SYNTHESIS, CHARACTERIZATION AND APPLICATION OF NANOMATERIALS BASED ON NOBLE METALLIC NANOPARTICLES AND ANTHOCYANINS

1Liliana Olenic, 2Ioana Chiorean
1National Institute for Research and Development of Isotopic and Molecular Technologies, 67-103 Donat Street, 400293 Cluj-Napoca, Romania
2Faculty of Mathematics and Informatics, Babeş-Bolyai University, 1 Kogălniceanu Street, 400023 Cluj-Napoca, Romania

Abstract- Natural extracts from European Cornel fruits have been found to be a significant source of natural antioxidants with health benefits for human body. In the present work we focused on using these extracts for preparing new nanomaterials based on metallic nanoparticles, taking in consideration that these metals have been used for a long time as treatment in medical fields. We have developed a synthesis method which permits to obtain the noble metal nanoparticles having anthocyanins from natural extract as ligands. We have characterized them by UV-Vis spectroscopy, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). Equilibrium constants of reactions between gold/silver nanoparticles and organic molecules as ligands have been determined in two ways and have been found of 104 orders of magnitude, values that suggest a good association between them. Some new medical products (creams) based on gold/silver-natural extracts have been applied on skin lesions appeared due to the psoriatic illness. The creams prepared have been used on a lot of 31 human subjects with skin lesions and after two months of treatment, a good anti-inflammatory effect has been observed. The good medical results have been confirmed by statistical studies.

Keywords - Nanomaterials, Colloids, Binding Constants Determination

I. INTRODUCTION
After a long period of time of using chemically processed foods and synthetic drugs, humanity witnesses now a “return to nature” which is becoming more and more pressing. Herbal remedies are used for thousands of years to treat certain diseases. In the last decade, an increasing interest in phenolic compounds from plant extracts is observed, due to their protection forms against different diseases, such as: cardiovascular, cancer etc [1]. The anthocyanins (which are numbered between polyphenols) have important antioxidants and anti-inflammatory properties [2-3]. The majority of biological active components from plant extracts are poorly absorbed in organism so their action is very slow [4]. Nowadays, nanomaterials having unique optical, physical and biological properties are becoming very important in many areas of science and were prepared using different methods [5-7]. In Medicine, used as therapeutics [8-9], they have been physicochemical tailored, function of the desired application. Metallic or nonmetallic nanomaterials based on plant extracts are now very much studied [10-24]. Metallic nanomaterials potentiate the action of the active compounds from plant and improve their efficacy and activity. The gold salts have been used in Medicine to treat arthritis and, newest, they are used in medical cardiac implants; silver salts have antibacterial [25], antithrombotic [26], antiviral and antifungal effects. They are known to have low toxicity in humans. The extraordinary ability of the human body to recover has been a challenge for researchers to find a way to copy its mode of action using self-assembling organic molecules technologies [27-28]. Many years passed from the introduction of concepts “nanotechnology” and “molecular nanotechnology” by Feynman in 1959, respectively by Drexler in 1986, until obtaining commercial nanoproducts (2000s). In Medicine, some years have passed before the metallic nanomaterials have been reserved used, because of the concern about the effect of nanoparticles would have had on human health [29]. In recent years the silver/gold colloidal have been promoted as dietary supplements against disease. When they are used in Medicine, the researchers tried to use only eco-friendly methods (by applying “green chemistry”) to obtain nanoparticles bound with organic natural molecules. Some studies have been made on nanomaterials based on polyphenols from fruit extract such as on anthocyanin dyes which are known as powerful antioxidants with health benefits for human body. A high antioxidant activity has been found in Cornelian Cherry fruits which is a significant source of natural antioxidants (polyphenols as anthocyanins and flavonoids) [30-33].

Our research group is involved in studying some creams for improving psoriatic lesions, based on natural extracts from plants growing in Romania. These creams are meant to be an alternative to the present existing medication based on cortisone or other synthetic drugs. Since now, we studied the anti-inflammatory effects of plants from different family, such as: Adoxaceae [34] and Cornaceae (which is the subject of the present paper). The original work is focused on the obtaining of some new metallic nanomaterials (gold/silver...
nanoparticles) functionalized with natural extracts from native fruits of European Cornel (Cornaceae family). In contrast with the majority of existing methods, the extracts are prepared in water, at room temperature, and have been obtained according to Crisan et al [34]. This procedure permit the anthocyanins molecules from fruits extract to bound to metallic nanoparticles. The nanomaterials are introduced in some creams and used on psoriatic lesions on humans patients. The metallic reduction has been made directly in the presence of natural extract. All the important factors in obtaining the metallic nanoparticles have been closely observed (pH, temperature, reactants ratio and solutions concentrations) [35]. The formation of gold/silver nanoparticles have been investigated and confirmed by UV-Vis spectroscopy (Surface Plasmon Resonance appearance). Transmission Electron Microscopy (TEM) has been used to observe the morphology of the nanomaterials and Dynamic Light Scattering (DLS) to find the average hydrodynamic size and zeta potential. Fourier Transform Infrared Spectroscopy (FTIR) has been used to analyze the functional groups present in plant extract [19].

Fourier Transform Infrared Spectroscopy (FTIR) has been used to analyze the functional groups present in organic compounds. By X-Ray Diffraction (XRD), the structure of metallic nanoparticles has been determined. Differential Scanning Calorimetry (DSC) indicates the thermal behaviour of nanomaterials. The organic molecules from the natural extracts act both as reductant and as stabilizer. From the UV-Vis spectra, the new complex formation may be seen. The equilibrium constants of the reactions between gold/silver nanoparticles and organic molecules have been determined in two ways.

We applied new hybrid nanomaterials, after their anti-inflammatory and cytotoxic effects have been studied on psoriatic skin lesions. The obtained results permitted to state that they have good anti-inflammatory effect. Consequently, some dermatological creams have been prepared and used in a medical trial on 31 subjects with clinical diagnosis of psoriasis. Patients enrolled in the study signed an informed consent form and the study has been approved by the Ethical Committee of the University of Medicine and Pharmacy “Iuliu Hatieganu” Cluj-Napoca. In order to observe the existing benefit of the creams based on the hybrid nanomaterials, some comparisons with a cortisone cream and with an emollient one have been made. The obtained data have been statistically studied.

II MATERIALS AND METHODS

Materials
The chemicals and reagents used have been of analytical grade and purchased from Merck Germany (chloroauric acid-HAuCl₄·3H₂O, silver nitrate-AgNO₃, NaOH). Double distilled water has been used throughout the experimental work.

The European Cornel fruits were collected from Cluj-Napoca town, Romania.

Apparatus
The morphology and size distribution of nanoparticles have been examined by TEM with a JEOL-JEM 1010 instrument (JEOL Inc).

An UV-Vis spectrophotometer Shimadzu at the range of 300-800 nm has been used to get the spectral analysis.

DLS measurements were carried out with a Brookhaven Instruments Corp. goniometer and laser light scattering system in colloidal suspensions in water. Acquisition time was set at 90 seconds, a laser radiation wavelength of 632.8 nm was used and the angle at which data acquisition was performed is 90°.

A D8 Advance Diffractometer with CuKα1 radiation (λ=15.4056 °A) and Ge (111) monochromator has been used to observe the crystalline nature of nanoparticles.

A JASCO 610 spectrometer (spectral domain 5000–500 cm⁻¹; the resolution was 4 cm⁻¹ with the sample as KBr pellets) has been used to study the interaction of organic molecules with metallic nanoparticles.

The DSC experiments were carried out with a Shimadzu DSC-60 differential scanning calorimeter using Shimadzu TA-WS60 and TA60 2.1 version system software for data acquisition and analysis.

The study of the lesions at “histological” level has been performed by using the non-invasive, high frequency ultrasound device Dermascan C 20 MHz (Cortex Technology, Denmark).

Methods

Nanomaterials preparation
Natural extracts from C have been obtained according to Moldovan et al [36]. It is important to notice that the fruits which give the natural extracts have to be younger than 1 year. 5 grams of C fruits are introduced in an Erlenmayer with 100 ml double distilled water and the extraction procedure has to be made at room temperature, under stirring conditions for 30 minutes. The anthocyanins depreciate at high temperature. The concentration of total anthocyanins is determined by differential pH method [37].

The gold/silver nanoparticles have been obtained by metallic ions reduction with the natural fruits extract. The pH of natural extracts solutions is brought at 7.5 with 1M NaOH solution. The concentrations of the used solutions have been: 0.03 M HAuCl₄·3H₂O, 0.06 M AgNO₃, natural extract with 25x10⁻³ mM total anthocyanins. For synthesis, in 200 ml boiling distilled water, 6.6 ml solution of metallic ion and 16.6 ml natural extract have been added. Immediately, the nanoparticles are obtained and a change in color is observed. Heating is stopped and the stirring continues until the solution begins to cool. The metallic nanoparticles have been kept at refrigerator until used.

III RESULTS AND DISCUSSIONS

Nanomaterials analysis

For XRD, FTIR and DSC analyses, the nanomaterials have been twice washed by centrifugation. The centrifugation has been performed at 15,000 rpm for 10 minutes, at 4°C. The nanomaterials have been dried at room temperature on watch glasses.

TEM analysis
The TEM images (Figure 1 A, B) indicate the morphology, the size and dimensions of nanoparticles (13-52 nm for AuNPs-C and 9-82 nm for AgNPs-C), and they have an almost spherical or elongated shape. After a month, the metallic nanoparticles begin to deposit. The data obtained for diameters of nanoparticles are represented by histograms for each type of nanomaterial (Figure 1 C). The average diameter of the nanoparticles was calculated manually on 100 nanoparticles.
Calculation of metallic nanoparticles concentrations

Supposing that the nanoparticles have a round form and the same dimension (considering the medium diameter 19 nm for gold and 22 nm for silver), based on the experimental results according with the used solution quantities and their concentrations, the molar concentrations of metallic nanoparticles solutions have been computed: 3.54 nM respectively 5.33 nM for gold/silver nanoparticles.

DLS analysis

The size and size distribution of gold/silver nanoparticles have been also studied by DLS. The dimensions obtained for gold/silver nanoparticles are higher than those obtained by TEM with 30-40 nm (the mean values are 69 nm for AuNPs-C and 84 nm for AgNPs-C) because by DLS, the hydrodynamic diameters is determined (Figure 2).

Zeta potential measurements

The zeta potential of AuNPs-C respectively AgNPs-C in aqueous solution was found to be -24.2 mV and -23.8 mV, respectively. The measurements have been made for 4 weeks and have been found to be stable. These data for zeta potential indicates capping of NPs by negatively charged groups. Even the potential values are higher than -30 mV, the nanomaterials are stable for a period of one month.

XRD analysis

The XRD spectra reveal that gold/silver nanomaterials are crystalline in nature. The observed diffraction peaks appear at 2θ = 38.14°, 44.31°, 64.56°, 77.64°, 81.85°, which are identical with those reported for standard gold/silver metal (Figure 3). The following dimensions have been obtained for crystallites size: \( D_{\text{AuNPs-C}} = 16.6 \text{ nm} \); \( D_{\text{AgNPs-C}} = 7.7 \text{ nm} \). Due to the fact that a nanoparticle may contain more crystallitles, the dimensions observed in TEM images are higher than those obtained by XRD studies (Figure 3).

UV-Vis spectroscopy

In Figure 4, the UV-Vis spectra of the metallic nanomaterials can be seen. The boiling solution containing the reactants gets, in 1 min., a red purple color for AuNPs-C, and a strong yellow color for AgNPs-C. The absorption peaks appear at 532 nm for AuNPs-C and at 408 nm for AgNPs-C.

Determining the equilibrium constants

In order to measure the interaction strength between the anthocyanins as ligands and metallic particles (AuNPs, AgNPs), a sensing experiment characterized by UV-Vis spectroscopy has been performed. The data are presented in Figure 5. The surface plasmon absorption band of metallic nanomaterials is sensitive to the ligand molecule which stabilized the nanoparticles. The spectral changes observed in figure are used to determine the equilibrium constants of the process according with Benesi-Hildebrand (Figure 5 A1, A2), irrespectively Thomas et al (Figure 5 B1, B2) methods.
The choice of the method has been made according with the absorbance variation after getting the isosbestic point.

Fig. 5 UV-Vis spectra of AuNPs-C after the exchange of citrate molecule with organic molecule from natural extract (A1,B1) and AuNPs-C obtained by direct synthesis with natural extract (A2,B2) and the dependence of 1/ΔΑ vs 1/c, where c=[natural extract]. Preparation of 30-40 nm in diameters AuNPs-sodium citrate: 49.5 ml bidistilled water: 0.5 ml HAuCl\textsubscript{4}: 0.5 ml sodium citrate [29]

In the present paper, we have determined these constants by two procedures: one, in which the metallic nanoparticles have been obtained in the presence of sodium citrate [40] (Preparation of 30-40 nm in diameters was performed with AuNPs-sodium citrate: 49.5 ml bidistilled water: 0.5 ml HAuCl\textsubscript{4}: 0.5 ml sodium citrate) and after that the citrate molecules have been changed with organic molecules from natural extract and the other one, in which the metallic nanoparticles have been prepared by direct reduction in the presence of natural extracts. The isosbestic point which appears in all cases confirms the existence of complexation equilibrium. The computed equilibrium constant for silver nanoparticles with both methods has given the same value, around 36,000 M\textsuperscript{-1}. For gold nanoparticles, the values are around 35,000M\textsuperscript{-1} by the first procedure and 91,400 M\textsuperscript{-1} by the second procedure. These equilibrium constants of 10\textsuperscript{4} magnitude values suggest a good association between the metallic nanoparticles and the organic compound [41-42].

FTIR spectroscopy

The interaction of organic molecules with gold/silver nanoparticles was investigated by FTIR spectroscopy (Figure 6). For nanomaterials, the stretching vibrations \nu_{\text{OH}} characteristic to associated OH-groups are shifted to higher values (3396 cm\textsuperscript{-1} for natural extracts and 3426/3435 cm\textsuperscript{-1} for gold/silver nanomaterials) with about 20 cm\textsuperscript{-1}. This indicates that more H linkages are broken. C=O and C=C stretching vibrations in extracts are at 1718 cm\textsuperscript{-1} and 1594 cm\textsuperscript{-1}. After the reduction of Au\textsuperscript{3+}/Ag\textsuperscript{+} at Au\textsubscript{0}/Ag\textsubscript{0} with anthocyanins, the peaks are shifted and intensively reduced in intensity (1719 cm\textsuperscript{-1} and 1629 cm\textsuperscript{-1}). This takes place probably due to the binding of natural molecules from C at the surface of the metallic nanoparticles [25]. The vibrational frequencies for glucosidic units appear between 1000-1250 cm\textsuperscript{-1}. These bands suggest that the anthocyanins are the organic molecules bounded as ligands at gold/silver nanoparticles [34].

The FTIR spectra confirmed the interactions between gold/silver nanoparticles and organic molecules from natural extract.

Fig. 6 FTIR spectra of nanomaterials and C

DSC analysis

Thermal effects on nanomaterials have been measured by DSC under nitrogen flow stream. DSC analysis of AuNPs-C and AgNPs-C has been compared with those obtained for
corresponding natural extract. Figure 7, for C shows three endothermic peaks at 118.83 °C, 154.39 °C and 203.26 °C for C which indicates the decomposition of organic molecules. Figure 7 shows, for AgNPs-C, two endothermic peaks at 116.87 °C, 151.32 °C corresponding to the decomposition of ligand organic molecules and one exothermic peaks at 303.08 °C which, probably, is due to crystalline rearrangement by sintering of silver aggregates. After the protective organic molecules layer is thermally destroyed between 100-200 °C the metallic surfaces of silver nanoparticles come into contact and the sintering process starts at 260 °C [43-46]. For AuNPs-C it can be observed two exothermic peaks at 275.83 °C and 346.23 °C which indicates some crystalline rearrangements in nanomaterial. No endothermic peaks are observed. The fact that there is a difference in the thermal behavior of the two nanomaterials suggests significant differences in the interaction of metals with organic ligand, fact that will be studied in some future works.

**TABLE 2** **STATISTICAL RESULTS FOR CREAM WITH AuNPs-C**

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**TABLE 3** **STATISTICAL RESULTS FOR CORTISONE CREAM**

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**TABLE 4** **STATISTICAL RESULTS FOR EMOLLIENT CREAM**

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Due to the fact that in each case the data are perfect correlated, the mean values are significant and correct conclusions may be stated. As the information in the tables indicates, the emollient cream has absolutely no effect on the psoriatic lesions (as expected) and the best anti-inflammatory effect has been obtained with the cortisone cream, both at epidermis and derma level. But the creams based on metallic nanomaterials, also have some anti-inflammatory effect, mainly at dermis level. We also note that the AuNPs-C cream gives better results than AgNPs-C cream. The results are important from medical point of view, because it is well known that the cortisone creams has good anti-inflammatory effect, but many other secondary effects and cannot be used a long period of time.

**IV CONCLUSIONS**

The present study demonstrated that gold/silver nanoparticles are formed in the presence of natural extract from European Cornel fruits. The formation of noble metal nanoparticles are observed from the changes in colours (from yellow to intense red in case of AuNPs-C and from colourless to yellow for AgNPs-C) and the appearance of the surface plasmon resonance of metallic nanoparticles in UV-Vis spectra. Good equilibrium constants in both cases demonstrate the formation of the complex between metallic nanoparticles and organic molecules. The nanomaterials are stable for one month and they are applicable in medicine as creams for psoriatic lesions.

**ACKNOWLEDGMENT**

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areas-PN II”, developed with the support of ANCS, CNDI-UEFISCDI, Romania.

ABBREVIATIONS

European Cornel, AuNPs-C the gold nanomaterial based on European Cornel, and AgNPs-C the silver nanomaterial based on European Cornel.

REFERENCES


