

SPECTROFLUOROMETRIC DETERMINATION
OF MERCURY (II) IN RIVER WATERS WITH
RHODAMINE B

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The effect of mercury (II), in the presence of iodide, on the fluorescence of the aqueous solution of rhodamine B was studied by means of the spectrofluorometric method. The optimum conditions in respect to the concentrations of mercury and iodide, the pH values and the effect of some salts on rhodamine B fluorescence quenching were determined. By the same method small amounts of mercury were measured in the waters from the Sava river. The results agree well with the values obtained by the method of atomic absorption spectroscopy.

In clear natural and unpolluted waters mercury appears as sulphate, chloride or hydroxide (1). In the waters of the lakes and rivers with pH values from 4 to 7 and pCl from 3 to 5 the complexes HgCl_2 , HgOHCl or Hg(OH)_2 can be found (2). In waste and polluted waters mercury is mostly present as HgS and sulphide-anion complexes as HgS_2H_2 , HgS^- and HgS_2^{2-} . These inorganic compounds of mercury are easily dissolved ($5 \times 10^{-4} \text{ mol dm}^{-3}$), except HgS , which is poorly soluble ($K = 10^{-50.96}$) (1, 3). The solubility of mercury increases with increasing pH values. Up to pH 5.5 the total mercury concentration depends on the concentration of HgS_2H_2 , in the pH range from 5.5 to 6.5 it depends on the concentrations of HgS_2H_2 and HgS_2H^- , in the pH range from 6.5 to 7.5 on the concentration of HgS_2H^- , between pH 7.5 and 8.5 on the concentrations of HgS_2^- , and over pH 9 it depends only on the concentration of HgS_2^{2-} (4).

Earlier investigations showed that the fluorescence quenching of the xanthene dyes can be used for the determination of small amounts of heavy metals (5—8).

As a xanthene dye rhodamine B, in aqueous solutions, shows a tendency of aggregating and forming a dimer with the increasing of the concentration (9). The dimer can be described as a linear combination of the product of one ground and one excited state of the isolated molecule (10). The formation of dimeric rhodamine is promoted in aqueous rhodamine B by addition of LiCl. At increasing LiCl concentration more H₂O molecules are found to surround the Li⁺ and Cl⁻ ions, and as the number of the remaining water molecules contributing to dissolve rhodamine B decreases, the effective rhodamine B concentration increases (11). The fluorescent quantum efficiency of aqueous rhodamine B was found to be greatly affected by dimerization, since the rhodamine B dimer in water was recognized as contributing little to fluorescence (12, 13).

The spectrofluorometric method for the direct determination of mercury (II) in river waters on the principle of rhodamine B fluorescence quenching is described in the present work. The results are compared with the values of mercury (II) obtained by atomic absorption spectroscopy.

MATERIAL AND METHODS

Rhodamine B (Merck), Bi(NO₃)₃ x 5 H₂O, 3 CdSO₄ x 8 H₂O, CuSO₄ x 5 H₂O, KBr, MgCl₂ x 6 H₂O, NaCl, Na₂NPO₄, NaI, NaNO₂, C₆H₅Na₃O₇ x 2 H₂O, Ni(NO₃)₂ x 6 H₂O, ZnCl₂ (Kemika), Fe₂(SO₄)₃ x 9 H₂O (Laphona), CrCl₃ x 6 H₂O, FeSO₄ x 7 H₂O, HgCl₂, KSCN, MnCl₂ x 4 H₂O, Pb (CH₃COO)₂ x 3 H₂O (Merck), NaNO₃ (Carlo Erba) were all analytical grade.

As solvent in the preparation of samples of waters for the fluorescence measurements chloroform (Kemika) — analytical grade reagent — was used without additional purification.

KMnO₄, SnCl₂ x 2 H₂SO₄, HNO₃, H₄CINO (Kemika) without purification were used as analytical grade reagents in the preparation of samples of waters for the determination of mercury by atomic absorption spectroscopy.

Samples of surface waters were taken from the river Sava near Zagreb at the following sites: before the first collector (a), between the first and the second collector (b), before the second collector (c), and after the second collector of the drainage system of New Zagreb City near the railway bridge near Jakuševac (d). All samples were taken at a distance of 2 m from the river bank, at a depth of approximately 0.5 m. The samples of waters were taken twice, in autumn (November 15, 1981) and in winter (March 15, 1982).

The absorption spectra at 293K were recorded on a Perkin-Elmer spectrophotometer, Model 202. Fluorescence as well as the excitation and emission spectra were measured on a Perkin-Elmer M—3000 fluorescence spectrometer equipped with separate monochromators for excitation and emission scanning, and with digital displays of wavelengths and emis-

sions. As a light source for fluorescence measurements served a xenon discharge lamp pulsed at line frequency. Slit-widths of 5 nm for excitation and of 10 nm for emission were chosen, and 10 x 45 mm quartz cells were used. The pH values of the solutions were adjusted with a pH-Meter M—28, Radiometer, Copenhagen.

The quantity of mercury was determined with a Perkin-Elmer atomic absorption spectrometer, Model 2380 at a wavelength of 253.6 nm, and at slit-width of 0.7 nm. The preparation of samples included a concentration step and u. v. irradiation.

A standard solution of mercury (II) (1×10^{-3} mol dm $^{-3}$) was prepared by dissolving 0.0679 g HgCl $_2$ in redistilled water and made up to 250 ml. This stock solution was diluted 100 times to a working standard solution of mercury (II) (1×10^{-5} mol dm $^{-3}$). Such extremely dilute solutions of mercuric salts had to be prepared immediately before use, since the salts are quickly adsorbed on the glass of the vessels.

A stock solution of sodium iodide (1×10^{-1} mol dm $^{-3}$) was prepared by dissolving 1.4989 g NaI in redistilled water and made up to 100 ml. A standard working solution of sodium iodide (1×10^{-3} mol dm $^{-3}$) was prepared by diluting the stock solution just before use.

A buffer solution with pH 2.0 was prepared according to the procedure in Ph. Jug. II.

Stock solutions, 5×10^{-2} mol dm $^{-3}$, of the salts were prepared by dissolving corresponding quantities of the salts in redistilled water.

Bi (NO $_3$) $_3$ was prepared by the addition of a necessary volume of concentrated HNO $_3$. By diluting stock solutions standard working solutions for the investigations of the effect of rhodamine B on fluorescence quenching were prepared. The rhodamine B solution consisted of 5×10^{-6} mol dm $^{-3}$ of rhodamine B and 5×10^{-4} mol dm $^{-3}$ of NaI.

A standard solution of rhodamine B (1×10^{-3} mol dm $^{-3}$) was prepared by dissolving 0.0479 g of rhodamine B in redistilled water and made up to 100 ml. A solution of 1×10^{-4} mol dm $^{-3}$ of rhodamine B was taken as the standard working solution.

For the determination of calibration curve, 100 ml glass beakers were used and the following solutions with a total volume of 10 ml were prepared:

- the blank solution for adjusting the instrument contained 5 ml of the buffer solution of pH 2, 0.5 ml of 1×10^{-3} of NaI, and 4.5 ml of redistilled water;
- the control solution for determining the maximum value of fluorescence intensity contained 5 ml of the buffer solution, 0.5 ml of 1×10^{-2} mol dm $^{-3}$ of NaI, 0.5 ml of 1×10^{-4} mol dm $^{-3}$ of rhodamine B, and 4.0 ml of redistilled water;
- the solutions for determining the calibration curve of mercury (II) contained 5 ml of the buffer solutions, 0.5 ml of 1×10^{-2} mol

dm^{-3} of NaI, 1 ml of 1×10^{-4} mol dm^{-3} of rhodamine B, 0.1–1.0 ml of 1×10^{-6} mol dm^{-3} of HgCl_2 , and were made up to a total volume of 10 ml with redistilled water. The intensity of fluorescence of these solutions was measured on the fluorescence spectrometer in the quartz cells at excitation and emission wavelengths of 350 and 585 nm.

The examined samples of the river water were filtered over the Whatman filter paper No. 1. The organic substances present in the filtered water were removed by extraction with chloroform in a separatory funnel.

In the previously prepared samples of water mercury (II) was determined according to the following procedure: in a 100 ml glass beaker 5 ml of the buffer solution, 0.5 ml of 1×10^{-2} mol dm^{-3} of NaI, 0.5 ml of 1×10^{-4} mol dm^{-3} of rhodamine B, and 4.0 ml of the analysed water were added, and mercury (II) was measured. Depending on the quantity of mercury, the samples of waters were concentrated by evaporation on a water bath to the concentrations that fitted the calibration curve of mercury.

Mercury was determined by atomic absorption spectroscopy according to the procedure of BITC (14).

RESULTS AND DISCUSSION

The effect of sodium iodide concentration on rhodamine B fluorescence intensity

The investigation of the effect of the concentration of sodium iodide on the intensity of fluorescence of 5×10^{-6} mol dm^{-3} of rhodamine B has shown that the concentrations lower than 5×10^{-3} mol dm^{-3} of sodium iodide by no means influence fluorescence intensity.

A concentration of 2.5×10^{-1} mol dm^{-3} of sodium iodide quenches fluorescence by 50%, while 90% fluorescence quenching is effected by a concentration of 1.4 mol dm^{-3} of sodium iodide (Figure 1).

Figure 2 shows the absorption curves of the monomeric form of rhodamine B and the change of this form to dimer due to increased concentration of sodium iodide. All spectra pass through an isobestic point at 535 nm, which confirms the equilibrium of the monomer-dimer of rhodamine B. Although curve B in Figure 2 shows the monomer form of rhodamine B, the fluorescence is found to be 10% of that shown by curve A, as well as by curves C, D and E, which are dimeric forms.

Evidently, dimerization is a consequence of the increased effective concentration of rhodamine B because of the water molecules surrounding the Na^+ and I^- ions, as predicted earlier (11). However, in this case the high rhodamine B fluorescence quenching is not a consequence of the monomer-dimer change.

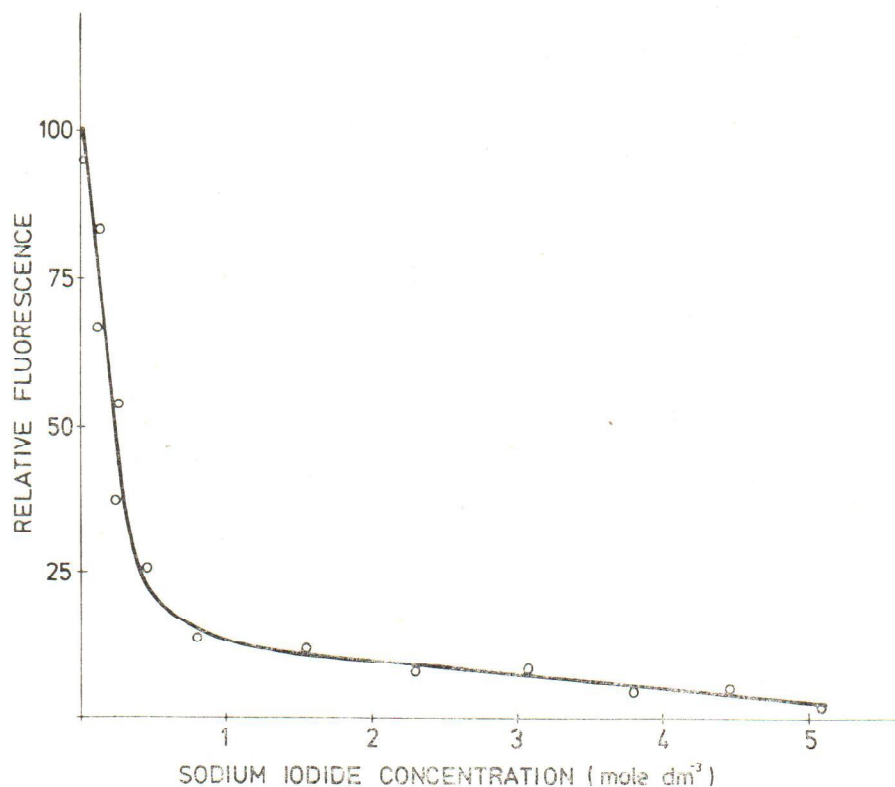


Fig. 1 — The effect of sodium iodide concentration on fluorescence intensity of rhodamine B ($5 \times 10^{-5} \text{ mol dm}^{-3}$)

The effect of concentration of some salts on rhodamine B fluorescence intensity

Table 1 shows the concentrations of some salts and their effect on the fluorescence intensity of $5 \times 10^{-5} \text{ mol dm}^{-3}$ of rhodamine B in the presence of $5 \times 10^{-4} \text{ mol dm}^{-3}$ of sodium iodide. It is seen that all salts, except HgCl_2 , do not influence the fluorescence intensity of rhodamine B even in much greater concentrations than is the case in the examined samples of water.

The effect of sodium iodide concentration on fluorescence intensity of rhodamine B in an aqueous solution with mercury (II)

Mercury (II) by itself has no influence on the intensity of rhodamine B fluorescence. Mercury (II) quenches rhodamine B fluorescence only

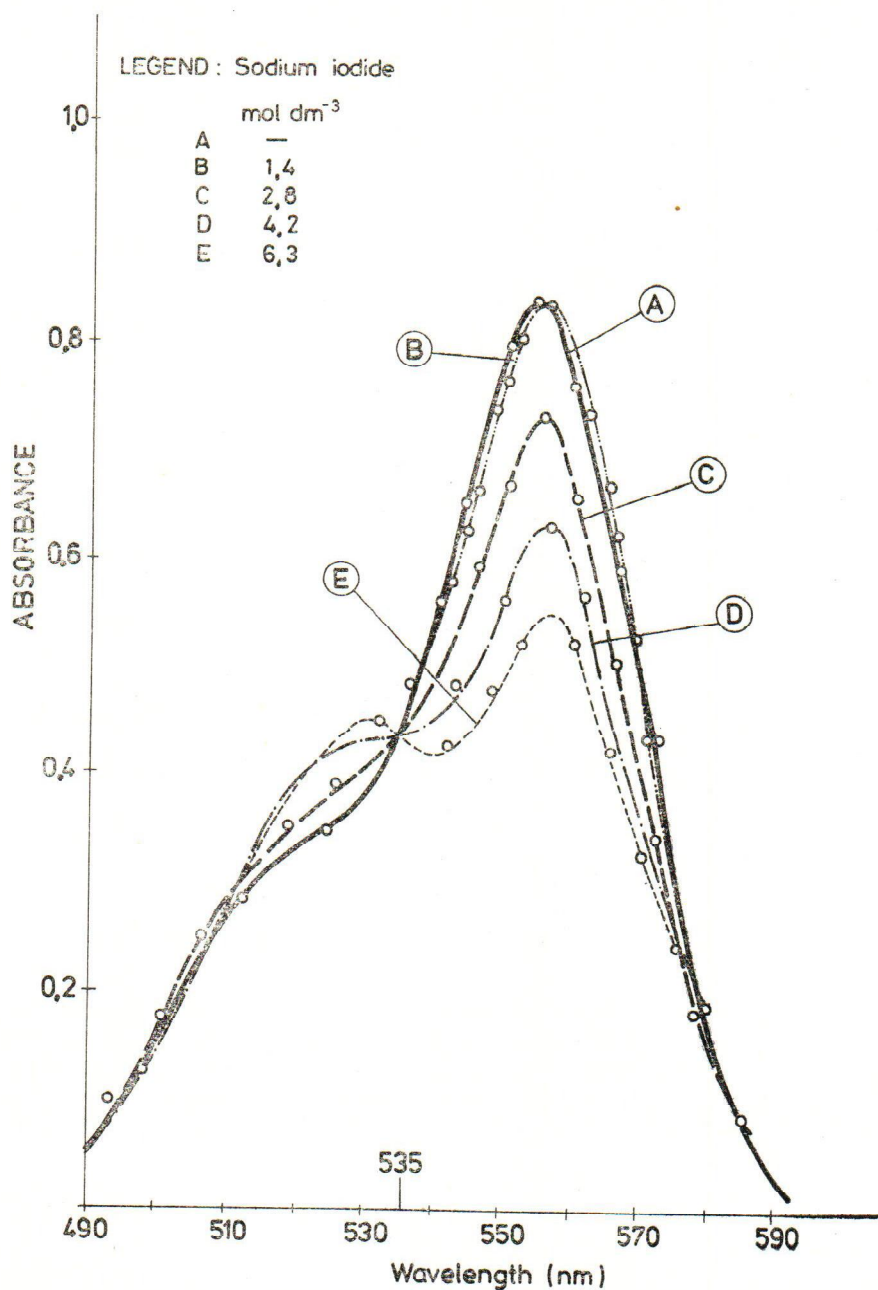


Fig. 2 — The effect of different concentrations of sodium iodide on absorption spectra of aqueous solutions of rhodamine B (1×10^{-5} mol dm⁻³)

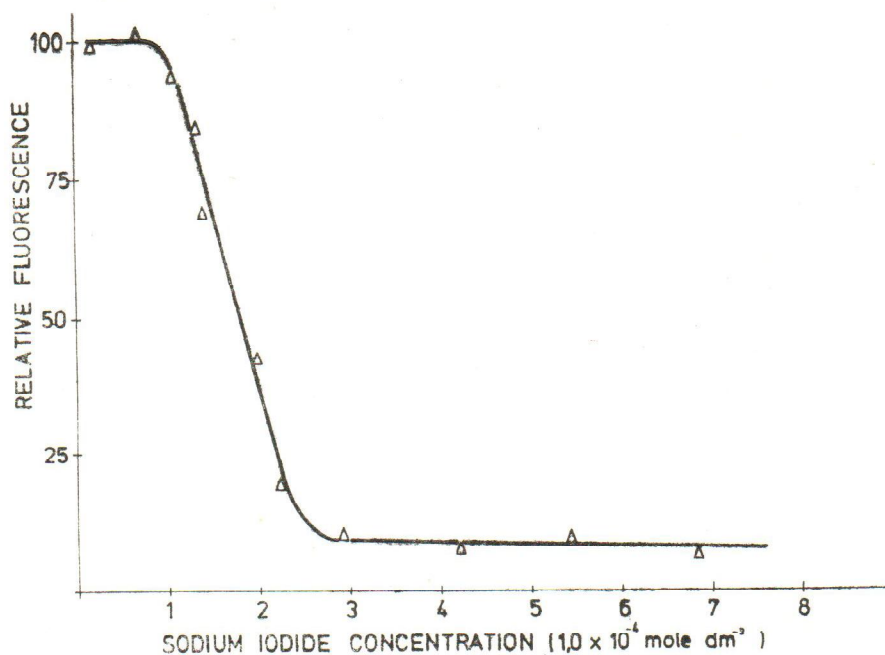


Fig. 3 — The effect of sodium iodide concentration on fluorescence intensity of rhodamine B ($5 \times 10^{-6} \text{ mol dm}^{-3}$) in the presence of mercury (II) $1 \times 10^{-6} \text{ mol dm}^{-3}$

in the presence of iodide ions. Figure 3 shows the effect of a mercury (II) concentration of $1 \times 10^{-6} \text{ mol dm}^{-3}$ on the fluorescence quenching of $5 \times 10^{-6} \text{ mol dm}^{-3}$ rhodamine B. Optimum effects were obtained in the presence of the sodium iodide concentrations between 3×10^{-4} and $1 \times 10^{-3} \text{ mol dm}^{-3}$.

The effect of pH on rhodamine B fluorescence quenching

Our investigations have shown that the pH considerably influences rhodamine B fluorescence quenching (Figure 4). Complete fluorescence quenching was obtained between pH values 1 and 3.5, while pH values higher than 6.5 had no influence on fluorescence quenching.

The effect of mercury (II) concentration on rhodamine B fluorescence quenching

The effect of mercury (II) concentration on rhodamine B fluorescence quenching was investigated under optimum conditions: the concentration of sodium iodide of $5 \times 10^{-4} \text{ mol dm}^{-3}$, of rhodamine B of $5 \times 10^{-6} \text{ mol dm}^{-3}$ and pH 2.0.

Table 1
The effect of concentrations of some salts on rhodamine B fluorescence intensity

Salts	Concentration (mol dm ⁻³)	Relative fluorescence %
None*	—	100.0
HgCl ₂	1.0 x 10 ⁻⁶	51.4
Bi(NO ₃) ₃ x 5 H ₂ O	5.0 x 10 ⁻⁴	97.0
3 CdSO ₄ x 8 H ₂ O	1.2 x 10 ⁻³	99.4
CoSO ₄ x 7 H ₂ O	7.0 x 10 ⁻³	98.5
CrCl ₃ x 6 H ₂ O	1.0 x 10 ⁻³	99.3
CuSO ₄ x 5 H ₂ O	2.4 x 10 ⁻³	98.8
FeSO ₄ x 7 H ₂ O	1.3 x 10 ⁻³	99.1
Fe ₂ (SO ₄) ₃ x 9 H ₂ O	2.5 x 10 ⁻⁴	95.9
KBr	1.5 x 10 ⁻²	101.4
KSCN	1.3 x 10 ⁻³	98.8
MgCl ₂ x 6 H ₂ O	1.2 x 10 ⁻²	98.6
MnCl ₂ x 4 H ₂ O	4.0 x 10 ⁻²	100.8
NaCl	7.0 x 10 ⁻²	101.5
Na ₂ HPO ₄	6.0 x 10 ⁻²	102.0
NaNO ₂	8.0 x 10 ⁻³	99.2
NaNO ₃	2.3 x 10 ⁻³	101.3
C ₆ H ₅ Na ₃ O ₇ x 2 H ₂ O	5.0 x 10 ⁻²	101.2
Ni (NO ₃) ₂ x 6 H ₂ O	4.0 x 10 ⁻³	98.2
Pb (CH ₃ COO) ₂ x 3 H ₂ O	1.0 x 10 ⁻²	99.5
ZnCl ₂	5.0 x 10 ⁻³	99.7

* The solution of 5 x 10⁻⁶ mol dm⁻³ of rhodamine B and 5 x 10⁻⁴ mol dm⁻³ of NaI

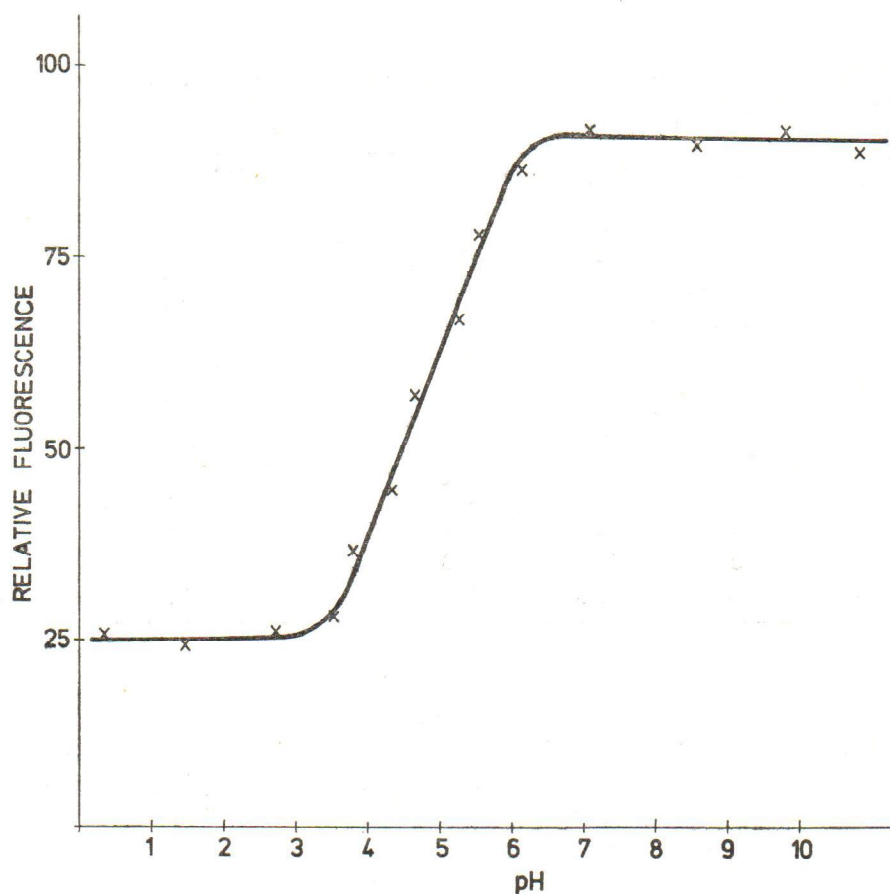


Fig. 4 — The effect of pH on fluorescence intensity of rhodamine B ($5 \times 10^{-6} \text{ mol dm}^{-3}$) and sodium iodide ($5 \times 10^{-4} \text{ mol dm}^{-3}$)

Figure 5 shows that in the mercury (II) concentration range from 1×10^{-8} to $1 \times 10^{-7} \text{ mol dm}^{-3}$ the fluorescence quenching of the aqueous solution of rhodamine B is between 10 and 50%, and that the quantity of mercury in 1.0 ml of this reaction mixture is between 20.0 and 200.0 ng.

Mercury (II) content in examined samples of river water

Table 2 gives the results of the determination of mercury in the filtered samples of water from the river Sava as average values of the

Table 2

Comparison of the results (mean of three measurements, $\mu\text{g dm}^{-3}$) obtained by different procedures for determining the content of mercury in river water

Sampling site	Autumn		Winter	
	Spectrofluorometric method	Atomic absorption spectrometry	Spectrofluorometric method	Atomic absorption spectrometry
a	0.27	0.85	0.60	0.54
b	1.21	1.39	0.96	1.18
c	1.13	1.33	1.00	0.97
d	1.40	1.22	1.18	1.26

Table 3

Recovery of mercury added to river water containing $1 \mu\text{g dm}^{-3}$ mercury

Experiment No.	$\mu\text{g dm}^{-3}$ Added	Hg^{2+} Found	Recovery %
1	1	1.83	91.5
2	2	3.18	106.0
3	3	3.95	98.8
4	4	4.79	95.8
5	5	6.32	105.3
6	6	7.40	105.7
7	8	8.76	97.3
8	9	10.11	101.0
9	10	10.88	98.9

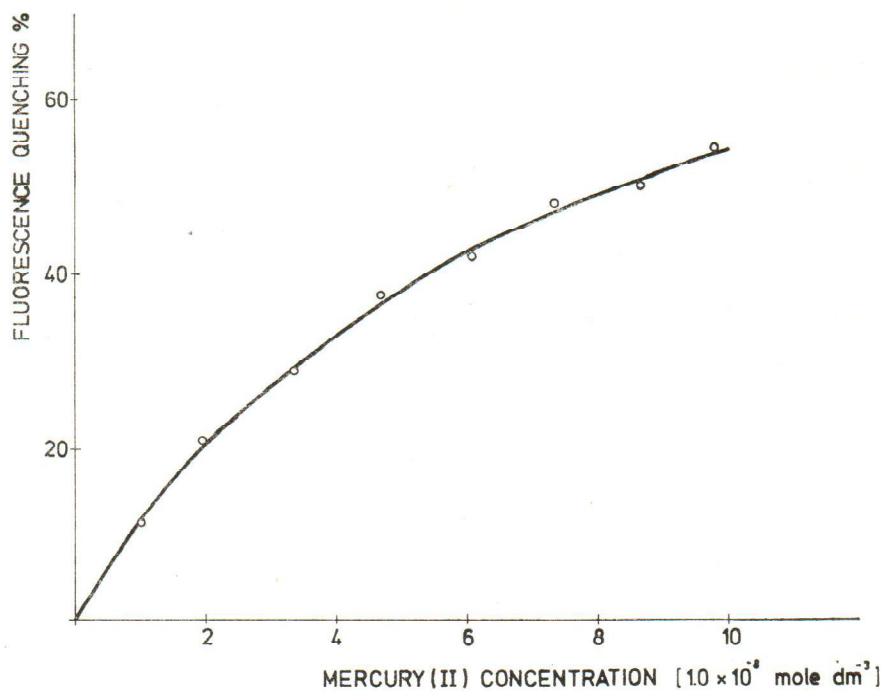


Fig. 5 — Calibration curves of mercury (II)

three measurements per sample. The results of spectrofluorometric measurements agree well with those of atomic absorption spectrometry.

Recovery test

The recovery of mercury added to river water was from 91.5—105.7% as shown in Table 3.

We hope that the described method will find its application in the toxicological-chemical analyses owing to its great sensitivity, reproducibility of the results and availability of the equipment.

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Sažetak

SPEKTROFLUOROMETRIJSKO ODREĐIVANJE ŽIVE (II) U RIJEČNIM VODAMA POMOĆU RODAMINA B

Proučavan je utjecaj žive (II) u prisutnosti jodida na pad intenziteta fluorescencije vodene otopine rodamina B spektrofotometrijskom metodom. Istraženi su i utvrđeni optimalni uvjeti u odnosu na koncentraciju žive, jodida, vrijednosti pH, te utjecaj nekih drugih soli na gašenje fluorescencije rodamina B. Ovom metodom određene su male količine žive u vodi rijeke Save, a rezultati se dobro slažu s vrijednostima koje su dobivene metodom atomske apsorpcijske spektroskopije.

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