# Complex Formation of Uranyl Acetate with Tetracycline and Its Utilization for Their Microdetermination

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**Abstract** □ Conductometric and spectrophotometric (covering the visible, UV, and IR ranges) studies as well as microanalyses of uranyl complexes with tetracycline revealed the existence of the 1:1 complex species. The mean stability constant of the 1:1 complex, as determined spectrophotometrically, amounted to $1.2 \times 10^5$. This finding permits the use of the procedure for the microdetermination of tetracycline using $UO_2^{+2}$ ion or vice versa.

**Keyphrases** □ Uranyl acetate—complex formation with tetracycline, analysis of both antibiotic and uranyl ion □ Tetracycline—complex formation with uranyl acetate, analysis of both antibiotic and uranyl ion □ Complex formation, uranyl acetate and tetracycline—analysis of both antibiotic and uranyl ion □ Spectrophotometry—analysis, tetracycline and uranyl ion from antibiotic–uranyl acetate complex

Several tetracycline antibiotics are known including tetracycline, chlortetracycline, and oxytetracycline. The high affinity of chlortetracycline and oxytetracycline for cations of heavy metals was detected (1) and the parent substance, tetracycline, was found to chelate with heavy metal cations such as $Fe^{+3}$, $Al^{+3}$, $Cu^{+2}$, $Ni^{+3}$, $Co^{+2}$, $Zn^{+2}$, and $Mn^{+2}$, forming the 1:1 and 1:2 (metal ion–ligand) complexes (2). The stability constants of these chelates were shown to increase in the same order; values of log $K$ ranging from about 9.9 to 4.4 were recorded, of the same order of magnitude as those corresponding to the chloro and oxy derivatives. Complexes of tetracyclines with the uranyl ion have not been studied frequently, but Ishidate and Sakaguchi (3) reported on complexes of the chloro derivative.

Several spectrophotometric methods, involving chelation with different ions, have been described for the determination of tetracycline. A ferric chloride colorimetric method was described (4). Tetracycline was determined using sodium hydroxide, where a yellow solution was produced, yielding an absorption maximum at 380 nm (5, 6). Colored materials have been also reported due to the interaction of tetracycline with several metal compounds including zinc chloride (7), zirconium oxychloride (8), thorium nitrate (9), and ammonium molybdate (10).

The present report concerns physicochemical studies, including conductometric, spectrophotometric, and microanalytical determinations, on uranyl–tetracycline complexes. Because of the pharmaceutical importance of tetracycline, a spectrophotometric method for its microdetermination using $UO_2^{+2}$ was investigated. Application to microdetermination of the uranyl ion was also studied.

## EXPERIMENTAL

**Materials and Reagents**—Tetracycline was prepared according to the procedure recommended by Duggar (11).

The uranyl–tetracycline (1:1) complex was prepared by mixing the organic reagent with uranyl acetate, both dissolved in a water–dioxane mixture [dielectric constant *(D)*, 30], and the complex separated out by evaporation under vacuum. The solid was filtered and washed thoroughly with redistilled water and then with pure dioxane to ensure complete removal of excess reagents. The uranyl–tetracycline complex was recrystallized from acetone, yielding brown crystals.

**Procedures**—For conductometric titrations, a solution of tetracycline in a water–dioxane mixture ($3 \times 10^{-3}$ *M*) and a uranyl acetate solution ($3 \times 10^{-4}$ *M*) in the same solvent were used. Both were previously thermostated at 25°. The reagent was added using a microburet to 20 ml of the uranyl acetate solution under a purified nitrogen atmosphere. After each addition the solution resistance was measured using an alternating current conductivity bridge[1] of >±0.02% accuracy. An audiofrequency electron tube oscillator was utilized as a source of alternating current (1000 Hz). A sensitive oscilloscope[2] served as a null indicator.

Spectrophotometric studies of the uranyl–tetracycline complexes in the visible, UV, and IR ranges were carried out at room temperature using a recording spectrophotometer[3]. The formation and composition of the uranyl complexes were detected by applying the slope ratio method as previously described (12).

Solutions of precipitated uranyl complex with tetracycline in the same solvent were also studied spectrophotometrically in the visible and UV ranges. The IR absorption spectra of the complexes studied were recorded[4] using the KBr disk technique.

The precipitated uranyl–tetracycline complex was also chemically analyzed[5] for uranium, carbon, and hydrogen.

## RESULTS AND DISCUSSION

**Conductometric Measurements**—In Fig. 1, the specific conductance values, $k$, calculated from resistance measurements and

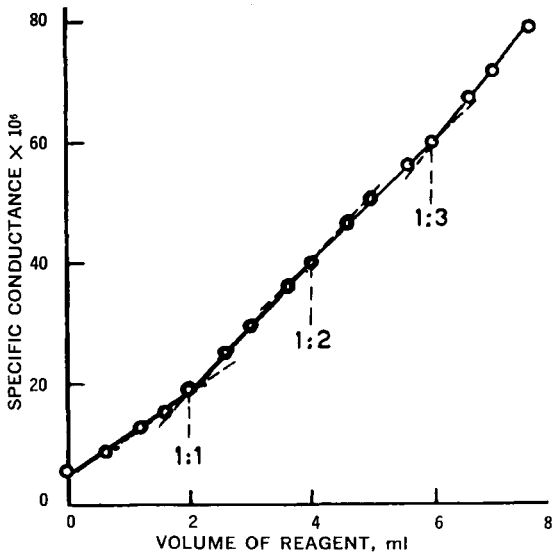


**Figure 1**—*Conductometric titration of* $UO_2^{+2}$ *ions* ($3 \times 10^{-4}$ M) *against tetracycline* ($3 \times 10^{-3}$ M).

[1] Leeds and Northrup Co.
[2] Hartmann and Braun AG, Frankfurt/Main, West Germany.
[3] Beckman DK.
[4] Perkin-Elmer 337 spectrophotometer.
[5] In the Microanalytical Unit, University of Cairo, Giza, Egypt.

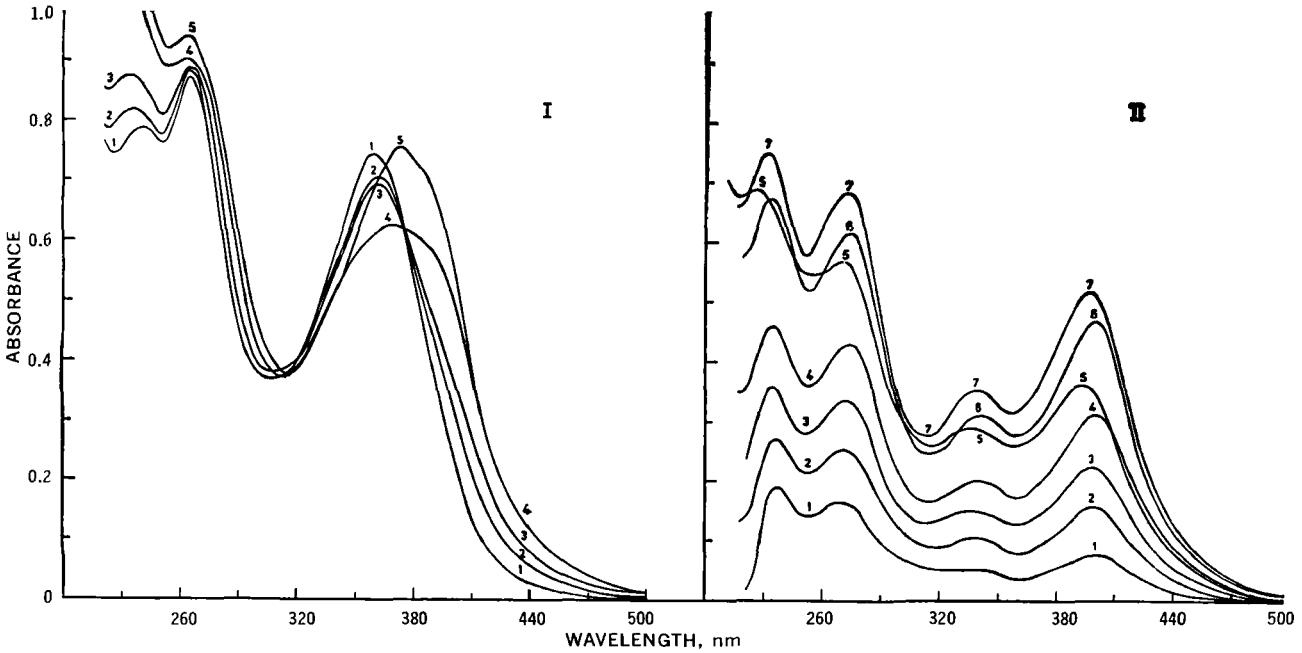


**Figure 2**—*Absorption spectra of uranyl acetate–tetracycline complex formed by the slope ratio method. Key: I, uranyl variable; and II, reagent variable.*

corrected for solvent conductance and volume changes are plotted against the volume of the ligand. The plot shows three breaks at the 1:1, 1:2, and 1:3 (uranyl–ligand) compositions. It reveals also a considerable increase in the conductance with an increase in the ligand concentration. This may be explained if it is assumed that the ligand behaves as a bidentate, using the carbonyl and the hydroxy groups in coordination with the uranyl cation. The $H^+$ ions released due to the participation of the OH group increase the conductance. Such an increase appears to be greater in the formation of the 1:2 complex. It is apparent that increased acidity is more effective in increasing conductance and overcomes the decrease of conductance due to partial balance of the charge of the central metal ion of the 1:2 species. In the same sense, the formation of the negatively charged 1:3 complex causes increased conductance at a greater rate than the 1:2 species. Further addition of excess reagent than required for the 1:3 species is accompanied by increased conductance, which may be plausibly attributed to partial acid dissociation of the free ligand. This effect is supposedly enhanced by masking the dissociated ligand in the 1:3 complex, thereby shifting the equilibrium toward further dissociation.

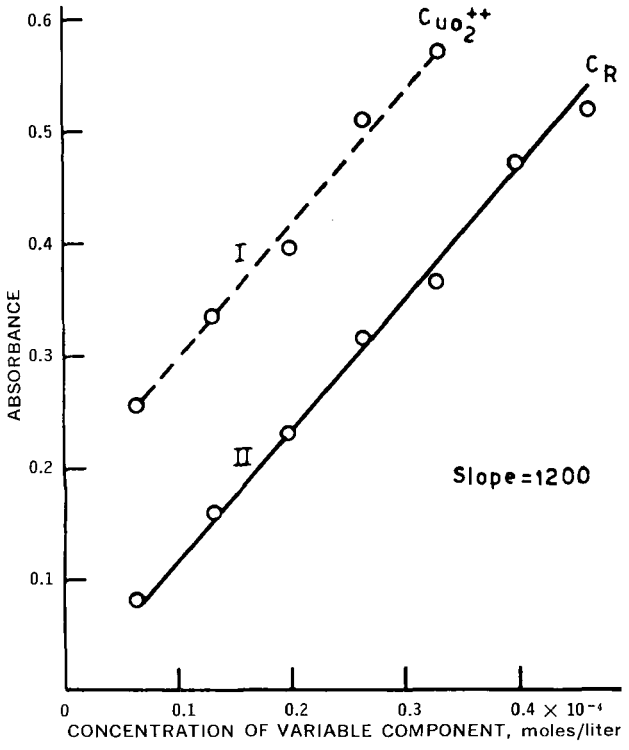


**Figure 3**—*Composition of uranyl–tetracycline complex by the slope ratio method (at* $\lambda_{404}$*).*

**Spectrophotometric Studies**—*Spectra of Uranyl–Tetracycline Complex Formed in Solutions*—The maximum absorption of uranyl acetate observed in pure dioxane occurs at 425 nm as was found previously (13). By applying the slope ratio method, plots of absorbance against wavelength are obtained (Fig. 2). Pure tetracycline yields $\lambda_{max}$ 349 nm; the corresponding uranyl complex shows a characteristic band maximum at $\lambda$ 404 nm. Absorption–concentration curves taken at that band maximum are given in Fig. 3. The slope ratio of uranyl to ligand is 1, revealing the major stability of the 1:1 species.

*Absorption Spectra of Precipitated Complex*—The complex prepared as described under *Experimental* separates as brown crystals. The spectra and chemical composition of the complex in the 1:1 mole ratio, as indicated by conductometric and spectrophotometric measurements, were studied to ascertain the behavior manifested in solutions of different reagents. For this purpose, the spectra of the complex, together with pure uranyl and pure tetracycline, were scanned in the UV, visible, and IR ranges.

The spectra in the 200–500-nm range are shown in Fig. 4. The spectra of the solutions of the complex in dioxane show three electronic transitions at $\lambda_{max}$ 404, 350, and 270 nm. These transitions are shifted from those of pure uranyl acetate and have significantly greater intensities. The peaks at the maximum absorption of the complex are closely comparable with those obtained by admixing solutions of the pure reagents at the same mole ratio.

The absorption maxima, $\lambda_{max}$, together with the molar absorptivities of the complex are shown in Table I and are compared to those of pure reagents.

The IR spectra of tetracycline and its uranyl complex are shown in Fig. 5. The structure of tetracycline is complicated; it is difficult to assign all of the bands observed with certainty. However, the IR spectrum shows an intense broad band covering the 3700–2000-$cm^{-1}$ region. In this region the $\nu_{OH}$, $\nu_{N-H}$, and $\nu_{C-H}$ are expectedly included. It also shows two carbonyl bands at 1630 and 1670 $cm^{-1}$, which can be assigned to the hydrogen-bonded ketone (14, 15) and amide-CO (16) groups, respectively.

The IR spectrum of the complex shows the absence of the high

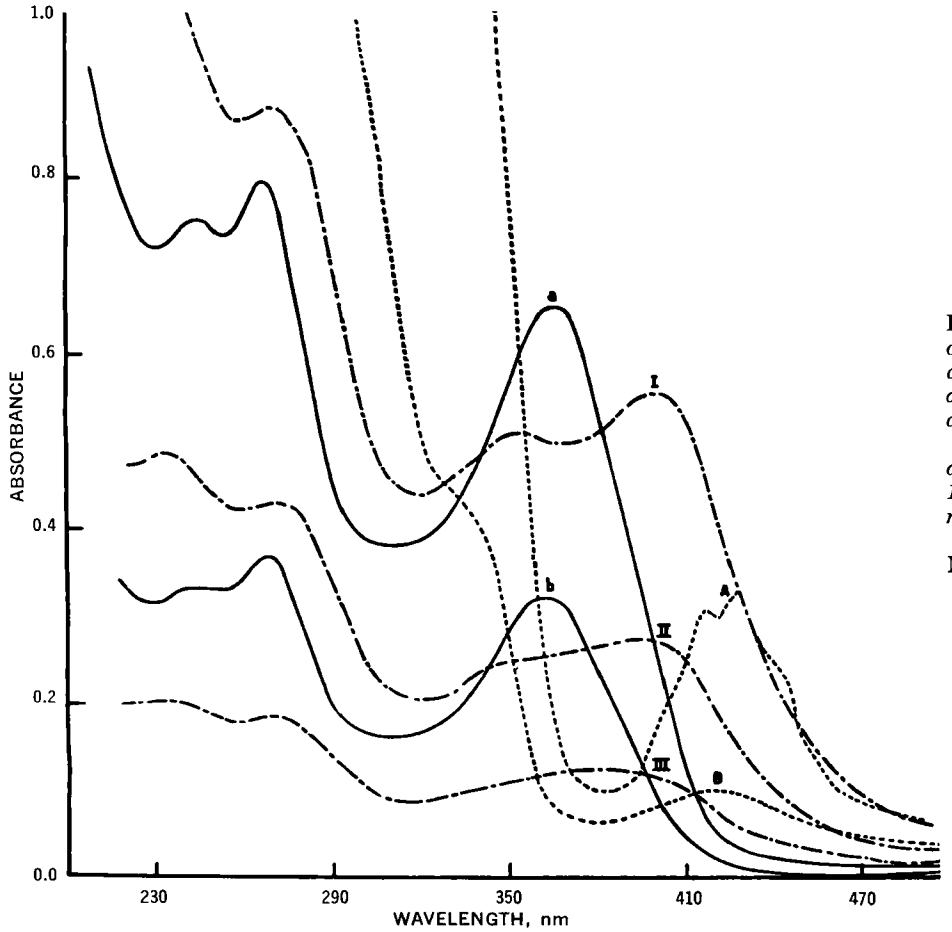


**Figure 4**—*Absorption spectra of uranyl acetate, tetracycline, and their complex in water–dioxane mixture. Key: ---, uranyl acetate, A = 6.25 × 10⁻³ M, B = 10⁻³ M; ——, tetracycline, a = 0.4 × 10⁻⁴ M, b = 2 × 10⁻⁵ M; and — - —, uranyl–tetracycline complex (1:1), I = 1 × 10⁻⁴ M, II = 0.5 × 10⁻⁴ M, and III = 0.2 × 10⁻⁴ M.*

frequency carbonyl stretching vibration at 1670 $cm^{-1}$. This suggests that the amide-CO group is involved in chelation with the $UO_2^{+2}$ ion. The broad band within the 3000–3600-$cm^{-1}$ range is considered to be due to a hydrogen-bonded hydroxy group (17).

*Chemical Microanalyses of Precipitated Complex*—The results of microanalyses of the precipitated recrystallized brown complex are: U, 29.6%; C, 33.6%; and H, 3.8%. These results are in good agreement with the calculated data for the complex composition $UO_2R{\cdot}3H_2O{\cdot}OH^-$, which are: U, 30.3%; C, 33.7%; and H, 3.8%.

Based on chemical analysis, it may be shown that the hydrogen content of the complex species and the difference in weight corresponding to the unanalyzed part of the product, principally oxygen, suggest the presence of 3 $H_2O$ molecules in the precipitated complex. This suggests a 1:1 complex. The presumption of the presence of water molecules is confirmed by the IR data. The assigned structure (Structure I) is in agreement with the octahedral structure proposed by Szöke (18), who was able to build up a stereomodel which reflects the most suitable two-dimensional projection in the form of a regular hexagon. The uranyl axis stands perpendicular to this plane.

The stability constant *(K)* of the uranyl–tetracycline (1:1) complex is determined by the limiting logarithmic spectrophotometric method (19) using data obtained by the slope ratio method as described previously. A mean $K$ value of $1.2 \times 10^5$ was found.

**Spectrophotometric Microdeterminations**—Complex formation between $UO_2^{+2}$ ion and tetracycline was used for the microdetermination of both by a spectrophotometric method. For the determination of $UO_2^{+2}$ ion, varying concentrations of the uranyl ion are added to a constant concentration of the ligand, as in the slope ratio method. The absorbances are scanned at different wavelengths, using the solvent (water–dioxane mixture, $D = 30$) as a blank. Plots of absorbances, corrected for excess tetracycline, against concentration of the metal ion (Fig. 6) indicate that the suitable wavelengths for such determination are $\lambda_{446}$ and $\lambda_{440}$ nm. The absorbance is a linear function of the metal-ion concentration in accordance with Beer's law at concentrations up to about $3.0 \times 10^{-5}$ $M$, but at higher concentrations the absorbance deviates from Beer's law. The lowest concentration of $UO_2^{+2}$ estimated with considerable accuracy, within the resolution of the instrument, corresponding to significant absorbance values, amounts to $6.6 \times 10^{-6}$ $M$ (about 2 ppm).

Because of the medical importance of tetracycline, the microdetermination was confirmed by carrying out another experiment in

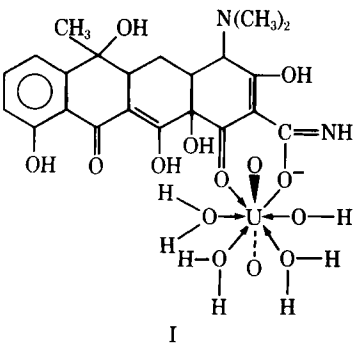


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**Table I**—Absorption Maxima and Molar Absorptivities

| Compound | $\lambda_{max}$, nm | log ε, $mole^{-1}$ $cm^{-1}$ |
|---|---|---|
| Uranyl acetate | 428 | 1.72 |
| | 416 | 1.69 |
| | 335 | 2.64 |
| Tetracycline | 364 | 4.22 |
| | 270 | 4.28 |
| Uranyl–tetracycline complex | 404 | 3.74 |
| | 350 | 3.71 |
| | 270 | 3.94 |

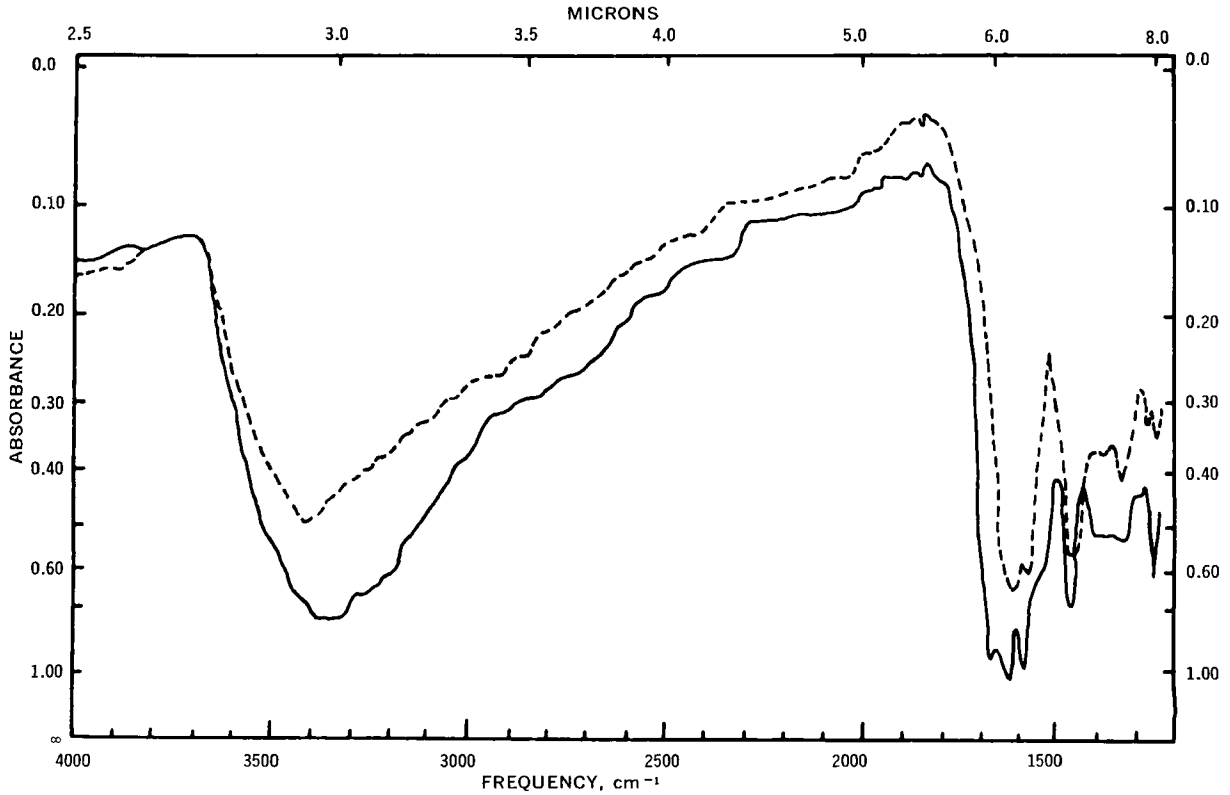


**Figure 5**—*IR absorption spectra of tetracycline and its complex with $UO_2^{+2}$ ions. Key: ——, pure tetracycline; and ---, uranyl–tetracycline complex.*

which the ligand was added in different proportions to a fixed proportion of the uranyl ions. The plots of absorbance, corrected for excess $UO_2^{+2}$ ion, against concentration of tetracycline at different wavelengths, corresponding to the peaks (Fig. 7) show that at $\lambda_{404}$ and $\lambda_{350}$ nm, a linear relationship exists to concentrations of about $5.0 \times 10^{-5}$ and $3.0 \times 10^{-5}$ *M*, respectively. The most suitable wavelength for such determination is $\lambda_{404}$ nm. The highest concentration of tetracycline estimated within the range of applicability of Beer's law was about 22 ppm, while the lowest concentration determined with considerable precision was about $6.6 \times 10^{-6}$ *M* (3 ppm).

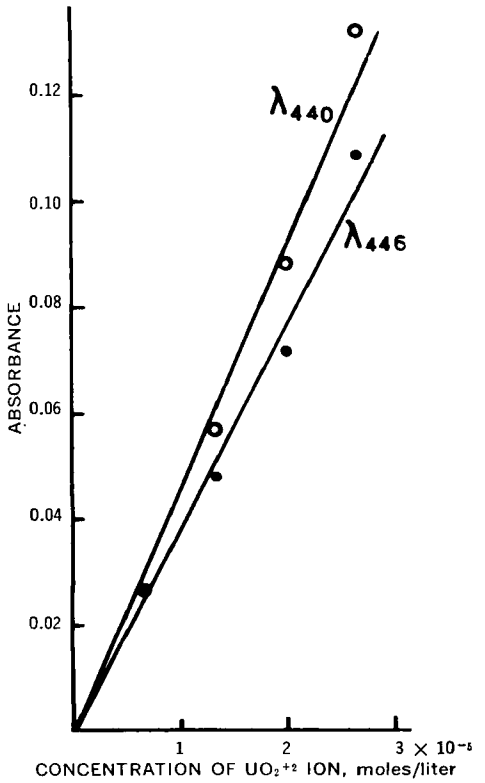


**Figure 6**—*Spectrophotometric microdetermination of $UO_2^{+2}$ ion with tetracycline, corrected for excess tetracycline.*

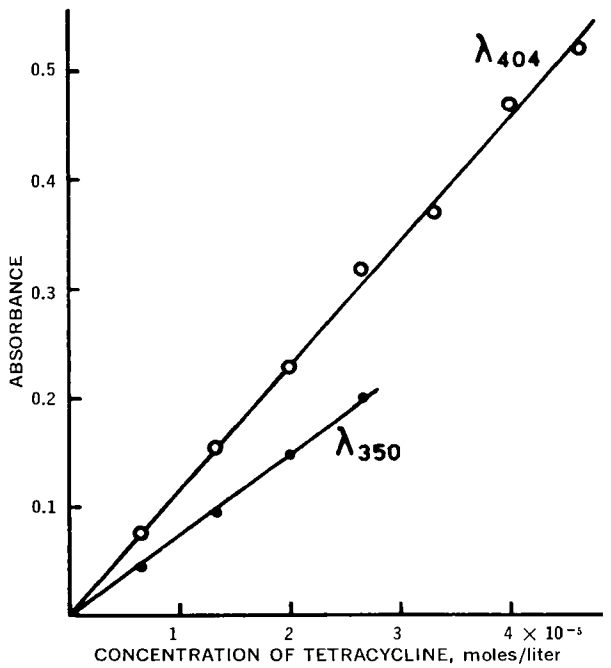


**Figure 7**—*Spectrophotometric microdetermination of tetracycline with $UO_2^{+2}$ ion, corrected for excess uranyl.*

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# Sodium Heparin Determination: Comparison of an Instrumental Method with the USP Method


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**Abstract** □ An instrumental method for determining sodium heparin in aqueous solutions is described that has the advantages over the USP grading procedure of being simpler and quicker but just as reproducible. It is based on the principle that heparin concentration in solutions can be determined by measuring the recalcification clot time of heparinized sheep plasma by mechanical means. The concentration is read from a standard curve of clot time *versus* concentration of standard heparin.



**Keyphrases** □ Heparin—determination using instrumental method, compared to USP method □ Sodium heparin—determination using instrumental method, compared to USP method □ Blood clotting time—used in instrumental method for determination of sodium heparin in aqueous solutions, instrumental method compared to USP method


The USP (1) procedure for sodium heparin assay is rather cumbersome in both operation and calculation. This paper describes an instrumental method which offers ease of operation, calculation, and reliability.

The USP procedure is based on the increase in recalcification clotting time of sheep plasma with increasing concentration of heparin. The test is performed by comparing grades of clotting in assay samples with grades of clotting in a set of standards. Both sets of samples must be prepared within 20 min of each other, and the end-points are checked 1 hr after addition of the calcium chloride reagent.

With the instrumental method, standardized sheep plasma is used to develop a standard curve of known sodium heparin concentrations plotted against clotting time measured by the instrument. From this standard curve, sodium heparin concentrations can be read directly using clotting time obtained with assay samples.

The instrumental method differs from the USP method by measuring the time at which the clot forms rather than by grading the extent of clotting after 1 hr. The principle of the instrument is shown in Fig. 1.

## EXPERIMENTAL

Each test vial contains a magnetic stainless steel ball. A drive motor moves the vial up and down in the reaction well. Before a clot forms, the stainless steel ball is held stationary by two calibrated permanent magnets as the vial moves. While in this stationary position, the ball interrupts a light beam directed through the vial at a photocell. As the vial moves up and down relative to the suspended ball, the test fluids flow back and forth around the ball, ensuring continuous and uniform mixing. When calcium chloride solution is added to the moving vial, an automatic timing device is activated. When clot formation occurs, the ball is pulled out of the magnetic field. Displacement of the ball more than 0.8 mm in either direction from its original suspended position permits the light beam to strike the photocell, stopping the timer.

The nominal bore diameter for the vial is 0.505 cm. The spherical nominal diameter of the stainless steel ball is 0.475 cm, having a weight of 0.4 g. The nominal clearance of the ball suspended in the vial is 0.015 cm on either side. Silicone oil standards of varying viscosities are used to factory calibrate each instrument. In brief, a master clot timing instrument is initially set up by using the most viscous plasma sample obtainable. A set point for the magnetic field is established with the viscous plasma. Silicone oil standards are prepared with the master instrument, which enable future adjustments to be made on subsequent production instruments.

One silicone oil standard has a viscosity that enables the photocell system to trip intermittently a timing motor relay circuit. This