(37) A. K. Cho, B. Lindeke, B. Hodsdon, and D. J. Jenden, *ibid.*, 45, 570 (1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 19, 1976, from the *Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143,* and the *University of Washington, Seattle, WA 98195.*

Accepted for publication September 21, 1976.

Supported by grants from the Seattle Foundation (W. A. Garland and W. F. Trager), the Rubenstein Fund of the School of Pharmacy, University of Washington (W. A. Garland), and National Institutes of Health Training Grant 5-T01-GM00728 (S. D. Nelson) and in part by National Institutes of Health Grant GM-16496. The authors also thank J. Einch, Arizona State University, for making available his MASS PLOT computer program.

[x] To whom inquiries should be directed (at the University of Washington).

# Application of Ion-Pair Methods to Extraction of Fluorouracil from Aqueous Fluids

ANDRÉ P. De LEENHEER,*[x], MARIE-CLAIRE COSYNS-DUYCK *, and P. MAURICE VAN VAERENBERGH [‡]

**Abstract** □ The polar molecule, fluorouracil, is a monoanion at pH 10 and may be quantitatively extracted from aqueous solutions with quaternary alkylammonium ions into an organic solvent such as dichloromethane as the ion-pair. Extraction constants of fluorouracil with the tetrapentylammonium ion in dichloromethane or dichloromethane–1-butanol (9:1) and with the tetrahexylammonium ion in dichloromethane were determined. Slope analysis demonstrated that association of the ion components in the aqueous phase occurred as the side reaction. A column ion-pair extraction technique, using tetrapentylammonium as the counterion and dichloromethane as the eluting phase, was developed and allowed quantitative transfer of fluorouracil to the organic solvent. The applicability of this method was shown by determining plasma levels of fluorouracil in cancer patients to whom 1 g of active substance was administered intravenously.

**Keyphrases** □ Fluorouracil—ion-pair extraction from aqueous solutions with quaternary alkylammonium ions into organic solvents □ Ion-pair extraction—fluorouracil from aqueous solutions with quaternary alkylammonium ions into organic solvents □ Antineoplastic agents—fluorouracil, ion-pair extraction from aqueous solutions with quaternary alkylammonium ions into organic solvents

Few methods have been published on the determination of fluorouracil in biological materials. A microbiological assay and a direct spectrophotometric method were used to determine plasma fluorouracil levels in cancer patients (1). Neither of these methods is sufficiently specific. Procedures using GLC were reported (2, 3), and a sensitive and highly specific mass fragmentographic method was recently described (4). However, in all of these techniques, the isolation of fluorouracil from aqueous biological materials into an organic solvent remains a weak point.

Dialysis and subsequent evaporation to dryness (2) and double-phase extraction with 2-propanol–ether (3, 4) were utilized. However, the first method is cumbersome and time consuming, and the two-phase extraction suffers from low specificity. A more polar system is required for extraction of the highly polar fluorouracil, a pyrimidine derivative.

The purpose of the present investigation was to study ion-pair systems, using quaternary alkylammonium counterions, for the extraction of fluorouracil from an aqueous matrix to an organic phase.

## EXPERIMENTAL

**Apparatus**—A pH meter[1] equipped with a combination electrode[2], a magnetic stirrer[3], and a mechanical shaking apparatus[4] were used. Spectrophotometric measurements were performed with an automatic UV–visible double-beam instrument[5] using 10-mm quartz cells.

**Chemicals and Reagents**—Fluorouracil[6] was used as received. Dichloromethane[7], tetrapentylammonium iodide[8], and tetrahexylammonium bromide[8] were analytical grade. Tetrapentylammonium and tetrahexylammonium hydrogen phosphate solutions, about 0.15 and 0.01 *M*, respectively, were prepared as follows. To the 0.2 *M* aqueous solutions of the halides was added an equivalent amount of silver oxide, and the mixtures were continuously stirred for 24 hr. After filtration, concentrated phosphoric acid was added to pH 10. The solutions were extracted three times with small volumes of dichloromethane (phase ratio, aqueous–organic, 10:1) to remove the unreacted iodide and bromide salts (5). The true concentrations of the tetraalkylammonium ions were determined by an ion-pair extraction method with picric acid (6).

Cellulose[7] for column chromatography was purified in a column by washing with ethanol and dichloromethane until the absorbance of the eluate at 269 nm was constant at 0.010. Carbonate buffer, pH 10 and ionic strength 0.1, was prepared for back-extraction experiments by mixing 25.0 ml of 1 *M* $NaHCO_3$ with 50.0 ml of 0.5 *M* $Na_2CO_3$ and diluting to 1000 ml. A concentrated carbonate buffer for plasma determinations was made by dissolving 2.1 g of sodium hydrogen carbonate and 2.6 g of sodium carbonate in 40 ml of water.

All other substances were analytical grade and were used without purification. Mutually saturated solvents were used throughout the experiments.

**Extraction Constants**—Constants for ion-pair extraction and ion-pair association in the aqueous phase were determined by a partition technique. The partition experiments were performed in centrifuge tubes using 20-ml volumes of aqueous and organic solutions. These solutions were mechanically shaken at room temperature for 30 min to establish equilibrium. After centrifugation, the phases were separated and the absorbance of the aqueous phase, $A_1$, was measured at 269 nm.

A 10.0-ml aliquot of the organic phase was subsequently reequilibrated with 10.0 ml of 0.01 *M* $NaClO_4$ in carbonate buffer, pH 10, by shaking for 15 min at room temperature. (A shorter time for establishing the equilibrium was used because of the much higher extraction constant of

[1] Radiometer Titrator TTT 1c, Copenhagen, Denmark.
[2] Radiometer GK 2321 C, Copenhagen, Denmark.
[3] Cenco Instruments N.V. 16632 B, Breda, The Netherlands.
[4] Wilten and Co., Berchem, Belgium.
[5] Pye Unicam SP 1800, Cambridge, England.
[6] Donated by N.V. Produits Roche, Brussels, Belgium.
[7] E. Merck AG, Darmstadt, West Germany.
[8] Eastman Kodak, Rochester, N.Y.

the perchlorate ion-pair in the organic phase.) After centrifugation to separate the two layers, the absorbance at 269 nm of this aqueous phase, $A_2$, was determined. Distilled water (20 ml) was treated identically to the fluorouracil solutions, and the resulting phases were used as the blank in the reference cell for both spectrophotometric measurements.

According to the described method, three complete series of experiments were performed:

1. To 10 ml of 0.004 *M* fluorouracil (previously adjusted to pH 10 with 1 *M* NaOH) was added 10.0 ml of tetrapentylammonium hydrogen phosphate solution of known concentration (aliquots of the stock solution of 0.138 *M* tetrapentylammonium hydrogen phosphate were diluted with appropriate volumes of 0.075 *M* $NaH_2PO_4$), and the aqueous phase was equilibrated with 20.0 ml of dichloromethane as the organic phase. Counterion stock and phosphate solution had the same ionic strength of 0.225, which remained constant throughout the experiments.
2. Same as Series 1, but dichloromethane–1-butanol (9:1) was used as the organic phase.
3. Same as Series 1, but a $2.06 \times 10^{-4}$ *M* fluorouracil solution, a $5 \times 10^{-3}$ *M* tetrahexylammonium hydrogen phosphate stock solution, and dichloromethane as the organic phase were used.

**Plasma Samples**—Ten cancer patients received 1 g of fluorouracil by intravenous injection in the right arm. Blood samples were drawn into heparinized syringes from the left arm by venous puncture at various intervals for 30 min after administration. Plasma was separated from the red blood cells by centrifugation and frozen until assayed.

**Determination of Fluorouracil in Plasma**—Volumes of 1.0 ml of plasma, 1.0 ml of 0.15 *M* tetrapentylammonium counterion, and 200 $\mu$l of pH 10 carbonate buffer were mixed thoroughly with 4 g of cellulose in a 100-ml beaker until dry. The adsorbent was packed in a glass column ($1 \times 50$ cm) and eluted with 10 ml of dichloromethane. The first 5 ml of eluate was collected in 10-ml screw-capped centrifuge tubes.

To the organic phase, 3 ml of 0.2 *M* $NaClO_4$ in pH 10 carbonate buffer was added, and the phases were allowed to equilibrate by shaking for 15 min at room temperature. After centrifugation, the aqueous phase was measured spectrophotometrically at 269 nm against 0.2 *M* $NaClO_4$ in pH 10 carbonate buffer. For each patient, a plasma sample collected just before the fluorouracil administration was used as a blank.

## RESULTS AND DISCUSSION

As indicated by its high water solubility [11.78 g/liter (2)], fluorouracil is a highly polar compound. Its extraction from an aqueous medium with usual double-phase techniques requires the use of polar systems [ether–lipophilic alcohols (8:2)] (3, 4). However, such systems possess a rather low selectivity. Because fluorouracil is an ionizable substance [*i.e.*, a weak diprotic acid ($H_2FU$) having a $pKa_1 = 8.0$ and a $pKa_2 = 13$ (7)], the possible application of an ion-pair extraction method was investigated. In aqueous solutions buffered at pH 10, 99.0% of fluorouracil exists as the monoanionic species $HFU^-$. Experiments were carried out to investigate the feasibility of extracting fluorouracil as a 1:1 ion-pair into a dichloromethane phase with tetrapentyl- or tetrahexylammonium as monocationic counterions.

The extraction of fluorouracil as an ion-pair from an aqueous phase buffered at pH 10 into an organic phase may be represented by Scheme I:

$$HFU^-_{aq} + Q^+_{aq} \rightleftharpoons HFU^-Q^+_{org}$$

*Scheme I*

where $HFU^-_{aq}$ represents the monoanionic species of fluorouracil in the aqueous phase; $Q^+_{aq}$ is the counterion, *i.e.*, the tetraalkyl (pentyl or hexyl) ammonium ion in the aqueous phase; and $HFU^-Q^+_{org}$ is the ion-pair in the organic phase.

The ion-pair extraction constant, $E$, is defined by:

$$E = \frac{[HFU^-Q^+]_{org}}{[HFU^-]_{aq}\,[Q^+]_{aq}} \qquad \text{(Eq. 1)}$$

where $[\,]_{aq}$ and $[\,]_{org}$ are the concentrations of the species indicated in the aqueous and organic phases, respectively.

However, to examine the influence of side reactions on the extraction, a conditional extraction constant, $E^*$, is used:

$$E^* = \frac{C_{HFU^-Q^+_{org}}}{C_{HFU^-_{aq}}\,C_{Q^+_{aq}}} = E\,\frac{\alpha_{HFU^-Q^+_{org}}}{\alpha_{HFU^-_{aq}}\,\alpha_{Q^+_{aq}}} \qquad \text{(Eq. 2)}$$

where $C$ is the actual concentration, and $\alpha$ is the activity coefficient that compensates for side reaction(s) of the species indicated.

The concentration of the monoanionic species of fluorouracil in the aqueous phase, $C_{HFU^-_{aq}}$, is measured by spectrophotometry at 269 nm ($A_1$), using the molar absorption coefficient $\epsilon = 4.12 \times 10^3$ liters/mole cm. The concentration of the ion-pair in the organic phase, $C_{HFU^-Q^+_{org}}$, is estimated by an indirect procedure. Because the spectral properties of the ion-pair $HFU^-Q^+_{org}$ in the organic phase were not known accurately, a back-extraction was performed with 0.01 *M* $NaClO_4$ in pH 10 carbonate buffer. However, the perchlorate anion is a much stronger ion-pair former than the monoanion of fluorouracil [*i.e.*, $E_{ClO_4^-Q^+}$ is much larger than $E_{HFU^-Q^+}$ (8)], and the equilibrium will be displaced far to the right as shown by Scheme II.

$$HFU^-Q^+_{org} + ClO^-_{4aq} \rightleftharpoons ClO^-_4Q^+_{org} + HFU^-_{aq}$$

*Scheme II*

Therefore, all fluorouracil originally present as an ion-pair in the organic phase will be quantitatively transferred to the aqueous phase, $HFU^-_{aq}$. Spectrophotometry at 269 nm of the latter yields $A_2$, which allows calculation of $C_{HFU^-Q^+_{org}}$ (using the molar absorptivity $\epsilon = 4.12 \times 10^3$ liters/mole cm) for substitution in Eq. 2. Finally, the concentration of the counterion in the aqueous phase, $C_{Q^+_{aq}}$, is obtained from Eq. 3 by difference between the initial concentration of counterion, $C_Q{}^0$, and the measured ion-pair concentration, $C_{HFU^-Q^+_{org}}$:

$$C_{Q^+_{aq}} = C_Q{}^0 - C_{HFU^-Q^+_{org}} \qquad \text{(Eq. 3)}$$

Substitution of the concentration terms in Eq. 2 by absorbance values (Lambert–Beer $C = A/\epsilon$ for 1.0-cm pathlength) and expansion with Eq. 3 give:

$$E^* = \frac{A_2/\epsilon}{A_1/\epsilon\,(C_Q{}^0 - A_2/\epsilon)} \qquad \text{(Eq. 4)}$$

which may also be written:

$$E^* = \frac{A_2\epsilon}{A_1(C_Q{}^0\epsilon - A_2)} \qquad \text{(Eq. 5)}$$

and which is used experimentally for calculation of the conditional extraction constants $E^*$.

Data for fluorouracil ion-pairs, as determined using three sets of experimental conditions, are given in Table I. Within each experimental series, the value found for $E^*$ increases with the decreasing initial concentration of counterion $C_Q{}^0$. This effect strongly suggests the existence of a side reaction, specifically the association of the ion-pair components in the aqueous phase (9–11), which is defined in Scheme III.

$$HFU^-_{aq} + Q^+_{aq} \rightleftharpoons HFU^-Q^+_{aq}$$

*Scheme III*

For this equilibrium, the apparent association constant, $K_a$, is given by:

$$K_a = \frac{[HFU^-Q^+]_{aq}}{[HFU^-]_{aq}\,[Q^+]_{aq}} \qquad \text{(Eq. 6)}$$

where $[HFU^-Q^+]_{aq}$ is the concentration of the fluorouracil ion-pair in the aqueous phase.

Furthermore, if association of the ion-pair components in the aqueous phase occurs as the only side reaction, the following values for the activity coefficients $\alpha$ ($\alpha = C/[\,]$, where $C$ is the actual concentration and $[\,]$ is the theoretical concentration of the species subscribed) should be assigned and substituted in Eq. 2. Then $\alpha_{HFU^-Q^+_{org}} = 1$, since the actual and theoretical concentrations of the ion-pair in the organic phase are equal; *i.e.*, $C_{HFU^-Q^+_{org}} = [HFU^-Q^+]_{org}$ (assuming no side reaction occurs in the organic phase); $\alpha_{Q^+_{aq}} = 1$ because there is always an excess of tetraalkylammonium counterion, $Q^+$, present in the aqueous phase or its actual and theoretical concentrations are practically equal, *i.e.*, $C_{Q^+_{aq}} \approx [Q^+]_{aq}$; and $\alpha_{HFU^-_{aq}}$ or the activity coefficient of the fluorouracil monoanion in the aqueous phase is then given by Eq. 7:

$$\alpha_{HFU^-_{aq}} = \frac{C_{HFU^-_{aq}}}{[HFU^-]_{aq}} = \frac{[HFU^-]_{aq} + [HFU^-Q^+]_{aq}}{[HFU^-]_{aq}} \qquad \text{(Eq. 7)}$$

which can be rearranged by use of Eq. 6 to the following form:

$$\alpha_{HFU^-_{aq}} = 1 + K_a[Q^+]_{aq} \qquad \text{(Eq. 8)}$$

Substituting the values for $\alpha$ in Eq. 2 yields the expression for $E^*$, which is valid when association of the ion-pair components in the aqueous phase occurs as the only side reaction:

$$E^* = \frac{E}{\alpha_{HFU^-_{aq}}} = \frac{E}{1 + K_a[Q^+]_{aq}} \qquad \text{(Eq. 9)}$$

**Table I—Determination of Extraction Constants of Fluorouracil Ion-Pairs [a]**

| $C_Q^0$, $M \times 10^{-3}$ | $C_{HFU^-_{aq}}$, $M \times 10^{-3}$ | $C_{HFU\text{-}Q^+_{org}}$, $M \times 10^{-3}$ | $C_{Q^+_{aq}}$, $M \times 10^{-3}$ | $E^*$, $M^{-1}$ | $1/E^*$, $M \times 10^{-3}$ | $D = E^* C_{Q^+_{aq}}$ |
|---|---|---|---|---|---|---|
| First Series: Tetrapentylammonium and Dichloromethane; $C^0_{fluorouracil} = 2.048 \times 10^{-3}\ M$ | | | | | | |
| 69.0 | 0.34 | 1.89 | 67.11 | 82.8 | 12.1 | 5.58 |
| 34.5 | 0.54 | 1.68 | 32.82 | 94.8 | 10.6 | 3.11 |
| 17.25 | 0.86 | 1.36 | 15.89 | 99.5 | 10.1 | 1.58 |
| 8.63 | 1.22 | 1.00 | 7.63 | 107.4 | 9.3 | 0.82 |
| Second Series: Tetrapentylammonium and Dichloromethane–1-Butanol (9:1); $C^0_{fluorouracil} = 2.011 \times 10^{-3}\ M$ | | | | | | |
| 75.0 | 0.0455 | 1.97 | 73.03 | 593 | 1.69 | — |
| 37.5 | 0.0600 | 1.95 | 35.55 | 914 | 1.09 | — |
| 18.75 | 0.0941 | 1.90 | 16.85 | 1,198 | 0.83 | — |
| 9.38 | 0.158 | 1.86 | 7.52 | 1,565 | 0.64 | — |
| Third Series: Tetrahexylammonium and Dichloromethane; $C^0_{fluorouracil} = 0.103 \times 10^{-3}\ M$ | | | | | | |
| 2.85 | 0.00300 | 0.100 | 2.75 | 12,120 | 0.0825 | — |
| 1.43 | 0.00494 | 0.0982 | 1.33 | 14,950 | 0.0669 | — |
| 0.71 | 0.00833 | 0.0947 | 0.62 | 18,340 | 0.0545 | — |
| 0.36 | 0.0156 | 0.0874 | 0.27 | 20,750 | 0.0481 | — |

[a] $C^0$ is the initial concentration of the compound indicated and taken through the experiment; $C$ is the actual concentration of the species indicated after reaching equilibrium.

By taking the reciprocals, Eq. 9 is linearized to:

$$\frac{1}{E^*} = \frac{1}{E} + \frac{K_a}{E}[Q^+]_{aq} \qquad \text{(Eq. 10)}$$

Thus, if the assumption of association of the ion-pair components in the aqueous phase is correct, slope analysis of the experimental data according to Eq. 10, namely the reciprocal of the conditional extraction constant, $1/E^*$, *versus* the concentration of the counterion in the aqueous phase, $[Q^+]_{aq}$ (which is approximated by the actual concentration $C_{Q^+_{aq}}$), should yield straight lines. The experimental data points of the three systems considered all fit Eq. 10, supporting the validity of the hypothesis. By using the same relationship, the theoretical extraction constant $E$ and the association constant $K_a$ of each series were derived: 109 and 5 $M^{-1}$, tetrapentylammonium and dichloromethane; 1855 and 30 $M^{-1}$, tetrapentylammonium and dichloromethane–1-butanol (9:1); and 21,761 and 298 $M^{-1}$, tetrahexylammonium and dichloromethane. Although the two latter systems afforded a high extraction efficiency of 97–98%, they were less selective and their applicability was limited to fluorouracil extracts that had undergone a preliminary cleanup.

The tetrapentylammonium and dichloromethane system yielded values for $E^*$ that approached $E = 109\ M^{-1}$ as the concentration of the counterion, $C_{Q^+_{aq}}$, decreased. Thus, under appropriate experimental conditions, the effect of the side reaction identified would be negligible. For example, when the activity coefficient of the fluorouracil monoanion in the aqueous phase, $\alpha_{HFU^-_{aq}}$, equals 1.01, $E^*$ approximates $E = 109\ M^{-1}$ (see Eq. 9, first term). When solving Eq. 8 for the activity coefficient $\alpha_{HFU^-_{aq}} = 1.01$ (chosen) and the association constant $K_a = 5\ M^{-1}$ (found), the highest concentration of tetrapentylammonium counterion in the aqueous phase that might be used would be 0.002 $M$.

However, under such conditions, the presence of higher concentrations of competitive anions, comparable to much higher extraction constants (*e.g.*, the chloride ion), would probably exhaust the tetrapentylammonium counterion and prevent the extraction of fluorouracil, even at rather low concentrations. Therefore, the feasibility of the ion-pair extraction of fluorouracil with the tetrapentylammonium and dichloromethane system under circumstances where the influence of the association phenomenon should be minor will not be investigated further. As mentioned previously, a low numerical value for the theoretical extraction constant $E$ (= 109 $M^{-1}$) was obtained for the tetrapentylammonium and dichloromethane system. Evidently, this value entirely governs the quantitative aspect of the ion-pair extraction.

In general, the percentage of the ion-pair extraction, $P$, is given by:

$$P = \frac{100}{1 + \frac{1}{D}\frac{V_{aq}}{V_{org}}} \qquad \text{(Eq. 11)}$$

and:

$$D = \frac{C_{HFU\text{-}Q^+_{org}}}{C_{HFU^-_{aq}}} \qquad \text{(Eq. 12)}$$

or by combining Eqs. 12 and 2:

$$D = E^* C_{Q^+_{aq}} \qquad \text{(Eq. 13)}$$

where $D$ is the partition ratio of the fluorouracil monoanion between the organic and aqueous phases, and $V_{aq}/V_{org}$ is the ratio of the volumes of the aqueous phase to the organic phase.

The extraction is considered quantitative when $P$ numerically equals 99%. Thus, in the present study where the ratio $V_{aq}/V_{org} = 1.00$, $D$ should be 100. By using Eq. 13 (rearranged for the conditional extraction constant $E^*$) and simplifying for approximating actual and theoretical counterion concentrations in the aqueous phase ($C_{Q^+_{aq}} = [Q^+]_{aq}$), Eq. 10 (which takes into account the only side reaction observed) can be written:

$$\frac{1}{D} = \frac{K_a}{E} + \frac{1}{E} C_{Q^+_{aq}} \qquad \text{(Eq. 14)}$$

where $C_{Q^+_{aq}}$ is the actual concentration of the tetrapentylammonium counterion in the aqueous phase. Plotting the experimental points for the first series (Table I) according to Eq. 14 (the reciprocal of the partition

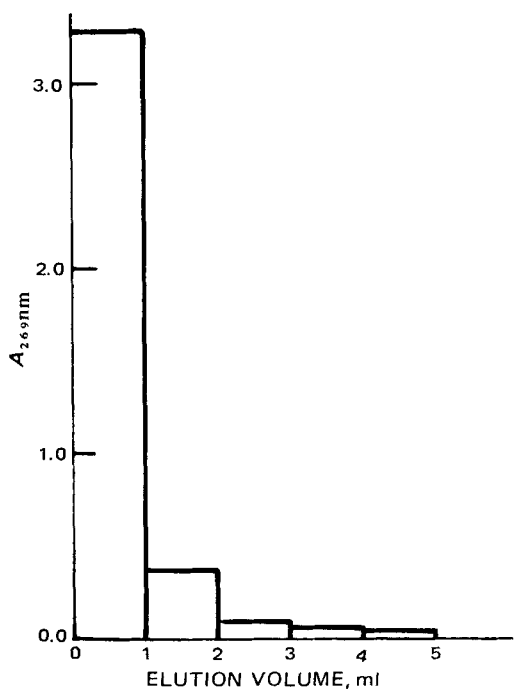


**Figure 1**—*Elution pattern of fluorouracil with column ion-pair extraction.*

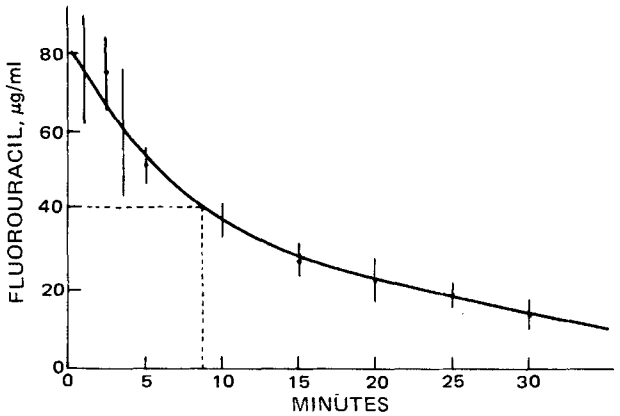


**Figure 2**—*Plasma fluorouracil concentration (1 g iv total amount) in 10 cancer patients.*

ratio $1/D$ as a function of the concentration of the tetrapentylammonium counterion in the aqueous phase ($C_{Q^+_{aq}}$) afforded a straight line, establishing that, for $D = 100$, $C_{Q^+_{aq}}$ should be about 1 $M$. However, the highest counterion concentration that could be prepared was about 0.15 $M$. Thus, the water solubility of tetrapentylammonium hydrogen phosphate is insufficient to meet the necessary 1 $M$ calculated value. Therefore, as follows from Eq. 11, the unique solution of this problem was to increase the ratio of volumes employed, $V_{org}/V_{aq}$.

This solution was realized by adsorbing an aliquot of aqueous sample, buffered at pH 10 and containing the fluorouracil monoanion, with an excess of the tetrapentylammonium counterion onto an adsorbent such as cellulose. The mixture was packed in a glass column, and the ion-pair $HFU^-Q^+$ eluted with the necessary volume of dichloromethane. Figure 1 shows the elution pattern of fluorouracil at an initial concentration of 100 μg/ml; all fluorouracil was recovered in the first 5 ml of dichloromethane.

Recovery studies were performed by preparing a blank plasma pool to which fluorouracil was added, covering the range of 0–100 μg/ml. Each sample, *i.e.*, 0, 10, 20, 30, 40, 50, 60, 80, and 100 μg/ml, was taken through the complete procedure three times. The recovery as calculated from all results was 99.6 ± 0.6%, but appreciable absorbance values (up to 0.500) were measured for the blank sample. These values were due to unidentified interferences from cellulose (that could not be removed entirely prior to analysis) or from compounds coextracted from plasma. This result, together with the low molecular absorptivity of fluorouracil, set the detection limit of the ion-pair column extraction method at about 10 μg/ml of plasma.

To demonstrate the applicability of the ion-pair column extraction to blood samples, 1 g of fluorouracil was administered intravenously to 10 cancer patients. At 1, 2.5, 3.5, 5, 10, 15, 20, and 30 min after injection, blood samples were collected. Results of the analysis of each plasma sample are presented as a mean plasma level curve in Fig. 2. The fluorouracil concentration leveled off rapidly, from more than 100 μg/ml (at 1 min) to about 10 μg/ml (at 30 min) with a biological half-life of 9 min. These data correspond to those reported earlier (3, 4). The determination of plasma fluorouracil levels below 10 μg/ml is an analytical problem currently being investigated.

Although the quantitative ion-pair extraction of fluorouracil in the concentration range of 0.1–10 μg/ml was feasible, combining the ion-pair extraction with GLC was not successful. In fact, extractive methylation, *i.e.*, simultaneous extraction and methylation of the fluorouracil monoanion in the ion-pair form, produced chromatograms wherein the *N,N'*-dimethyl derivative of fluorouracil was overlapped by multiple interfering peaks originating from irreproducible thermal degradation reaction(s) of the residual counterion (tetrapentylammonium or tetrahexylammonium cation) in the organic phase. An attempt was made to solve this problem by purification of the *N,N'*-dimethyl derivative through selective extraction with ether (12) and by column chromatography on magnesium silicate[9] (13), alumina[10], or a cation-exchange resin[11]. None of these approaches gave satisfactory results. This fundamental problem has been recognized (14, 15), and it may be the major reason that the combination of ion-pair extraction with GLC has not been utilized.

## REFERENCES

(1) B. Clarkson, A. O'Connor, L. Winston, and D. Hutchison, *Clin. Pharmacol. Ther.*, **5**, 581 (1964).

(2) J. J. Windheuser, J. L. Sutter, and E. Auen, *J. Pharm. Sci.*, **61**, 301 (1972).

(3) J. L. Cohen and P. B. Brennan, *ibid.*, **62**, 572 (1973).

(4) C. Finn and W. Sadée, *Cancer Chemother.*, **59**, 279 (1975).

(5) K. Gustavii and G. Schill, *Acta Pharm. Suec.*, **3**, 259 (1966).

(6) *Ibid.*, **3**, 241 (1966).

(7) I. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199, 369 (1952).

(8) K. Gustavii, *Acta Pharm. Suec.*, **4**, 233 (1967).

(9) G. Schill, *ibid.*, **2**, 13 (1965).

(10) B. A. Person and G. Schill, *ibid.*, **3**, 281 (1966).

(11) R. Modin and G. Schill, *ibid.*, **4**, 301 (1967).

(12) J. A. F. de Silva, I. Bekersky, C. V. Puglisi, M. A. Brooks, and R. E. Weinfeld, *Anal. Chem.*, **48**, 1 (1976).

(13) J. M. Rosenfeld and V. Y. Taguchi, *ibid.*, **48**, 4 (1976).

(14) M. Ervik and K. Gustavii, *ibid.*, **46**, 39 (1974).

(15) E. Ehrsson, *ibid.*, **46**, 922 (1974).

## ACKNOWLEDGMENTS AND ADDRESSES

Received July 6, 1976, from the **Laboratoria voor Medische Biochemie en voor Klinische Analyse, Faculteit van de Farmaceutische Wetenschappen, and the ‡Kliniek voor Radiotherapie en Kerngeneeskunde, Faculteit van de Geneeskunde, Rijksuniversiteit, Academisch Ziekenhuis, 135 De Pintelaan, 9000-Gent, Belgium.*

Accepted for publication October 8, 1976.

Supported by Grant 20 452 from the National Foundation for Medical Research (F.G.W.O.).

The authors thank Professor G. Schill, University of Uppsala, Uppsala, Sweden, for training in his laboratories and S. Eksborg and P. O. Lagerström for helpful discussions.

ˣ To whom inquiries should be directed.

[9] Florisil 60-100 U.S. mesh, BDH Chemical Co., Poole, England.
[10] Neutral alumina AG 7, Bio-Rad, Richmond, Va.
[11] Cation-exchange resin AG 50W-X8, Bio-Rad, Richmond, Va.