance (A_{308}–A_{360}) of 0.025 (*SD* = 0.004). This figure, hereafter referred to as "cell correction," was deducted from all net absorbance readings. The baseline drift is presumably due to the cell composition or the long pathlength used and not to their being unmatched, because the drift remained constant on reversing the cell positions.

Plasma concentrations of warfarin were calculated using Eq. 1:[2]

$$\text{plasma concentration} = \frac{A_{308}-A_{360} - (\text{cell correction})}{0.183} \quad \text{(Eq. 1)}$$

TLC Assay—This assay is similar to that reported by Lewis and Ilnicki (8). Estimation of warfarin by UV absorption was, however, substituted for fluorescence. The problem of fluorescence fading with exposure of acetone solutions of warfarin to UV light and the consequent difficulty in reassaying samples were therefore avoided [Corn and Berberich (7)]. At the plasma concentrations used in this study, the fluorescence technique seemed to offer no advantage over the method used.

Methodology in this assay was the same as in the modified O'Reilly assay up to the point of obtaining the 15 ml. of washed EDC extract.

Specificity was achieved in this assay by separating warfarin from other EDC extractable material, present after washing with phosphate buffer, by TLC. In all steps subsequent to obtaining 15 ml. of buffer-washed EDC extracts, contact of warfarin solutions with light was minimized. Glass containers were wrapped in aluminum foil. Volume reduction of solutions under nitrogen and plate develop-

[1] Eastman.
[2] For the derivation of these constants, see Tables I, II, and IV.

Table I—Statistics of Beer's Law Plots for Runs with Human Plasma Spiked with Warfarin[a]

Day	No. of[b] Samples	Statistics of Least-Squares Line Free to Pass through Any Intercept: Slope	*SE* of Slope	Intercept	*SE* of Intercept	Correlation Coefficient	Slope of Least-Squares Line Forced through the Origin
1	6	0.168	0.0128	−0.006[c]	0.0288	0.989	0.166
2	6	0.181	0.0029	−0.010[c]	0.0054	0.9995	0.178
3	6	0.180	0.0029	−0.007[c]	0.0054	0.9995	0.177
4	6	0.200	0.0032	−0.024	0.0072	0.9995	0.191
5	5	0.177	0.0028	−0.002[c]	0.0064	0.9996	0.185
6	5	0.183	0.0044	−0.002[c]	0.0107	0.9992	0.182
	Av.	0.182					0.180[d]
	SE	0.0043					0.0035
	95% *CI*	0.171 to 0.193					0.171 to 0.189
	Pooled data	0.182	0.0031	−0.009[c]	0.0067	0.9954	0.179

[a] Using 7.5-cm. pathlength cells in the Cary 14 recording spectrophotometer (modified O'Reilly assay method). [b] Concentrations used were 0.1, 0.2, 0.5, 1, 2, 3, and 4 mcg. warfarin/ml. [c] Not significantly different from zero. [d] Average not including Day 1 (which was inordinately low) is 0.183.

ment were done in the absence of light, and application of solutions to TLC plates was done in a minimum of light.

It has been shown in this laboratory that EDC and acetone solutions of warfarin are light sensitive. Reproducible results cannot be obtained in this assay, in which warfarin must remain in the solution for longer periods, unless these precautions are taken.

The 15-ml. washed EDC extract was reduced to dryness in a 50-ml. wide-mouthed centrifuge tube at room temperature under nitrogen. The tube was washed down with 3, 2, and 1 ml. of EDC and taken to dryness each time to concentrate material at the bottom of the tube. The contents were redissolved in a minimum volume of EDC, quantitatively transferred by capillary tube to 250-μ thick silica gel GF 254[3] chromatography plates (20 × 20 cm.), and developed in EDC–acetone (9:1).

Warfarin could be clearly detected at the lowest plasma concentration by its quenching of plate fluorescence under a wide-range UV lamp. The use of quenching on fluorescing plates is more sensitive than warfarin fluorescence on normal plates and eliminates the need to expose the plates to ammonia vapor before detection.

The spot at R_f 0.50–0.54 due to warfarin was scraped off into a 15-ml. centrifuge tube with a small spatula and eluted by agitating with acetone (3 × 1.5 ml.) on a vortex mixer, centrifuging at 2000 r.p.m., decanting, and finally washing the walls of the tube down with 1 ml. of acetone, centrifuging, and decanting again. Plate areas from blank plasmas at identical R_f values to warfarin were identically treated to yield reference solutions in the spectrophotometer.

The acetone solution was taken to dryness under nitrogen at room temperature. The warfarin was redissolved by vigorous agitation in 2.5 *N* sodium hydroxide (5 ml.) for 10 min. on a vortex mixer. After centrifuging at 2000 r.p.m. to precipitate any remaining silica gel, the clear supernatant was measured as before and the plasma warfarin concentration was calculated using Eq. 2:[2]

$$\text{plasma concentration} = \frac{A_{308}-A_{360} - (\text{cell correction})}{0.167} \quad \text{(Eq. 2)}$$

In the use of both assays, all the plasma samples from one phase of the study for a particular subject were assayed at the same time.

The developed chromatograms from dosed subjects were identical to those from warfarin-spiked plasma. Spots at R_f 0.91, 0.85, and 0.25 were identical to blank plasma in all cases. A spot at R_f 0.1 was also observed in blank plasma in 18 out of 24 cases. No other spots could be detected.

The 4′—OH, 6—OH, and 7—OH metabolites have R_f values of 0.22, 0.18, and 0.23, respectively, in this system. These R_f values are not affected when chromatographing the metabolites in combination with the parent drug.

Assay Standardization—On six different days, human plasma was spiked with warfarin at 0.1–4.0 mcg./ml. concentrations. The absorbances obtained in the modified O'Reilly assay gave rise to the statistics given in Table I. Multiple samples of plasma spiked at one concentration only were also assayed using 1-cm. cells in a Beckman DB recording spectrophotometer. The results of these assays, and also equivalent figures for a 7.5-cm. light path, are given in Table II.

As a consequence of these data, it was decided to divide the observed net absorbance of final extracts in the modified O'Reilly assay by 0.183 to obtain the plasma warfarin concentration.

Table III lists recoveries of warfarin from thin-layer plates at five different concentrations on 4 separate days. The average recovery was 91.7%. Therefore, the expected net absorbance/concentration ratio in the TLC assay is 0.183 × 0.917 = 0.168. The actual ratio obtained when pooled plasma was spiked with warfarin was 0.167 (Table IV). Therefore, the figure 0.167 was substituted for 0.183 in the TLC assay.

In both assays the sensitivity compared to the original O'Reilly assay (1) has been increased by a factor of

$$\frac{4}{2} \times \frac{7.5}{1} \times \frac{4}{5} = 12 \quad \text{(Eq. 3)}$$

where the fractions represent plasma volumes, cell pathlengths, and sodium hydroxide volumes, respectively.

Clinical Study—Six healthy adult human volunteers, four male and two female, weighing between 120 and 200 lb. and between 21 and 30 years of age, were selected. All had normal vital signs and screening laboratory values. No barbiturates or other enzyme-inducing agents were permitted to be taken for 30 days preceding initiation of the study. No medication, apart from warfarin and menadiol diphosphate (tetrasodium salt),[4] was permitted for a period of 7 days before commencement to the end of the study. The study was arranged as a two-phase crossover design. In each phase each subject ingested orally a 25-mg. dose of warfarin sodium as five 5-mg. tablets or one 25-mg. tablet. The tablets were swallowed whole. Subjects were fasted overnight and for 4 hr. postadministration of drug. Eight fluid ounces of water was taken within 1 hr. after arising and also when the tablets were ingested. No other beverage, water, or food was taken until 4 hr. after dosage. After this period, food and liquids were allowed *ad libitum*.

Twenty milliliters of blood was taken from a forearm vein just before dosing (0 hr.) and at 1, 4, 8, 12, 24, 48, 72, and 96 hr. after dosing. The blood was drawn into one or two Vacutainer tubes containing EDTA as the anticoagulant. After centrifuging, the plasma was aspirated off into a second tube and quick-frozen at −18° until required.

To reduce the possibility of hemorrhage, each subject was administered a 10-mg. dose of menadiol diphosphate (2 × 5-mg. tablets) 1 day before, on the same day, and 3 days following warfarin dosage in each phase of the crossover study. This compound and its metabolites have been shown in this laboratory not to interfere with the O'Reilly or the TLC assay for warfarin. The plasma concentrations, arranged according to the experimental design, will be reported in a forthcoming publication.

Materials—As a standard, USP reference standard warfarin acid was solubilized as the sodium salt by dissolving in 0.1 *M* phosphate buffer, pH 7.5. A stock solution of concentration 100 mcg./ml. was kept refrigerated, and aliquots were taken and brought to room temperature before use. Plasma used for assay standardization and for controls was obtained from the blood bank, University of Michigan Medical Center.

[3] Merck & Co., Inc., Rahway, NJ 07065

[4] Synkayvite, Hoffmann-La Roche, Nutley, NJ 07110

Table II—Net Absorbance–Concentration Ratios (A_N/C) Observed on Different Days with Modified O'Reilly Assay[a]

Day (No. of Samples)	Conditions				A_N/C		
	Plasma Vol., ml.	EDC Vol., ml.	NaOH Vol., ml.	Warfarin Concn., mcg./ml.	Observed Average	Coefficient of Variation, %	Expected Average A_N/C for 7.5-cm. Cell[b]
1(5)	4	20	4	5	0.0305	4.52	0.183
2(5)	4	20	4	10	0.0261	2.61	0.157
3(5)	4	20	4	10	0.0320	3.53	0.192
4(5)	4	20	4	10	0.0308	1.66	0.185
5(3)	4	10	4	5	0.0339	1.36	0.203
6(4)	4	20	2	5	0.0634	1.74	0.190
7(5)	4	20	2	5	0.0607	1.29	0.182
8(5)[c]	4	20	2	5	0.0580	1.03	0.174
						Av.	0.183

[a] Using 1-cm. pathlength cells in a Beckman DB spectrophotometer. [b] Expected average A_N/C value for the final assay adopted (4 ml. plasma, 20 ml. EDC, backextracted into 5 ml. NaOH, and absorbance read in 7.5-cm. pathlength cells) based on results obtained. [c] Assays on Day 8 were run by a different analyst than those on Days 1–7.

4′—OH, 6—OH, and 7—OH metabolites of warfarin were donated.[5] All other chemicals and solvents were reagent grade and were not further purified before use.

RESULTS

Because this report is concerned only with the sensitivity and comparison of results of two methods of assay, no specification of plasma levels of warfarin obtained from the five 5-mg. or the one 25-mg. dosage is made here.

In the modified O'Reilly assay, the average net absorbance ($A_{308} - A_{360}$ − cell correction) of final extracts from subjects' zero-hour plasmas, using water in the reference cell, was 0.216 with a *SD* of 0.056. When corrected for cell pathlength and plasma and sodium hydroxide solution volumes, this figure is equivalent to an average of 0.018 absorbance unit compared with 0.010 reported by O'Reilly *et al.* (1). The average net absorbance of final extracts from unspiked blood bank plasma was 0.261, with a *SD* of 0.024, yielding an O'Reilly equivalent of 0.022.

In the TLC assay, the average net absorbance of final extracts from subjects' zero-hour plasmas was 0.155 with a *SD* of 0.030 and an O'Reilly equivalent of 0.013. The average net absorbance of final extracts from unspiked blood bank plasma was 0.158 with a *SD* of 0.035 and an O'Reilly equivalent of 0.013.

Hence, both assays gave higher blanks than reported by O'Reilly *et al.* (1). A reduction in the net absorbance of the blanks was obtained by the TLC purification step.

In Fig. 1 the plasma concentration of warfarin measured by the modified O'Reilly method is plotted on the ordinate against the corresponding plasma concentration of warfarin measured by the TLC method for the 93 plasma samples analyzed by both methods. The slope of the least-squares line forced through the origin is 1.00; this is the exact theoretical value for equivalence of the assays.

For the differences (TLC assay − O'Reilly assay), there were 44 positive values, 2 zero values, and 47 negative values. These differences are plotted against the averages, namely (TLC assay + O'Reilly assay)/2, in Fig. 2. It appears that the differences are independent of plasma concentration in the range studied. The average absolute value of the difference was 0.17 mcg./ml. with a *SD* of 0.143 mcg./ml. If the average of the two assays on each plasma sample is taken as the true concentration of warfarin, the average deviation of an assay value from the true concentration is 0.085 mcg./ml.

From Fig. 3 it may be seen that the UV spectra of final extracts from both assays are essentially identical. However, since the metabolites of warfarin have spectra similar to the parent compound (9), small amounts of metabolite would not be detected by this method. This evidence is, therefore, of minor importance.

Elimination half-lives of warfarin were estimated from terminal plasma concentrations of warfarin from each of the 12 sets of plasma samples assayed by both methods. The method of least squares was applied to the logarithms of the plasma concentrations of each set, which appeared to be randomly distributed about a straight line when plotted against time; the slopes of the least-squares lines were divided into the logarithm of 2 to obtain the half-lives. In Fig. 4 the elimination half-life estimated from plasma samples assayed by the modified O'Reilly method is plotted on the ordinate against the elimination half-life estimated from plasma samples assayed by the TLC method on the abscissa. The least-squares line free to pass through any intercept had a slope of 0.99 and an intercept of 2.5, which was not significantly different from zero. The least-squares line forced through the origin had a slope of 1.0, and this line is drawn through the points in Fig. 4. The average half-life of 37.4 hr. obtained with the modified O'Reilly assay was not significantly different from the average half-life of 35.2 hr. obtained with the TLC assays by a paired *t*-test ($t = 0.95, p > 0.25$).

DISCUSSION

O'Reilly (10) showed that the binding strength of warfarin metabolites to crystalline human albumin was 7- to 17-fold less than that of unchanged warfarin. He suggested that the introduction of the polar hydroxyl groups by metabolism greatly decreases the hydrophobic binding of warfarin metabolites, not only to albumin but also to the receptor sites for the unchanged drug; this allows for their ready renal clearance and accounts for the loss of anticoagulant activity. This may explain why warfarin metabolites are present in urine but not in plasma in measurable amounts.

Since the TLC assay employed measures only unchanged warfarin in plasma, from the data presented it may be concluded that the original and the modified O'Reilly assays are specific for unchanged warfarin in plasma. Results reported here strongly support the countercurrent distribution studies of O'Reilly *et al.* (1), which indicated the specificity of his assay method. If warfarin metabolites are present in plasma, they must be present in minute amounts and, in such trace amounts, would not invalidate the O'Reilly assay as a

Table III—Recoveries of Warfarin from TLC Plates

No. of Samples[a]	% Recovery of Warfarin	
	Average	*SD*
5	92.7	1.1
5	92.1	0.64
5	90.2	1.8
5	91.7	2.9

[a] All five samples in each case were different concentrations of warfarin. The range of amounts spotted was 1.66–20.9 mcg.

Table IV—Standardization of the TLC Assay Using Pooled Plasma Spiked with USP Reference Standard Warfarin

Day	No. of Samples	Concentration Range, mcg./ml.	A_N/C		
			Average	*SD*	Range
1	6	0.25–3.0	0.168	0.011	0.151–0.182
2	4	2.0 only	0.166	0.005	0.162–0.174
		Av.	0.167		

[5] Dr. Karl Paul Link and Fred W. Deckert, Department of Biochemistry, University of Wisconsin.

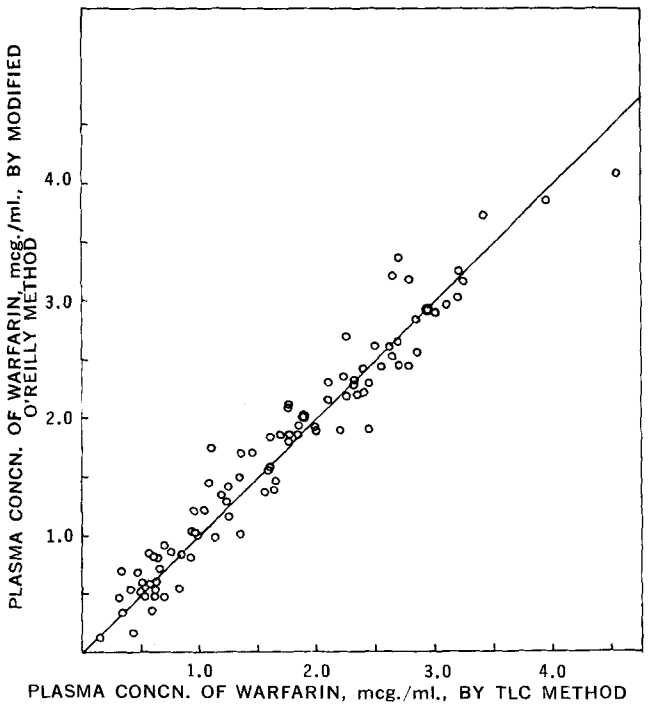

Figure 1—*Plot of plasma concentration of warfarin measured by the modified O'Reilly method against plasma concentration of warfarin measured by the TLC method.*

specific method. It is feasible that warfarinlike products detected on thin-layer plates after spotting extracts from plasma by Lewis and Ilnicki (8) were degradation products which arose from the action of light on warfarin during their TLC assay procedure. However, if these authors were using a very high dose of warfarin, then the possibility of an increase in the activity of hydroxylating enzymes at high plasma concentrations of drug, causing a disproportionate increase in plasma metabolites, would also be a feasible explanation for their results.

If a metabolite also had a long half-life in the body, its concentration in blood could build up after multiple doses of drug to levels that would invalidate the conclusions reached following administration of single doses of drug.

SUMMARY

Ninety-three plasma samples obtained from human volunteers, following ingestion of 25-mg. single oral doses of warfarin sodium, were independently assayed by a modification of the method of O'Reilly *et al.* (1) and by a TLC method. There was no detectable evidence of the presence of warfarin metabolites on any of the TLC plates developed from extracts of plasma from subjects dosed with warfarin in this study. The UV spectra of final extracts from both assays were essentially identical. Comparison of the 93 pairs of plasma concentrations arising from the application of the two assay methods on the same plasma samples and the 12 pairs of elimination half-lives of warfarin, estimated from terminal plasma concentrations, measured by the two assay methods indicated that the assays gave equivalent results. It is concluded that the original and the modified O'Reilly assay methods are specific for unchanged warfarin in plasma in the concentration range of 0.1–4.5 mcg. warfarin/ml. plasma observed in this study.

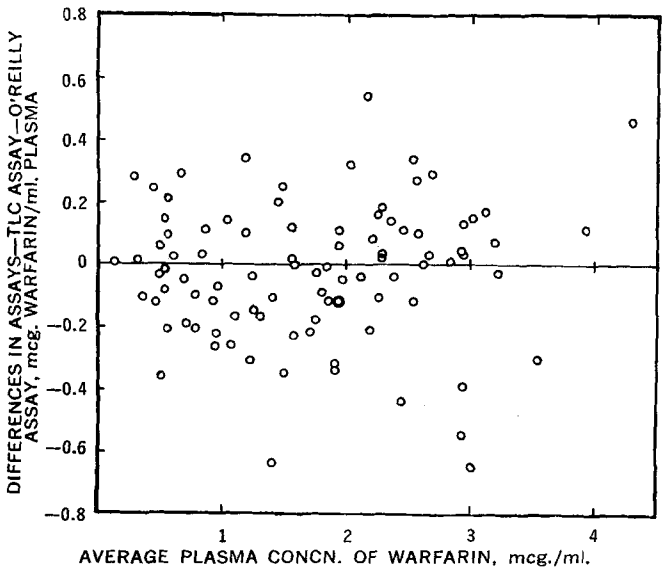

Figure 2—*Plot of difference between assays against average of assays.*

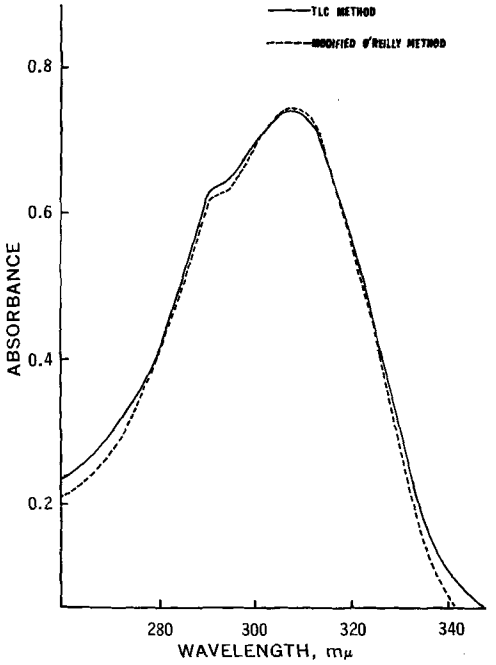

Figure 3—*UV spectra of final extracts from TLC and modified O'Reilly assays.*

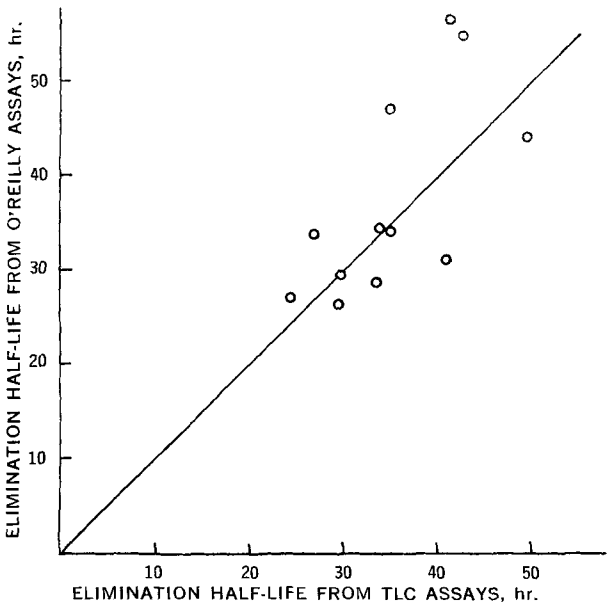

Figure 4—*Plot of the elimination half-life of warfarin estimated from modified O'Reilly assays against the elimination half-life of warfarin estimated from TLC assays.*

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* Present address: Department of Pharmacology (Program in Investigative Clinical Pharmacology), University of Michigan.

Salt Effects in Aqueous Solutions of Urea

RICHARD E. LINDSTROM and ALEXANDER R. GIAQUINTO

Abstract ☐ It is suggested, when working with drug–urea–water systems, that more than passing consideration be given to the effect or effects produced by addition of a fourth component, as, for example, when an acid is used to adjust pH. Data are cited which indicate that these effects are measurable and often predictable. Solubility data for methyl salicylate and methyl benzoate are given as functions of varying acid, salt, and urea concentrations. These data represent the equilibrium solubilities of the esters in the various systems at 30°. The solubilities were obtained by sampling and subsequent determination of the ester concentration using UV spectrophotometry. A mathematical model was derived which permits a quantitative evaluation of salt effects in urea solutions. The theoretical calculations, based on this model, were found to be in good agreement with the experimental values observed for neutral and pH 1 solutions of methyl salicylate and methyl benzoate in urea. Extension of these findings to an earlier investigation indicates that significant error in interpretation of solubility in urea may result if these salt effects are disregarded.

Keyphrases ☐ Urea aqueous solutions—salt effects ☐ Methyl salicylate and benzoate solubility—urea–water mixture ☐ Electrolyte, HCl concentration effects—methyl salicylate and benzoate solubility ☐ UV spectrophotometry—analysis

The introduction of a fourth component into a urea–drug–water system will produce changes in both the physical and chemical properties of the system. Understandably, the inclination is to disregard or minimize the resultant effects, especially when the rationale of the investigation appears to be unaltered by doing so. However, it is imperative that some quantitative estimate be obtained before following this premise, since the magnitude of these effects may be large enough to require reassessment of the object of the experiment.

Work on systems involving urea–water mixtures are particularly prone to questionable assumptions. For example, during their study of the solubility of salicylic acid in urea solutions, various authors (1–3) sought to preclude ionization of the salicylic acid by making the solutions pH 1 with strong acid. In two instances, the workers made the solutions 0.1 *N* in H^+. Taking note of observed irregularities in this matter, however, Feldman and Gibaldi (1) were careful to adjust the pH of each solution to 1. These two different procedures result in systems that are not equivalent, a fact that may have been responsible for the differences in observations and conclusions. The strong acid which was added in these studies is certain to exhibit a characteristic influence on the solubility of any additional solutes. Whether this influence is significant and measurable in multicomponent mixtures of urea, water, drug, and salt is typical of the problem which should concern the investigator.

Wetlaufer *et al.* (4) demonstrated that salt effects do exist in urea solutions. These workers found that the solubility of skatole in aqueous urea–sodium chloride solutions was measurably less than in solutions of the same urea concentration but containing no sodium chloride. Additionally, it was estimated that this "salting out" approximated that observed when the solubility of skatole in water and in water–sodium chloride solution was compared. Thus, it would seem that the salt effects are not only measurable but also are relatively independent of the urea concentration. In that event, one is in a favorable position to quantify similar effects in urea solutions in general.

THEORETICAL CONSIDERATIONS

The phenomena of "salting in" and "salting out" have been described empirically by the Setschenow equation (5):

$$\log S^\circ/S = KC \qquad \text{(Eq. 1)}$$

where S° and S are the molar solubilities of a nonelectrolyte in pure water and in a solution containing C moles/l. of electrolyte, respectively. The symbol, K, is an empirical salt parameter, which is characteristic of both the electrolyte and nonelectrolyte species.