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STUDY AN APPLICATION POSSIBILITY OF THE FLAVONOIDS FOR THE SYNTHESIS OF COPPER NANOPARTICLES

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ABSTRACT

The method of biochemical synthesis of metals nanoparticles in inverse micelles using natural biologically active compounds, from flavonoids groups as reductants has been substantiated. An extraction of rutin from green tea and onion peel was carried out. Quantitative content of rutin in investigated objects was determined by photocolorimetric method. A synthesis of copper nanoparticles in inverse micelles by biochemical method has been carried out.

KEYWORDS: synthesis, flavonoids, rutin, copper nanoparticles, inverse micelles.

INTRODUCTION

In the last decade, there is a rapid development of the research field called nanotechnology, which considers dispersed systems consisting of nanometer-sized objects. In the development of modern nanotechnology a research of metal nanoparticles plays a significant role. This is due, primarily, to a wide range of possibilities for their practical application in various fields of technology, as well as in biology and medicine, where the specific properties of both nanoparticles and modified materials are used. The investigation possibility of the metal nanoparticlesproperties, development of variants for their practical application, to a large extent depend on the method of production, which in many cases determines their structure, dimensions, physical and chemical properties, and, most importantly, their stability - lifetime in nano-dimensional state.

Methods of metal nanoparticles synthesis represent approaches of inorganic, organometallic and organic synthesis with the phase formation processes in colloidal or similar systems. Analysis of published data [3] showed that the most prospective method for obtaining metal nanoparticles is the method of biochemical synthesis in inverse micelles. A feature of the production of nanoparticles by biochemical synthesis is the use of non-conventional reducers - plant pigments of the group of flavonoids. These substances possess known structures and concentrations, that allows to influence the formation of nanoparticles by varying the relevant parameters, and provides a higher reproducibility of the results. Incidentally the technological simplicity and efficiency are saved, as well as high stability of the nanoparticles. It provides several advantages that are important for practical applications of metal nanoparticles.

MATERIALS AND METHODS

Flavonoids represent a largest class of plant pigments of low-molecular polyphenolic compounds, the basis of which is a three ring structure, two aromatic rings connected to each other by heterocyclex containing oxygen, also called the pyran ring. In interest in flavonoids is great due to a broad spectrum of their biological effect and antioxidant activity. Flavonoids are found in citrus peel, onion, green tea, as well as in fruits, flowers, herbs [1-3]. In addition, flavonoids are more effective reducing agents and, at the same time, less toxic and safer agents than traditional chemical reducing agents.

In the method of biochemical synthesis as natural reducing agents three flavonoids from the subgroup of flavonol are most often used: quercetin, rutin and morin. These substances are known for their ability to chelate metal ions. For metal nanoparticles production, as a reductant we studied the possibility of application of rutin, extracted from local raw materials.

Rutin is contained in the composition of biologically active supplements and medications. Together with other flavonoids it is present in many plants and

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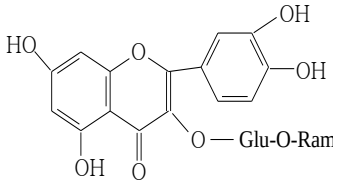
foods. According to the chemical structure the rutin represents 5,7,3 ', 4'-OH-3-ramnoglyukozid:



Initially, our aim was to study the possibility of extraction of rutin from natural raw materials. The objects of study were two samples of green tea (broken-leaf and large leaf) and onion peel. For flavonoids there is no universal method of extraction from plant materials, since they differ greatly in their solubility in water or organic solvents. In each case one should use the most appropriate method or combination of methods taking into account the properties of the extracted substances, properties of possible related compounds, and characteristics of plant raw materials.

In order to remove lipophilic admixtures from the raw materials by carbon tetrachloride. In order to determine the content of flavonoids in plant species they were preliminary processed. We carried out an extraction in Soxhlet apparatus by 70% solution of ethanol during 120 minutes. For the detection of various types of flavonoids qualitative reactions are used. They are needed to confirm the finding of a particular structure on the stage of identification of flavonoids.

RESULTS AND DISCUSSION

For the qualitative determination we used cyanidin reaction based on reduction of carbonyl groups and the formation of antocyanide by zinc dust in the presence of concentrated hydrochloric acid with formation of oxonium compound. To perform the cyanidin reaction the extracts were evaporated and zinc dust was added by 0.05 g. Then they were heated in a water bath up to boil. The presence of flavonoids in the samples was confirmed by the appearance of a bright red color of the liquid.

We also carried out a reaction with aluminum chloride. We added 2-3 drops of 5% solution of aluminum chlorideto one ml of the extract. The appearance of lemon-yellow color indicates the presence in the analyzed objects of flavonoids having two oxide groups. Furthermore, for the qualitative determination of the composition of the studied samples we used the method of downlink chromatography in system of 15% acetic acid. The chromatography was carried out using the chromatographic paper of "C" mark. As a developer we used a solution of aluminum chloride. In the chromatogram a yellow-brown spot was obtained. Judging by the color of the spots, after processing and determining Rf value (Rf for rutin = 0,62, Rf for quercetin = 0.90) we can conclude on the presence of flavonoids in the samples.

Quantitative determination of total flavonoids was performed by spectrophotometric method based on the use of the complex-formation reaction of flavonoids with aluminum chloride. An aliquot of the analyzed extract of 1 ml was placed in a preweighed measuring flask of 25 ml, and weighed. After that 4 ml of 5% solution of aluminum chloride was added. As a comparison solution, we used the aliquot of the analyzing extract with 70% ethanol solution, the volume of both flasks was adjusted to the mark. In the case of turbidity of the solutions they were filtered through filter paper. Optical density was measured in the range of 400 - 450 nm at the wavelength of absorption maximum in cuvettes with the absorbing layer thickness of 1 sm, in the working cuvette we placed the solution with added aluminum chloride, in the comparison cuvette - the reference solution. The absorption maximum is located in the region of 400 - 420 nm, so the rutin GSO was used as a standard.

To plot a calibration curve of the dependence of the optical density on the amount of rutinon the solution accurate weigh of GSO of rutin of about 0.05 g quantitatively was flask of 50 cm^3, 40 ml of 60% aqueous alcohol was added, then heated transferred to a volumetric to 5O - 6O ° C up to dissolve of rutin, then cooled to room temperature and brought up to the mark with 60% alcohol. After 30 minutes the optical dencity in the 400-450 nm range was measured. Calibration graph of the dependence of the optical density on the amount of rutin in the solution was a straight line passing through the beginning of the co-ordinates.

Formulae:

$$X = \frac{C \cdot F_p \cdot 10^5}{M} \quad (1)$$

where,

c - amount of rutin in the tested aliquot of extract corresponding to the measured optical density on the calibration graph, g/25 cm^3;

F_p - dilution factor;

10^5 - conversion factor in mg 100g;

M - mass of extract, g.

Table 1 The total flavonoids content in the samples

	Extract	Content $1 \cdot 10^{-3}$, mg/ ml
1	Onion peel	104,7
2	Green tea (bigleaf)	31,1
3	Green tea (broken-leaf)	63,4

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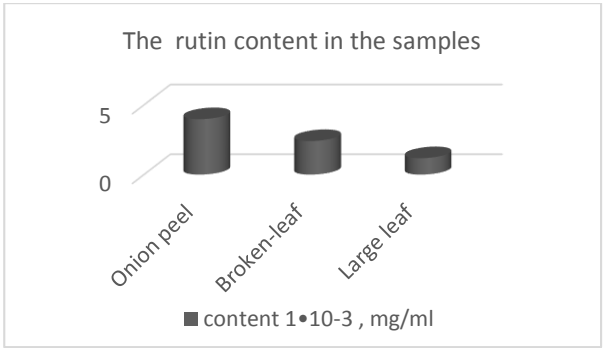
CONCLUSION

For quantitative analysis of the content of rutin in the investigated plant material, experiments on separate extraction of rutin were carried out according to the known method [2]. For the analysis we took 5 g of plant material, triturated in a porcelain mortar in the presence of alcohol. The tumble weighed was transferred to a Buchner funnel, and extracted by alcohol until complete discoloration of residual and dripping extract. The volume of the filtrate was adjusted to 100 cm^3 by alcohol and from this amount for the determination 25 sm^3 was collected in Wurtz flask. The alcohol was distilled to near dryness and the residue in the flask was treated by small portions of diethyl ether until a colorless extract (to remove quercetin, carotenoids and other ether-soluble substances).

The ether extracts were combined. The alcohol solution of rutin was brought up to 25 cm^3 by 80^0 alcohol. Colorimetric analysis was then performed on the device FEC-56M with a blue filter in a cuvette with a working length of 0,5 mm. The content of routine was found from the calibration curve. The results are shown in Figure 1.



To solve the applied problems using copper nanoparticles we, on the basis of the published data, have chosen the method of biological synthesis, based on the reduction of copper ions in inverse micelles by natural reducing agents from the group of flavonoids, namely routine.

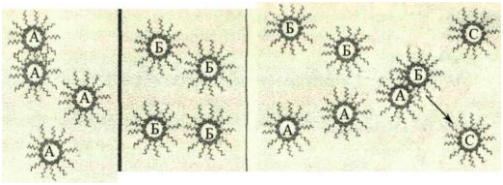


Fig.2. Scheme of nanoparticles synthesis in inverse micelles.

The synthesis was performed by standard procedure (Fig.2): a solution of bis-(2-ethylhexyl) sulfosuccinate, sodium salt (AOT) in isooctane, then based on it two micellar solutions were prepared by introducing a metal ion (A) and reducing agent (B)to aqueous solutions. Then, these solutions were mixed. After introduction of aqueous solution of the metal ions in micellar solution of flavonoids a synthesis of nanoparticles starts; the course of this process and its termination are reflected in corresponding changes in the optical absorption spectra. To prepare a micellar solution of rutin we used solution of AOT in isooctane with the concentration 0.135. For nanoparticles production the ammonium salt of copper solution was prepared by introducing aqueous ammonia solution in copper sulphate solution up to dissolion of the precipitate of metal hydroxides and the formation of complex ammonia-containing cations. Introduction of aqueous solutions of ammonium salts of copper in micellar solution has led to rapid changes in color, indicating the formation, first, complex of rutin with metal ions, and then the nanoparticles. The solution of copper nanoparticles is of cooper-red color; the absorption maximum is at 550 ± 5 nm, in the region,which is characteristicfor nanoparticlesabsorption.

Typical is presented in Figure 3.

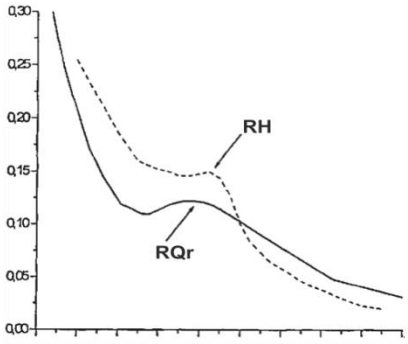


Fig.3. Absorption spectrum of Cu nanoparticles

For comparison, the band of Cu nanoparticles obtained in inverse micelles using conventional reducing agent (hydrazine) is shown. Compasion with the spectrum of the copper nanoparticles, obtained by reduction with hydrazine in reverse micelles shows that the peak position is almost identical, but in this case the band of nanoparticles is more pronounced, probably due to the higher degree of conversion of copper ions and, respectively, a lower optical density in the UV region of the

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spectrum due to decrease of the concentration of the reduced metal ions. The absorption at 800 nm in both cases is negligible. It shows that in the biochemical synthesis achieves as small degree of oxidation of nanoparticles by atmospheric oxygen as in using the conventional chemical reductant.

Thus, our studies have shown the use possibility of rutin extracted from local materials to be a reducing agent in the synthesis of copper nanoparticles in inverse micelles.

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