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Spectrophotometric determination of ascorbic acid in grapes with the Prussian Blue reaction

Nicoleta MATEI*[a], Simona DOBRINAS[a] and Gabriel Lucian RADU[b].

[a] *Department of Chemistry and Chemical Engineering, University "Ovidius" of Constantza, 124 Mamaia Blvd, 900527, Constantza, Romania*
[b] *Polytechnic University of Bucharest, Department of Analytical Chemistry and Environmental Engineering, 1-7 Polizu Street, 011061, Bucharest, Romania*

Abstract The objective of the present work was to adapt the Prussian Blue reaction for the determination of ascorbic acid. The procedure was successfully applied for the determination of ascorbic acid in red and white grapes (*Vitis vinifera* L.) just previous ingathering. In the present work was used the red and white grapes from Murfatlar vineyard: Mamaia, Cabernet Sauvignon, Merlot, Pinot Noir, Chardonnay, Sauvignon, Muscat Ottonel and Riesling Italian. The results were situated in the range of 0.67 – 1.79 mg vitamin C/100g product for red grapes and respectively 0.50 – 1.49 mg vitamin C/100g for white grapes.

Keywords: spectrophotometric determination, ascorbic acid, grapes, Murfatlar vineyard

1. Introduction

High consumption of fruits and vegetables has been associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts [1-7]. These protective effects are considered, in large part, to be related to the various antioxidants contained in them. An important antioxidant is ascorbic acid (vitamin C).

Since the discovery of the ascorbic acid (AA) by Szent-Gyorgvi in 1928, followed by the structural determination in 1933 and chemical synthesis from L-xylosone in 1933 and D-glucose in 1934 [8], vitamin C and its biosynthesis in plants and animals has aroused a lot of interest and passion among the scientists all over the world [9]. AA has numerous biological functions, which include the synthesis of collagen, hormones and neurotransmitters [10]. It is believed that the role of AA in disease prevention is due to its ability to scavenge free radical in the biological systems. AA is abundant in many fruits [8, 11].

Many analytical methodologies have been proposed for the determination of AA taking into account its chemical role. Redox processes are generally involved in these determinations and it is possible to minimize air oxidation by using techniques such as flow injection (FI). One of these techniques is proposed by Nobrega and Lopes [12] and are mainly based on electroanalytical detectors due the inherent redox chemistry of the analyte and reagents. Optical FI methods also involve redox reactions with AA in which a colored compound is formed or decomposed. Thus, the formation of ferroin complex from the reduction of Fe(III)-phenanthroline [13] and a method using choramine T in the presence of starch-KI solution [14] were adapted. Descolorimetry was also employed by reduction of Cerium (IV) [15] and by reduction of triiodide [16]. This last paper involves the online formation of iodine and the measurement of the inverse peaks caused by the excess of triiodide at 350nm or the triiodide/starch complex at 580nm.

The objective of the present work was the optimization of spectrophotometric method with Prussian Blue reagent for the determination of ascorbic acid. The formation of Prussian Blue is a classical qualitative test to detect Fe(II) using hexacianoferrate (III) as reagent [17]. The first step is the oxidation of iron (II):

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$$Fe^{2+} + [Fe(CN)_6]^{3-} \rightarrow Fe^{3+} + [Fe(CN)_6]^{4-}$$

The second step is the formation of hexacyanoferrate (II) ferric complex (Prussian Blue):

$$4Fe^{3+} + 3[Fe(CN)_6]^{4-} \rightarrow Fe_4[Fe(CN)_6]_3$$

The complex formed is highly insoluble [18]. Employing an excess of the complexing anion and Fe(III) as colorimetric reagents, a deep blue soluble compound is formed when Fe(III) is reduced to Fe(II) by ascorbic acid:

$$2Fe^{3+} + C_6H_8O_6 \rightarrow 2Fe^{2+} + C_6H_6O_6 + 2H^+$$

The excess of hexacianoferrate (III) avoids the occurrence of precipitation [17].

The reduction of Prussian Blue reagent by vitamin C is a pH dependent process. The acid pH was found as optimal for vitamin C determination. In the process of Prussian Blue reduction, alkaline cations are involved. They play the role of counter-ions necessary for the electroneutrality of the reduced solution. The potassium cations due to their size can easily penetrate into structure of Prussian Blue reagent, but larger cations can block the reduction process. Therefore, an excess of potassium ions in the reaction medium is necessary. The used KCl solution gives such conditions and no interferences from alkaline cations were observed.

The procedure was successfully applied for the determination of ascorbic acid in red and white grapes (Vitis vinifera) just previous ingathering. The samples used were from Murfatlar vineyard. Blessed with one of the most suitable natural settings, Murfatlar Vineyard is situated in the south-eastern part of Romania, between the Danube and the Black Sea, in the centre of the Dobrogea plateau. The vineyard stretches across a surface of more than 3000ha, covering the villages of Murfatlar, Valul lui Traian, Poarta Alba, and Siminoc [19]. In the present work were used the red and white grapes by Murfatlar area.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical-reagent grade and all solutions were prepared using distilled-deionized water. The weightings were made at a Metler Toledo analytical balance with ±0.0001g accuracy.

Reference solutions containing from $5.0 \cdot 10^{-5}$M to $4.0 \cdot 10^{-4}$M ascorbic acid (by Merck) were prepared immediately before use by dilution of a $2.5 \cdot 10^{-3}$M stock solution in a 0.014M nitric acid medium with deaerated water, because ascorbic acid oxidation is slower in acidic medium. The effect of acidity on sensitivity was evaluated using 0.014 and 0.14M nitric acid solutions.

The Fe(III) reagent was prepared by dissolving iron (III) chloride salt in distilled-deionized water in the following concentrations: $1.0 \cdot 10^{-4}$, $5.0 \cdot 10^{-3}$, $1.0 \cdot 10^{-3}$, and $1.0 \cdot 10^{-2}$M, for the evaluation of the effect of Fe(III) concentration on sensitivity. The hexacyanoferrate (III) solutions ($2.0 \cdot 10^{-3}$, $5.0 \cdot 10^{-3}$, $5.0 \cdot 10^{-2}$M) were prepared by dissolving the potassium salt in water.

Other solutions were prepared: KCl 0.1M by dissolving the salt in water, and respectively 0.01M HCl by dilution of concentrated hydrochloric acid in water, for the maintain the pH of solution and its adjustment.

2.2. Sample preparation

The procedure was successfully applied for the determination of ascorbic acid in red and white grapes (*Vitis vinifera*) just previous ingathering.

The samples used were making by squeezing 100 grain grapes after weighing. The homogeneity of the samples was made. It was harvest the grapes in the diagonal from plot of land of each variety. After that the grapes were handpick of the cluster, it was numbered 300 grain grapes and 100 grain grapes representatively it was choice for the homogeneity of the sample for each variety of grapes. The juice was filter out, and from the filtrate was made the determination of ascorbic acid after it was measured the total volume. From the red grapes a discoloration with coal (animal black) was made before determinations.

The samples used were red and white grapes by Murfatlar area. The varieties of red grapes used were: Mamaia, Cabernet Sauvignon, Merlot, Pinot Noir and white grapes used were: Chardonnay, Saugvignon, Muscat Ottonel and Riesling Italian.

2.3. Sample analysis

An UV-VIS Thermospectronic HR 200 (and instrument LTD) spectrometer from England with 10mm cells were used for measuring absorption spectra. For each sample three determinations were performed and average results were reported.

The proposed procedure was optimized by using a model of flow injection spectrometric ascorbic acid determination studied by Nobrega and Lopes [12]. First step was the reaction between $2.0 \cdot 10^{-3}$M Fe(III) chloride salt and the $2.0 \cdot 10^{-3}$M potassium hexacyanoferrate (III) solution. After that there were added the following reagents in order: 0.1M KCl, 0.01M HCl and respectively the samples. The final solution was made up to 50mL-calibrated flask with distilled-deionized water. It was waiting 10 minutes for the stabilizing the complex formed and after the absorbance of the complex was read at the 700nm.

2.4. Optimization of conditions for vitamin C determination

Optimal conditions for the determination of ascorbic acid were established. The conditions are assured by the composition of the KCl and HCl solutions. Also, for the optimization of this method were diversified the reagents concentrations and respectively the volumes. The Fe(III) reagent in the following concentrations: $1.0 \cdot 10^{-4}$, $5.0 \cdot 10^{-3}$, $1.0 \cdot 10^{-3}$, and $1.0 \cdot 10^{-2}$M, was used for the evaluation of the effect of Fe(III) concentration on sensitivity and respectively the hexacyanoferrate (III) solutions $2.0 \cdot 10^{-3}$, $5.0 \cdot 10^{-3}$, $5.0 \cdot 10^{-2}$M were prepared. Also, the ascorbic acid concentration was varied in the range: $1 \cdot 10^{-3}$-$1 \cdot 10^{-4}$M for procedure optimization.

The variation of volumes by the reagents at the concentrations mentioned bellow was presented in **Fig. 1.**

After this variations of reagents volumes was find optimum the $FeCl_3$ ($2.0 \cdot 10^{-3}$M) : $K_3[Fe(CN)_6]$ ($2.0 \cdot 10^{-3}$M) : KCl (0.1M) : HCl (0.01M) : AA ($2.5 \cdot 10^{-3}$M) =1:1:1:1:1 ratio and then was plotted the calibration curve. But in time, the absorbance values are unsteady and for this reason the absorbance values were read at different times after reagents homogenization. The analyte (ascorbic acid) reacts with the reagent (Prussian Blue) and converts it to a blue product. The absorbance of the formed solution increases over time and a steady-state signal is reached after ten minutes.

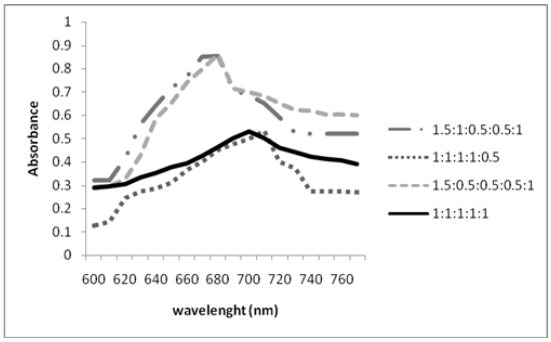

Fig. 1. The visible absorption spectrum for ascorbic acid varying the volume of reagents: $FeCl_3$: $K_3[Fe(CN)_6]$:KCl:HCl:AA (1.5:1:0.5:0.5:1; 1:1:1:1:0.5; 1.5:0.5:0.5:0.5:1 and respectively 1:1:1:1:1, mL) in the concentrations mentioned in the text.

2.5. Calibration curve

Considering that the Prussian Blue reaction had been employed only in qualitative tests, a first experiment was made to obtain the UV-Visible absorption spectrum of this compound. **Figure 2 (a)** shows the absorption spectrum for chromogenic reagents, Fe(III) mixed with the hexacyanoferrate (III) and a strong absorption band can be observed in the UV region and a weaker band with wavelength peak at 420nm, which is related to the formation of the brown complex hexacyanoferrate (III) ferric [17]. After adding KCl, HCl and ascorbic acid to this mixture, the absorption spectrum changed as can be seen in **Fig. 2 (b)** due the formation of Prussian Blue ($Fe_4[Fe(CN)_6]_3$) with an absorbance peak at 700nm.

The UV-VIS absorption spectrum optimum it was found for the Prussian Blue complex formed after mixing the $FeCl_3$:$K_3[Fe(CN)_6]$:KCl:HCl:AA solution in 1:1:1:1:1 ratio (in the concentration last-mentioned).

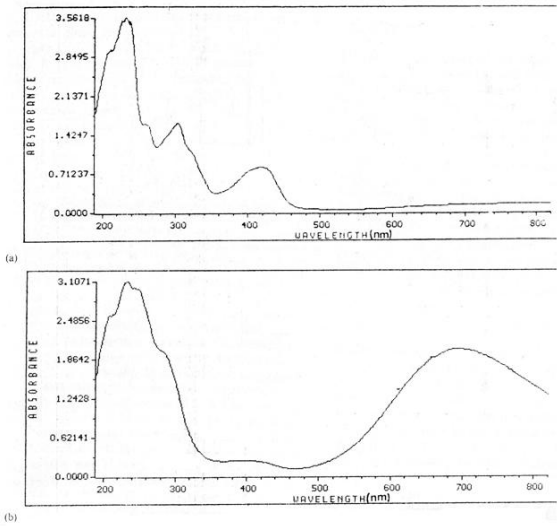

Fig. 2. UV-visible absorption spectra. (a) Chromatogenic reagents: Fe(III) plus hexacyanoferrate(III). (b) Chromatogenic reagents as for (a) plus KCl, HCl and ascorbic acid.

The ascorbic acid concentrations were varied for standardization of the curve. The standardization curve was presented in **Fig. 3.**

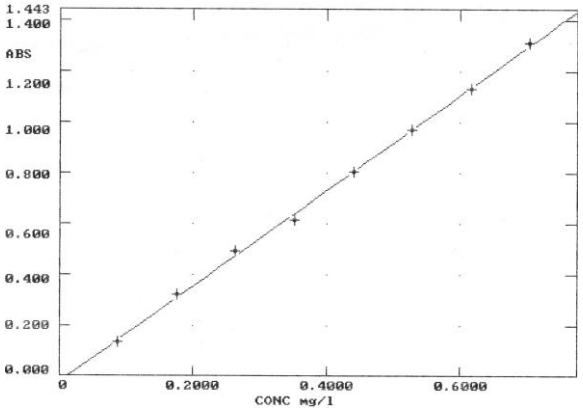

Fig. 3. The calibration curve of ascorbic acid with a correlation coefficient of 0.9994.

The calibration graph was linear over the range 0.0880-0.7040mg/L ($5\cdot10^{-5}$-$4\cdot10^{-4}$M) with a correlation coefficient of 0.9994.

2.6. Precision

Precision was primarily expressed as a percentage relative standard deviation (RSD%). It was discussed only repeatability.

Repetability was demonstrated by measuring a standard solution of ascorbic acid concentration (0.704 mg/L) by six times.

3. Results and Discussions

It was evaluated the effect of pH on the chromogenic reaction and it was found that the 3.5 value is optimum for mention the stability of the reaction.

The effect of Fe(III) concentration was also evaluated. In the proposed methodology, Fe(III) concentration should be higher than ascorbic acid concentration. Additionally, the sensitivity and reproducibility should not be negatively affected. Low concentrations of Fe(III), such as $1.0x10^{-4}$M, caused a strong decrease in sensitivity.

However, higher concentrations of Fe(III), such as $5.0\cdot10^{-3}$M, caused a loss of linearity.

Considering the compromise between sensitivity and linearity, all further measurements were made employing a $2.0\cdot10^{-3}$M Fe(III) solution. Taking into account these same effects, hexacyanoferrate (III) concentration was fixed at $2.0\cdot10^{-3}$M.To avoid the precipitation of the blue compound formed [17], the Fe(III) and hexacyanoferrate (III) solutions was mixed in 1:1 ratio.

The acidity effect was evaluated by changing the concentrations of KCl and HCl solutions. When 0.01M HCl was used, the sensitivity decreased, and this effect can be related to higher protonation of AA in this medium, since the reducing agent is the ascorbate anion [20]. Best results were attained by using a solution containing 0.01M HCl and 0.1M KCl (in 1:1 ratio). Also, the AA stock solution was prepared in acidic medium 0.14M HNO_3, by the same reason. This is also suitable for sample preparation because AA oxidation is slow in acid medium. Also, it was evaluated the time effect by the complexation reaction. 10 minutes was enough for the complex reduction by the AA.

The results obtained for repeatability (RSD%) for the six measurements of a standard solution was 3.56%, much lower than those in Horwitz equation: RSD< $2^{(-0,5\lg c)}$, for the working range (0.0880

mg/L - 0.7040 mg/L), for which RSD% should be lower than 16%.

The method was applied for the AA determination in grapes in the day of ingathering. Grapes juices sampled from *Vitis vitifera* varieties: Mamaia, Cabernet Sauvignon, Merlot, Pinot Noir of red grapes and Chardonnay, Saugvignon, Muscat Ottonel and Riesling Italian of white grapes were analyzed according to eight different groups. Grapes are cultivated in 2007, and the juices are manufactured just before determination.

In **Fig.4** were presented the results obtained for AA determination in white grapes and respectively in **Fig. 5** for red grapes by two methods: titrimetric and spectrometric.

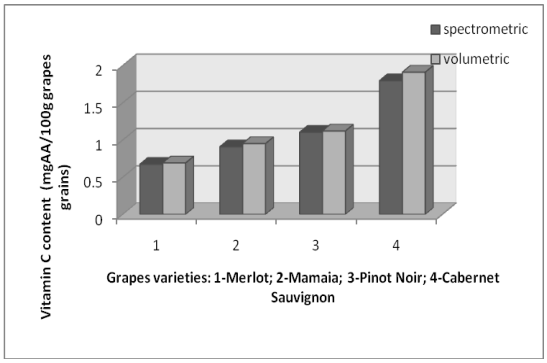

Fig. 4. Vitamin C content by white grapes (mgAA/100g grapes grains)

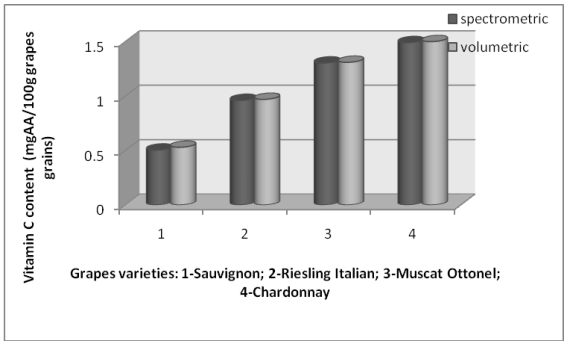

Fig. 5. Vitamin C content by red grapes (mgAA/100g grapes grains)

Results obtained by the proposed procedure are in good agreement with those encountered in the literature [21-28]. For comparison of results a standard titrimetric method for ascorbic acid determination was used based on the titration with potassium bromated-bromide solution in the acid medium [29, 30].

The results for AA concentration in grapes obtained in this study are lower than those reported in the Romanian literature (50mgAA/L) [21, 22].

Dani, C. et al. [23] were determined the vitamin C for eight *Vitis labrusca* juices-white or purple, from organically-or conventionally-grown grapes, and obtained in pilot or commercial scale. The results for AA determination in grapes by Brazil region were between 4.4 and 57.2 mg AA% in 2004 year and the results of our study were situated in the range of 0.50 – 1.49 mg vitamin C/100g for white grapes.

0.67 – 1.79 mg vitamin C/100g was recorded for Murfatlar red grapes while Guo et al. [24] was measured the vitamin C content of red rose grapes by pulp (0.49mmol/100g), peel (11.02mmol/100g) and seed (55.54mmol/100g) commonly consumed in China (100mg vitamin C is equivalent to 1.51mmol FRAP value as determined – FRAP = method using the ferric reducing antioxidant power assay).

4mg% ascorbic acid content in red rose grapes was cited from China Food Composition 2002, compiled by Institute of Nutrition of Food Safety, China CDC Beijing [25].

The vitamin C content in the Canada commercial pasteurized red grape juice was found 0.082mg/ml [26] unlike the vitamin C content of grapes by SUA (0.5mgAA/100g) [27]. Those results are lower than the results of vitamin C concentration by Murfatlar region.

Jain et al. was presented that the vitamin C concentration in grapes juice by India was 3.8mg/100mL of ascorbic acid [28].

4. Conclusions

The objective of the present work was to adapt the Prussian Blue reaction for the determination of ascorbic acid. The formation of Prussian Blue is a classical qualitative test to detect Fe(II) using hexacianoferrate (III) as reagent. The procedure was successfully applied for the determination of ascorbic acid in red and white grapes (*Vitis vinifera)* just previous ingathering. The samples used were from Murfatlar vineyard. Results obtained by the proposed procedure are in good agreement with those established using a previously proposed

procedure and with those encountered in the literature.

The proposed method was simple and precise for ascorbic acid determination from grapes and can be used without any pre-treatment of the sample.

5. References

* E-mail address: nmatei@univ-ovidius.ro

[1]. M. T. Tarrago-Trani, K. M. Phillips and M. Cotty, Journal of Food Composition and Analysis, **26,** 12, (2012).
[2]. Yang Li, Dan Li, Cheng-Yuan Ma, Chao-Ying Liu, Hui-Ding, Zhong-Mei Wen and Li-Ping Peng, Nutrition, **28**, 5, 504, (2012).
[3]. D. Feskanich, R. G. Ziegler, D. S. Michaud, E. L. Giovannucci, F. E. Speizer, W. C. Wilett and G. A.n Corditz, Journal of the National Cancer Institute, **92**, 1812, (2000).
[4]. R. Reiss, J. Johnston, K. Tucker, J. M. DeSesso and C. L. Keen, Food and Chemical Toxicology, **50** (12), 4421, (2012).
[5]. A. D. Haegele, C. Gillette, C. O'Neil, P. Wolfe, J. Heimendinger, S. Sedlacec and H. J. Thompson, Cancer Epidemiology Biomarkers & Prevention, **9**, 421-425 (2000).
[6]. D. P. Singh, J. Beloy, J. K. McInerney and L. Day, Food Chemistry, **132** (3), 1161, (2012).
[7]. K. B. Michels, E.Giovannucci, K. J. Joshipura, B. A. Rosner, M. J. Stampfer, C. S. Fuchs, G. A. Colditz, F. E. Speizer and W. C. Willett Journal of the National Cancer Institute, **92,** 1740, (2000).
[8]. M. B., Davies, J. Austin and D. A Partridge, *Vitamin C: Its Chemistry and Biochemistry*. The Royal Society of Chemistry, Cambridge, UK, 1991.
[9]. A. B. Wolucka and M. Van Montagu, Phytochemistry, **68**, 2602, (2007).
[10]. G. Block, Age, **16,** 55, (1993).
[11]. J. Pincemail Degrune, F. Voussure, S. Malherbe, N Paquot and J-O. Defraigne, Nutrition clinique et methabolisme, **21,** 66, (2007).
[12]. A.J., Nobrega and S.G. Lopes, Talanta**, 43**, 971, (1996).
[13]. S. M Sultan, A. M. Abdennabi, and F. O. E. Suliman Talanta, **41,** 125, (1994).
[14]. M., Vacarcel, F. Lazaro, A. Rioos and M. D. Luque de Castro, Analyst, **41,** 111, (1986).
[15]. S. M., Sultan Talanta, **40,** 593, (1993).
[16]. J. Hernandez-Mendez, A. Alonso Mateos, M. J. Almendral Parral, and C. Garcia de Maria, Anal. Chim. Acta **184,** 24, (1986).
[17]. A. I Vogel: *Textbook of Macro and Semimicro Qualitative Inorganic Analysis*, 5th edn. Longman, London, 1979.
[18]. J. Lurie: *Handbook of Analytical Chemistry*, Mir Publishers, Moscow, 1975.
[19]. www.murfatlar.com
[20]. J. L. A Martins and J. Barbosa da Costa, J. Chem. Educ. **65**, 176 (1988).
[21]. V. Costea *Tratat de oenologie,* Vol. 1, Editura Ceres, Bucureşti, 1985, pp. 409.
[22]. N. Pomohaci, C. Sârghi, V. Stoian, V.V. Cotea, M. Gheorghiță and I. Năməloşanu, Oenologie, Vol. 1, *Prelucrarea strugurilor şi producerea vinurilor*, Editura Ceres, Bucureşti, 2000, pp. 135.
[23]. C. Dani, L. S Oliboni, R. Vanderlinde, D. Bonatto, M. Salvador and J. A. P.Henriques, Food and Chemical Toxicology, **45,** 2574, (2007).
[24]. C. Guo, J. Yang, J. Wei, Y. Li, J. Xu and Y. Jiang, Nutrition Research, **23,** 1719, (2003).
[25]. China Food Composition 2002, *Institute of Nutrition of Food Safety*, China CDC Beijing: Peking University Medical Press, 2002**,** pp. 82.
[26]. Y. Wu, G. S. Mittal and M. W. Griffiths, Biosystems Engineering, **90** (1), 1, (2005).
[27]. L. P Leong and G. Shui, Food Chemistry, **76,** 69, (2002).
[28]. A. Jain, A. Chaurasia and K. Verna, Talanta, **42,** (6), 779, (1995).
[29]. N. Matei, S. Birghila, S. Dobrinas and P. Capota, Acta Chimica Slovenica, **51,** 169, (2004).
[30]. N. Matei, V. Magearu, S. Birghila and S. Dobrinas, Revista de Chimie, **55,** (5), 294, (2004).

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