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Note

## Quantitative Determination of Submicro Amounts of Uranine in Subterranean Rivers

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Owing to its intense colour, uranine is very often used for following the course of underground waters, particularly subterranean rivers. The qualitative identification of uranine does not present any particular difficulty, even in the case of very small concentrations. Ultra-violet light causes a strong fluorescence, so that  $5 \cdot 10^{-5}$  g. uranine/l. can easily be detected.

There is however a method which enables the detection of the extremely small concentrations of uranine with the naked eye (Fig. 1.). A beam of intense white light is directed into the glass containing an uranine solution of low concentration, the glass placed in front of a black screen, and the green colour

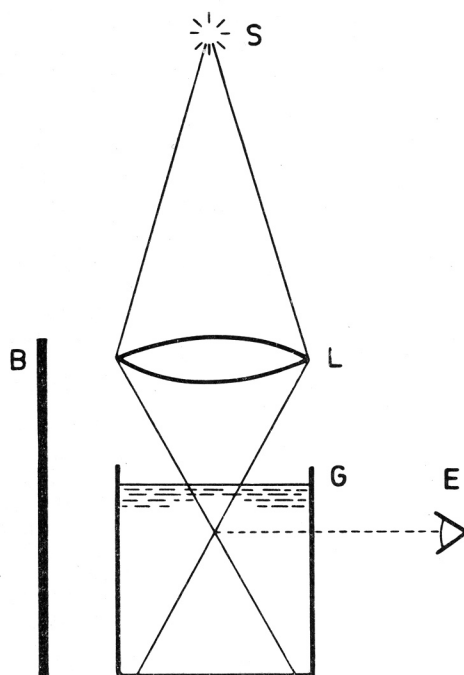


Fig. 1. Detection of submicro quantities of uranine in water solution: S — source of intense white light, B — black screen, L — lense, G — glass containing the tested solution, E — eye.

of uranine can then be observed. The limit of sensitivity in this case may reach  $5.10^{-7}$  g. uranine/l. This method is often used to prove the beginning and the end of the outflow of the uranine in subterranean rivers.

The total quantity of uranine which has flowed out can be calculated if its concentration and the average flow are known. The quantitative determination of the uranine concentration is usually carried out spectrophotometrically, measuring the extinction at the convenient wavelength. The limit of sensitivity in such a case is approximately  $5.10^{-4}$  g. uranine/l.

Using the fluorometric method a considerably higher sensitivity can be obtained,<sup>1</sup> up to  $2.10^{-8}$  g. uranine/l. This method, however, has the disadvantage that, where natural waters are encountered, particles of organic matter cause a bluish fluorescence, which considerably reduces the sensitivity of determination.

When investigating large subterranean streams it often happens that the concentration of uranine drops below the limit of sensitivity of these methods, making thus quantitative determination impracticable. In such cases the concentration can be determined by a new method, which is based on the fact that the limit of sensitivity for qualitative identification of uranine as shown in Fig. 1. is clearly defined and reproducible.

#### EXPERIMENTAL

A slightly modified version of the *Malux 250* slide projector, with a 250 W bulb was used as the source of light. The lenses of the projector were so arranged that a focused beam of a highly intense white light was obtained at a distance of about five centimeters. The projector was mounted on a support in a vertical position, while the glass cell containing the solution of uranine of unknown concentration, was placed against a black screen. In order to prevent any effect of surrounding light and enable the eyes to adapt themselves to a maximum sensitivity, the work on the apparatus was performed in a dark chamber.

Under the condition described above, it was possible to prove the presence of uranine at the concentration of  $5.10^{-7}$  g./l. A solution containing  $4.10^{-7}$  g. uranine/l. however, did not show a green fluorescence any longer.

A higher sensitivity can be obtained if an optimum pH value is maintained in the solution. It is a well known fact that the intensity of fluorescence increases with the increase of alkalinity, reaching its maximum at approximately pH 13. This pH value was obtained by using a 0.1 M sodium hydroxide solution for dilution instead of water. The limit of sensitivity at this pH value is  $10^{-7}$  g. uranine/l. A further increase of sensitivity can be obtained by adding organic solvents which are miscible with water, but do not dissolve uranine appreciably<sup>2</sup>.

By applying the two factors mentioned above — that is, the optimum pH value and the addition of acetone — a sensitivity of  $10^{-8}$  g. uranine/l. is obtained. If dioxane instead of acetone is used, an even higher sensitivity can be obtained. At the pH 13 in 50% dioxane solution, the lowest perceptible concentration is  $5.10^{-9}$  g. uranine/l. All these data refer to an uranine solution in distilled water.

Now, in a solution of an unknown uranine concentration a point can be reached when on diluting that solution the green colour just disappears. In this moment the limit of sensitivity is reached and the concentration of such a solution is  $5.10^{-9}$  g. uranine/l. From this figure and from the known initial and final volume of the solution (the solution being diluted with mixture of 0.1 M sodium hydroxide and dioxane from the burette) the initial concentration of uranine in the solution can be calculated.

$$V_{\text{initial}} \times C = (V_{\text{initial}} + V_{\text{added}}) \times 5.10^{-9} \quad (1)$$

If in equation (1)  $V_{\text{initial}}$  is the volume of the solution of uranine,  $V_{\text{added}}$  is the volume of sodium hydroxide dioxane solution added, and  $C$  is the initial concentration

of uranine in the sample, the unknown concentration can be calculated from the equation (2):

$$C = \frac{(V_{\text{initial}} + V_{\text{added}}) \times 5 \cdot 10^{-9}}{V_{\text{initial}}} \quad (2)$$

Thus any unknown concentration of uranine, exceeding  $5 \cdot 10^{-9}$  g./l. can be determined by titrating a solution of unknown concentration with sodium hydroxide dioxane solution, until the end point, defined by the sensitivity limit, is reached.

The precision of this method varies in dependence upon the initial concentration of uranine and is of an order of magnitude of 5—10%. If the initial concentration is higher, a greater volume of sodium hydroxide — dioxane mixture must be added, and then the error of determining the end point is smaller. However, since, as a rule, overtitration is involved, the error is reproducible to some extent, and can be reduced to about 2—5%.

For natural waters the sensitivity limit is slightly different. For water from the municipal water supply it is  $6 \cdot 10^{-9}$ , and for water taken from the Sava river  $7 \cdot 10^{-9}$  g.uranine/l. The precision of determination was essentially the same as with distilled water.

If great initial concentration of uranine are used, the volume after the titration is too large. Therefore the initial solution must first be diluted (e. g. 1 : 1000) and the uranine is then determined in the described way.

In conclusion it should be added that the sensitivity of this method can be further increased by the extraction of uranine from the diluted solution. G. Trümpler<sup>3</sup> was one of the first who studied the adsorption of uranine on charcoal. Other adsorbents such as talc, alumina, infusorial earth etc., have been also employed, and the adsorbed uranine was desorbed with an alcoholic sodium hydroxide solution.

In order to examine the possibility of applying the extraction to increase the sensitivity of our method, five liters of uranine solution with  $10^{-11}$  g./l. were prepared. The solution was passed through column filled with alumina. 25 ml. of a 1 : 1 mixture containing 0.1 M sodium hydroxide and ethyl alcohol were used for desorption. The eluate was completely evaporated on a water bath and the residue dissolved in 5 ml. (1 : 1) dioxane and water mixture. Using the method described above, in such a solution  $10^{-8}$  g.uranine/l. were found. These results therefore show that the extraction and desorption of uranine can be carried out quantitatively.

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#### IZVOD

#### Kvantitativno određivanje submikrokoličina uranina u vodama ponornica

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Submikro količine uranina u vodama ponornicama mogu se kvantitativno odrediti razrjeđivanjem uzorka i određivanjem razrjeđenja kod kojega nestaje zelena fluorescencija. U prisustvu dioksana i kod pH 13 (bijelo upadno svjetlo i crna pozadina) zelena fluorescencija nestaje kod koncentracije  $5 \cdot 10^{-9}$  g.uranina/l. Nestanak boje je dobro definiran i reproducibilan. Početna koncentracija uranina može se izračunati iz početnog i konačnog volumena otopine. Metoda se može primijeniti za određivanje koncentracije u području od približno  $10^{-5}$  do  $10^{-9}$  g.uranina/l., a tačnost mjerenja varira od 2—10%, u ovisnosti o početnoj koncentraciji uzorka. Vrlo male koncentracije uranina mogu se odrediti kombinacijom metode ekstrakcije i gore opisane metode.

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