Optimization of Gold Nanoparticles Synthesis using Design of Experiments Technique

Article *in* Revista de Chimie -Bucharest- Original Edition- · August 2017

DOI: 10.37358/RC.17.7.5707

8 authors, including:

Adina Turcu-Stiolica
Universitatea de Medicina si Farmacie Craiova
119 PUBLICATIONS **571** CITATIONS
SEE PROFILE

Claudiu Nicolicescu
University of Craiova
57 PUBLICATIONS **101** CITATIONS
SEE PROFILE

Optimization of Gold Nanoparticles Synthesis using Design of Experiments Technique

ADINA TURCU STIOLICA[1], MARIANA POPESCU[1]*, MARIA VIORICA BUBULICA[1], CARMEN NICOLETA OANCEA[1], CLAUDIU NICOLICESCU[2], COSTEL VALENTIN MANDA[1], JOHNY NEAMTU[1], OCTAVIAN CROITORU[1]

[1] University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, 4 Petru Rares Str., 200349, Craiova, Romania

[2] University of Craiova, Faculty of Mechanics, 1 Calugareni Str., 220037, Drobeta Turnu Severin, Romania

Gold nanoparticles are considered the newest drug carriers for different diseases. Therefore it is appropriate continuous optimization of their preparation. In this study, gold colloids with an average size of 1 - 26 nm were obtained by the reduction of tetrachloroauric acid with trisodium citrate. The nanomaterials were characterized by UV-Vis spectroscopy and dynamic light scattering technique. In addition, zeta potential was measured for samples synthesized in order to determine the stability of the colloids. A Two-level Full Factorial design was chosen to determine the optimum set of process parameters (chloroauric acid concentration and sodium citrate concentration) and their effect on various gold nanoparticles characteristics (size and zeta potential). These effects were quantified using Design of Experiments (DoE) with 5 runs and 1 centerpoint. The selected objective and process model in this investigation are screening and interaction. Findings from this research show that to obtain particles larger than 35 nm, it is recommended to increase sodium citrate concentration, at low chloroauric acid values. These conditions will help to achieve smaller zeta potential, too.

Keywords: gold nanoparticles, full factorial design, ultraviolet-visible spectroscopy, dynamic light scattering, zeta potential

Gold nanoparticles (GNPs) are considered by major pharmaceutical companies as a drug delivery agent of the future for the treatment of cancer and other diseases [1-8]. Even the cosmetic industry develop today new products based on noble metal nanoparticles like gold, silver or platinum [9-12]. However, at present are not known in detail the interaction mechanisms between nanoparticles and living cells. But is known that some parameters such as nanoparticle's shape, size, concentration or electric charge, can influence this interaction (13-20]. Thus, it is necessary to find strategies that allow synthesis of optimal nanoparticle size, shape, concentration, electrical charge so that GNPs will be harmless for human body.

Nanoparticles size can be determined by several methods, such as scanning/transmission electron microscopy (SEM/TEM), atomic force microscopy (AFM), dynamic light scattering (DLS) or UV-Vis spectroscopy. Among the techniques of nanoparticles characterization the most commonly used are dynamic light scattering (DLS) [21-23] and UV-Vis spectroscopy [24–27]. These techniques are increasingly used for nanoparticles characterization in many fields of science and industry [28, 29].

DLS measures the light scattered from the laser that passes through a colloid. Next, the modulation of the scattered light intensity as a function of time is analyzed, and the hydrodynamic size of particles can be determined [30, 31].

In case of UV-Vis spectroscopy, the intensity of light that is passing through the sample is measured. The optical properties of gold nanoparticles change when particles aggregate. The surface plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red-shift to longer wavelengths. Thus UV-Visible spectroscopy can be used for monitoring the stability of nanoparticle solutions. In addition, the stability can also be evaluated by measuring the zeta potential. Briefly, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed nanoparticle. Colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate [32]. Namely, zeta potentials from 0 to ± 25 mV indicate instability, while zeta potentials higher than ± 25 mV indicate stability [33].

In this investigation, statistical experimental design MODDE 9.1 was employed to optimize the process parameters for the enhancement of GNPs preparation [34]. The colloidal gold nanoparticles have been prepared employing standard chemical reduction methods. The concentration of solutions of starting material ($HAuCl_4$) and the reducing agent (sodium citrate) were selected as the main parameters that influence the physical properties of GNPs, namely size and zeta potential. Gold nanoparticles were characterized by DLS method and UV-Vis spectroscopy.

Experimental part

Synthesis of Gold Nanoparticles

Tetrachloroauric acid trihydrate 99.5% ($HAuCl_4 \times 3H_2O$), trisodium citrate dihydrate 99.0% ($C_6H_5Na_3O_7 \times 2H_2O$) were purchased from Sigma Aldrich. Double distilled water was used throughout the course of this investigation.

All glassware used in the preparation and storage of gold nanoparticles was cleaned with *aqua regia* (3:1 HCl:HNO_3), rinsed with distilled water and oven dried.

As mentioned above colloidal gold particles are prepared by old methods developed by Frens [36], by reduction of chloroauric acid ($HAuCl_4$) with sodium citrate at high temperature. Briefly, 100 mL of the appropriate concentration of $HAuCl_4$ aqueous solution (0.2 mM, 0.6 mM, 1 mM) was refluxed while stirring vigorously in a 250 round-bottom flask equipped with a condenser. Next, 10 mL of sodium citrate solution (0.3 - 1.5 % by weight) was quickly added, refluxed for 15 min, and allowed to cool to room

* email: mariana.popescu@umfcv.ro; m_tatucu@yahoo.com; Phone: 0351 443 507

temperature while stirring. The addition of sodium citrate to the auric solution resulted in a color change from pale yellow to red burgundy.

Measurement Techniques

Dynamic Light Scattering

The particle size distributions were measured using a BROOKHAVEN 90 PLUS instrument equipped with a 15 mW solid state laser and a scattering angle of 15° respectively, 90°. The range size of the 90 PLUS is < 1 nm up to 6 µm and has a BI-ZETA accessory in order to study both zeta potential and particle size in the same instrument. Measurement parameters were as follows: a measurement temperature of 25°C, a medium viscosity of 0.890 cP and a medium refractive index of 1.330. From all the suspensions, 2.5 mL were taken and placed in a rectangular cuvette. The measurement time was one minute five times.

UV-Vis Spectroscopy

Particle size of the synthesized gold colloids was evaluated by monitoring the visible absorption band using a JASCO V-500 UV-Vis spectrophotometer.

Experimental Design Approach

The approach to implementing experimental design in this study was investigated over screening experiments, used to identify a subset of the original process variables that have significant effects on the final gold nanoparticles properties: the size and zeta potential. Also, the main interactions between factors that determine the synthesis are followed. A two-level full factorial design, interaction model, was used to evaluate the preliminary significance of the process variables, as well as the interactions effects between them, using MODDE 9.1 software. An interaction model is more complex than a linear model and may therefore fit more intricate response functions. The factors (chloroauric concentration ($HAuCl_4$) and sodium citrate concentration) were investigated at two levels that are +1 (high) and -1 (low). This experimental design was used to identify the factors which have the most significant effects on the resulting gold colloid properties, such as: nanoparticles size and zeta potential. With this screening we want to find out which factors are the dominating ones and what are their optimal ranges. The two experimental process parameters were selected, each one at two levels and these are indicated in table 1.

The responses (nanoparticles size and zeta potential) were measured in sequential order. The design matrices of experiments, in coded and real values, obtained according to the full factorial design, interaction model, are shown in table 2 and table 3, respectively. The run order of experiments was run in a completely randomized order, in order to assure that uncontrolled factors did not affect the final results obtained.

Results and discussions

DLS and UV-Vis characterization of gold nanoparticles

The formation of gold nanoparticles is confirmed by using DLS technique and UV-Vis spectroscopy. Color changes appear after the completion of the reaction. It is well known that gold nanoparticles exhibit dark purple or red wine color based on the shape, size and aggregation of the colloidal nanoparticles. Analysis of the obtained results indicate that in the investigated colloids gold nanoparticles with an average size of 1 - 26 nm are present. The particle size distribution for sample 5, for example, is shown in figure 1.

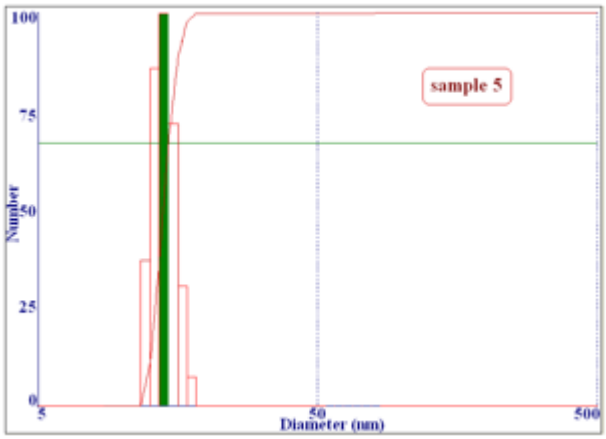

Fig. 1 Size distributions of gold nanoparticles measured by DLS technique for sample 5

To evaluate the particle charge and stability, we employed the zeta potential approach. It is known that nanoparticles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability [33]. Our gold suspensions show high values of zeta potential (from -27 mV to -95 mV), therefore, it can be stated that these gold dispersions are quite stable.

UV-Vis spectroscopy also confirms the information obtained from DLS measurements. This technique can be a useful tool in monitoring of nanoparticles size, providing information about particles aggregation and concentration [36]. The position of the surface-plasmon resonance (SPR) is affected by multiple factors, like environment dielectric

No.	Factors	Symbol	Level (-1)	Level (+1)
1	$HAuCl_4$ conc. (Conc. 1)	X_1 (%)	0.2	1
2	Citrate conc. (Conc. R)	X_2 (%)	0.3	1.5

Table 1
EXPERIMENTAL FACTORS AND LEVEL OF FACTORS

Table 2
DESIGN MATRIX FOR SCREENING FULL FACTORIAL DESIGN EXPRESSED AS CODED VALUES

No. experiment	X_1	X_2	Interaction X_1*X_2
1	-	-	+
2	+	-	-
3	-	+	-
4	+	+	+
5	0	0	0

Table 3
DESIGN MATRIX FOR SCREENING FULL FACTORIAL DESIGN EXPRESSED AS REAL VALUES OF FACTORS

Exp. no.	Run no.	$HAuCl_4$ (mM/L) X_1	Sodium Citrate (%) X_2
N1	3	0.2	0.3
N2	5	1	0.3
N3	2	0.2	1.5
N4	1	1	1.5
N5	4	0.6	0.9

properties [37-39], physical or chemical interactions on particles surface [40], surface charge [41], interparticle distance [42, 43], and aggregation [44].

Moreover, the SPR band can be highly indicative of nanoparticle stability [45]: their aggregation can be monitored by colour change or variation in the SPR band position and shape. A broad peak indicates that the particles are polydispersed. A sharp peak indicates that the particle sizes are uniform. In our experiments the characteristic SPR bands with the wavelengths varying between 519 and 540 nm were observed (fig. 2). The SPR bands are narrow so the gold colloids are fairly monodisperse.

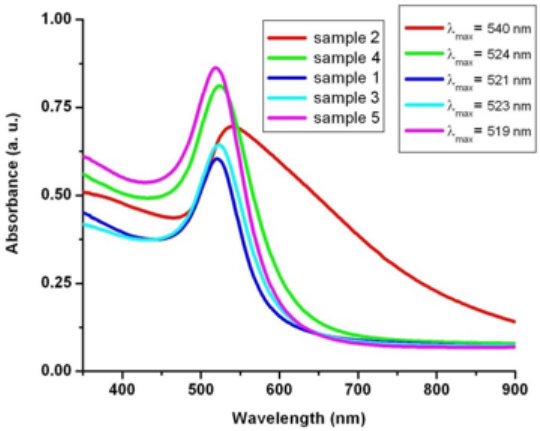

Fig. 2 The UV-Vis spectra for gold nanoparticles (samples 1-5)

Experimental design and optimization

To screen the most significant process parameters that affect the properties of gold nanoparticles, the design of experiments was based on a full-factorial design, with main interaction effects, with two influencing variables, at two levels. The experiments were carried out according to the design matrix shown in table 4 in a fully randomized order to avoid any systematic error.

Sample	Size (nm)	Z (mV)
N1 (sample 1)	13	-65
N2 (sample 2)	1	-27
N3 (sample 3)	26	-95
N4 (sample 4)	20	-65
N5 (sample 5)	14	-51

Table 4
DESIGN MATRIX FOR SCREENING FULL FACTORIAL DESIGN, EXPRESSED AS REAL VALUES OF RESPONSES

Statistical analysis of the obtained data was performed using MODDE 9.1 software [34]. Analysis of the regression coefficients of the linear polynomial models describing the relationship between the responses and the two factors are presented in the following section. The condition number is a tool that can be used to evaluate the performance of our experiment design prior to its execution. Our condition number is 1.187<3, so it's a very good screening design.

Development of Nanoparticles Size and Zeta potential Models

As a result of analyzing the measured five responses using MODDE software, the insignificant model terms ($p<0.05$) were automatically eliminated. The analysis of experimental data generated through DoE consists of three primary stages.

The first stage, evaluation of raw data – histograms, focuses on a general appraisal of regularities and peculiarities in the data. In regression analysis it is advantageous if the data of the response variables are normally distributed or nearly so. The all histograms don't need a logarithmic transformation, they show a *bell shaped* normal distribution, like in the figure 3.

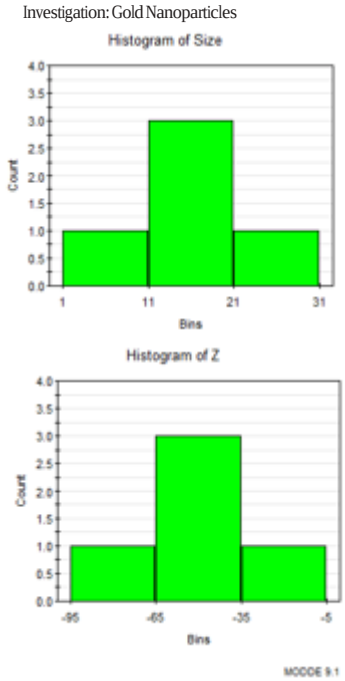

Fig. 3 Histogram of Nanoparticles Size and Zeta Potential

The second stage, regression analysis and model interpretation, involves the actual calculation of the model, linking the factors and the responses together, and the interpretation of this model. Parameter R^2 is a measure of how well the regression model can be made to fit the raw data, but R^2 alone is not a sufficient indicator for probing the validity of the model. A much better indication of the validity of a regression model is given by the Q^2 parameter, called the goodness of prediction, and estimates the predictive power of the model. It reflects the final goal of modeling – predictions of new experiments. For a model to pass this diagnostic test, both R^2 and Q^2 should be high and preferably not separated by more than 0.2-0.3 units. A substantially larger difference constitutes a warning of an inappropriate model. The two $Q^2>0.5$ demonstrated both good models. The R2/Q2 parameters are displayed in Figure 4, like a summary of fit plot, and table 5, like a summary list.

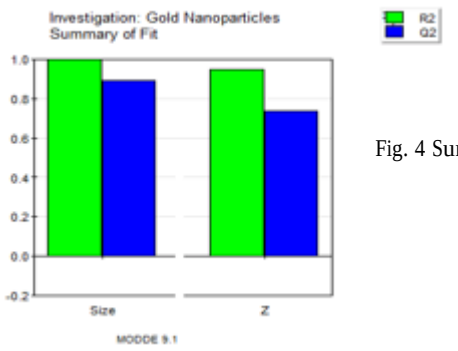

Fig. 4 Summary of fit plot

Table 5
SUMMARY LIST

	R2	Q2
Size~	0.999	0.894
Zeta Potential~	0.946	0.739

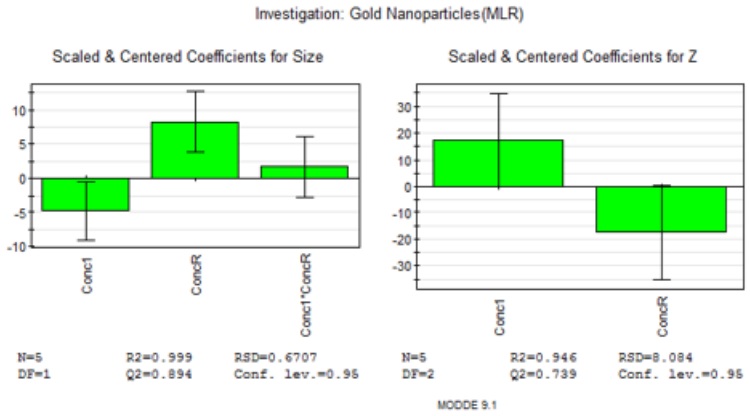

Fig. 5 Regression coefficients of Size model and Z model

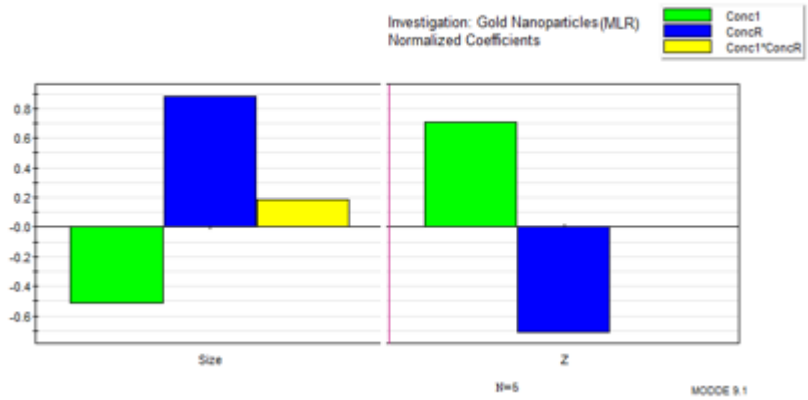

Fig. 6. Coefficient Overview Plot

Model interpretation with coefficient plot plays an important role in the data analysis. For nanoparticles size model we can see an important interaction between the two concentrations. We can see this doesn't happened for zeta potential model. We now have a simpler model with better predictive ability.

We can clearly see the positive and negative effects over the two responses and the interactions between them in figure 6.

The ANOVA results and its lack of fit test for our responses have been analyzed. In ANOVA, two F-tests are made: the first test assesses the significance of the regression model and when $p<0.5$ this test is satisfied. Our models are statistically significant as $p=0.046$ for size model and $p=0.034$ for zeta potential model (table 6).

The ANOVA plot helps as to say that the model is significant at the 5% level if the third bar (RSD*sqrt(F(crit))) is smaller than the first (SD-regression) in the figure 7. In the ANOVA plot the regression component is compared with the residual component and 3 bars are displayed: SD-Regression shows the variation of the response explained by the model, RSD (Residual Standard Deviation) shows the variation of the response not explained by the model and RSD*sqrt(F(crit)) shows second bar multiplied by the square root of the critical F.

Size	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
Total	5	1442	288.4			
Constant	1	1095.2	1095.2			
Total Corrected	4	346.8	86.7			9.31128
Regression	3	346.35	115.45	256.628	**0.046**	10.7448
Residual	1	0.449873	0.449873			0.670726
	N = 5	Q2 =	0.894	Cond. no. =	1.187	
	DF = 1	R2 =	0.999	RSD =	0.6707	
		R2 Adj. =	0.995			
Z	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
Total	5	20805	4161			
Constant	1	18361.8	18361.8			
Total Corrected	4	2443.2	610.8			24.7144
Regression	2	2312.48	1156.24	17.6908	**0.034**	34.0036
Residual	2	130.717	65.3585			8.08446
	N = 5	Q2 =	0.739	Cond. no. =	1.154	
	DF = 2	R2 =	0.946	RSD =	8.084	
		R2 Adj. =	0.893			

Table 6
ANOVA TESTS

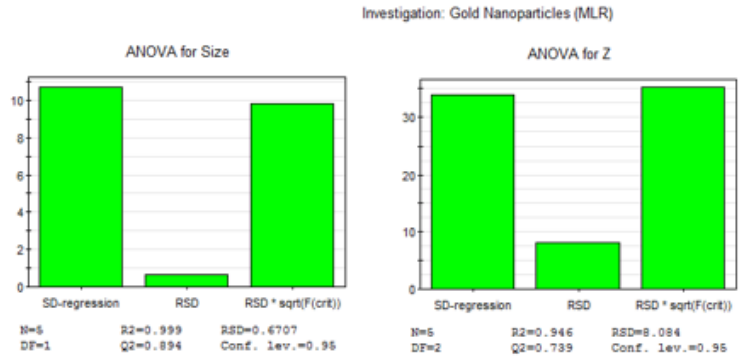

Fig. 7 ANOVA Plot

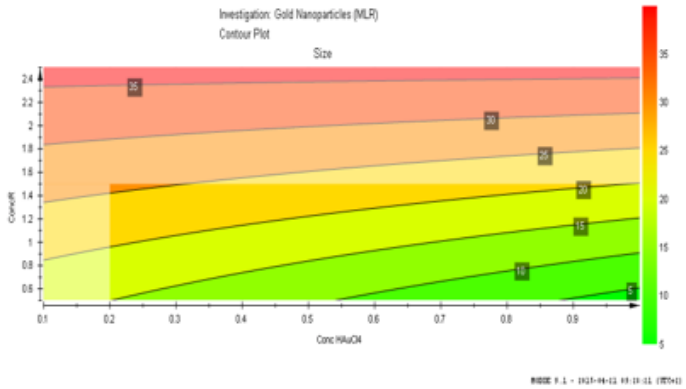

Fig. 8 Response contour plot of Nanoparticles Size

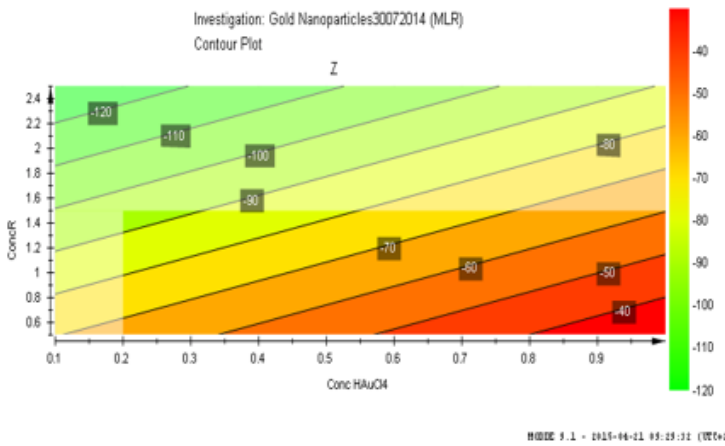

Fig. 9 Response contour plot of Zeta potential

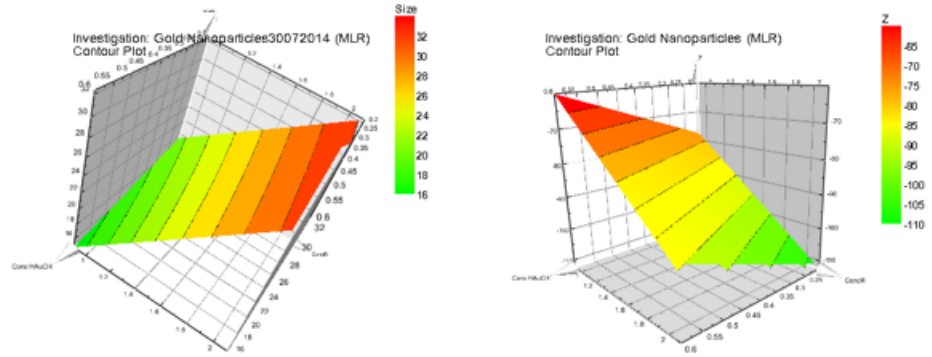

Fig. 10 Two response contour plots of Gold nanoparticles Size and Zeta potential

The optimal regression model has been acquired so we can carry out the third stage of the data analysis, use of regression model; the model obtained is utilized to predict the best point at which to conduct verifying experiments.

Figures 8-10 show a response contour plot created with the factors sodium citrate concentration and chloroauric acid concentration.

To maximize the size, we should position new (verifying) experiments in the upper left-hand corner, that is, with greater value of sodium citrate concentration and low chloroauric concentration. We can see that the 95% confidence interval indicates that at this point smaller values of zeta potential are obtainable. The other points correspond to extrapolated factor settings outside the model calibration domain. All predicted points unanimously indicate that even better nanoparticles size values are obtainable outside the explored experimental region. Hence, we should select one of these proposed recipes and carry out the verifying experiment.

Conclusions

In this study, we have synthesized a series of gold dispersions by the classical technique of the reduction of tetrachloroauric acid by trisodium citrate at different reactant concentrations.

The nanoparticle properties were determined by dynamic light scattering technique and UV-Vis spectroscopy. The effects of different factors such as reactant concentrations on the particle size and charge as well as the relationship between the properties and the factors were investigated. Statistical analysis was performed using MODDE 9.1 software.

The results show that full factorial 2^2 design is useful for screening few numbers of factors in few experiments and to analyze their influence on gold nanoparticles properties. Small nanoparticles can be obtained using the following chemical process parameters: greater chloroauric concentration and smaller sodium citrate concentration.

Findings from this research show that to obtain particles larger than 35 nm, for example, it is recommended to increase sodium citrate concentration, at low chloroauric acid values. These conditions will help to achieve smaller zeta potential, too.

Acknowledgments: This work has received financial support of the project The optimization of methods for the synthesis of gold nanoparticles based on supercritical fluids, project no. 1437/23.12.2014.

References

1. DAVIS, M.E., CHEN Z., SHIN D.M., Nat. Rev. Drug. Discov., **7**, 2008, p. 771
2. SHI, J., VOTRUBA, A.R., FAROKHZAD, O.C., LANGER, R., Nano Lett., **10**, 2010, p. 3223
3. DREADEN, E.C., MACKEY, M.A., HUANG, X., KANG, B., EL-SAYED, M.A., Chem. Soc. Rev., **40**, 2011, p. 3391
4. PARK, J.H., VON MALTZAHN, G., RUOSLAHTI, E., BHATIA, S.N., SAILOR, M.J., Angew. Chem. Int. Ed. Engl., **47**, 2008, p. 7284
5. ZHANG, L., CHAN, J.M., GU, F.X., RHEE, J.W., WANG, A.Z., RADOVIC-MORENO, A.F., ALEXIS, F., LANGER, R., FAROKHZAD, O.C., ACS Nano, **2**, 2008, p. 1696
6. MEDINA, S.H., EL-SAYED, M.E.H., Chem. Rev., **109**, 2009, p. 3141
7. MAEDA, H., Adv. Enzyme Regul., **41**, 2001, p. 189
8. KAUL, G., AMIJI, M., Pharm. Res., **19**, 2002, p. 1061
9. SHARMA, S., BHUMIKA, S., SHARMA, A., Int. J. Pharm. Sci., **4**, 2012, p. 57
10. KIM, J., HONG, C., KOO, Y., CHOI, H., LEE, K., Biol. Pharm. Bull., **35**, 2012, p. 26011. SCHUELLER, R., ROMANOWSKI, P., Cosmetics and Toiletries, Available at: http://www.specialchem4cosmetics.com/services/articles.aspx?id=957
12. CALINESCU, I., MUSTATEA, G., GAVRILA, A.I., DOBRE, A., POP, C., Rev. Chim. (Bucharest), **65**, 2014, p. 15
13. PICA, A., FICAI, D., GURAN, C., Rev. Chim. (Bucharest), **63**, 2012, p. 459
14. HADARUGA, D. I., HADARUGA, N. G., BANDUR, G. N., RIVIS, A., COSTESCU, C., ORDODI, V. L., ARDELEAN, A., Rev. Chim. (Bucharest), **61**, 2010, p. 669
15. BUNGHEZ, I. R., DUMITRESCU, O., SOMOGHI, R., IONITA, I., ION, R. M., Rev. Chim. (Bucharest), **66**, 2015, p. 1112
16. EL BADAWY, A.M., SILVA, R.G., MORRIS, B., SCHECKEL, K.G., SUIDAN, M.T., TOLAYMAT, T.M., Environ. Sci. Technol., **45**, 2011, p. 283
17. SURESH, A.K., PELLETIER, D.A., WANG, W., MORRELL-FALVEY, J.L., GU, B., DOKTYCZ, M.J., Langmuir, **28**, 2012, p. 2727
18. LEE, K.J., BROWNING, L.M., NALLATHAMBY, P.D., XU, X.H., Chem. Res. Toxicol., **26**, 2013, p. 904
19. SILVA, T., POKHREL, L.R., DUBEY, B., TOLAYMAT, T.M., MAIER, K.J., LIU, X., Sci. Total. Environ., **468-469**, 2014, p. 968
20. SHANG, L., NIENHAUS, K., NIENHAUS, G.U., J. Nanobiotechnol., **12**, 2014, 1-11.
21. KHLEBTSOV, B.N., KHLEBTSOV, N.G., Colloid J., **73**, 2011, p. 118
22. JANS, H., LIU, X., AUSTIN, L., MAES, G., HUO, Q., Anal. Chem., **81**, 2009, p. 9425
23. ZANETTI-RAMOS, B.G., FRITZEN-GARCIA, M.B., OLIVEIRA, C.S., Mater. Sci. Eng., **29**, 2009, p. 638
24. ZIMBONE, M., CALCAGNO, L., MESSINA, G., BAERI, P., COMPAGNINI, G., Mater. Lett. **65**, 2011, p. 2906
25. BHUI, D.K., BAR, H., SARKAR, P., SAHOO, G.P., DE, S.P., MISRA, A., J. Mol. Liq., **145**, 2009, p. 33
26. HAO, E., SCHATZ, G.C., HUPP, J.T., J. Fluoresc., **14**, 2004, p. 331
27. CREIGHTON, J.A., EADON, D.G., J. Chem.Soc. Faraday Trans., **87**, 1991, p. 3881
28. BRAR, S.K.,VERMA, M., Trends Anal. Chem., **30**, 2011, p. 4
29. SATO-BER´RU, R., RED´ON, R., V´AZQUEZ-OLMOS, A., SANIGER, J.M., J. Raman Spectrosc., **40**, 2009, p. 376
30. KOPPEL, D.E., J. Chem. Phys., **57**, 1972, p. 4814
31. BERNE, B.J., PECORA, R., Dynamic light scattering: with applications to chemistry, biology and physics, Dover, New York, 2000, p. 223
32. CAO, G., Nanostructures and Nanomaterials – Synthesis, Properties and Applications, Imperial College Press, London, 2004, p.15
33. Zeta potential analysis of nanoparticles, San Diego, 2012, CA, NanoComposix.com; available at: http://50.87.149.212/sites/default/files/nanoComposix Guidelines for Zeta Potential Analysis of Nanoparticles.pdf
34. ERIKSSON, L., JOHANSSON, E., KETTANEH-WOLD, N., WIKSTRORN, C., WOLD, S., Design of Experiments – Principles and Applications, Umetrics Academy, 2000
35. FRENS, G., Nature, 1973, **241**, p. 20
36. AMENDOLA, V., MENEGHETTI, M., J. Phys. Chem. C, **113**, 2009, p. 4277
37. JAIN, P.K., LEE, K.S., EL-SAYED, I.H., EL-SAYED, M.A., J. Phys. Chem. B, **110**, 2006, p. 7238
38. LEE, K.S., EL-SAYED, M.A., J. Phys. Chem. B, **110**, 2006, p. 19220
39. KELLY, K.L., CORONADO, E., ZHAO, L., SCHATZ, G.C., J. Phys. Chem. B, **107**, 2003, p. 668
40. HÖVEL, H., FRITZ, S., HILGER, A., KREIBIG, U., VOLLMER, M., Phys. Rev. B, **48**, 1993, p. 18178
41. JULURI, B.K., ZHENG, Y.B., AHMED, D., JENSEN, L., HUANG, T.J., J. Phys. Chem. C, **112**, 2008, p. 7309
42. JAIN, P.K., EL-SAYED, M.A., Nano Lett., **7**, 2007, p. 2854
43. KLAR, T., PERNER, M., GROSSE, S., VON PLESSEN, G., SPIRKL, W., FELDMANN, J., Phys. Rev. Lett., **80**, 1998, p. 4249
44. NORMAN, T.J., GRANT, C.D., MAGANA, D., ZHANG, J.Z., LIU, J., CAO, D., BRIDGES, F., VAN BUUREN, A., J. Phys. Chem. B, **106**, 2002, p. 7005
45. AVVAKUMOVA, S., Gold Nanoconjugates: Preparation, Characterisation and Biological Applications, PhD Thesis, 2012, Università degli Studi di Milano.

Manuscript received: 27.06.2016