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# Development and Validation of a Method Spectrophotometric for Quantify GNPs and GNPs-Lanreotide *In vitro*.

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**Abstract**

We development and validate one analytical method, easy and economic for quantify GNPs and conjugate of peptide lanreotide (LAN) and GNPs. The parameters of specificity, linearity, exactness and precision were evaluated. We evaluated parameters of analytical method specific, linearity, and precision. The applied analytical method proved to be lineal, precise, specific and exact in the range of the studied concentrations.

***Key words**: Gold-NPs, Gold-NPs-LAN, UV-vis spectrophotometry.*

## INTRODUCTION

The gold colloid or nanoparticles of gold (GNPs) indicate in the treatment of rheumatic arthritis and agent of treatment cancer, on the one case the studied activity indicate in adult people of 40 years, not in adult to 60 years or more or in children. The gold colloids were used remote époques and the union organic biomolecules diversify yours use. The stability of gold nanoparticles is good and yours use are their assembly of multiple types involving materials science, the behavior of the individual particles, size-related electronic, magnetic and optical properties (quantum size effect), and their applications to catalysis, biology and medic use[1]. Fascinating aspects are the optoelectronic properties of GNPs related to the surface Plasmon absorption, reflecting the collective oscillation of the conducting electrons of the gold core, a feature relevant to the quantum size effect. NLO applications of GNPs are also rapidly growing. The combination of photonics discipline with biology and medicine has already been demonstrated by the seminal work on GNPs-DNA assemblies and is very promising for future biomolecular manipulations and applications, such as labeling, detection, and transfer of drugs, including genetic materials[2]. The spectroscopy UV-vis is instrument powered in the variety analytic problems. One inconvenient is specifying in organic compounds for the interference in the degradation products, their present spectra similar the analytical compound and the degradation product. Another problem is the concentrations determinations their shorts and to meet below the sensibility limit of analytic method or experimental error[3]. Validation is establishing one process for to obtain document tests their method analytic is trust for produce results expect. The analytical parameters have been considerate for in the validation of analytical method are exactitude, linearity, precision, specify, detection limit, quantification limit, tolerance and robustness[4, 5].In this case for one first time to realize of development of spectrophotometric analytical method and the validation their same for determine gold colloids nanoparticles (GNPs) and to join the peptide lanreotide (LAN-GNPs) *in vitro*[6, 7]. The conjugate GNPs-LAN this investigation phase and this use toward for therapeutic cancer, therefore is important this studies and the analytic method appropriate to determinate in biological fluids [8-13].

## MATERIAL AND METHODS

The sample of gold colloid was used for development this work, gold monoscattered nanoparticles was synthesized in our laboratory by the reduction reaction between $HAuCl_4.3H_2O$ was boiled with trisodium citrate in an aqueous solution for 15 min to form a colloidal suspension in CICATA-IPN, the same the GNPs-LAN conjugate. The compound chemist standard of gold colloid was buying as standard of gold colloid the commerce *Sigma-Aldrich*, Corp. (St. Louis, MO, USA). All reagents used were analytic quality. The general process was reference Turkevich-Frens synthesis aqueous medium when gold monodispersed nanoparticles were synthesized for reduction of $HAuCl_4.3H_2O$ with sodium citrate tribasic ($Na_3C_6H_5O_7.2H_2O$) was boiled for 20 minutes to form a colloidal suspension. By changing the molar ratio of sodium citrate tribasic to $HAuCl_4.3H_2O$ in the solution, monodispersed Au colloids particles was examined using a UV-vis spectrum, which yielded a strong surface plasma resonance at 523

nm, characteristic of monodispersed colloidal Au. The particle size was 5 ± 0.1 nm, as confirmed by transmission electron microscopy (TEM) and dynamic light dispersion (DLS) [14-19]. For GNPs synthesis was weight 34 mg of cloroauric acid ($HAuCl_4.3H_2O$) and carried volume of mark of 100 ml, of also solution to take 90 ml and carried boil to addition 5 ml of solution of sodium citrate tribasic 32 mM and the reaction was boiled was 20 minutes. After 20 minutes of reaction, the heating was turned off and the solution allowed cool at the room temperature. The solution was then transferred into a 3 ml quartz cuvette, and their extinction coefficients were measured using a UV-vis of simple beam of light *Thermo Spectronic Helios λ*, constitute deuterium and tungsten bulbs for excitation source, and photodiode detector in the 190-900 nm wavelengths range. In this study of linearity was prepared one calibration curve triplicate with standard solutions of gold colloid and conjugate solution (GNPs-LAN) in the intervals concentrations since 40 as 200 µmol/ml, was calculate the determination coefficient linear ($r^2$), will report major or equal at 0.98 and the variation coefficient minor or equal at 3.0%, to establish in the Pharmacopeia of USA (USP 29)[20, 21]. Was carried reading in the same spectrophotometer UV-vis, wavelengths of 523 nm. To where use quartz cuvettes of diameters one cm. In their study of exactness to utilize recover method, to prepared solutions with concentrations different of GNPs-citrate. Five replicas model to use for five different concentrations: 40, 80, 120, 160 and 200 µmol/ml, and determinate percent recover, the standard deviation and the variation coefficient. To determinate beside the Cochran test for verify if the variation of concentration generate significant differences in the results or validation parameters and the Student test (t) for determinate significant differences between average recoveries of 100%. The reproducibility was to realize one calibration curve for triplicate, utilize two analysts and consecutive different three days with same spectrophotometer for calculate variation coefficient, the specifications are minor or equal 3 %. To application Fisher test and Student t tests to determinate differences between two results variant the analysis conditions. The repeatability was to realize the calibration curve for triplicate studies only analyst the same day with spectrophotometer UV-vis. Limit under detection of calibration curve to take of concentration minor solution, later to dilute all concentrations middle to concentration successively as arrive to detection row spectrophotometer and to taking respective dates of absorbance in the spectrophotometer UV-vis. The results interpolate the calibration curve and determinate of limit under detection to compare theory dates and experimental dates. For GNPs conjugation with lanreotide peptide was taking 5 ml of syntheses product GNPs-citrate and to dissolve with 3 ml of peptide LAN: lanreotide [β-(2-naphthyl)-D-Alanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl-L-cysteinyl-L-threoninamide, cyclic (2→7)-disulfide] (0.1M). The volumetric matrix glass keeps marking volume of 20 ml with deionization water. To continued fit solution the pH = 9 with potassium carbonate solution 0.1 N. The dates of absorbance were reading in the spectrophotometer UV-vis during three months one wave longitude of 529 nm. Student *t* and F distribution were used as the statistical strategy with a significance level $p<0.05$. Cochran test is statistic test to utilized for measurements dates with three or mayor independent samples in the experimental models where population be of use same control where exist the one previous period and the later period where the nominal scale. The date calculates in the Cochran test Q to distribute the same quadratic $\chi^2$ test. This test was utilize to determination of variability of results with concentration for evaluate exactness.

## RESULTS

In the Figure 1 show the size nanoparticle was obtain beginning from reduction of $HAuCl_4.3H_2O$ with sodium citrate tribasic in the microphotograph to obtain for electronic microscopic high resolution (TEM). The results was obtain in the specify studies of analytic method to employ standard solutions of gold colloids nanoparticle GNPs and conjugate complex GNPs-LAN respectively shows in the Figures 2 and 3.

*Linearity*

In the Figure 4 shows several spectrums of absorption was obtain in several solutions with several concentrations of GNPs-citrate to evaluate of linearity the analytic method. Linearity function and the graphic profile were showing in the Figure 5. In the table 1 to shows the results of linearity.

*Accuracy and precision of analytic method*

In the table 2 and 3 was report the results of the study of the accuracy, repeatability and precision, and reproducibility respectively. The analysis of precision and reproducibility was analyzed for two

analysts for three days to obtain the variation coefficient in the interval 1 – 3.4, these shows in the table 4.

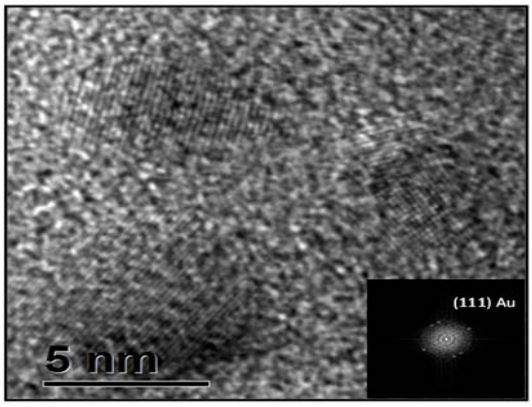


**Fig. 1. Small gold nanoparticles of about 5 nm size.**

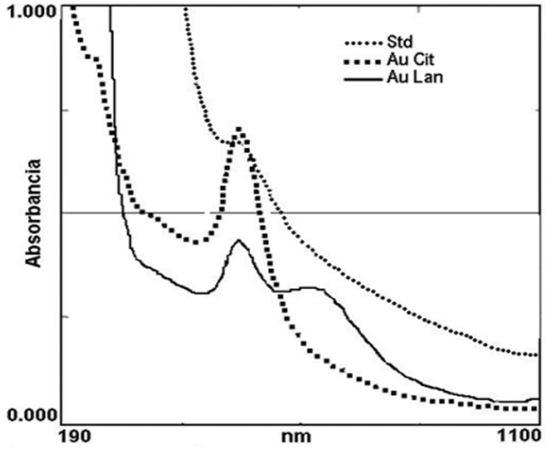


**Fig. 2. Specify: optical spectrum of gold solution STD (0.722), GNPs-Citrate solution (0.850) and GNPS-citrate diluted solution (0.788) at $\lambda_{max}$ = 523 nm.**

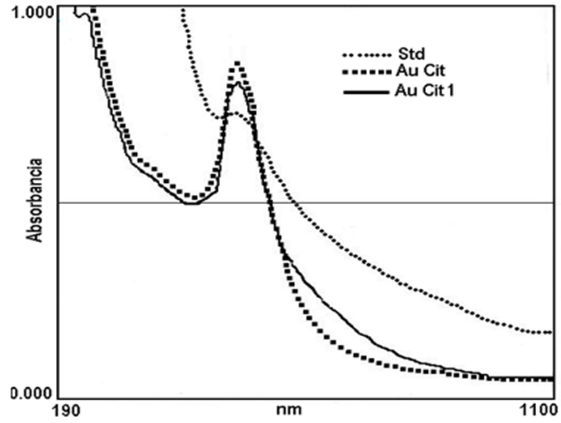


**Fig. 3. optical spectrum of gold solution STD (0.586), GNPs-Citrate solution (0.699) and GNPs-LAN-Mercapto-etOH solution (0.361). The inset shows a plot of Plasmon resonance wavelength $\lambda_{max}$ = 523 nm and $\lambda_{max}$ = 529 of GNPs-Citrate and GNPs-LAN-Mercapto-etOH respectively.**

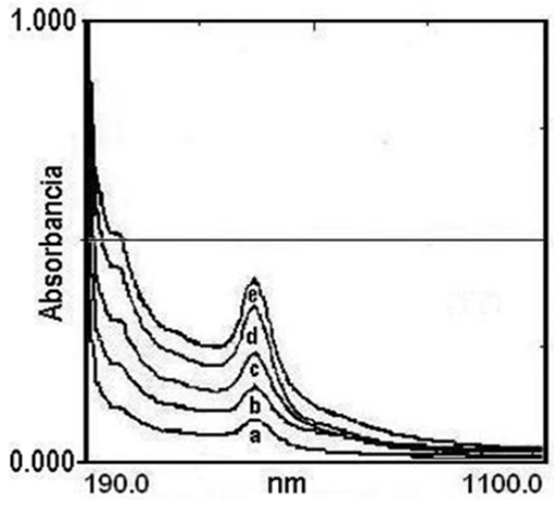


**Fig. 4. Linearity: optical spectrums UV-vis of gold nanoparticles with concentrations differents, a) 40 µM, 0.137; b) 80 µM, 0.311; c) 120 µM, 0.495; d) 160 µM, 0.632; e) 200 µM, 0.778, $\lambda_{max}$ = 523 nm.**

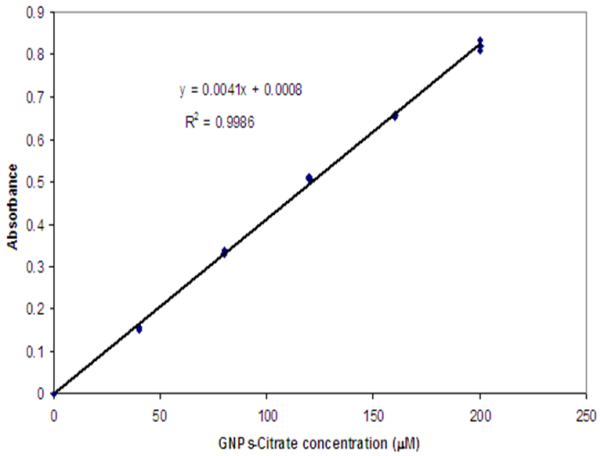


**Fig. 5. Calibration curve of GNPs-Citrate by the proposed analityc method.**

**Table1: Regression analysis of calibration curve (GNPs-Citrate).**

| *Linearity parameters* | *Values* |
|---|---|
| Absorbance maximum: resonance Plasmon (nm) | 523 |
| Linearity range (µmol/ml) | 40-200 |
| Correlation coefficient ($R^2$) | 0.9986 |
| Regression equation | Y=0.0041 X + 0.0008 |
| Slope | 0.004 |
| Intercept | 0.0008 |
| Limit of detection (µmol/ml) | 3.61 |
| Limit of quantitation (µmol/ml) | 7.32 |

**Table2: Precision and repeatability of GNPs-Citrate.**

| *GNPs C(µM)* | *PR1(%)* | *PR2(%)* | *PR3(%)* | *PR4(%)* | *PR 5(%)* | *PRA(%)* | *Sb(%)* | *CV(%)* |
|---|---|---|---|---|---|---|---|---|
| 40 | 105.9 | 97.1 | 95.3 | 97.9 | 97.1 | 98.66 | 4.1 | 4.2 |
| 80 | 98.7 | 99.6 | 100.1 | 97.1 | 100.1 | 99.11 | 1.26 | 1.27 |
| 120 | 101.1 | 102.2 | 102.8 | 97.8 | 102.6 | 101.3 | 2.0 | 1.98 |
| 160 | 100.1 | 103.1 | 102.6 | 98.2 | 99.6 | 100.92 | 2.04 | 2 |
| 200 | 98.6 | 101.9 | 103.4 | 94.8 | 97.6 | 99.26 | 3.4 | 3.42 |

GNPs C = nanoparticles;GNPs-Citrate concentration; PR= rate of analytic recovery GNPs-Citrate; PRA= rate of analytic recovery average; Sb= STD deviation; CV= variation coefficient. In this conditions PRA= 99.85 % and % CV= 2.6.

**Table 3: Recovery of GNP-Citrate.**

| *GNPs C(µM)* | *PA1(%)* | *PA2(%)* | *PA3(%)* | *PA4(%)* | *PA5(%)* | *PRA(%)* | *Sb(%)* | *CV(%)* |
|---|---|---|---|---|---|---|---|---|
| 40 | 101.34 | 101.34 | 104.85 | 104.85 | 104.85 | 103.44 | 1.92 | 1.85 |
| 80 | 103.10 | 105.15 | 104.44 | 105.44 | 105.15 | 104.05 | 1.73 | 1.66 |
| 120 | 103.10 | 103.68 | 103.49 | 103.49 | 103.1 | 103.37 | 0.26 | 0.25 |
| 160 | 99.14 | 99.29 | 99.58 | 99.58 | 99.43 | 99.4 | 0.19 | 0.19 |
| 200 | 100.28 | 98.64 | 98.41 | 98.41 | 98.53 | 98.85 | 0.80 | 0.81 |
| PRA(%) | 101.82 | | | | | | | |
| Sb | 0.98 | | | | | | | |
| CV (%) | 1.00 | | | | | | | |

GNPs C = nanoparticles GNPs-Citrate concentration; PR= rate of analytic recovery GNPs-Citrate; PRA= rate of analytic recovery average; Sb= STD deviation; CV= variation coefficient. In this conditions PRA= 101.82 % and % CV= 1.00.

**Table 4: Reproducibility of GNP-Citrate.**

| | *Analyst 1* | | | *Analyst 2* | | |
|---|---|---|---|---|---|---|
| | *Day 1(% rate)* | *Day 2(% rate)* | *Day 3(% rate)* | *Day 1(% rate)* | *Day 2(% rate)* | *Day 3(% rate)* |
| | 95.1 | 104.82 | 96.4 | 96.23 | 96.40 | 99.95 |
| | 101.67 | 107.31 | 101.14 | 101.81 | 103.21 | 103.50 |
| | 102.87 | 105.13 | 102.12 | 104.3 | 102.71 | 102.52 |
| | 98.09 | 104.94 | 98.62 | 101.04 | 98.77 | 99.07 |
| | 100.12 | 105.77 | 100.07 | 96.85 | 98.41 | 98.18 |
| Media | 99.57 ±3.0 | 106±1.18 | 99.67± 2.24 | 100.04 ±3.43 | 99.90±2.93 | 100.64 ±2.26 |
| CV (%) | 3.09 | 1.12 | 2.24 | 3.42 | 2.94 | 2.26 |

## DISCUSSION

The results was obtain for specifies compound studies of spectrophotometric analytic method to show in the Figure 2, to suggest not exist interferences in determination of GNPs nanoparticles to obtain en the synthesis of Turkevich-Frens and the absorbance maxim to correspond resonance Plasmon, $\lambda_{max}$ = 523 nm of gold colloid [22-24]. The calibration curve was result lineal in the analyzed range; the correlation coefficient (0.99) and the determination coefficient (0.99) to meet in the accept criterions of line function. The test of linearity was obtain response factors were similar himself and nears in the value slope, this to take of variation coefficient of response factors (3.2%) to linearity expression to result minor that 5%, therefore to fulfill of parameters of linearity. The values relative Sb was minor of 2%, that which demonstrate fit linearity. To apply the proportionality test to obtain that systematic error was small, the confidence limits contain of cero. In the significance statistic test of variance of origin ordinate (b), was to obtain one values of "t" test of 0.49 minor that of tabulate "t" test (2.13) therefore this indicate the proportionality condition[25-28].

In the selection range for accuracy studies, the values of percentage recover at inside limits to establish for spectrophotometer analytic methods (98-102%) and the value of variation coefficient was minor of 3%.

To influence of concentration factor related of result variability connect accuracy with Cochran test was obtain that G calculate (0.00017) was minor that tabulate G (0.71)in the probability of 0.05; K=5 and n=5; therefore , the variances of concentrations was used were equivalents, to indicate that employed concentrations not affect of variability this same.

To realize the signification test between recovery test average and the 100% of recovery was obtain one value calculate "t" (0.43) minor that tabulate "t" (2.13), to confirm good accuracy of analytic method since average recovery was not different significantly of 100%.

In the studies of repeatability realized in 5 concentrations level, for one analyst, same day with 3 replicas; to determinate variation coefficients fits in alls concentration levels. Therefore to

demonstrate of good precision of analytic method, thereby the dates to included in the establish limits of variation coefficients.

The values to obtain in the Fischer test for precision studies halfway was demonstrate that not exist significant differences between precisions obtained for analyst neither precisions obtained between different days, for probability of 0.05 %, since of value calculate "F" was 0.55 minor that value tabulate "F" (3.06). To realize "t" student test the value calculate was 0.48 results was minor that tabulate value (2.13) for probability of 0.05, therefore to demonstrate that no exist important differences between averages with significant level of 5%.

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