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HISTORY OF ANALYTICAL CHEMISTRY

The 125th Anniversary of the Griess Reagent

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Abstract—The creation of the Griess reagent for nitrites is an example of the first directed synthesis of an organic reagent in analytical chemistry. In connection with the jubilee of the reagent, some aspects of the use of diazotization and azo coupling reactions for determining nitrites and nitrates are considered. It is shown that the Griess reaction is widely used at present; however, the components of this reaction and the methods for determining nitrites are continuously improved.

A mixture of sulfanilic acid and 1-naphthylamine is most often used for detecting nitrite ions. The appearance of red coloration of different intensities points to the presence and approximate concentration of nitrite ions. The reactions involving the Griess or the Griess–Ilosvay reagents are also used for the quantitative determination of nitrite ions in various samples, particularly, in environmental samples, including nitrous gases. The appearance of coloration is due to diazotization and azo coupling reactions. Nitrites diazotize sulfanilic acid, and then azo coupling with 1-naphthylamine is carried out in sulfuric or nitric acid solutions. The resulting azo compounds are used as analytical forms in determining nitrite ions. The reactions and reagents used are name ones (Griess' reaction, Griess' or Griess–Ilosvay's reagents); all of them are attributable Peter Griess. The name of Griess should be mentioned not only in the context of the 125th anniversary of the reagent, which was virtually the first directionally synthesized organic reagent he proposed, but also in connection with the 175th anniversary of the birth of its author, who was among the first to synthesize organic reagents for chemical analysis, although examples of the use of naturally occurring organic substances in chemical analysis are known since great antiquity. It is true that, much earlier than Griess, K. Scheele also demonstrated that the oxalic acid he synthesized could be used as a reagent for calcium.

Johann Peter Griess (1829–1888) was a German organic chemist, one of the founders of the azo and diazo dye industry. In 1856, he began work at an aniline dye plant, where he started to develop the fundamentals of the diazotization reaction [1]. On April 24, 1858, Griess reported the discovery of diazo compounds; this work became the starting-point in the development of the chemistry of azo dyes [2, p. 7]. At that time, organic chemistry was intensely developed. One of the achievements of chemistry at that time was the discovery that the valence of carbon is four; the structural theory of organic compounds had not yet been developed. Elemental analysis was the only tool that allowed an organic chemist to gain information about the structure of isolated naturally occurring compounds or of the products of chemical transformations. Assuming that two hydrogen atoms of a benzene ring were replaced by two nitrogen atoms, Griess chose the name "diazo" for the substances he synthesized [2, p. 20; 3]. The first method Griess used to diazotize picramic acid consisted of passing nitrogen oxides synthesized by the reduction of nitric acid with starch or arsenic acid into an ethanolic amine solution [4]. The diazo salt of picramic acid was found to be a stable compound, and the hydroxy group was substituted for the diazo group to give picric acid only upon heating. Other researchers did not observe the intermediate formation of diazo salts, since the azo salts formed in diazotization were unstable, especially upon heating.

The use of different amines as diazo and azo components; the effect of the concentrations of the reactants, including mineral and organic acids, water, and organic cosolvents; the mechanisms and kinetics of individual stages; and some historical aspects are considered in detail in monograph [2].

In 1879, Griess proposed the reaction of sulfanilic acid with 1-naphthylamine in the presence of nitrites and sulfuric acid and mentioned that it could be used for the identification of nitrites [5]. This composition was later named *Griess' reagent*.

Griess found, and a number of organic chemists later confirmed, that the diazotization of aromatic amines (RNH_2) at 0°C in mineral acid solutions could be expressed by the following equation:

$$ArNH_2 + 2HX + NaNO_2 = ArN_2^+X^- + NaX + 2H_2O, \quad (1)$$

where X is Cl^-, Br^-, NO_3^-, HSO_4^-, etc.

In acid media, the reacting species and the products of reaction (1) are almost completely dissociated, and the amine is in equilibrium with the ammonium ion $ArNH_3^+$. The difference in the basicity of amino groups in sulfanilic acid and 1-naphthylamine is due to the

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effect of the aromatic rings of benzene and naphthalene and of the sulfo group. As a result, of the two compounds bearing amino groups, sulfanilic acid is diazotized first. Sulfanilic acid diazotate then reacts with 1-naphthylamine with the replacement of the hydrogen atom in the 4-position of the naphthalene ring rather than in the amino group of 1-naphthylamine:

$$ArN_2^+X^- + H_2N–C_{10}H_7 \Rightarrow ArN=N–C_{10}H_6NH_2 + HX. \quad (2)$$

It should be noted that Eqs. (1) and (2) do not account for the protonation of amino groups and the ionic state of the reactants.

Using the reaction of diazotoluene with aniline as an example, Griess showed that the product of this reaction was identical to the product of the reaction between diazobenzene and *p*-toluidine [6]:

$$H_3C–C_6H_4–N=N–NH–C_6H_5 \Leftrightarrow H_3C–C_6H_4–NH–N=N–C_6H_4. \quad (3)$$

The reaction type was later confirmed by Griess [7] and other researchers [8] based on the tautomerism of diazoamidobenzene and amidoazobenzene. This type of tautomerism was also observed in the products of the azo coupling of nitric acid solutions of aniline and phloroglucinol and in the products of the azo coupling of *p*-toluidine and tartaric acid solution of phloroglucinol [9]. Tautomerism (3) was later dubbed *ptototropy.*

It is essential that reaction (1) proceeds smoothly in the presence of two equivalents of mineral acid; the nature of the mineral acid affects the kinetics of diazotization. Therefore, hydrohalic acids or alkali halides (diazotization catalysts) are substances of choice in reaction (1). The presence of equimolar amounts of diazo and azo components in the initial mixture is of no less importance. These features were revealed later [2].

Being an organic chemist, Griess pointed only to the possibility of using a mixture of sulfanilic acid and 1-naphthylamine for the identification of nitrite ions or nitrous acid. The sensitivity of this reaction was insufficient.

In 1889, Lajos Ilosvay, Professor of General Chemistry at Budapest Technical University (1851–1936), proposed replacing the sulfuric acid in the Griess reagent with acetic acid and dissolving 0.5 g of sulfanilic acid and 0.05 g of 1-naphthylamine in 150 mL of dilute acetic acid [10].

This mixture was proposed for the detection of nitrates after their reduction with zinc to nitrites; later it was labelled *Griess–Ilosvay's reagent*. According to [11], the Griess–Ilosvay reagent is prepared as follows: 0.6 g of sulfanilic acid is dissolved in 100 mL of 20% HCl; 0.48 g of 1-naphthylamine is dissolved in 100 mL of 13% HCl; the solutions are mixed; 272 g of $CH_3COONa \cdot 3H_2O$ is added and dissolved in the mixture, and the solution is diluted to 1 L. *Griess–Romeyne's reagent* is also known: it is a dry mixture of sulfanilic acid, 1-naphthylamine, and tartaric acid in the ratio 1.0 : 0.1 : 8.9 [11].

Today, one can hardly discern Griess' contributions to the theory of organic chemistry, the synthesis of azo compounds, particularly of azo dyes, and analytical chemistry. In any case, an analytical reagent for determining nitrite and nitrate ions and the basis for organic analytical reagents for a great number of metal ions are associated with the name of Griess.

One and the same azo compound is often used for technical purposes in dyeing and as a reagent.

It might be noted that, depending on solubility, nature, and specific features of their interaction with materials to be dyed, azo dyes are divided into the following groups [12]: pigments; fat-, alcohol-, and acetone-soluble; disperse; basic, cationic, and acid; lacquer; chrome (mordant); direct; reactive; and metal-containing dyes.

The Griess reaction is the first example of the directed synthesis of an organic reagent that includes an analyte and thus differs from, e.g., other azo compounds bearing functional analytical groups and forming complexes with metal ions. Another, more recent example of the synthesis of an organic reagent including an analyte is the synthesis of Methylene Blue from dimethyl-*p*-phenylenediamine and sulfide ions in the presence of an oxidant. Methylene Blue is used for the photometric determination of sulfide ions.

The Griess reaction is also used for determining nitrate ions after their reduction to nitrite ions with different reducing agents [11–14]. Instead of the classical components of the Griess reaction, the following substances are used as diazo components: sulfanilic acid derivatives; 8-aminoquinoline; sulfanylamide; *p*-aminoacetophenone; *p*-aminophenylmercaptoacetic acid; *o*-, *m*-, and *p*-nitroanilines; and 2-nitro-4-aminotoluene. The following compounds are used as azo components: dimethyl-1-naphthylamine; *m*-phenylenediamine; benzidine; *N*-(naphthyl)ethylenediamine; dimethylaniline; 2-naphthol; 2,5-dihydroxybenzoic acid; dihydric phenols: resorcinol, pyrocatechol; pyrocatechol-3,5-disulfonic acid (tiron), and chromotropic acid; a mixture of diphenylamine and *p*-diaminodiphenylsulfone; and a mixture of *p*-nitroaniline and azulene [15].

To obtain reproducible results, it is necessary to strictly follow not only the conditions of single stages but also use exact concentrations of the components and measure absorbance at constant time intervals. With the Griess reagent, the optimum pH_{opt} of solutions is between 1.7 and 3.0; absorbance is measured at 520 nm; $\varepsilon = 4.00 \times 10^4$ [16]. The method is selective. Two-hundredfold amounts (in mg/L) of $C_2O_4^{2-}$, CO_3^{2-}, and SiO_3^{2-}, 120-fold amounts of Co, 100-fold amounts of CN^-, 80-fold amounts of Cr(VI) and Pt(IV), and <40-fold amounts of Cr(III) and Sn(IV) do not interfere with determination. Fe(III), Sb(III), Bi, Ce(III),

Au(III), Hg(II), Ag, Pb, and V(V), substances that react with NO_2^- with the liberation of nitrogen (urea, thiourea, and sulfamic acid), reducing agents (I^-, S^{2-}, $S_2O_3^{2-}$, while SO_3^{2-}), and strong oxidants are interferents.

The Griess–Ilosvay reagent has the following shortcomings: the necessity of changing acidity in going from diazotization (acid medium) to azo coupling (weakly alkaline medium); the oxidizability of 1-naphthylamine in air; its high toxicity and carcinogenic properties; insufficient stability of azo compound solutions in storage; and the necessity of maintaining low temperatures at the diazotization stage. This causes problems with the use of the reagent in test methods and in some current methods in which the resulting azo compound serves as the detected species. The sensitivity of the photometric version of nitrite determination is insufficient when small samples or low concentrations are used.

At present, some of the disadvantages of the Griess–Ilosvay reagent have been eliminated. A procedure based on the diazotization of *p*-nitroaniline (instead of sulfanilic acid) and its coupling with chromotropic acid (instead of 1-naphthylamine) was proposed in [17]. The resulting compound is 7-(4-nitrobenzazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid (*chromotrope 2B*). Studies of the diazotization kinetics of *o*-, *m*-, and *p*-nitroanilines, 2-nitro-4-aminotoluene, 4-aminotoluene-2,5-disulfonic acid, 3-amino-5-sulfosalicylic acid, and 2-aminothiazole and their coupling with pyrocatechol, resorcinol, tiron, sulfosalicylic acid, or chromotropic acid showed the advantages of the use of chromotrope 2B as the final product [18]. The merits of such an analytical form are its high molar absorption coefficient (3.84×10^4), and the possibility of conducting both stages of the synthesis at the same pH (1.0–2.5) and at room temperature. Chromotrope 2B and the initial substances used in the synthesis are nontoxic and water-soluble.

The reaction was used for the determination of nitrite ions in grape wine [19], soil, snow, and tap water [20], and in cucumbers and tomatoes [21]. In addition to the conventional photometric procedure, it was proposed that nitrites be determined by continuous flow analysis with photometric detection [22] and by polarography [19]. To enhance the selectivity of determination, chromotrope 2B was adsorbed on an AV-17 anion exchanger or on Silochrome S-120 silica gel, and nitrite ions in seawater were determined by diffuse reflectance spectroscopy and chromaticity measurements [23]. The detection limits for nitrite ions in diffuse reflectance spectroscopy and in chromaticity measurements of total color difference were 1.2 and 0.6 μg/L, respectively.

Reaction in the test version was also used for determining nitrate ions after their reduction to nitrite ions in a blister cell [24, 25].

Most azo compounds bear basic or acidic functional groups and are pH indicators [26]. The reagents bearing salt-forming –OH, $–AsO_3H_2$, $–PO_3H_2$, –COOH, and other groups in *o*,*o*' positions with respect to the azo group comprise the second, very ample group of reagents. Of these, mono- and bisazosubstituted chromotropic acid derivatives (Arsenazo I, Arsenazo III, Chlorphosphonazo III) [27], heterocyclic azo compounds (PAN, PAR, 5-bromo-PAR) [28], and *o*,*o*'-dihydroxyazo compounds (Lumogallion IREA, Magneson IREA, nitrosulfophenol M, Eriochrome Black T, picramine–epsilon) [29] stand out. These reagents are widely used in complexometric titration as metal indicators and in photometry, solid-phase spectroscopy [30, 31], diffuse reflectance spectroscopy and chromaticity measurements [32], test methods [33], flow-injection analysis, HPLC [34], and capillary electrophoresis as reagents.

A few of the most illustrative examples of the use of the Griess reaction for detecting and determining nitrite and nitrate ions are given above. This reaction is widely used in the routine analysis of various samples, particularly, of environmental samples. There are many fewer works on the improvement of procedures and studies of the kinetics of the reaction with different diazo and azo components. In contrast, the use of the Griess reaction for the synthesis of organic analytical reagents of the azo series remains of current interest.

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