

## A New Method of Labelling Rose Bengal with $^{131}\text{I}$ and $^{125}\text{I}$ \*

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Differences in the composition of rose bengal influence the results of liver chromoexcretion tests. Preparation of pure rose bengal, i. e. tetraiodotetrachlorofluorescein (TITCF) and its labelling with  $^{131}\text{I}$  and  $^{125}\text{I}$  in a suitable medium is discussed. Yields of the exchange reaction between non-radioactive TITCF and radioactive elementary iodine in ethanol—ether solution are good. Dependencies of radiochemical yield and radiochemical purity on reaction time and temperature are established. The dependence of radiochemical yield on concentration of elementary iodine is evaluated.

Rose bengal labelled with iodine  $^{131}\text{I}$  is at present one of the most widely used radioactive diagnostic substances. In the most cases it has been prepared by the exchange reaction between non-radioactive rose bengal and radioactive sodium iodide in aqueous medium at pH 5 according to J. Liebster and O. Andrysek [1]. Rose bengal, as it is evident from several literary data [2—5], usually is not a pure compound but it consists of a number of coloured substances. In previous communications [6, 7] we demonstrated considerable differences in the composition of commercial preparations of rose bengal. With our coworkers, J. Brousil and P. Jirounek, we proved that these differences affected the results of liver chromoexcretion tests [8].

In the present work we investigated the stability of pure rose bengal i. e. of tetraiodotetrachlorofluorescein (TITCF), which we obtained according to M. Jirsa [5] by adsorption chromatography on alumina. We found that in acid medium this compound is gradually transformed to other three compounds. Much more complicated mixture is formed from TITCF in alkaline medium while TITCF is relatively stable in neutral solution. The compounds mentioned above, originate also during exchange reaction with iodine  $^{131}\text{I}$  at pH 5 as it can be seen from following results of paper chromatographic examination. The scheme of typical chromatogram of the reaction mixture is represented in Fig. 1. The first spot is TITCF, the other spots are compounds formed in the course of the exchange reaction. On the basis of counting chromatograms with scintillation counter we determined the relation between the amounts of originating compounds (expressed in percentage) and time of heating. This dependence is shown in Fig. 2.

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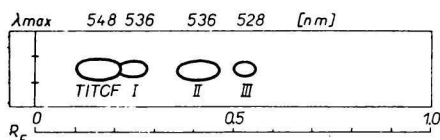


Fig. 1. The scheme of typical paper chromatogram of mixture resulting by heating TITCF with acetate buffer (pH 5) on boiling water bath.

Paper Whatman No. 3 developed with ethanol—21 % ammonia—water (10 : 8.1 : 81.9); descending technique.

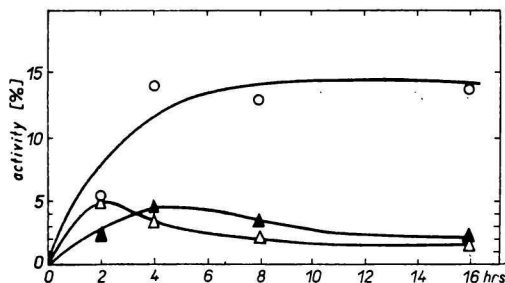


Fig. 2. The dependence of the amounts of compounds originating from TITCF (expressed as percentage of total radioactivity of coloured substances) on time of heating.

○ compound II  
 △ compound III  
 ▲ compound I

The situation is even worse when we have to label TITCF with iodine  $^{125}\text{I}$ . Sodium thiosulfate, which is usually added to sodium iodide  $^{125}\text{I}$  solution as stabilizing agent, on the one hand decreases the rate of exchange reaction and on the other hand increases the amount of by-products formed. The oxidation of thiosulfate with elementary iodine or with hydrogen peroxide leads to a higher radiochemical yield but it does not result in the limitation of by-products formation.

Thus, it is impossible to obtain pure labelled TITCF using this method even though we work with extremely pure initial material.

The aim of our further investigation was:

first to find a suitable method for separation of both non-exchanged inorganic iodide and coloured by-products from labelled TITCF,

second to develop a labelling method that could not give a possibility of by-products formation.

We succeeded to solve the first task by the application of the adsorption chromatography. The mixture after exchange reaction was evaporated, dissolved in 96 % ethanol and placed on the top of a column packed with deactivated alumina. The selection of optimum conditions for chromatography will be published elsewhere [9]. The column was eluted with 96 % ethanol. By single operation we were able to separate labelled TITCF from inorganic iodine as well as from the other coloured by-products.

In the course of the second task we elaborated labelling method which made use of the exchange reaction between non-radioactive TITCF and radioactive elementary iodine in ethanol—ether solution. First we determined the dependence of radiochemical yield and radiochemical purity on reaction time and

temperature. The mixture of 0.5 ml of potassium iodide (1 mg/ml) and sodium iodide  $^{125}\text{I}$  was oxidized with potassium iodate, elementary iodine  $^{125}\text{I}$  was extracted with 3 ml of ether and mixed with the solution of disodium salt of TITCF in 96 % ethanol. The mixture was heated under reflux, samples were taken after 2, 4, 8, and 24 hours, mixed with the same volume of diluted sodium thiosulfate solution, and paper chromatographed in ethanol—ammonia—water system [2]. Distribution of radioactivity in 4 hours' sample is presented in Fig. 3, from which it is evident that formation of by-products does not occur under

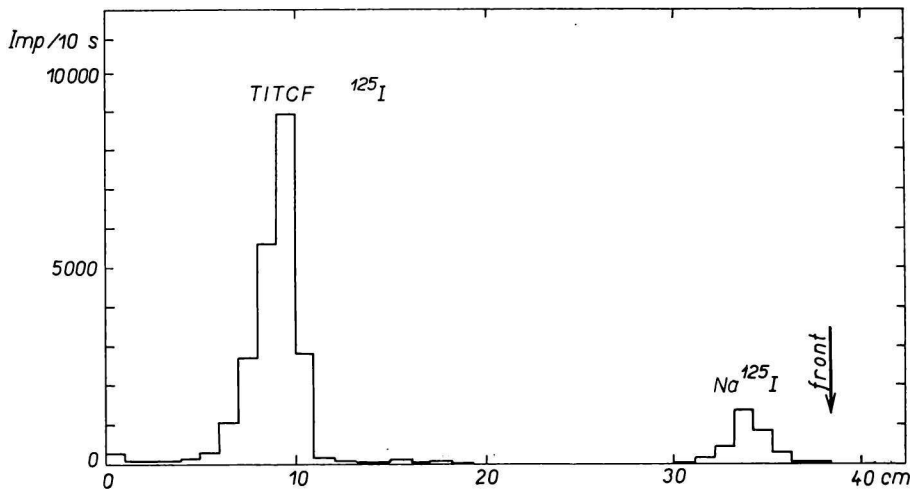
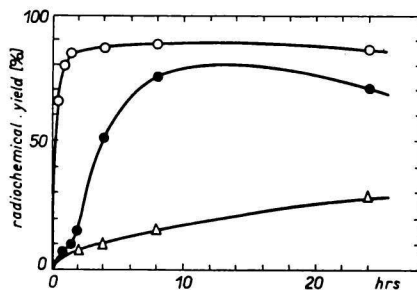


Fig. 3. The distribution of radioactivity in the sample after 4 hours' heating TITCF with iodine  $^{125}\text{I}$  in ethanol—ether solution.

these conditions. The first peak corresponds to labelled TITCF, the second one to inorganic iodide. Radiochemical yields for each reaction time were calculated. This dependence is expressed by the upper curve in Fig. 4. For comparison we also investigated the dependence of radiochemical yield on reaction time for the reaction carried out at room temperature. The result is expressed by the

Fig. 4. The dependence of radiochemical yield on reaction time for exchange reaction of TITCF with elementary iodine  $^{125}\text{I}$  in ethanol—ether solution.

- TITCF (di-Na salt); boiling
- TITCF (acid form); boiling
- △ TITCF (di-Na salt); room temperature



lower curve. The third curve is an expression of the same dependence as the upper curve with exception that acid form of TITCF was used instead of the disodium salt. Small amount of by-products originated under these conditions.

From the upper curve on this figure we can see that about 85 % labelled TITCF are present in the reaction mixture after 4 hours of heating. Apart from radioactive inorganic iodide the reaction mixture does not contain other radiochemical impurities.

Thus, the whole labelling was carried out in the following way. Solution of radioactive elementary iodine in 3 ml of ether obtained as above was added to the solution of 0.1 g of TITCF in 20 ml of 96 % ethanol. The mixture was heated under reflux for 4 hours, the solvent distilled off and the solid residue dissolved in diluted sodium hydroxide. Non-exchanged radioactive iodine was removed by repeated precipitation, the pH of final solution of labelled TITCF was adjusted to near 7, and its radioactivity was measured by the ionization chamber. Radiochemical yields were mostly above 80 %.

When previous method is used, two factors decrease radiochemical purity of labelled TITCF: slightly acidic acetate buffer and sodium thiosulfate solution in which radioactive iodide is supplied. The effect of reaction medium is completely excluded by using the new method. As far as sodium thiosulfate is concerned, it can sometimes interfere even with the new labelling method. When we worked with relatively great volumes of iodide-125, as for example in the case of low-specific activity isotope, we often obtained low radiochemical yields. To make this method suitable even for such cases we investigated also the dependence of radiochemical yields on concentration of elementary iodine. The graphical expression of the results is shown in Fig. 5. The different curves correspond to following concentrations of iodide carrier in the mixture before the oxidation to elementary iodine: 2.5 mg, 5 mg, 10 mg, and 20 mg. From this

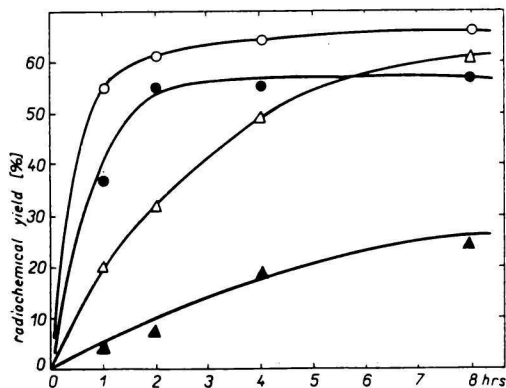


Fig. 5. The dependence of radiochemical yields on concentration of elementary iodine in reaction mixture.

- ▲ 2.5 mg of KI carrier added to  $\text{Na}^{125}\text{I}$  before oxidation to iodine
- △ 5 mg of KI carrier added to  $\text{Na}^{125}\text{I}$  before oxidation to iodine
- 10 mg of KI carrier added to  $\text{Na}^{125}\text{I}$  before oxidation to iodine
- 20 mg of KI carrier added to  $\text{Na}^{125}\text{I}$  before oxidation to iodine

figure it is apparent that radiochemical yield increases up to certain level with increasing concentration of the carrier. On the basis of further experiments we assumed following explanation of this rather surprising phenomenon: During oxidation of iodide, a certain portion of sodium thiosulfate is decomposed into colloidal sulfur which is extracted simultaneously with iodine into ether layer and transferred into medium in which the exchange reaction occurs. There it converts the portion of elementary iodine into iodide, which does not participate in the exchange reaction. Thus, the greater is the concentration of iodine, the greater portion of radioactivity is used for labelling. On the other hand the rate of the exchange reaction is decreased by diluting the isotope with non-radioactive carrier. The real yield is dependent on the combination of these factors.

The present method enabled us to obtain a product with higher radiochemical purity when compared with previous methods. This method was also found suitable in the preparation of labelled diiodopyridoneacetic acid, which we obtained in a higher yield in comparison with the former technique.

#### NOVÝ SPŮSOB OZNAČOVANIA BENGÁLSKEJ ČERVENE IZOTOPOM $^{131}\text{I}$ ALEBO $^{125}\text{I}$

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Rozdielne zloženie bengálskej červene ovplyvňuje výsledky chromoextraktívnych pečeňových skúšok. Preto sa hľadajú spôsoby prípravy čistej bengálskej červene, t. j. tetrajódtetrachlórfluoresceínu (TITCF), označenej izotopom  $^{131}\text{I}$  alebo  $^{125}\text{I}$ . Výťažky výmennej reakcie medzi nerádioaktívnym TITCF a rádioaktívnym elementárnym jódom v zmesi etanol—éter sú dobré. Stanovila sa závislosť rádiochemického výťažku a rádiochemickej čistoty od času a teploty, ako aj vzťah medzi rádiochemickým výťažkom a koncentráciou elementárneho jódu.

#### НОВЫЙ СПОСОБ ПРИГОТОВЛЕНИЯ ТЕТРАИОДТЕТРАХЛОРОФЛУОРЕСЦЕ- ИНА, МЕЧЕНОГО ИЗОТОПОМ $^{131}\text{I}$ ИЛИ $^{125}\text{I}$

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Различный состав тетраиодтетрахлорофлуоресцеина влияет на результаты хромоэкстракционных опытов на печени. Поэтому ищутся способы приготовления чистого тетраиодтетрахлорофлуоресцеина, меченого изотопом  $^{131}\text{I}$  или  $^{125}\text{I}$ . Выходы обменной реакции между нерадиоактивным тетраиодтетрахлорофлуоресцеином и радиоактивным

элементарным иодом в смеси этан—эфир являются хорошими. Была определена зависимость радиохимического выхода и радиохимической чистоты от времени и температуры, а также зависимость между радиохимическим выходом и концентрацией элементарного иода.

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