

Aging Study of Gold Nanoparticles Functionalized with Chitosan in Aqueous Solutions

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We studied the way in which stability and physicochemical properties of gold (Au) nanoparticles (NP) coated with chitosan are influenced by the molecular weight of chitosan and Au precursor concentration when samples are subjected to aging. Pharmaceutical use of AuNPs coated with chitosan is closely related to stability and physicochemical properties in relation to storage conditions and processing. For this purpose, series of AuNPs of different sizes were prepared by aqueous chemical reduction method using chitosan with various molecular weights as template. The physicochemical properties and stability at room temperature of AuNPs in aqueous solutions of chitosan have been investigated by following the temporal evolution of surface plasmon absorbance, Zeta potential, average dimension, and Au-chitosan interaction, after synthesis and through a period of 24 months. In our experiment, during the observed aging period, the AuNPs coated with chitosan presented a better colloidal stability, while using chitosan with medium molecular weight and medium concentration of Au precursor.

Keywords: chitosan-gold nanoparticles, aqueous chemical reduction method, template, stability, aging

Gold nanoparticles (AuNPs) have drawn interests due to their special characteristics such as size-tunable surface plasmon resonance (SPR), fluorescence, and easy-surface functionalization [1]. The NP can be widely used in biosensors, cell labelling and imaging, photo-thermal therapy [2-3], and drug delivery [4-11]. Generally, two factors significantly affect the structure and optical properties of NPs. The crystalline anisotropy is the first factor-in which nanocrystals with different shapes show different optical and electronic properties due to the surface modification in molecular environment [12]. The second is due to the high surface-to-volume ratio of NPs-modifying the surface with various organic or inorganic species is expected to remove the surface defects and enhance their optical properties (surface enhancement Raman scattering (SERS)) by increasing the distribution of quasi free electrons belonging to atoms in the surface. In addition, capping the nanoparticles with organic molecules, give rise to a barrier against aggregation and electronic passivation of the particles. This way, AuNPs designed for use in biomedicine as contrast agents or drug carriers are typically coated with molecules to prevent the particles flocculation and/or precipitation, which diminish their effectiveness. Concerning the preparation of AuNPs, many methods were developed such as chemical, sonochemical and sonoelectrochemical reduction [13-15].

In our study, AuNPs were synthesized sonochemically by reducing chloroauric acid (HAuCl_4) with chitosan (CHI). The chitosan acts both as reducing and stabilizing agent due to its cationic character [16-23]. As it is known, CHI is the second abundant polysaccharide next to cellulose in nature and has excellent biocompatible and biodegradable characteristics [24]. It exhibits low toxicity [25, 26], has

immune stimulating proprieties and, due to its unique polycationic character, quickly binds to negatively charged surfaces such as cellular membranes or anionic AuNPs [27]. Conditions of storage and handling of colloidal solutions are necessary to ensure optimal stability of NPs for a given period of time. If the conditions for synthesis depend on the pH and ionic strength of the solution, the instability may be due to factors NP external physical disturbance. In this study solutions were analyzed during aging processes through storing up to two years. All samples for this study were taken from stock solutions synthesized in the same physical conditions. Catalytic activity of AuNPs in CHI study showed that the mobility of NH and OH functional groups leads to increased activity to nanoparticle surface [28]. The result will be changing the size NP, and system stability fluctuation depending on the polymer molecular weight and concentration of precursor. The effects of these solutions can be seen as SPR due CHI environment.

Experimental part

Materials and methods

Chemicals and materials

The hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), acetic acid ($\text{CH}_3\text{-COOH}$) and four CHIs with various molecular weight-Practical Grade (PG), Medium Molecular Weight (MMW), Low Molecular Weight (LMW) and Oligo-saccharide Lactate (OSL)-were purchased from Sigma-Aldrich. PG having the highest molecular weight (MW) compared with OSL which has the lowest MW, the following values were obtained by Gel Permeation chromatography (GP): PG-Mn = 97.607 g/mol, Mw = 263.836 g/mol, PI = 2.70; MMW-Mn = 60.070 g/mol, Mw =

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= 213.907 g/mol, PI = 3.56; LMW-Mn = 30.421 g/mol, Mw = 114.374 g/mol, PI = 3.76. For OSL-Mn = 5.000 g/mol, Mw = 340.30 g/mol, as specified by the manufacturer. All solutions were prepared using Milli-Q deionized water (18.2 ohm).

Gold nanoparticles preparation

1 g HAuCl₄·3H₂O was placed in a volumetric flask of 1L, and then filled with deionized water to obtain a solution with a concentration of 2.53915 mmol HAuCl₄ (aqueous solution 10⁻³ M). From this solution, 196.9 mL were taken and put in a 500 mL volumetric flask, and then deionized water was added to obtain a HAuCl₄ (10⁻³ M) stock solution. The CHI stock solutions consist of 0.1% CHI (0.1 g/L) in 1% acetic acid (v/v) for each of the three types of CHI. Precursor solutions of Au NPs were prepared for each of the three types of CHI, by mixing 2, 4, 6, 8 and 10 mL of HAuCl₄ (1 mM) stock solution with 38, 36, 34, 32 and 30 mL respectively of CHI stock solution. The samples were named based on the HAuCl₄ concentration and type of CHI: 2PG, 4PG, 6PG, 8PG, 10PG; 2MMW, 4MMW, 6MMW, 8MMW, 10MMW; 2LMW, 4LMW, 6LMW, 8LMW, 10LMW and 2OSL, 4OSL, 6OSL, 8OSL, 10OSL respectively. The solutions were stirred and heated by applying an ultrasonic field of 20 kHz for 10 min, using a Sonoplus Bandelin device. After each step, samples were thermalized to room temperature (RT) of about 23°C, embedded in vials of 40 mL. After which, the vials were opened and suitable quantities for analysis were taken.

Aging process

To analyze the behaviour of colloidal solutions of AuNPs coated with CHI undergoing aging process, samples were kept at RT for more than 12 months. The analyses were performed three days after preparation (reference, R0) and then at 2, 3, 4, 7, 8 and 11 months after the NPs preparation.

Numerical molar mass (Mn), gravimetric molar mass (Mw) and polydispersity index (PI) were determined using a Varian PL-GPC 120 chromatograph.

The SPR collective effect of AuNPs was monitored by investigating the UV-Vis-NIR absorption spectra of NPs aqueous suspensions, in the range of 200-1100 nm, using a Hitachi U-2001 spectrophotometer. To compare the intensity of SPR transverse mode absorption band at 530 nm of AuNPs, spectra were recorded without samples

dilution. The intensity of absorption bands was considered proportional with the area under the curve band, from which baseline line was low. The NPs size distribution and Zeta potential of aqueous suspensions was evaluated with a Malvern Zetasizer Nano ZS, Zen-3500 model, at RT. The stability of AuNPs colloidal solutions was defined in accordance with the average Zeta potential (AZP) [29], average dimension (AD) and PI. The interaction of CHI with AuNPs was studied using FTIR Bomem MB Serier 104 spectroscopy, after synthesis and 24 month later.

Results and discussions

Gold nanoparticles

After 2-3 days, depending on the MW of CHI and the amount of HAuCl₄ precursor, the colour of solutions had changed until setting to different shades of pink-indigo-violet. All solutions prepared with OSL, regardless of the amount of Au precursor, precipitated after 10-12 days from preparation. Also solutions containing 8mL and 10mL HAuCl₄ formed microcrystals at the bottom of the vial, as a brown precipitate. Dimensional analysis by UV-Vis spectroscopy corroborated with colour aspect showed that as AuNPs size increases, the colour of the solution varies from pink-red (small NPs ~ 15 ÷ 20 nm) to blue-violet (large NPs ~ 160 ÷ 200 nm). The other solutions that did not precipitate were either solutions containing low concentrations of Au which are on the threshold of agglomeration or solutions with high concentrations of Au which are found at the threshold of light dispersion (table 1).

Analysis of size distribution and Zeta potential (fig. 1) shows that at low concentrations of precursor (sample 2PG) in the first phase we obtained NPs of about 10 nm and clusters of about 1000 nm. The fact that 2PG does not precipitate and the distribution of Zeta potential is almost centred on zero value, entitles us to consider that the NPs formed are not covered by CHI. Therefore, NPs are net negative charged and the clusters are formed by CHI containing early stages of AuNPs crystallization that have Au ions. The positive total charge compensates the negative charge of naked AuNPs. Assertions are substantiated by the fact that in the process of maturation (aging time process), after 2 months the AD decreased to almost half: AD = 504.00 nm, PI = 0.86 and AZP = 52.20. We suppose that the growth of crystallites and confinement of CHI

Sample	AD (nm)	PI	Effect
2PG	999.00	0.61	agglomeration
4PG	51.50	0.45	threshold of light dispersion
6PG	49.00	0.46	threshold of light dispersion

Table 1

STABILITY LEVELS OF AuNPs WITH CHI PG

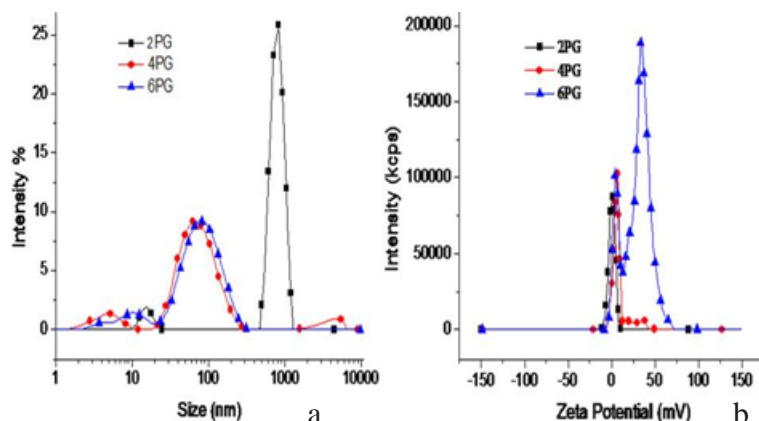


Fig. 1. The distribution of size (a) and Zeta potential (b) of Au-PG colloidal solutions after 3 days of preparation. Although 2PG it very unstable, the precipitation of NPs does not occur, because the clusters are made up of CHI, containing ions in the first stage of crystallization

clusters continued, increasing thus the PI and stability. As the concentration of Au precursor is bigger, the amount of NPs that grow from the beginning and also the amount of CHI that is involved in their coverage, increase.

FTIR analysis (fig. 2) showed the interaction of colloidal solutions with all 4 types of CHI with AuNPs. Characteristic spectrum of CHI shows the following characteristic bands [30-32]: amide I at 1650 cm^{-1} (C=O stretching), amide II at 1558 cm^{-1} (N-H deformation coupled with plan C-N stretching) amide III (C-N stretching coupled with N-H deformation in plan) and CH_2 wagging coupled with OH in plane deformation at 1317 cm^{-1} . Broad band observed at $3200\text{-}3450\text{ cm}^{-1}$ is given by the contribution of the various vibration: hydrogen bonds with OH (stretching, 3426 cm^{-1}), NH_2 asymmetric stretching vibration (3360 cm^{-1}) and NH stretching hydrogen bond $\text{NH}\cdots\text{O}=\text{C}$ (3293 cm^{-1}). The bands at 2873 cm^{-1} and 1375 cm^{-1} were attributed to CH stretching and CH_3 deformation symmetric respectively.

These AuNPs in solutions with CHI (fig. 2) causes the shift to lower wavelength bands of the functional groups. This result proves the involvement of amino groups in the interaction with the metal surface. Functional groups of CHI in contact with AuNPs form points, allowing stabilization of colloidal solutions.

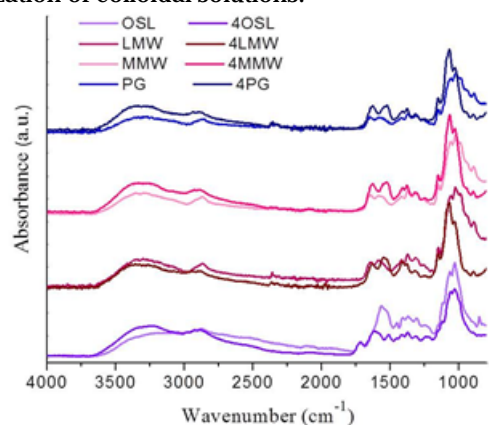


Fig. 2. FTIR spectra of the 4 types of CHI with and without AuNPs. Note the involvement of the amino group in the interaction with the surface of AuNPs

Analysis of aging process

Figure 3a, presents the change in stability for colloidal solutions undergoing time aging process. It is noticed that systems stability increases to 3 month reaching reasonable stability, after which gradually decreases to moderate stability at the end of the 7th month for all solutions with middle and high concentrations of Au precursor. The samples with low concentrations of Au precursor manage to reach in reasonable stability after 3 months and moderate decrease by the end of 11 months. These solutions have the longest stability, regardless of the molecular weight of chitosan. For solutions having high concentrations of Au precursor the measurements made at the end of the 11th month showed a tendency for dispersion, after that most samples precipitating. After 12 months, results showing a trend of precipitation process have been obtained for all the solutions with lower concentrations of Au precursor, but a law of instability process for all samples does not had been observed. As colloidal solutions become more stable, size distributions show a decrease in the AD and PI of NPs in nearly all samples. The situation changes when it shifts from moderate stability to threshold of light dispersion, AD and PI increase and AZP decreases, which proves the occurrence of clusters of AuNPs with different size, more or less covered by CHI and having net charge of opposite sign and which, in the next stage lead to flocculation-precipitation processes.

One month after preparation, the UV-Vis spectra of all colloidal solutions show a slight decrease of absorption band of SPR AuNPs transversal mode at 530 nm , accompanied by the shift to smaller wavelengths (fig. 4).

In the first three months, except the solutions with 6MMW and 6LMW, the absorbance of AuNPs transversal mode at 530 nm increases [33]. The probable reason is due to the increase of CHI functional groups interactions with metal atoms from the surface of NPs, as evidenced by the increase of solutions stability and the intensification of SERS effect [34].

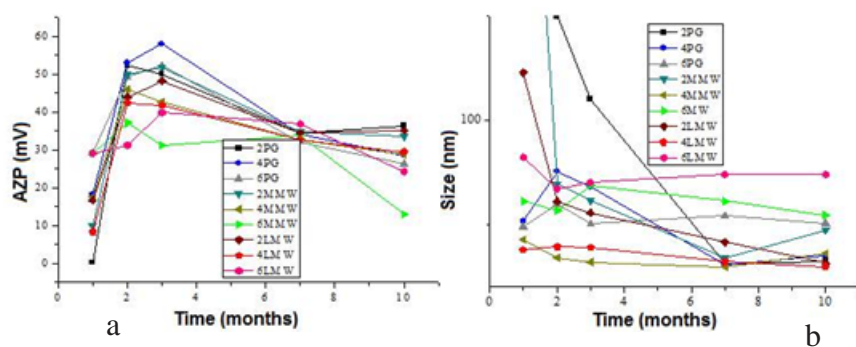


Fig. 3. Zeta potential (a) and size distributions (b) variation graphs during the aging time. Note that in the first three months of aging, most solutions improve their stability. Maintaining stability in time of the solutions depends on the concentration of precursor and not the mass of the polymer

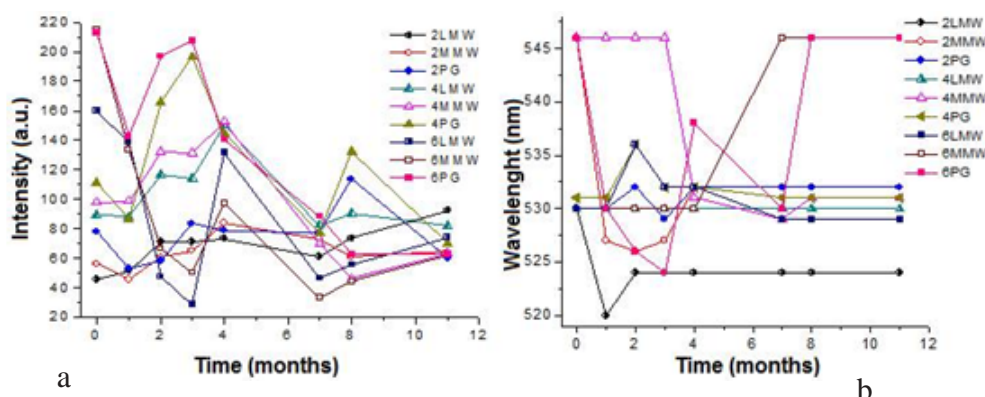


Fig. 4. The intensity of absorption bands of AuNPs SPR transversal mode at 530 nm (a) and corresponding wavelength (b) for all Au-CHI colloidal solutions during the aging process

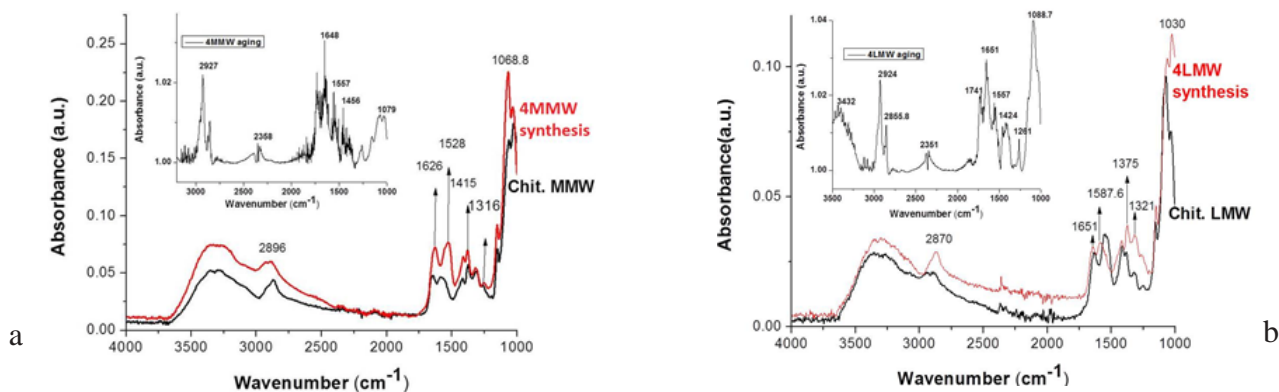


Fig. 5. FTIR spectra of freshly prepared samples 4LMW and 4MMW (a) and the same samples after 24 month of aging (b). Note the break of the hydrogen bridges with the solvent and the increase of the absorption bands due to the occurrence of vibration of CH bond valence

FTIR spectra of the samples 4LMW and 4MMW after 2 years from preparation (fig. 5) shows a band shift towards higher wavelengths in the functional groups. It is also noted that instead of broadband (3200-3450cm⁻¹), narrow bands occurred due to the breakage of hydrogen bridges with the solvent and the absorption bands increased due to the valence vibration of CH bonds (2850-2960 cm⁻¹) [30-32]. These changes suggest that the stability continues to fall until the end of the second year.

Conclusions

We studied the stability of AuNPs obtained by sonochemical reduction of HAuCl₄ in solution containing CHI with 4 different MW. We obtained solutions of AuNPs that do not precipitate immediately after preparation for concentrations of HAuCl₄ up to 8 mM. NPs dimensions do not evidently depend on the MW of CHI, but are sensible to Au precursor concentrations. The stability of colloidal solutions of AuNPs decreases as CHI MW is smaller, the solutions containing CHI with the lowest MW (OSL) precipitating after 2 weeks of preparation. It was found that CHI with the highest MW (PG) has the best stability for 1 year, these solutions preserving the NPs optical and physicochemical properties during this period, precipitating afterwards. During the observed aging period, the AuNPs coated with chitosan presented a better colloidal stability, while using chitosan with medium molecular weight and medium concentration of Au precursor.

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References

- 1.RATHER, M.A., SHARMA, R., GUPTA, S., FEROSEKHAN, S., RAMYA, V.L., JADHAO, S.B., PLoS One, **8**, 2013, p. e57094.
- 2.ZHANG, G.D., SUN, X.H., JASINSKI J., PATEL, D., GOBIN, A.M., Journal of Nanomaterials, 2012, Art. No. 853416.
- 3.BODA, D., NEGREI, C., ARSENE, A.L., CARUNTU, C., LUPULEASA, D., ION, R.M., Farmacia, **53**, 2015, p. 218.
- 4.HU, M., CHEN, J., LI, Z.Y., AU, L., HARTLAND, G.V., LI, X., MARQUEZ, M., XIA, Y., Chem. Soc. Rev., **35**, 2006, p. 1084.
- 5.ANKER, J.N., HALL, W.P., LYANDRES, O., SHAH, N.C., ZHAO, J., VAN DUYN, R.P., Nat. Mater., **7**, 2008, p. 442.
- 6.SOKOLOV, K., FOLLEN, M., AARON, J., PAVLOVA, I., MALPICA, A., LOTAN, R., RICHARDS-KORTUM, R., Cancer Res., **63**, 2003, p. 1999.
- 7.LINK, S., EL-SAYED, M.A., Int. Rev. Phys. Chem., **19**, 2000, p. 409.
- 8.PISSUWAN, D., VALENZUELA, S.M., CORTIE, M.B., Trends Biotechnol., **24**, 2006, p. 62.

- 9.ZHENG, J., ZHANG, C., DICKSON, R.M., Phys. Rev. Lett., **93**, 2004, Art. No. 077402
- 10.HUANG, X., EL-SAYED, I.H., QIAN, W., EL-SAYED, M.A., J. Am. Chem. Soc., **128**, 2006, p. 2115.
- 11.BUTU, A., RODINO, S., GOLEA, D., BUTU, M., BUTNARIU, M., NEGOESCU, C., DINU-PIRVU, C.E., Farmacia, **63**, 2015, p. 224.
- 12.WANG, Y., HERRON, N., J. Phys. Chem., **95**, 1991, p. 525.
- 13.LEFF, D.V., OHARA, P.C., HEATH, J.R., GELBART, W.M., J. Phys. Chem., **99**, 7036-7041 (1995)
- 14.OKITSU, K., MIZUKOSHI, Y., YAMAMOTO, T.A., MAEDA, Y., NAGATA, Y., Mater. Lett., **61**, 2007, p. 3429.
- 15.WANG, L., MAO, W., NI, D.D., DI, J.W., WU, Y., TU, Y.F. Electrochem. Commun., **10**, 2008, p. 673.
- 16.HUANG, H., YANG, X., Biomacromolecules, **5**, 2004, p. 2340.
- 17.POPESCU, V., SANDU, I.C.A., POPESCU, G., Rev.Chim. (Bucharest), **67**, no. 1, 2016, p. 74.
- 18.POPESCU, V., SANDU, I.C.A., POPESCU, G., Rev.Chim. (Bucharest), **67**, no. 4, 2016, p. 768.
- 19.POPESCU, V., SANDU, I.G., VASLUIANU, E., SANDU, I., MANEA, L.R., CAMPAGNE, C., Rev.Chim. (Bucharest), **65**, no. 12, 2014, p. 1439.
- 20.SUBLI, M.H., OMAR, M.F., SANDU, I.G., ZULKEPLI, N.N., ABDULLAH, M.M.A., SANDU, A.V., Mat. Plast., **51**, no. 4, 2014, p. 391.
- 21.TOFOLEANU, F., MINDRU, T.B., BRINZA, F., SULITANU, N., SANDU, I.G., RAILEANU, D., FLORISTEAN, V., HAGIU, B.A., IONESCU, C., SANDU, I., TURA, V., JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS, **10**, no. 12, 2008, p. 3512.
- 22.HALICIU, A.M., FOLESCU, R., ZUGUN, F., STRAT, L., POROCH, V., ZAMFIR, C.L., Rev. Chim. (Bucharest), **68**, no. 3, 2017, p. 624.
- 23.BOGZA, G.E., CHELARU, L., BITERE, E., POROCH, V., SULEA, D., COSTULEANU, M., Rev.Chim. (Bucharest), **67**, no. 11, 2016, p. 2295.
- 24.MUZZARELLI, R.A.A., Chitin. Pergamon Press, Oxford, New York, 1977.
- 25.HU, L., MAO, Z.W., GAO, C.Y., J. Mater. Chem., **19**, 3108-3115 (2009)
- 26.GÂRLEA, A., MELNIG, V., POPA, M.I., J. Mater. Sci. Mater. Med., **21**, 2010, p. 1211.
- 27.de CAMPOS, A.M., DIEBOLD, Y., CARVALHO, E.L., SANCHEZ, A., ALONSO M.J., Pharm Res., **21**, 2004, p. 803.
- 28.IIJIMA, M., KAMIYA, H., KONA Powder Part. J., **27**, 2009, p. 119.
- 29.RIDDICK, T.M., Control of Colloid Stability through Zeta Potential, Livingston Publishing, Wynnewood, PA, 1968.
- 30.POPESCU, V., SANDU, I.C.A., POPESCU, G., Rev.Chim. (Bucharest), **67**, no. 11, 2016, p. 2184.
- 31.POPESCU, V., VASLUIANU, E., FORNA, N.C., SANDU, I., BERCU, E., Rev.Chim. (Bucharest), **64**, no. 11, 2013, p. 1284.
- 32.VASLUIANU, E., POPESCU, V., GRIGORIU, A., FORNA, N.C., SANDU, I., Rev.Chim. (Bucharest), **64**, no. 10, 2013, p. 1104.
- 33.BHUMKAR, D.R., JOSHI, H.M., SASTRY, M., POKHARKAR, V.B., Pharm. Res., **24**, 1415-1426 (2007)
- 34.MOHD SULTAN, N., JOHAN, M.R., Scientific World Journal, 2014, Art. no. 184604.

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