

CCA-327

541.18.041:544.65

Original Scientific Paper

## Methorics of the Precipitation Processes. XIX. Fluorescence of Sodium Fluoresceinate, Rhodamine B and their Mixtures, as a Function of Hydrogen Ion Concentration in Water Solutions

Đ. Težak and B. Težak

Laboratory of Physical Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia, Yugoslavia

Received April 3, 1964

The fluorescence of sodium fluoresceinate and rhodamine B has been measured over a wide range of pH. The results obtained are in full agreement with the absorption measurements reported by previous authors<sup>8,13</sup>.

Precipitation maximum at about pH 3 is identical with the minimum of fluorescence intensity for the predominant form of fluorescein *i.e.* the neutral molecule. The precipitation limit at about pH 5 is characterized by the equilibrium between the neutral molecule and the monoanion; the discontinued curve at pH 7 represents the equilibrium between the monoanion and the dianion.

Fluorimetric curves of divalent cation of rhodamine B remain practically constant over a wide range of pH.

In the system consisting of sodium fluoresceinate and rhodamine B it has been shown that the phenomenon of isoelectric precipitation takes place by »equivalent body« formation. The concentrational relationship between the interacting ions is strongly influenced by the hydrogen ion concentration.

### INTRODUCTION

It was found to be of interest to examine the influence of the ionic state of a dye-molecule upon its precipitation from an aqueous acid or basic solution. Since the fluorescence may be used as a good indicator for characterizing such a system, the series of experiments were carried out with fluorescent solutions. In solutions of sodium fluoresceinate and rhodamine B the influence of the change of hydrogen ion concentration on fluorescent power of these solutions could be studied in a large gradient of concentration. Measurements were compared with the results obtained by previous authors. The absorption spectra and fluorescent properties of dilute and concentrated solutions of organic dyes were already investigated by Th. Förster<sup>6,7</sup>, V. S. Adamov<sup>1</sup>, N. Mataga<sup>9</sup>, V. Zanker<sup>14</sup>, N. Rozwadowski<sup>10</sup>, and others.

### EXPERIMENTAL

The fluorimetric measurements were performed by means of a set consisting of a B. Lange photoelectric fluorimeter, model G.A. 42, a Hg-lamp HQE 42, a violet filter for the isolation of the spectral band at 365 m $\mu$ , and a Multiflex galvanometer Lange MGF 4 (sensitivity  $6.85 \times 10^{-10}$  A/mm). An aqueous solution having the highest fluorescence intensity was used as the standard solution and its fluorescence intensity was arbitrarily set to  $G = 100$ .

The pH-values were measured with a glass electrode and a »Macbeth« pH-meter — Macbeth Corp. New York. Various amounts of nitric acid and sodium hydroxide were added to the aqueous solutions of the dyes in order to obtain different pH-values over the whole pH-range. Different pH-values in the system sodium fluoresceinate — rhodamine B (Fig. 4) were obtained by varying the concentrations of the dyes without adding any acid or base.

The charges of the particles formed were determined by ultramicroscopic electrophoretic observations.

### Materials

All chemicals used were of »AnalaR« grade (sodium fluoresceinate — Basler Drogerie Bohny & Cie A.G., Basel; rhodamine B — Merck, Darmstadt). The solutions were prepared with bidistilled water.

### RESULTS

The experimental results are shown on Figs. 1—5. Fig. 1. shows the relationship between the pH of the solution and the relative intensity of fluorescence (G) of sodium fluoresceinate. The different pH-values were obtained by adding sodium hydroxide or nitric acid. With decreasing dye-concentration the fluorescence intensity becomes smaller, but the shape of the curves remains almost the same. The number denoting each curve on

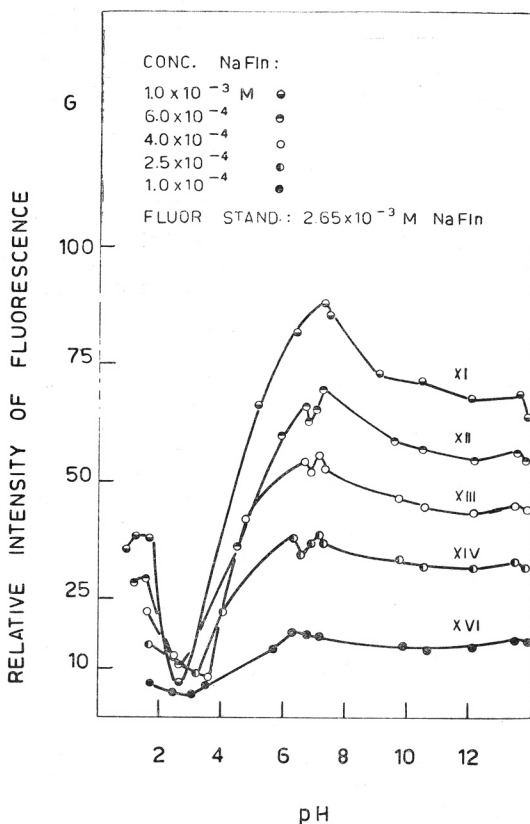


Fig. 1. Relative intensity of fluorescence of sodium fluoresceinate as a function of pH at various dye-concentrations.

Fig. 1 corresponds to the number of the same curve on Fig. 2. When putting each G-pH-curve in a separate plane corresponding to a constant dye-concentration and these planes are arranged in the order of decreasing dye-concentrations, a three-dimensional diagram as in Fig. 2. is obtained.

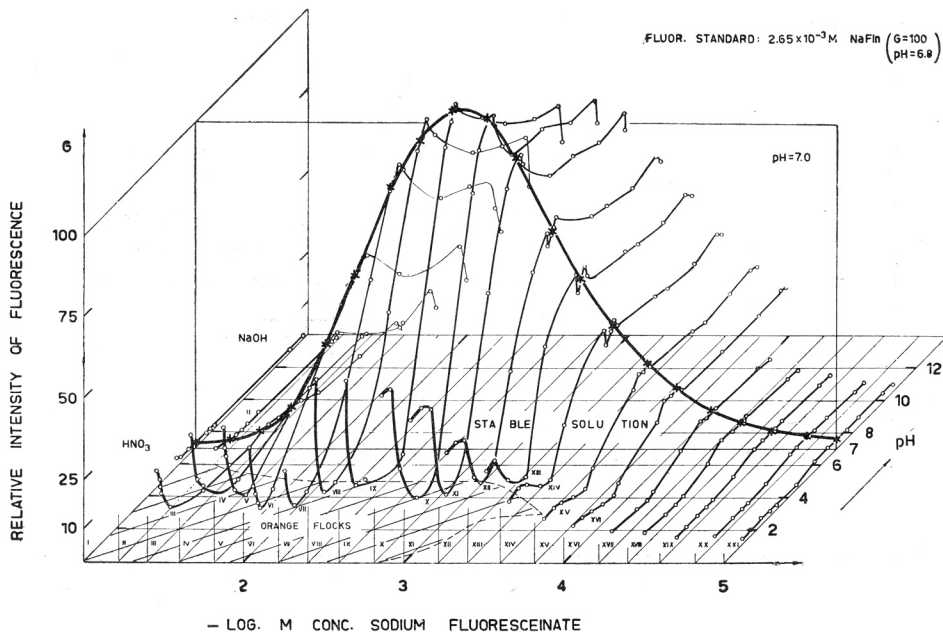


Fig. 2. Three-dimensional diagram: relative intensity of fluorescence of sodium fluoresceinate as a function of dye-concentration at various pH-values.

The three-dimensional diagram illustrates the change of fluorescence intensity of sodium fluoresceinate with the change of both pH and the dye concentration i.e. the whole pH-range was covered and the dye concentration was varied between  $10^{-1}$  and  $10^{-5}$  M/l.

The broken line on the ground-plane at pH 5 denotes the approximate limit of the precipitated neutral molecule of the fluorescein acid in the form of yellow-orange photosensitive flocks. This effect disappears as the concentration of the dye ( $10^{-4}$  M/l) becomes smaller. From its minimum at pH 5 the fluorescence intensity increases sharply with increasing pH reaching its maximum at pH 7. Above this value fluorescence intensity remains practically constant increasing only very slightly in the whole gradient of sodium fluoresceinate concentration; the systems are almost stable.

The interpolation of fluorescence intensity in the plane corresponding to pH 7 gives a typical fluorometric curve at constant pH.

Fig. 3. shows a similar curve for rhodamine B as was shown for fluorescein in Fig. 2. The dye-concentration was changed between  $4 \times 10^{-2}$  and  $6 \times 10^{-6}$  M/l over the whole pH range. The different pH-values were obtained by adding appropriate amounts of sodium hydroxide or nitric acid. In the pH range between pH 2—3 and pH 1 red flocks were precipitated for all rhodamine B concentrations higher than  $10^{-3}$  M/l. All systems having rhodamine B con-

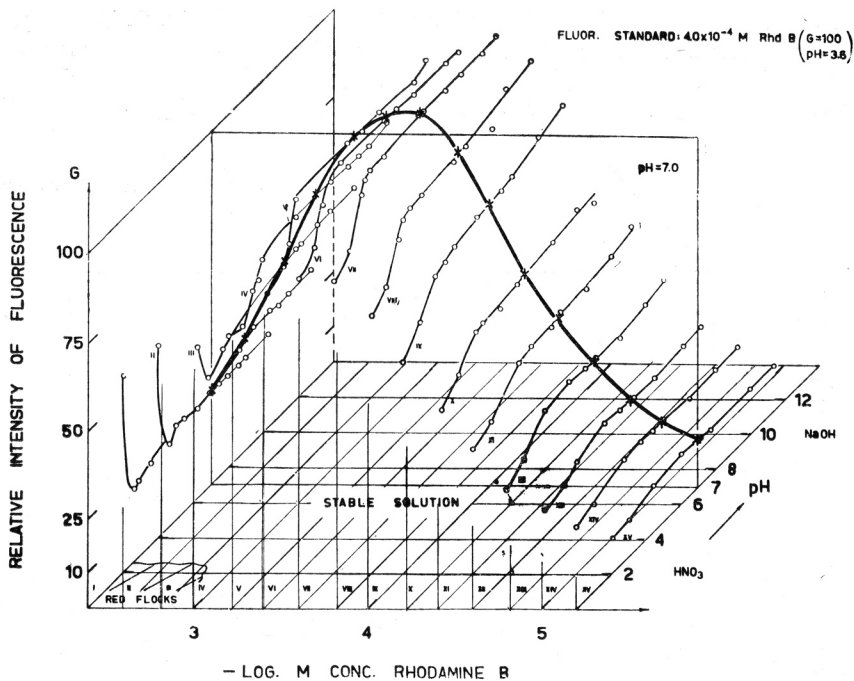


Fig. 3. Three-dimensional diagram: relative intensity of fluorescence of rhodamine B as a function of dye-concentration at various pH-values.

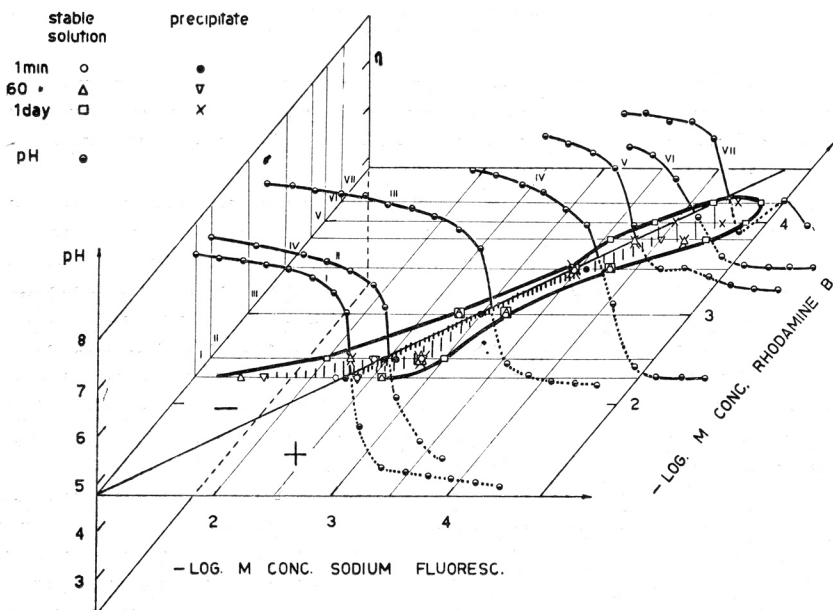


Fig. 4. Three-dimensional diagram: concentrational relationship in the system consisting of sodium fluoresceinate and rhodamine B. pH in dependence of excess dye concentration.

centration smaller than  $10^{-3}$  M/l and pH higher than 2—3 were stable. The fluorescence intensity remains almost unchanged over the pH-range between pH 12 and pH 4—5; a slight decrease of fluorescence intensity occurs at pH 2. As with sodium fluoresceinate the plane cutting pH-curves at pH 7 is also shown.

The precipitation region for the system sodium fluoresceinate-rhodamine B after 1 minute, 60 minutes and 1 day is shown in Fig. 4. Beyond the precipitation region, stable systems exist for the excess of sodium fluoresceinate or rhodamine B as well as in very dilute solutions. The pH-log  $M$  curves are shown in planes I—VII, their inflection points being close to pH 4.7. The straight line with the slope of  $45^\circ$  represents the theoretical equivalence line for the two dyes along which the precipitate is formed. This is especially true for the concentration of sodium fluoresceinate of  $10^{-3}$  M/l and that of rhodamine B of  $10^{-3}$  M/l, where the precipitation region is equally extended on both sides of the equivalence curve in each case *i.e.* 1 minute, 60 minutes and 1 day after the preparation of the systems. The precipitation region for more concentrated or more dilute systems is not symmetrically distributed. The charge of both precipitated and stable particles is always negative on the left of the equivalence curve and positive on the right of it (as measured after 1 day). The stable system (consisting of  $6 \times 10^{-4}$  M/l Na-

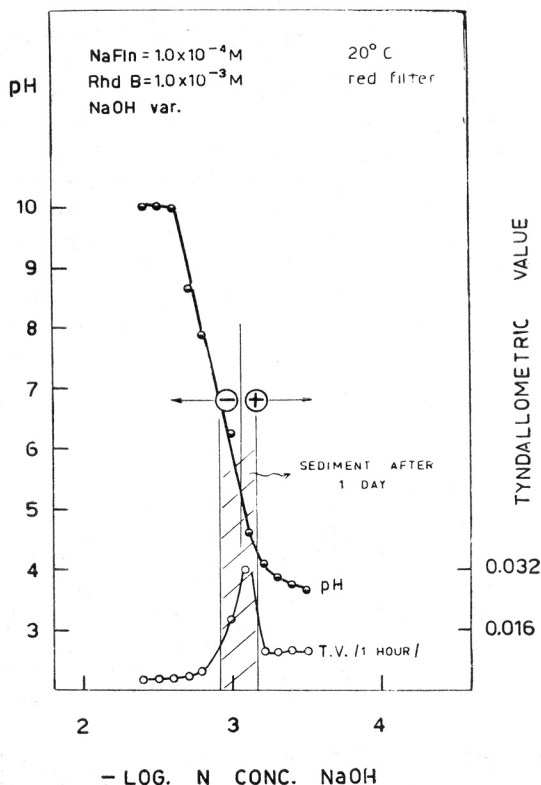


Fig. 5. Precipitation in the system consisting of sodium fluoresceinate and rhodamine B as a function of pH of the solution.

-fluoresceinate and  $1.5 \times 10^{-4}$  M/l rhodamine was coagulated with  $\text{KNO}_3$ ,  $\text{Ba}(\text{NO}_3)_2$ , and  $\text{La}(\text{NO}_3)_3$ . The coagulation values for uni-, bi-, and trivalent cations are in good agreement with the Schulze-Hardy rule.

Finally, the results for the systems of sodium fluoresceinate-rhodamine B in the presence of an acid or base are obtained for the following concentration-points:

- a)  $10^{-3}$  M/l RhdB,  $10^{-2}$  M/l NaFln (clear system),
- b)  $10^{-3}$  M/l RhdB,  $10^{-3}$  M/l NaFln (precipitated system),
- c)  $10^{-3}$  M/l RhdB,  $10^{-4}$  M/l NaFln (clear system).

In case a) the addition of acid was necessary in order to obtain pH 4.7; when approaching this value some fluorescein precipitated. In the case b) the precipitation occurred again in the neighbourhood of pH 5 and was continued to pH 3; only at very high pH-values there was no precipitation. The case c) is illustrated in Fig. 5. In the aqueous medium the system is clear (Fig. 4). On addition of some base slow precipitation occurs as illustrated by the tyndallometric curve (1 hour) and by precipitation region for 1 day. If the time amounts to less than 1 hour the systems are stable; later the precipitation region appears between pH 6.3 and 4.5 and does not spread after 1 day. Consistently, the charge is changed in the neighbourhood of pH 5.

#### DISCUSSION

In the three-dimensional diagrams (Figs. 2. and 3.) the relative intensity of fluorescence of sodium fluoresceinate and rhodamine B is shown as a function of the dye concentration and the pH of the solution. The plane cutting the G-pH curves at pH 7 gives the points of intersection illustrating corresponding G—dye—concentration curve. Taking into account that the pH-values of the pure solutions of rhodamine B and sodium fluoresceinate change: for rhodamine B from pH 3 to 7 for concentrations ranging from  $10^{-3}$  M/l to  $10^{-7}$  M/l, and for sodium fluoresceinate from pH 7.6 to 6.7 for concentrations ranging from  $10^{-1}$  M/l to  $10^{-4}$  M/l, such G—dye—concentration curves for a constant pH may be of interest. Since the pH-value of the fluorimetric standard used was 3.6 with rhodamine B and 6.8 with sodium fluoresceinate the relative intensity of fluorescence exceeds the limit of 100 in the plane set at pH 7 (Figs. 2. and 3.).

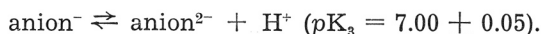
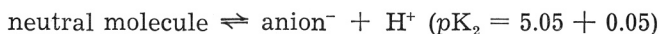
Bouchard<sup>2</sup> and Boutaric<sup>3,4</sup> investigated the fluorescent power of sodium fluoresceinate solutions as a function of hydrogen ion concentration. Our results are in complete agreement with theirs<sup>4</sup>. The fluorescent power quickly increases with increasing pH; having reached pH 8 it remains constant or decreases only slightly with further increase of pH.

The work of Lindquist<sup>8</sup> in which the maxima of monomeric forms of sodium fluoresceinate were obtained by graphical determination of pK-values of the dissociation equilibria of fluorescein may be also used for comparison. The absorption spectra of fluorescein show the predominance of the monomeric form in a slightly acid water solution. Lindquist found the predominance of the neutral molecule at pH 3.3, of the monoanion at pH 5.5 and of the dianion at pH 12.

In our measurements the minimum of the fluorescence intensity of the solution over the flocks was obtained at pH 3.3 (Fig. 2.); the precipitation limit at pH 5 (with high concentrations of sodium fluoresceinate); between

pH 7 and pH 12 the pH curves are almost parallel with the pH axis. According to Lindqvist in solutions containing  $10 \mu\text{M}/\text{l}$  of sodium fluoresceinate and having pH within the above range sodium fluoresceinate exists in the form of the dianion.

Using absorption data Zanker and Peter<sup>13</sup> have also applied a graphical method for the determination of pK-values of fluorescein corresponding to the following equilibria:



According to Zanker<sup>13</sup> the maximum of precipitation of the neutral molecule of fluorescein occurs between pH 2 and 3 and decreases towards pH 5 at which value it eventually stops (Fig. 2). Relative intensity of fluorescence increases with increasing pH up to pH 7. With further increase in pH values the fluorescence intensity remains almost constant. The sharp increase in G on all G—pH curves between pH 5 and 7 is due to the transition:  $\text{anion}^- \rightleftharpoons \rightleftharpoons \text{anion}^{2-} + \text{H}^+$ , and the constant values of G above pH show that the dianion is the predominant species. The process of dimerization also increases with increasing dye-concentration. This is reflected in the absorption spectrum in the presence of a high concentration of base<sup>7,9</sup>. In the case of rhodamine B the precipitation from stable solutions is reduced to a very narrow region, and the fluorescence intensity remains practically constant almost over the whole pH-range. It increases only very slightly just before reaching pH 5.

The two organic dyes: sodium fluoresceinate and rhodamine B (Fig. 4.) form a precipitation system with the isoelectric maximum in the region of equivalence. The isoelectric maximum is formed from particles representing »equivalent body« in the sense used by Fajans<sup>5</sup>. On both sides of the line representing the equivalency there are colloidal particles of opposite charges. The neutral electrolytes are influencing these systems according to the Schulze-Hardy rule. When comparing with the precipitation of inorganic systems e.g. precipitation of silver halides one can observe a difference<sup>11,12</sup> silver halides showing two concentration maxima besides one isoelectric maximum.

Since both dyes are either a weak organic acid or a weak organic base, the precipitation does not only depend on the relationship between the concentration of rhodamine B and that of sodium fluoresceinate, but, as shown in Fig. 5, also on pH of the medium. The precipitation takes place between pH 6.3 and 4.5 in the system which was clear before the addition of sodium hydroxide.

## REFERENCES

1. V. S. Adamov and L. T. Kantardzyhan, *Zhur. fiz. him.* **36** (1962) 376.
2. J. Bouchard, *J. chim. phys.* **33** (1936) 51.
3. A. Boutaric and C. Maraux, *Bull. soc. chim. France* **1948**, 952.
4. A. Boutaric and M. Roy, *Compt. rend.* **209** (1939) 162.
5. K. Fajans and T. Erdey-Grúz, *Z. physik. Chem.* **158** (1932) 97.
6. Th. Förster, *Naturwissenschaften* **366** (1949) 186.

7. Th. Förster and E. König, *Z. Elektrochem.* **61** (1957) 344.
8. L. Lindqvist, *Arkiv kem.* **16** (1960) 79.
9. N. Mataga, *Bull. Chem. Soc. Japan* **30** (1957) 375.
10. M. Rozwadowski, *Acta Phys. Polonica* **20** (1961) 1005.
11. B. Težak, E. Matijević, K. Schulz, J. Kratochvil, M. Mirnik, and V. Vouk, *Disc. Faraday Soc.* **1954**, No. 18, 63.
12. *Ibid.*, *General Discussion*, 180.
13. V. Zanker and W. Peter, *Chem. Ber.* **91** (1958) 572.
14. V. Zanker and W. Peter, *Z. physik. Chem. (Frankfurt)* **26** (1960) 159.

### IZVOD

#### **Metorika precipitacionih procesa. XIX. Fluorescencija natrijeva fluoresceinata, rodamina B i njihove smjese u ovisnosti o koncentraciji vodikovih iona u vodenim otopinama**

Đ. Težak i B. Težak

Mjerena je fluorescencija natrijeva fluoresceinata i rodamina B u širokom pH-području. Dobiveni rezultati se vrlo dobro slažu s apsorpcionim mjerenjima drugih autora<sup>8,13</sup>.

Precipitacioni maksimum kod pH 3 je identičan s minimumom fluorescencije za predominantnu formu fluoresceina u tom području, tj. neutralnu molekulu. Precipitaciona granica kod pH 5 karakterizira ravnotežu između neutralne molekule i monoaniona; prijelaz na krivulji fluorescencije kod pH 7 pokazuje ravnotežu između monoaniona i dianiona.

Fluorometrijske krivulje za divalentni kation rodamina B ostaju praktički konstantne kroz široko pH-područje.

Sistem Na-fluoresceinat — rodamin B pokazuje da izoelektrična precipitacija uključuje stvaranje »ekvivalentnog tijela«. Koncentracijski odnos između iona ispitanih boja strogo zavisi od koncentracije vodikovih iona.

Rad je izveden u okviru Instituta za fizikalnu kemiju Sveučilišta u Zagrebu, a uz pomoć Republičkog fonda za naučni rad.

FIZIČKO-KEMIJSKI INSTITUT  
PRIRODOSLOVNO-MATEMATIČKI FAKULTET  
ZAGREB

Primljeno 3. travnja 1964.