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Chemichromic azodye from 2,4-dinitrobenzenediazonium *o*-benzenedisulfonimide and γ-acid for monitoring blood parameters: structural study and synthesis optimisation

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Abstract

Azo coupling of 6-amino-4-hydroxy-2-naphthalenesulfonic acid (γ-acid) with 2,4-dinitrobenzenediazonium ion in alkaline solution gave a mixture of aminoazo and hydroxyazo dyes. The use of dry diazonium salt, stabilised as *o*-benzenedisulfonimide derivative, afforded a higher quantity of desired hydroxyazo dye compared to use of diazonium salt prepared *via* nitrosylsulfuric acid. The addition of a highly acidic diazonium salt solution determines a local pH-value decreasing. In the mixing zones the acid-base equilibrium corresponds to the "microscopic", lower pH-values and the aminoazo dye is formed. Only hydroxyazo dye was obtained when *N*-acetyl derivative of γ-acid was used as a coupling agent. In any case, the use of 2,4-dinitrobenzenediazonium *o*-benzenedisulfonimide allowed for an easier experimental procedure. ^{1}H NMR analysis, in particular ^{1}H–^{1}H COSY and ^{1}H–^{1}H NOESY techniques, elucidated the structure of dyes evidencing that coupling took place at the para-position to the hydroxy group in hydroxyazo dye and at usual ortho-position in aminoazo dye. Preliminary spectroscopic study showed that *p*-hydroxyazo dye gave change in colour in the physiological range of pH, an important requisite for its use in a sensor applicable for the monitoring of blood parameters. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The blood gas measurement is often required in modern diagnosis and during the treatment of critically ill patients. Blood gas measurement is a term that has come to stand for the measurement of pCO_2, pO_2 and pH. These blood parameters are regarded as critical measures in the clinical assessment of pulmonary and cardiovascular function. The current hospital practice involves the use of instruments placed in the hospital laboratory and is time-consuming and expensive. The placing of blood gas analyser instruments in or near surgical and other critical care units saves time but does

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not reduce the costs and the required specialised training of laboratory personnel.

A variety of more economical methods and, in particular, near patient blood gas measurements have been proposed. Direct blood contact measurements, made by sensors placed within the extracorporeal circulation system during open heart surgery or by bringing blood to sensors outside the patient's body, appeared very promising. In particular, the evolution of fibre-optic technology has been fundamental for the development of on-line blood gas measurements systems. Studies on useful indicators sensible to pH variation, dependent on luminescence [1] or absorptive [2] properties, in physiological range have been made by several investigators. Fibre-optic technology permits lighting chemichromic film and reading of the relative optical answer. If the chemichromic membrane is in direct contact with the blood, its variation in optical properties gives information about the pH of the physiological liquid.

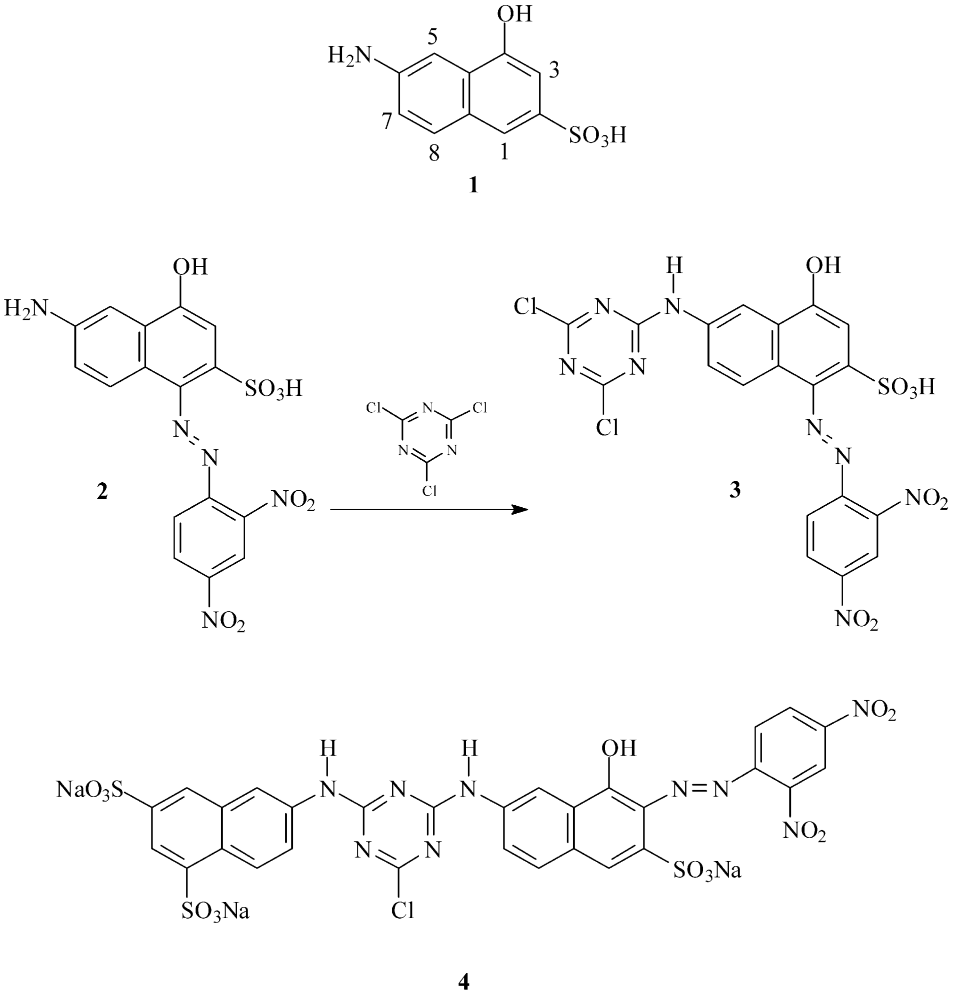
The same sensor may also be used to measure the concentration of gases (CO_2 or O_2) in blood. In this case, a membrane that is permeable to the gas to be measured is placed over a chamber to isolate it from the blood. The gas permeates the membrane and reacts with a liquid or liquid-containing media in the chamber to shift the pH, evidenced by the chemichromic film in contact with the liquid.

The choice of the indicator structure is fundamental because it has to allow: (i) the variation of optical properties over the physiological pH range (6.8–7.8), (ii) absorption of red LED light to guarantee the economy of device, (iii) the linking formation to useful membrane, (iv) photostability, (v) stability during the required gamma or ethylene oxide sterilisation process.

In a common project finalised to develop a sensor for the monitoring of blood parameters, azo dyes appeared particularly promising for meeting stated requirements. The pioneering work of Wenker [3] on indicator properties of azo dyestuff proved very informative. When diazonium salt of 2,4-dinitroaniline or 2,6-dinitroaniline, and relative sulfonic acids, reacts with 1-naphthol, and relative 6-, 7-, 8-sulfonic and 3,6-disulfonic acids, azo dyes are obtained showing changes in the absorption/transmission in the physiological pH range. On the contrary, when 1-naphtol-4-sulfonic acid is used as the coupling agent, azo dyes with colour change at higher pH, are isolated. Wenker suggested that para coupling operates in the former azo dyes and ortho coupling in the last one. The Wenker conclusion is in agreement with the general consideration that unwelcome halochromism in textile fabrics is shifted to the highest pH values if ortho coupling on naphthol systems occurs. The formation of a strong hydrogen bond, involving azo/hydrazone equilibrium, in *o*-hydroxyazo compounds causes them to be much weaker acids than their *p*-isomers [4].

One of the previous requirements is the possibility to immobilise the indicator to membrane. Because cellophane was chosen as membrane, reactive dyes containing azo chromogen appeared particularly interesting. So, our interest was focused on 6-amino-4-hydroxy-2-naphthalenesulfonic acid (γ-acid) **1** as coupling agent and, on the basis of Wenker data, structure **2** was hypothesised, whose amino group can react with cyanuric chloride converting the acid azo dye in reactive one **3** (Scheme 1).

A detailed literature search on the synthesis of dye **2** evidenced a work of Wolthuis et al. [5] where the same strategy has been performed for realising analogous sensors. Wolthuis et al. proposed structure **4** for their indicator, suggesting for the precursor acid azo dye that ortho orientation occurred during the coupling of 2,4-dinitrobenzenediazonium salt on γ-acid **1**. The study of the change in colour with the variation of pH solution justifies the use of dye **4** for this kind of sensor but the relative structure is doubtful because the ortho substitution in azo chromogen is incoherent with spectroscopic properties that the authors reported [5]. The interesting spectroscopic data but the uncertainty in the structure and the complete lack of structural and preparative information suggested we perform a study on the synthesis of dye **2**. The classical procedure gave a low yield of the desired product, whose structure was elucidated by ^1H–^1H COSY and ^1H–^1H NOESY techniques, so a study for optimising the relative synthesis was also performed.



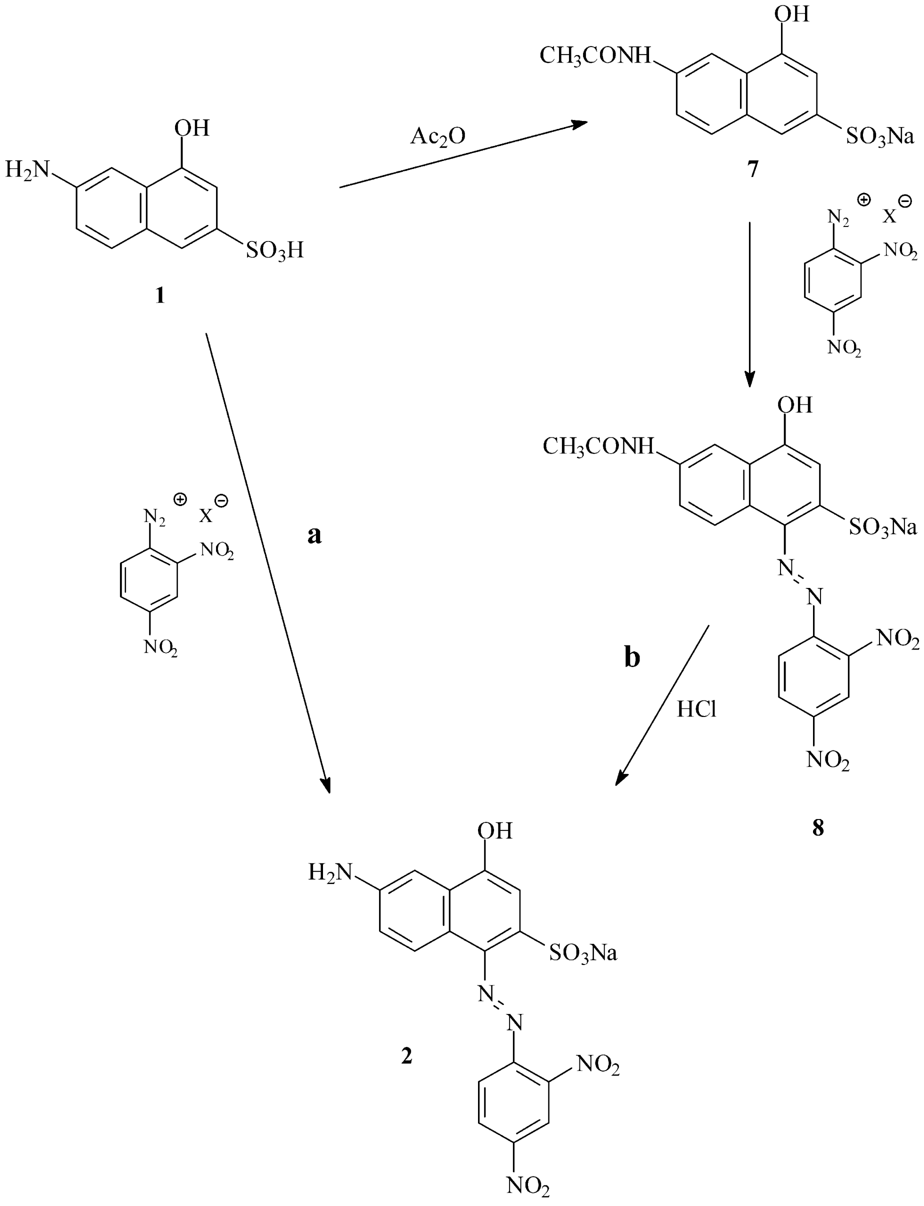
Scheme 1.

2. Results and discussion

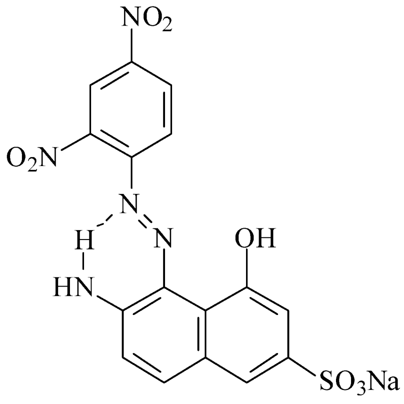
2.1. Synthesis

The γ-acid is largely used as intermediate for the synthesis of commercial azo dyes. The regioselectivity of the attack of diazonium salt to γ-acid is tuneable acting on the pH of reaction medium owing to co-presence of amino and hydroxy groups on the coupling agent. At moderate acidic conditions (pH 4–5) the rate of azo coupling dominates in the molecular segment containing the amino group while at pH 8 the substitution predominates at ring which carries the hydroxy one [6]. In basic conditions, the ortho/para isomer ratio depends on steric and electronic factors acting in the reaction intermediate, and electrophilic character of diazonium ion [6,7]. With no particular electrophilic diazonium ions, substitution takes place mainly at ortho position to hydroxy group. This was observed for benzenediazonium, 3-trifluoromethyl and 4-nitrobenzenediazonium salts [8–11]. No experiment is reported in the literature about the reaction of γ-acid with the strongly electrophilic 2,4-dinitrobenzenediazonium salt.

In Scheme 2, two different approaches are described for obtaining dye **2**. In method **a**, the 2,4-dinitrobenzenediazonium salt was added to the basic (pH 10) solution of γ-acid. In a first experiment, the diazonium salt of the weak base 2,4-dinitroaniline was prepared by nitrosylsulfuric acid following a usual procedure [12]. Because the high acidity of diazonium salt solution could produce local variations of pH in the reaction medium, high dilution and very slow dropwise addition of equimolar diazonium and coupling agent solutions to a buffered (pH 10) solution were used, with efficient mixing and checking pH by pHmeter. Zollinger et al. applied the same experimental conditions in the reaction of γ-acid with 4-nitrobenzenediazonium ion to minimise local variation of pH [8]. In our experimental conditions, a mixture of two dyes was obtained. After chromatographic separation, the ^{1}H NMR



Scheme 2.



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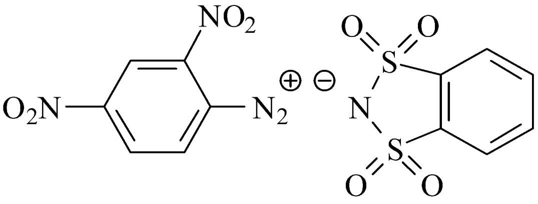

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Scheme 2. *(continued.)*

analysis showed that in this conditions dye **5** (aminoazo dye), usually obtained in acid conditions, is the main product (yield 50%) while dye **2** (*p*-hydroxyazo dye) was obtained in lower quantity (16% yield). Analogously, Zollinger et al. observed in a kinetic study that the azo coupling of γ-acid with 3-trifluoromethyl- and 4-nitrobenzenediazonium ion in concentrated aqueous alkaline solutions gave both aminoazo and ortho hydroxyazo compounds, and the relative increase of the aminoazo dyes was attributed to a micromixing effect [8]. Similar effect can be invoked in the present conditions, i.e. the addition of a highly acidic diazonium salt solution determines a local pH-value decreasing. In the mixing zones the acid-base equilibrium corresponds to the "microscopic", lower pH-values and the aminoazo dye (**5**) is formed. Our experimental expedients (high dilution, slow dropwise adding, buffering of coupling solution, stirring) were not enough to compensate for the micromixing effect, charged evidently with the high reactivity of 2,4-dinitrodiazonium ion. The formation of ortho hydroxyazo dye can not be excluded but, from a preparative point of view, we can conclude that it is not present in recoverable quantity in the previous experimental conditions. This is in agreement with the observation that strongly electrophilic diazonium ions, as 2,4-dinitrobenzenediazonium salt, essentially couple with 1-naphthol-3-sulfonic acid in the para position to hydroxy group [13]. Finally, the ^{1}H NMR analysis evidenced the non-equivalence of amino hydrogen's suggesting a hydrogen bond with the azo group as Zollinger et al. reported for similar dyes [8].

The low yield in dye **2** and, in particular the high dilution incompatible with large scale preparation, justified the research of alternative procedures. In a second set of experiments, the 2,4-dinitroaniline was diazotised by a recent method that furnishes dry benzenediazonium salt, stabilised as *o*-benzenedisulfonimide salt **6**, also from deactivated bases without using nitrosylsulfuric acid [14]. The salt **6** has low solubility in water and its aqueous suspension, showing a moderate acidity (pH 5), was added to the basic (pH 10) solution of γ-acid. Also in this case a mixture of dyes **2** and **5** was obtained without recovery of *o*-hydroxyazo dye. An increased global yield was observed with a higher quantity for dye **2** (yield 48%) than dye **5** (yield 28%). The higher global yield can be attributed to the higher concentration of reaction medium that favours the recovery of dyes partially soluble in water. Inversion of hydroxyazo and aminoazo dyes ratio was obtained but the tedious required step to eliminate dye **5** suggested the development of an alternative procedure.

In method **b** (Scheme 2) the amino group of γ-acid **1** was deactivated by acetylation with acetic anhydride obtaining the *N*-acetyl γ-acid sodium salt derivative (**7**). The following coupling step on intermediate **7** was performed with 2,4-dinitrobenzenediazonium ion prepared both via nitrosylsulfuric acid and as salt **6**. Both experiments gave only dye **8** and in comparable yields but the use of dry 2,4-dinitrobenzenediazonium *o*-benzenedisulfonimide allowed to work in higher concentrations of reaction medium and with lower inorganic salts that can co-precipitate and contaminate dye. As the last step of method **b**, acid hydrolysis of acetamide group restores the amino group of dye **2**, needed for obtaining a reactive dye by reaction with cyanuric chloride.

2.2. Structural analysis

Lyčka et al. used ^{1}H NMR technique for the structural analysis of dyes obtained by coupling of benzenediazonium chloride with 7-amino-4-hydroxy-2-naphthalenesulfonic acid (J-acid), 5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid (H-acid), and 6-amino-4-hydroxy-2-naphthalenesulfonic acid (γ-acid) [10]. ^{1}H–^{1}H NOESY experiments were particularly useful. This bidimensional NMR technique offers a means of determining spatial relationships between nuclei in a molecule by allowing the observation of Nuclear Overhauser Effect (NOE) cross peaks between neighboring nuclei [15]. Lyčka et al. observed a cross peak between the doublet of H_8 and a singlet that can be assigned to H_1 unambiguously, suggesting that benzenediazonium ion attacked position 3 of naphthalene ring.

The same experiment was performed for the coupling agent **1**, showing the expected cross peak between the doublet at 7.94 ppm of H_8 and the singlet (lightly broad) at 7.63 ppm of H_1. On the contrary, no cross peak was evidenced in the ^{1}H–^{1}H NOESY spectrum of dye **2** between the doublet at 8.29 ppm of H_8 and the alone singlet at 7.07 ppm, that can be assigned to H_3. Except the singlet at 7.07 ppm signal, all signals of ^{1}H NMR were easily assigned by their multiplicity and ^{1}H–^{1}H COSY experiments (Fig. 1). As a consequence, the attack of 2,4-dinitrobenzenediazonium ion on γ-acid involved the position 1 (para substitution) of naphthalene ring.

Similarly, the substitution position for dye **5** was assessed. By ^{1}H–^{1}H COSY experiment the signals of benzene and naphthalene protons were identified. The absence of a naphthalene doublet with meta coupling constant already suggested that the attack of diazonium ion was directed to 5 position. The ^{1}H–^{1}H NOESY experiment (Fig. 2) confirmed this hypothesis showing a cross peak correlation between the doublet at 7.95 ppm of H_8 and the singlet at 7.57 ppm, that was assigned to H_1. As a consequence, the remaining singlet at 7.30 ppm was attributed to H_3, suggesting for dye **5** the reported structure.

A preliminary spectroscopic study showed that dye **2** changed colour in the physiological range of pH (Fig. 3), confirming the applicability of this dye for monitoring blood parameters and that Wolthuis et al. [5] erroneously assigned the structure of dye **4**, for which para substitution has to be invoked.

3. Experimental

The ^{1}H NMR (400 MHz) spectra were recorded on a Jeol EX400 NMR spectrometer in DMSO-d_6 solution (3%) using DMSO as reference ($\delta_H = 2.52$ ppm). The two-dimensional homonuclear proton NOESY and COSY experiments were performed with a spectral width of 4800 Hz over 1024 data points. The mixing time of ^{1}H–^{1}H NOESY was set at 50 ms. The spectrum (16 scans) was obtained after multiplying the data with a sine square bell function in both dimensions.

3.1. Synthesis procedures

2,4-Dinitroaniline (Aldrich) was used as received. 6-Amino-4-hydroxy-2-naphthalenesulfonic acid monohydrate (**1**, γ-acid, Fluka) [8] and 6-acetamido-4-hydroxy-2-naphthalenesulphonic acid sodium salt (**7**, *N*-acetyl γ-acid sodium salt) [16] were respectively purified and prepared following literature methods. 2,4-dinitrobenzenediazonium *o*-benzenedisulfonimide (**6**) was prepared accordingly to literature method [14] and supplied by the research unit, co-ordinated by Prof. Iacopo Degani.

3.1.1. Method **a:** experiment 1

γ-Acid (0.51 g, 2.0 mmol) were solubilised in 100 ml of water containing 0.16 g (4.0 mmol) of

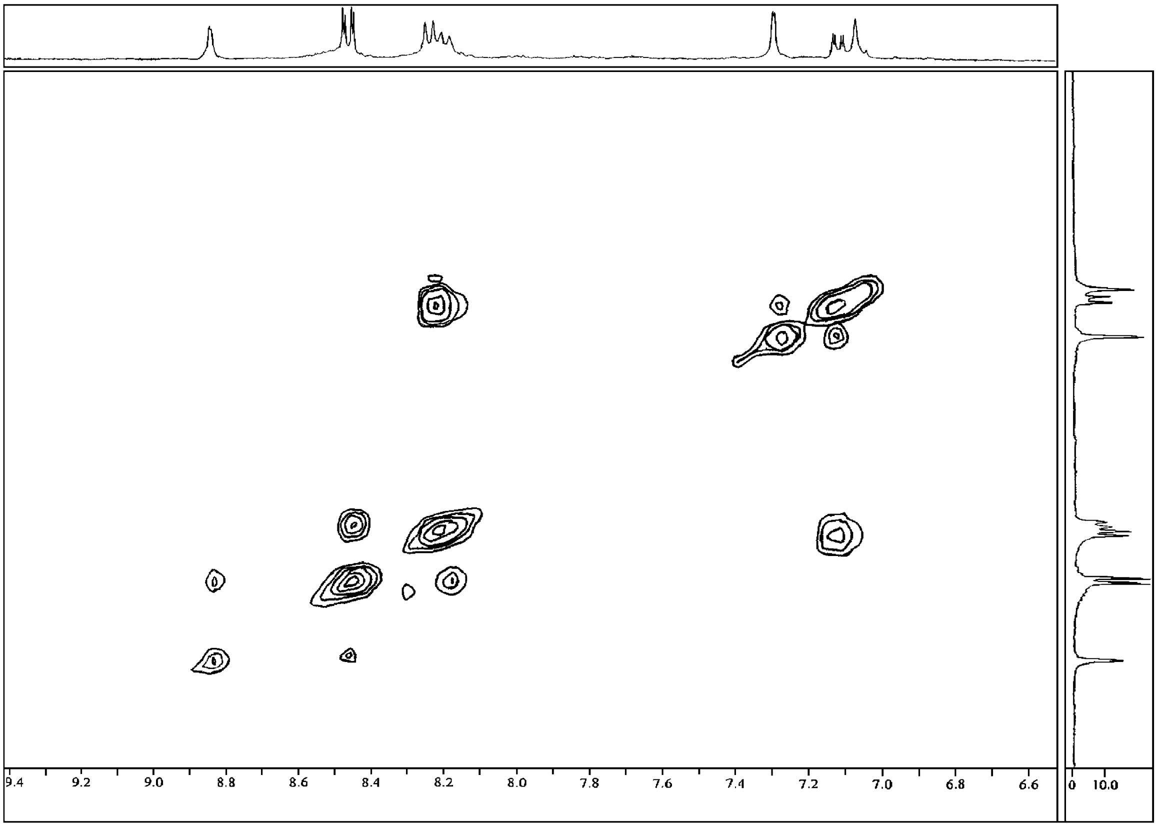

Fig. 1. Aromatic portion of ^1H–^1H COSY spectrum of dye **2**.

sodium hydroxide. 0.37 g of 2,4-dinitroaniline (2.0 mmol) was diazotised by nitrosylsulfuric acid accordingly to the literature [12]. The solution of diazonium salt (10 ml) was diluted to 100 ml with cold water. The coupling agent and diazonium salt solutions were added by two different burettes at the same time to 1 l of buffer solution (pH 10) maintaining the temperature at 0–5 °C and checking the pH by pH meter. The buffer solution was prepared mixing 500 ml 0.05 M $NaHCO_3$, 107 ml 0.1 M NaOH, 164.3 ml 1 M KCl and diluting to 1 l [8]. After 2 h of stirring at 0–5 °C, conc. H_2SO_4 was added until neutrality and the resulting mixture was lyophilised overnight. The solid was treated with 50 ml of cold water and filtered. The crude product was purified by flash chromatography on silica gel using BuOH:EtOH:NH_3:pyridine (4:1:3:2) as eluent, affording 0.14 g of dye **2** (yield 16%), and 0.43 g of dye **5** (yield 50%).

*3.1.2. Method **a**: experiment 2*

γ-Acid (5.1 g, 0.020 mol) were solubilised in 80 ml of water containing 1.60 g of NaOH (0.040 mol) of sodium hydroxide and the required volume of 1.0 M NaOH to obtain pH 10 of reaction medium. During 30 min, 8.3 g (0.020 mol) of 2,4-dinitrobenzenediazonium *o*-benzenedisulfonimide (**6**) were added as suspension in 40 ml of water to ice-cold basic solution of coupling agent under efficient stirring, continuously checking the pH of reaction medium by pH meter and maintaining 10 value by dropwise addition of NaOH 0.1 M. After 1,5 h of stirring, 37% HCl was added to neutralise the reaction medium. The precipitated solid was filtered and dried under vacuum. The crude solid was purified by Flash Chromatography as described in experiment 1, affording 4.77 g of dye **2** (yield 48%) and 2.77 g of dye **5** (yield 28%).

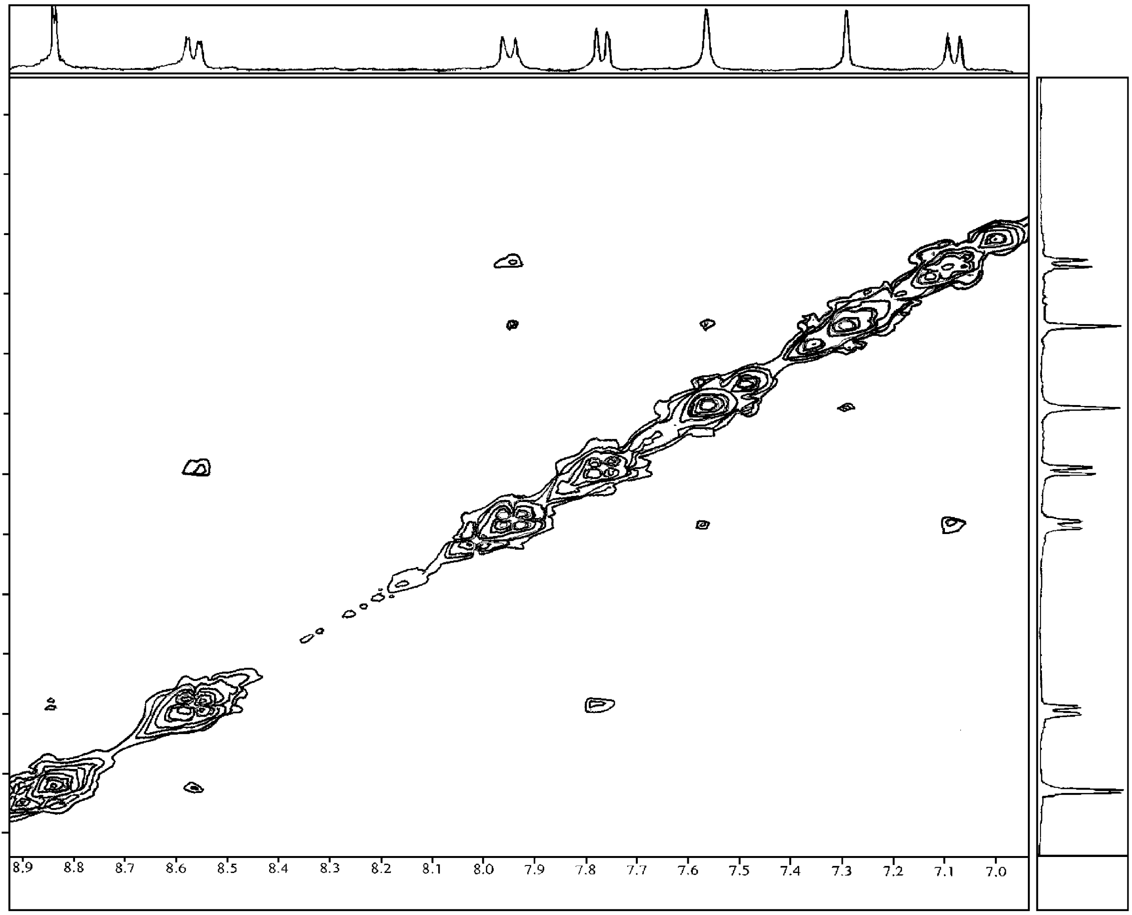

Fig. 2. Aromatic portion of ^1H–^1H NOESY spectrum of dye **5**.

3.1.3. Method *b*: experiment 1

N-acetyl γ-acid sodium salt (12.13 g, 0.040 mol) (**7**) and equimolar quantity of NaOH were dissolved in 160 ml of buffer solution at pH 10, prepared as reported previously. 7.32 g of 2,4-dinitroaniline (0.040 mol) was diazotised by nitrosylsulphuric acid according to the literature procedure [12] obtaining a final aqueous solution of 100 ml that was added dropwise at 0–5 °C to coupling solution, checking continuously the pH of reaction medium by pHmeter and maintaining 10 value by dropwise addition of 0.1 M NaOH. After 2 h of stirring at 0–5 °C, conc. H_2SO_4 was added until neutrality. The solid was filtered and crystallised from water obtaining 16.71 gr of dye **8** (yield 84%).

3.1.4. Method *b*: experiment 2

N-acetyl γ-acid sodium salt (12.13 g, 0.040 mol) of (**7**) and equimolar quantity of NaOH were dissolved in 160 ml of water; 0.1 M NaOH solution was added to obtain pH 10. 16.6 g (0.040 mol) of 2,4-dinitrobenzenediazonium *o*-benzenedisulfonimide (**6**) were added to 100 ml of water. The ice-cold suspension of diazonium salt was added to the basic solution of coupling agent in 30 min at 0–5 °C under efficient stirring, checking continuously the pH of reaction medium by pHmeter and maintaining 10 value by dropwise addition of 0.1 M NaOH. After 1,5 h of stirring, 37% HCl was added to neutralise the reaction medium. The precipitated solid was filtered and crystallised from water, obtaining 14.92 g of dye **8** (yield 75%).

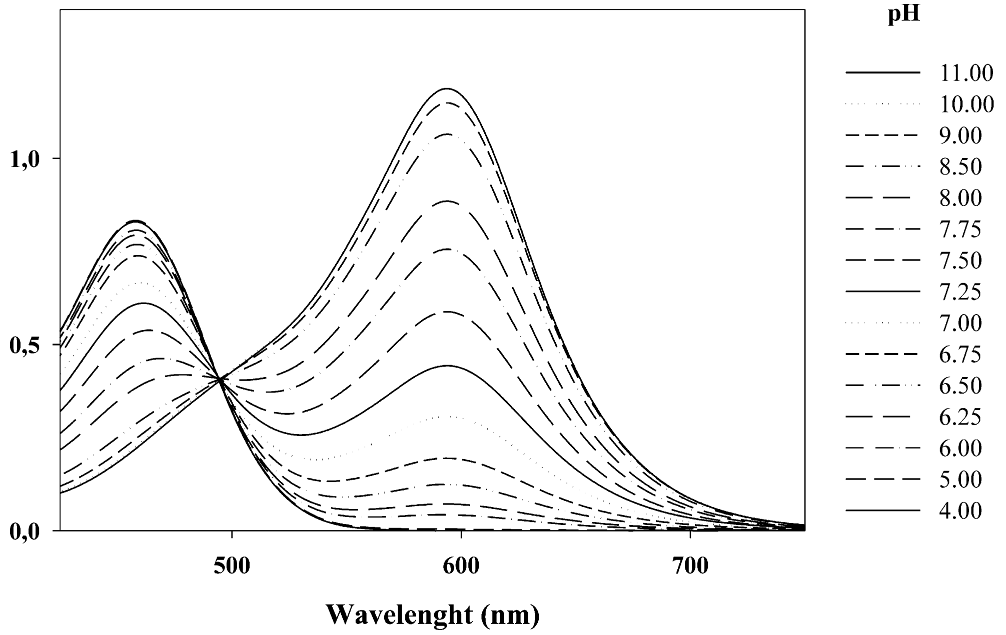

Fig. 3. Absorption spectra of dye **2** in various buffered pH solutions.

3.1.5. *Method* ***b****: hydrolysis of dye* ***8*** *acetamide group*

A mixture of 10 g (0.02 mol) of dye **8**, 42 ml of 37% HCl, 150 ml of water was stirred at 100 °C for 8.5 h. After cooling at 5 °C and neutralisation with 10 M NaOH, filtration afforded 6.10 g of chromatographically pure red solid. ^{1}H NMR analysis confirmed that it was equivalent to the product synthesised by method **a** and identified as dye **2** (yield 67%).

3.1.6. *Synthesis of dye* ***5*** *in acid solution*

γ-Acid (5.1 g, 0.020 mol) were solubilised in 80 ml of water by addition of 1.60 g of NaOH (0.040 mol). Glacial acetic acid was added to aqueous solution of γ-acid until 4.5 pH was obtained. A solution of 2,4-dinitrobenzenediazonium sulfate (0.020 mol), prepared according to the literature procedure [12] with a final volume of 50 ml, was added dropwise at 0–5 °C to suspension of γ-acid, checking continuously the pH of reaction medium by pHmeter and maintaining 4,5 value by addition of sodium acetate. After 15 h of stirring, filtration gave a blue-black solid that was crystallised from water affording 3.92 g of a dye that resulted from ^{1}H NMR analysis equivalent to a product identified as dye **5**, chromatographically isolated from mixture obtained by method **a** (yield 43%).

3.2. *^{1}H NMR data*

3.2.1. *6-Amino-4-hydroxy-2-naphthalenesulfonic acid (**1**)*

^{1}H NMR: 7.21 (*d*, 1H, J_{H3-H1} = 2.1 Hz, *H-3*), 7.37 (*dd*, 1H, J_{H7-H8} = 8.7 Hz and J_{H7-H5} = 2.2 Hz, *H-7*), 7.63 (*s*, 1H, *H-1*), 7.94 (*d*, 1H, J_{H8-H7} = 8.6 Hz, *H-8*), 8.00 (*d*, 1H, J_{H5-H7} = 2.0 Hz, *H-5*).

3.2.2. *6-Amino-1-(2,4-dinitrophenylazo)-4-hydroxy-2-naphthalensulfonic acid, sodium salt (**2**)*

^{1}H NMR: 7.07 (*s*, 1H, *H-3*), 7.10 (*dd*, 1H, J_{H7-H8} = 8.8 Hz and J_{H7-H5} = 2.4 Hz, *H-7*), 7.26 (*d*, 1H, J_{H5-H7} = 2.2 Hz, *H-5*), 8.15 (*d*, 1H, $J_{H6'-H5'}$ = 9.4 Hz, *H-6'*), 8.29 (*d*, 1H, J_{H8-H7} = 8.8 Hz, *H-8*), 8.44 (*dd*, 1H, $J_{H5'-H6'}$ = 9.4 Hz and $J_{H5'-3'}$ = 2.7 Hz, *H-5'*), 8.82 (*d*, 1H, $J_{H3'-5'}$ = 2.6 Hz, *H-3'*), 13.98 (*s*, 1H, *OH*).

3.2.3. *6-Amino-5-(2,4-dinitrophenylazo)-4-hydroxy-2-naphthalensulfonic acid, sodium salt (**5**)*

^{1}H NMR: 7.09 (*d*, 1H, J_{H7-H8} = 9.2 Hz, *H-7*), 7.30 (d, 1H, J_{H3-H1} = 1.8 Hz, *H-3*), 7.57 (*s*, 1H, *H-*

1), 7.79 (*d*, 1H, $J_{H8-H7} = 9.2$ Hz, *H-6′*), 7.95 (*d*, 1H, $J_{H6'-H5'} = 9.1$ Hz, *H-8*), 8.57 (*dd*, 1H, $J_{H5'-H6'} = 8.9$ and $J_{H5'-H3'} = 2.1$ Hz, *H-5′*), 8.84 (*d*, 1H, $J_{H3'-H5'} = 1.9$ Hz, *H-3′*), 10.10 (broad *s*, 1H, *NH*) 11.65 (broad *s*, 1H, *NH*) 12.29 (*s*, 1H, *OH*).

3.2.4. 6-Acetamido-1-(2,4-dinitrophenylazo)-4-hydroxy-2-naphthalensulfonic acid, sodium salt (8)

^{1}H NMR: 2.11 (*s*, 3H, $COCH_3$), 7.11 (*s*, 1H, *H-3*), 7.98 (*dd*, 1H, $J_{H7-H8} = 8.9$ and $J_{H7-H5} = 2.2$ Hz, *H-7*), 8.25 (*d*, 1H, $J_{H6'-H5'} = 9.4$ Hz, *H-6′*), 8.28 (*d*, 1H, $J_{H5-H7} = 2.0$ Hz, *H-5*), 8.40 (*d*, 1H, $J_{H8-H7} = 8.9$ Hz, *H-8*), 8.47 (*dd*, 1H, $J_{H5'-H6'} = 9.2$ and $J_{H5'-H3} = 2.6$ Hz, *H-5′*), 8.84 (*d*, 1H, $J_{H3'-H5} = 2.6$ Hz, *H-3′*), 10.31 (*s*, 1H, *CONH*), 14.05 (*s*, 1H, *OH*).

Acknowledgements

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References

[1] (a) Saari LA, Seitz WR. pH sensor based on immobilised fluoresceinamine. Analytical Chemistry 1982;54(4):821–3; (b) Wolfbeis OS, Fürlinger E, Kroneis H, Marsoner H. Fluorimetric analysis 1. A study on fluorescent indicators for measuring near neutral ("physiological") pH-values. Fresenius Z Anal Chem 1983;314:119–24.

[2] (a) Peterson JI, Goldstein SR, Fitzgerald RV, Buckhold DK. Fiber optic pH probe for physiological use. Analytical Chemistry 1980;52(6)864–9;
(b) Edmonds TE, Flatters NJ, Joner CF, Miller JN. Determination of pH with acid-base indicators: implications for optical fibers probes. Talanta 1988;35(2)103–7;
(c) Chang SH, Druen SL, Garcia-Rubio LH. Modelling and analysis of fibre optic pH sensors: effect of the ionic strength. SPIE Proceedings "Advances in fluorescence sensing technology II" 1995;2388:540–51.

[3] Wenker H. Indicator properties of dinitroaniline azo dyestuffs. Ind Eng Chem Anal 1935;27:40–1.

[4] Zollinger H. Color chemistry; synthesis properties and applications of organic dyes and pigments. 2nd ed. Weinheim: VCH; 1991 136.

[5] Wolthuis RA, McCrae DA, Saaski EW, Hartl JC, Mitchell G. Development of a medical fibre-optic pH sensor based on optical absorption. IEEE Trans Biomed Eng 1992;39(5):531–7.

[6] Zollinger H. Diazo chemistry I: aromatic and heteroaromatic compounds. Weinheim: VCH; 1994 351–2.

[7] Zollinger H. Color chemistry. synthesis properties and applications of organic dyes and pigments. 2nd ed. Weinheim: VCH; 1991 119–23.

[8] Kaminski R, Lauk U, Skrabal P, Zollinger H. 198. Mechanism of azo coupling rections (XXXIII). pH-dependence and micromixing effects on the product distribution of couplings with 6-amino-4hydroxy-2-naphthalenesulfonic acid. Evidence for N-coupling of a naphthylamine derivative. Helv Chim Acta 1983;66:2002–17.

[9] Skrabal P, Zollinger H. Mechanism of azo coupling reactions: part XXXV pH-dependence and ortho/para ratio in coupling reactions of aminohydroxynaphthalenesulfonic acids. Dyes and Pigments 1988;9:201–7.

[10] Lyčka A, Jirman J. Two-dimensional 1H-, 13C- and 15N-NMR spectra of azodyes derived from J-acid, H-acid and gamma acid. Dyes and Pigments 1987;8:315–25.

[11] Langfeld HW, Ionitã S. Das verhalten einiger amino-naphhtholsulfosäuren in der azokupplungsreaktion. Rev Roumaine Chem 1983;28:151–67.

[12] Fierz-David HE, Blangey L. Fundamental processes of dye chemistry. New York: Interscience; 1949. p. 247–8.

[13] Gatterman L, Liebermann H. Über die konstitution der von den sulfosäuren des α-naphthylamins und α-naphthols sich ableitenden azofarbstoffe mit negativen substituenten. Ann Chem 1912;393:198–214.

[14] Barbero M, Degani I, Dughera S, Fochi R. Halo-de-diazoniations of dry arenediazonium o-benzenedisulfonimides in the presence or absence of an electron transfer catalyst. Easy general procedures to prepare aryl chlorides, bromides, and iodides. J Org Chem 1999:3448–53.

[15] Claridge TDW. High-resolution NMR techniques in organic chemistry. Elsevier: Amsterdam; 1999 [chapter 4].

[16] Bucherer HT, Wahl R. Über die 2,5,1-amino-naphtolsulfonsäure (A-Säure) und ihre derivative. J Prakt Chem 1921;103:129–62.