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Mendeleev Commun., 2020, **30**, 339–341

Mendeleev Communications

Cellulose-tethered tetradentate chelating agent for pyridoxine determination

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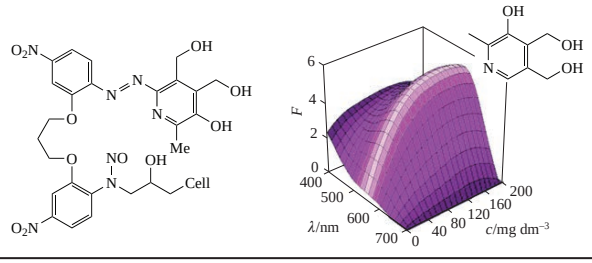
DOI: 10.1016/j.mencom.2020.05.026

The test method of determination of the pyridoxine (vitamin B_6) containing hydroxyaryl group comprises its azo coupling with chromogenic indicator diazo test strip, a reagent with functional diazoaryl group attached to the cellulose carrier through 1,3-diphenoxypropane bridge. This test strip reaction affords cellulose-tethered tetradentate chelating agent capable of forming chromogenic blue-violet complex with Cu^{II} ion.

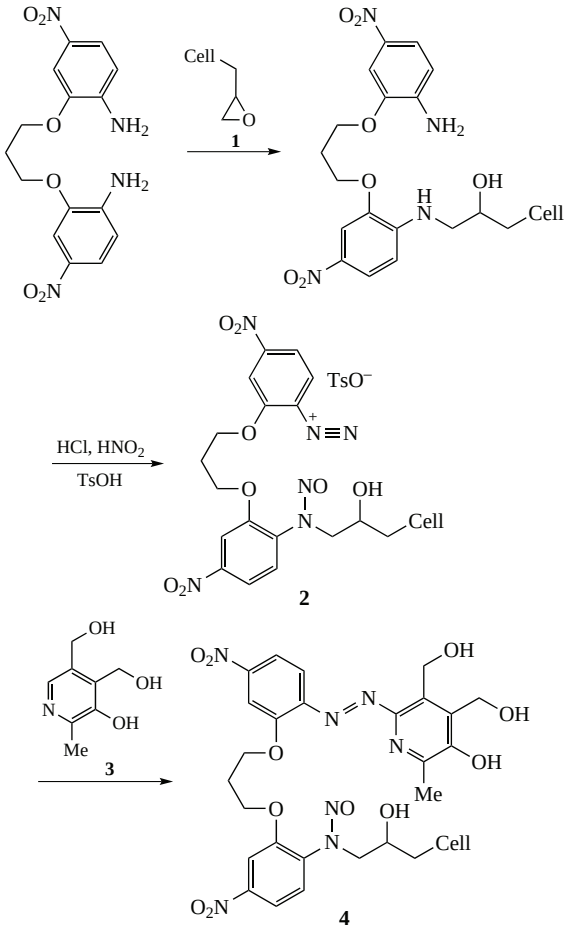


Keywords: indicator test strip, test method, azo coupling, pyridoxine, solid phase reagent, copper(II) complexes.

Pyridoxine (vitamin B_6) and its derivatives play an important role in body metabolism.[1] The known methods for the determination of pyridoxine involve the use of chemical indicators such as diazo compounds in combination with physicochemical methods of analysis. Semi-quantitative determination of pyridoxine in urine was carried out by its preliminary separation *via* electrophoresis on paper with the formation of complexes of red-violet azo compounds. The determination of pyridoxine in the presence of vitamins was performed by the preliminary azo coupling with diazotized 4-aminobenzenesulfonic acid in the micellar medium of *N*-cetylpyridinium chloride. The selectivity of the determination was achieved due to the differential-kinetic approach based on the inhibiting effect of boric acid,[2] and also to the regime of spectrophotometric flow-injection analysis.[3] The multi-commutation analysis mode based on the reaction of combined oxidation of pyridoxine and *N*,*N*-diethylbenzene-1,4-diamine with hexacyanoferrate(III) with a sensitivity of 0.1 mg dm^{-3} (ref. 4) and 0.06 mg dm^{-3} with pre-concentration on a chromatographic column.[5] These methods are characterized by the complexity of implementation and high cost of equipment. Determination of pyridoxine by the azo coupling with diazotized 4-nitroaniline in the presence of cetyltrimethylammonium bromide was carried out with high sensitivity of 0.2 mg dm^{-3}, however, this method was not selective.[6] Electrochemical determination of vitamin B_6 in tablets employing an ion-selective membrane electrode was not tested on real pharmaceutical formulations.[7] Determination of pyridoxine is hampered by phenol and aniline being sensitive to azo coupling reaction.[8] The spectrophotometric methods of determination by means of diazo reagents entering with pyridoxine into the azo coupling reactions are classified as the group.[9–14] Test methods on the basis of the cellulose carrier for determination of medicines were not still developed.[15,16] The method of determination of vitamin B_6 using a solid phase mineral sorbent with bromphenol blue as an indicator and Fe^{II} ions is not selective.[17]

The problem of selective determination of pyridoxine requires simple and reliable express methods of its analysis suitable for conditions of clinical laboratory and also for non-laboratory conditions of pharmacy. The purpose of this work was to develop a selective test method for the chemical analysis of pyridoxine through its template azo coupling with a chromogenic solid phase diazo reagent in the presence of metal ions. The chemical transformations occurring in the course of pyridoxine tethering based on epoxypropylated cellulose **1** are outlined in Scheme 1. Test strip reagent indicatior paper (TS RIP)-Diazo **2** entered into an azo coupling with pyridoxine **3** with the formation of RIP-Azo-Pyridoxine **4**. The attachment of the N=N group at the pyridoxine molecule can occur only to the position 6 of the pyridine ring *para* to OH group. The experimental details[18,19] are described in Online Supplementary Materials.

Compound **4** being a tetradentate reagent is capable of reacting with metal ions to afford complexes manifesting bathochromic shift, namely, Ag^{I} brown, Cu^{II} blue-violet, Co^{II} and Zn^{II} dark brown colour (Figure 1). The reaction of pyridoxine with TS RIP-Diazo **2** and Cu^{II} is remarkable since the bathochromic shift occurs with colour transitions from light yellow to red-orange in the first stage and then to blue-violet in the second stage in a wide pH range of 2−7; the colour reaction is characterized by stability, high speed and high sensitivity. This allowed us to use TS RIP-Diazo **2** and this test-reaction for determination of pyridoxine in its real pharmaceutical formulations, produced in the form of tablets, by sorption of pyridoxine



Scheme 1

from the sample by a test strip and subsequent determination by the diffuse reflectance spectra. To this, the tablet was powdered, pyridoxine hydrochloride was extracted with deionized water (100 ml for every 5 mg specified in the drug instructions). The reflection spectra of test-strips were recorded for these ranges (0.1–10 and 3–70 mg dm^{-3}) at the test reaction time of 5–20 min

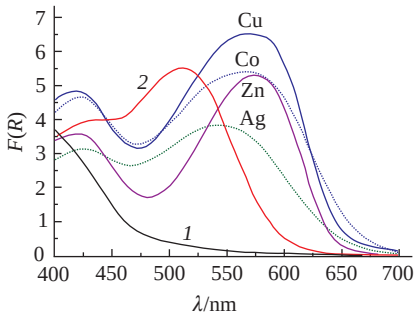


Figure 1 Diffuse reflectance spectra of TS: (*1*) RIP-Diazoamine, (*2*) RIP-Azo-Pyridoxine **4**, and its complexes with Cu^{II}, Co^{II}, Zn^{II} and Ag^{I}.

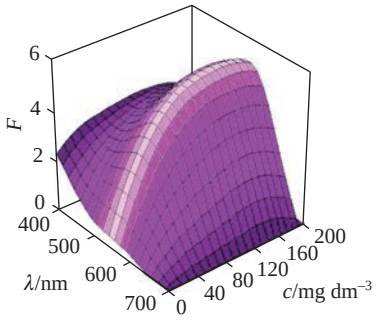


Figure 2 3D Diffuse reflectance spectra of TS RIP-M products of a two-step interaction of a TS RIP-Diazo **3** with pyridoxine microquantities and Cu^{II} for 0–200 mg dm^{-3} concentration range.

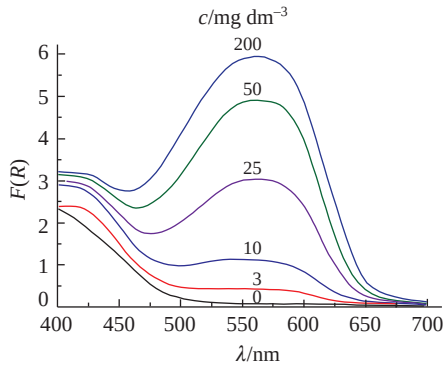


Figure 3 Diffuse reflectance spectra of TS RIP-M products of a two-step interaction of a TS RIP-Diazo **3** with pyridoxine microquantities and Cu^{II}.

(Figures 2, 3). The first concentration range (0.1–10 mg dm^{-3}) was the most suitable for these objects of analysis. According to the reflection spectra at the wavelengths 540–570 nm (see Figures 2, 3), analytical calibrations were carried out using the test-strip RIP-Diazo **2** in the presence of Cu^{II} ions to determine the content of pyridoxine hydrochloride. For the calibration curve, a wavelength of 570 nm was selected to determine the content of pyridoxine hydrochloride in tablets of pharmaceutical formulations (Table 1).

It is important to note that the significant content of excipients and fillers in these tablets did not interfere with the analysis. Phenol and aniline also formed azo compounds with RIP-Diazo, conventionally designated as RIP-Azo-Phenol and RIP-Diazoamine. These azo compounds of light yellow colour with maxima of reflection spectra (as the *F* function) in the ultraviolet region (see Figure 1) do not form coloured compounds with all studied metal ions, and thus do not interfere at the determination of pyridoxine in a tested amount of up to 2 wt%. During the analysis in the absence of copper, the results were overestimated. Since the indicator is covalently immobilized on cellulose carrier, it is possible to pre-concentrate pyridoxine to any desired c_{min}; the time and aliquot are set experimentally depending on the desired c_{min}. Other vitamins and medications, such as

Table 1 Determination of the content of pyridoxine hydrochloride in vitamin B$_6$ pharmaceutical formulations at 570 nm; P = 0.95, n = 5.

Pharmaceutical formulation	Company/Country	Quantity of active substance per 1 tablet (optional drug) according to the instructions	Found in 1 tablet	
			mg	s_r
Milgamma Compositum	WORMAG PHARMA Gmbh & Co. Kg/Germany	Pyridoxine hydrochloride 100 mg (benfotiamine 100 mg)	82.1	0.18
Pyridoxine hydrochloride	'OZON'/Russia	Pyridoxine hydrochloride 10 mg	10.4	0.12
Lysobact	'Bosnalijek'/Bosnia and Herzegovina	Pyridoxine hydrochloride 10 mg (lysozyme hydrochloride 20 mg)	8.6	0.15

ascorbic or folic acid, tetracycline, *etc.* also do not interfere with the determination of pyridoxine.

In summary, an approach to creating a selective test method for the determination of pyridoxine, active in the azo coupling reaction using an indicator test strip containing a functional diazoaryl group covalently bound to a cellulosic carrier through 1,3-diphenoxypropane bridge, has been proposed. As a result of the test reaction of azo coupling with pyridoxine on the indicator strip, a tetradentate coordination group was formed, which includes the diester fragment, azo group, and the amino group from the detected pyridoxine. Due to this, the product of combining an indicator strip with pyridoxine could give selective colour reactions with metal ions, observed visually or by means of a mini spectrophotometer (at 570 nm): c_{min} = 0.2 ppm, at P = 0.95 and n = 5, s_r = 0.12–0.18.

The study was partially carried out within the framework of the State Assignment of the N. S. Kurnakov Institute of General and Inorganic Chemistry, RAS, in the field of fundamental scientific research.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.05.026.

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Received: 26th December 2019; Com. 19/6097