Luminescence-Based Detection of Microquantity of Aromatic Nitro Compounds

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Abstract

A two-stage method of converting aromatic nitro compounds into luminophors is proposed. It includes catalytic reduction of the nitro aromatic compound by molecular hydrogen followed by the interaction of the formed aromatic amine with a fluorogenic reagent (fluorescamine, *ortho*-phthalaldehyde). Efficient reduction is provided by using palladium catalysts on supports with developed surface, such as barium sulphate, C_{60} fullerene, and microcapillary glass plates. Luminescence of the final products is observed in the regions λ = 490–520 and 450–460 nm under excitation in the regions λ = 390–410 and 340–350 nm for the fluorescamine and *ortho*-phthalaldehyde, respectively.

INTRODUCTION

Luminescence-based methods of investigation possessing exclusively high sensitivity and selectivity [1] find increasingly wide application in chemistry, biology, medicine, different branches of technology. One of the most important application areas of luminescence analysis is becoming the monitoring of environmental pollution. Aromatic (poly)nitro compounds (APNC/ANC) belong to the class of the most dangerous toxic agents. These compounds are also known as the basis of various explosive assemblies. Their timely detection in environmental constituents, including air sampled from industrial enterprises, airports, railway stations, metro, *etc.* is an urgent problem of modern days and provides a need in sensitive and fast-time methods to detect low concentrations of these compounds.

The methods of determining these compounds which exist at present [2, 3] are not always satisfactory from the viewpoint of sensitivity, selectivity, fast operation or reliability. Because of this, we think that luminescence analysis methods are the most suitable for solving the indicated problem. Since APNC and ANC do not belong to luminophors, the task was to transform them into luminescent species. For this purpose, we examined the possibility to apply highly sensitive fluorescence methods of amine determination developed in the 70-ies of the XX century [4–7] to aromatic nitro compounds after their preliminary reduction.

The fluorescence methods of amine detection [4–7] are based on their interaction with fluorogenic reagents including 4-phenylspiro[furan-2(3H),1-phthalan]-3,3-dione (fluorescamine, or flurame) [4, 5], *ortho*-phthalaldehyde (OPA) [6, 7] and their derivatives [8]. This reaction results in the formation of strongly fluorescent products like pyrrolinones I and isoindoles II (Fig. 1).

EXPERIMENTAL

Initial reagents

All solvents of "ch." (pure) or "kh.ch." (chemically pure) grade were additionally purified

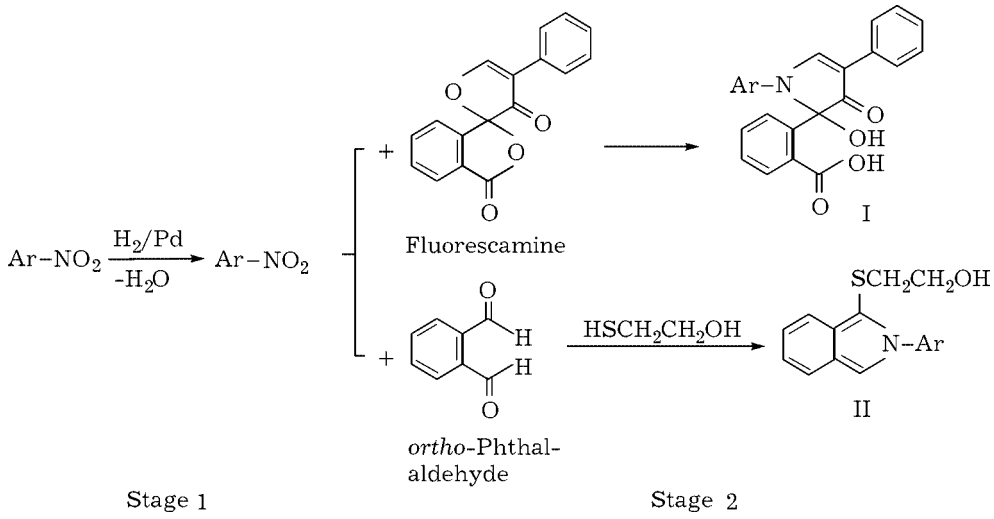

Fig. 1. Scheme of conversion of aromatic compounds into luminophors. Stage 1 – catalytic reduction to amine; stage 2 – fluorogenic reaction.

by distillation before using. Dehydrated acetone and ethanol were obtained according to standard procedures [9]. Fresh borate buffer solution was prepared before each experiment using the corresponding titrants. Nitro aromatic compounds of the "ch." grade were recrystallized four times from the preliminarily distilled ethanol. Other reagents of the "kh. ch." grade were used without additional purification. The $Pd/BaSO_4$ (5 %) and Pd/C catalysts were kindly submitted by the Institute of Catalysis, SB RAS. The Pd_nC_{60} catalysts were synthesized according to the reaction of ligand exchange [10] between the fullerene C_{60} and Pd(0) complex. The latter was synthesized according to the procedure [11] from palladium chloride and dibenzylideneacetone [12].

Dibelzylideneacetone $C_6H_5–CH=CH–CO–CH=CH–C_6H_5$ (DBA) was synthesized by condensation of benzaldehyde and acetone [12]. A half of a mixture prepared of 4 ml of freshly distilled benzaldehyde and 0.7 ml of acetone was added under careful mixing to the solution containing sodium hydroxide (4 g) in 40 ml of water and 32 ml of ethanol at the temperature of 20–25 °C. After 15 min, the rest mixture of benzaldehyde was added. The entire process was conducted under intensive stirring. After 20 min, bright-yellow precipitate was separated on a filter, washed with water and dried in the air. The product was purified by recrystallization from ethylacetate, m.p. 112 °C.

Tris(dibenzylideneacetone)-dipalladium (0) ($Pd_2(DBA)_3$) was synthesized using the procedure described in [11]. Palladium chloride (1.05 g) was added at 50 °C to a mixture containing 4.6 g of DBA and 3.9 g of sodium acetate in 150 ml of methanol. The mixture was stirred for 4 h at 40 °C and then cooled to achieve complete precipitation of purple-red product. The precipitate was filtered, washed with water and acetone, and purified in vacuum. In order to obtain $Pd_2(DBA)_3 \cdot CHCl_3$, 3.39 g of the $Pd_2(DBA)_3$ complex was dissolved in 120 ml of hot chloroform. The solution was filtered; 170 ml of diethyl ether was added to it. The precipitated purple crystals were filtered, washed with ether and dried in vacuum; m.p. 122–124 °C.

The C_{60} fullerene was obtained using a laboratory set-up according to a modified procedure [13] by plasma arc vaporization of graphite rods in the atmosphere of helium. The product was separated by extraction with benzene (toluene) followed by chromatographing on a column with carbon sorbent (eluted with benzene). The purity of the fullerene was examined by means of spectrophotometry (λ = 330 nm) and IR spectroscopy (1429, 1183, 577, and 528 cm^{-1}).

In order to synthesize fullerene-palladium complexes Pd_nC_{60} (n = 3–6) as catalysts, 150 ml of benzene solution containing 73 mg of C_{60} fullerene (0.1 mmol) was added to 150 ml of the benzene solution containing 230–370 mg of the $Pd_2(DBA)_3 \cdot CHCl_3$ complex (0.22–0.36 mmol).

TABLE 1

Composition and yield of the Pd_nC_{60} product depending on the ratio of initial reagents

$Pd_2(DBA)_3 \cdot CHCl_3$: C_{60}	Product	Yield, %
0.2 : 1	$Pd_{0.94}C_{60}$	78
0.4 : 1	$Pd_{1.11}C_{60}$	89
0.8 : 1	$Pd_{1.68}C_{60}$	88
1.6 : 1	$Pd_{2.98}C_{60}$	82
2.0 : 1	$Pd_{3.68}C_{60}$	91
3.0 : 1	$Pd_{5.21}C_{60}$	87
4.0 : 1	$Pd_{6.30}C_{60}$	90

The fine black precipitate was centrifuged, separated by filtering, carefully washed with benzene, then with ether. The product was dried in vacuum at the temperature of 200 °C. The composition and yield of the fullerene-palladium product for different ratio of the initial reagents are shown in Table 1.

Reduction of aromatic nitro compounds to amines

In the organic solvent medium. A typical example is the reduction of trinitrotoluene (TNT) to triaminotoluene (TAT) [14]. The 40 ml of alcoholic solution containing 300 mg (1.3 mmol) of TNT and 100–150 mg of the catalyst was poured into the reactor equipped with reflux condenser and a drying tube with calcium chloride; the reactor was preliminarily blown with hydrogen. Under intensive stirring, the flow of hydrogen was admitted at the flow rate of 60 ml/min to the reaction dredge through a gas tube with a sprayer. The process was conducted at the temperature of 40–45 °C. The reaction progress was checked by means of chromatography. The product, which can easily be oxidized in an aqueous-alcoholic medium, was rapidly separated from the reaction mixture, in which water is accumulated as a by-product. The solution was separated from the precipitate; the solvent was evaporated at reduced pressure and the temperature about 40 °C in the atmosphere of hydrogen supplied through a capillary.

The dry residue in the form of yellow crystals was recrystallized from chloroform, washed with ether + alcohol 1 : 1 mixture, then with ether, and dried in the evacuated desiccator above H_2SO_4. The product (TAT) is obtained as white crystals melting with decomposition at 121 °C (m.p. 118–121 °C [14]). The ^{1}H NMR (DMSO-d_6, HMDS, δ, ppm): 2.20 s (3, CH_3), 3.02 s (2, *para*-NH_2), 4.70 s (4, *ortho*-NH_2), 5.82 s (2, H_{arom}); UV (ethanol): 220 nm.

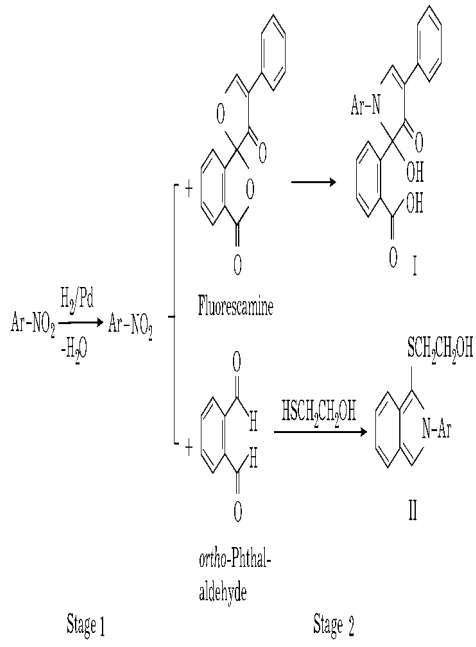

Fig. 2. Schemes of reactors for the catalytic reduction of aromatic nitro compounds by molecular hydrogen in the gas phase: *1* – vessel with drying agent; *2* – thermostat; *3* – catalytic cell; *4* – receiving vessel with the sorbing solution (fluorogenic agent dissolved in borate buffer with pH 9.2); *5* – nitro compound.

In gas. Aromatic nitro compounds were reduced in reactors shown in Fig. 2. In the reactor shown in Fig. 2, *a*, the Pd/C, $Pd/BaSO_4$, Pd_nC_{60} catalysts were deposited as dredges in a volatile organic solvent onto glass balls of about 1 mm in diameter, with which the glass container *3* (125 × 15 mm) was filled. In the reactor shown in Fig. 2, *b*, the catalyst (metallic palladium) was deposited on a fitted glass microcapillary plate (MCP) *3*. In both cases, local heating (90–170 °C) was provided with the help of glycerol bath or a specially coiled resistive heater *2*. Preliminarily dried hydrogen *1* was admitted into the reactor at the flow rate of 30 ml/min. A weighed portion of nitro compound *5* (some tenths of a milligram to several milligrams) was placed at the inlet of the container with the catalyst or directly on the Pd/MCP. The resulting aromatic amine was col-

lected either by freezing out in a liquid nitrogen trap or by dissolving in a sorbing solution, which was borate buffer *4*.

Reactions of aromatic amines with fluorogenic agents

These reactions were conducted with the help of fluorescamine and *ortho*-phthalaldehyde [4–7]. All solutions were prepared immediately before measurements; purified reagents and solvents were used. Amines, which are the products of reduction of the corresponding nitro compounds, were used both as individual compounds and as reaction solutions of the first stage (see Fig. 1) without isolation and purification. Below we describe a typical procedure of conducting the fluorogenic reaction for the product of TNT reduction, which is TAT.

The TAT solution was prepared by dissolving 0.7 mg of TAT in 20 ml of tetraborate buffer with pH 9.18 (C = 0.035 mg/ml, 2.55 10^{-4} M). The solution was diluted with a borate buffer till the required low TAT concentration up to 10^{-6} M. The fluorescamine solution was prepared by dissolving 2 mg of the reagent in 20 ml of dehydrated acetone (C = 0.1 mg/ml, 3.6 10^{-4} M). Then the acetone solution of fluorescamine was mixed with the TAT solution under examination at the volume ratio of 1 : 3. After 1 min, the excitation and luminescence spectra of the mixture under investigation were recorded.

The reaction with *ortho*-phthalaldehyde was conducted in the presence of potassium borohydride or mercaptoethanol as a reducing agent. The 90 ml of borate buffer (pH 9.2, 0.05 M) was added to the solution of 15 mg of OPA in 1 ml of ethanol; then the solution of 6 mg of KBH_4 in 1 ml of ethanol was added (or 1.5 ml of the solution of C_2H_5SH in ethanol, with a concentration of 5 μl/ml; the volume of the mixture was brought to 100 ml with the borate buffer. The concentrations of OPA and reducing agent in the resulting solution are equal to 1.1 10^{-3} M. After that, 1 ml of the solution was mixed with 3 ml of the TAT solution. After 5 min, excitation and luminescence spectra of the reaction mixture were recorded.

Instruments

The IR spectra were recorded with the Specord IR 75 spectrophotometer (KBr tablets), NMR spectra were recorded with the NMR spectrometer of the JEOL C. Luminescence spectra were recorded with the Fluorolog spectrometer of the Spex C. The reaction progress was monitored chlomatographically using the Milikhrom liquid chromatograph with spectrophotometric detector (λ = 240 nm, steel column 120 × 2 mm, stationary phase: nucleosil C_{18} with mean particle size 5 μm, mobile phase: dehydrated ethanol, 100 μl/min, sample volume: 3 μl; measurement time: 0.6 s).

RESULTS AND DISCUSSION

A necessary condition for the application of the fluorescence method of the determination of primary amines [4–7] to the analysis of aromatic nitro compounds is the rapid and complete reduction of the latter. The proposed two-stage conversion of aromatic mono-, di- and trinitro derivatives into luminophors is shown in Fig. 1. The method includes the stage (1) of reduction of nitro aromatic compound to amine by molecular hydrogen in the presence of a catalyst, and the stage (2) involving the interaction of the amine with fluorogenic agent, which is fluorescamine or *ortho*-phthal aldehyde, resulting in the formation of fluorescent products (I, II).

The reduction proceeds with high yield both in the organic solvent medium according to the procedure proposed by F. Hein and F. Wagner [14] and in the gas medium. The method proposed in [14] for the reduction of TNT to TAT by molecular hydrogen over $Pd/BaSO_4$ in dehydrated ethanol was spread over a broad range of aromatic compounds; however, it requires much time (5–6 h). We came across the problem to conduct the reduction of ANC in small concentrations quantitatively at high rate and in mild conditions. It was stated that these requirements are best met with the reduction in the gas phase at moderate temperature (40–170 °C) over catalysts with developed surface providing high interaction rate. We test-

TABLE 2

Efficiency of catalysts for the reduction of aromatic nitro compounds to amines

Catalyst	Reduction conditions	
	Organic solvent medium	Gas medium
$Pd/BaSO_4$	+	+
Pd/C	–	–
$Pd_{1.1}C_{60}$	–	–
$Pd_{1.7}C_{60}$	–	–
$Pd_{3.7}C_{60}$	+	+
$Pd_{5.2}C_{60}$	+	+
Pd/MCP	Not applied	+

ed palladium catalysts on such supports as barium sulphate, coal, C_{60} fullerene, and glass microcapillary plates (see Fig. 2). Among the tested catalysts ($Pd/BaSO_4$, Pd/C, Pd_nC_{60},, Pd/MCP), all the substances except palladium on carbon and Pd_nC_{60} with $n < 3$ exhibited high efficiency in the processes under investigation, both in the gas and in liquid medium (Table 2).

Reduction in the gas medium in the proposed version is characterized by high reaction rate, quantitative yield of the amine, purity of the product, convenience and easy governing, applicability to a wide range of aromatic nitro derivatives, which makes this process attractive from the viewpoint of analytical application. Compactness of the reactor with the fitted catalytic Pd/MCP cell should be specially stressed (see Fig. 2, *b*). For instance, more than 5 h was necessary in order to reduce TNT to TAT in dehydrated ethanol over the indicated catalysts (as monitored by LC), while the same process conducted in the gas-phase reactor (see Fig. 2, *b*) took several seconds or several tenths of a second. The same is observed in the case of mono- and dinitro derivatives of benzene and toluene.

The amine product was collected either by freezing it out in a nitrogen trap or excluding the isolation stage it was directed into a collector with the solution of fluorogenic agent in borate buffer with pH 8–9.5. After that, luminescence spectra were recorded. Maximal emission of the adducts with fluorescamine is observed in the region λ = 490–500 nm when excitation was performed in the region 390–410 nm. For the adducts of OPA/reducing agent, the corresponding spectral regions are 450–460 and 340–350 nm, respectively. An important feature of the fluorogenic reaction with *ortho*-phthalaldehyde is that without a reducing agent (C_2H_5SH or $K/NaBH_4$) the amine forms non-fluorescent products with OPA [6]. Because of this, the order of reagents mixing is important. The excitation and luminescence spectra of the products of addition of fluorescamine to *ortho*-toluidine and OPA to TAT are shown in Fig. 3.

The investigation of the interaction of fluorogenic agents with mono-, di- and triamino toluene revealed almost additive increase in the intensity of emission of the adducts with an increase in the number of NH_2 groups in the ring; the position of the maximum in emission is shifted to smaller values (Table 3). This may be due to steric factors. For example,

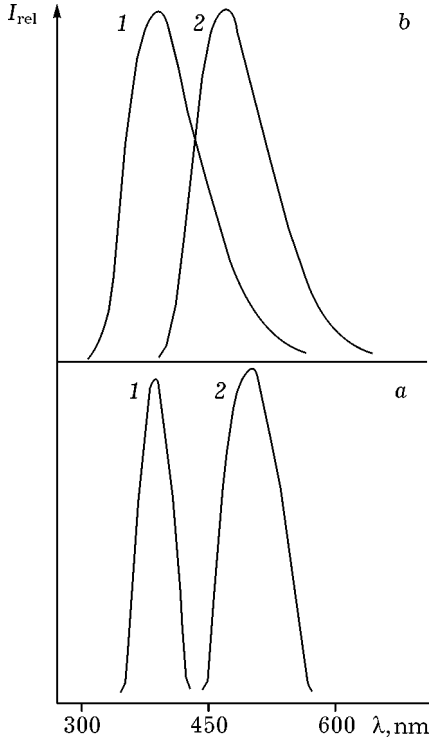

Fig. 3. Spectra of excitation (*a*) and luminescence (*b*) of the products of fluorescamine addition to *ortho*-toluidine (*1*) ($C_1 = 4.17 \cdot 10^{-5}$ M) and of OPA addition to triaminotoluene (*2*) ($C_2 = 1.28 \cdot 10^{-5}$ M).

TABLE 3

Maximal wavelength of excitation and emission of aminotoluene derivatives with fluorescamine

Amine	Number of NH_2 groups	λ_{max}, nm	
		excitation	emission
para-Toluidine	1	409	520–525
ortho-Toluidine	1	390	500–505
2,4-Diaminotoluene	2	400	510
2,4,6-Triaminotoluene	3	395	485–490

fluorophors based on *para*- and *ortho*-toluidine emit at λ_{max} = 520 and 505 nm, respectively. It is demonstrated that the intensity of emission for the final fluorophor is directly proportional to the amount of the initial nitro compound within the range 10^{-5}–10^{-9} moles (Fig. 4). The results were observed in experiments to be well reproducible, independent of a method of amine synthesis or kind of catalyst used. It is demonstrated that there is no necessity to isolate amine between the stages of reduction and the fluorogenic reaction. This points to the acceptability of the proposed two-stage method of converting mono-, di- and trinitro aromatic derivatives into luminophors in order to detect them by means of detecting their luminescence in air samples when they are present in concentrations in the indicated range.

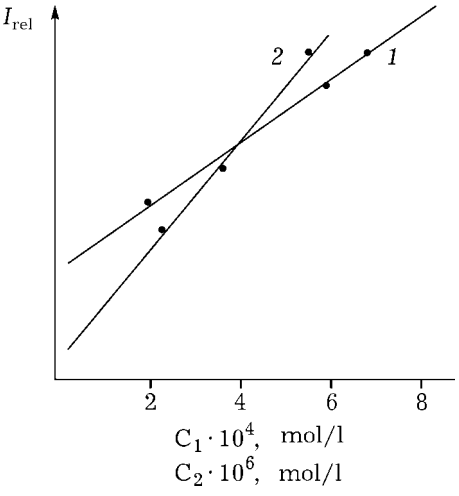

Fig. 4. Concentration dependence of the intensity of fluorescence of adducts of the reduced nitro compounds with fluorescamine: *1* – *ortho*-toluidine, *2* – 2,4,6-triaminotoluene.

CONCLUSION

The proposed method for the conversion of aromatic nitro and polynitro compounds into luminophors can be used as the basis for the operation of portable sensors recording the presence of the indicated compounds in various constituents of the environment. The method will help recording total content of nitro and amino derivatives.

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