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VALIDATION AND CHARACTERIZATION OF NANOPARTICLES AUNPS AND AUNPS-LAN CONJUGATE FOR CAPILLARY ELECTROPHORESIS

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AuNPs; AuNPs-LAN, spectrophotometry, capillary electrophoresis.

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ABSTRACT: The Ooctapéptido (LAN: 3-(2-Naphthalenyl)-D-alanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-lysyl-L-valyl-L-cysteinyl-L-threoninamide (2 → 7) disulphide acetate, angiopeptin acetate) cyclic covered gold nanoparticles (AuNPs) results in a complex (AuNPs-LAN) when reacted following the aqueous synthesis using Turkevich method for preparing colloidal gold nanoparticles followed by reaction of conjugation with the octapeptide LAN. The nanoconjugate was characterized by transmission electron microscopy high-resolution, UV-vis spectrophotometry, and by Zeta-potential. The objective of this study was to develop a method of capillary zone electrophoresis (CZE) to determine AuNPs and AuNPs-LAN *in vitro*. The reaction was I carried out by reduction of $HAuCl_4$ with trisodium citrate, based on the synthesis of Turkevich-Frens-Kimling for colloidal Au at 90 °C where the plasmon resonance was detected at a wavelength of 526 nm, previously reported from 1954, 1976 and 2006, wavelength UV-vis nanoconjugate was 531 nm in which prolongation observed in migration times. We also describe part of the validation (linearity and precision) by capillary electrophoresis, arguing that it is a suitable method to quantify the gold nanoparticles and AuNPs-LAN complex. We conclude that the CZE has greater sensitivity to determine AuNPs and AuNP-LAN, comparing to the method described by UV-Vis spectrophotometry, resulting in a good method for analysis and characterization of the nanoparticles and the conjugated compound. HRTEM necessary to use also is important to know the identification and characterization of these compounds to quantify them in biological fluids for biodistribution and pharmacokinetic studies.

INTRODUCTION: Applications of Gold colloidal nanoparticles (AuNPs) are of interest for their use how: labeling, tracing and imaging; sensing and detection and as active elements (heat mediation, optical sensitizing or delivery vehicles) [1, 2].

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General information about the application of gold nanoparticles in biological systems can be found in a number of articles [1-6]. For nanomedicine, particularly, strategies in the self-assembly of biocompatible materials into nanoscale or drug loaded packages with improved therapeutic efficacy is needed, because will permit doses control in administration of nanodrugs [7]. AuNPs have been widely used in analytical procedures because of their size dependent electrical properties, high electrocatalytic activity, and functional compatibility with biomolecules and polymers and others chemist species [8].

Due to their interesting properties, research on colloidal system in nanocrystals form has moved in the last years from fundamental research to first applications in science materials and life sciences how biomedicine [9-13]. It is therefore necessary to develop highly selective and sensitive optical methods for the detection of metal ions. The spectroscopic methods are based on the selective plasmonic resonance energy transfer (PRET) between metal–ligand conjugates and the single gold nanoplasmonic probe. Studies were performed by mentioning that PRET-based metal ion sensing could have applications in cellular imaging, biology systems and environmental monitoring used one spectophotometry method [12, 13].

The electronic and vibrational spectra of AuNPs-Lanreotide conjugate (AuNPs-LAN) were studied before and supported by experimental evidence in relation to gold nanoparticles and conjugated AuNPs-LAN coated and stabilized in the presence of PEG polymer through covalent bond, also mentioned in this study the development of the spectrophotometric method UV-vis used for quantification of gold nanoparticles conjugated AuNPs and AuNPs-PEG-LAN [14,15].

At this time we are reporting the development of the analytical method based on capillary electrophoresis to quantify the conjugated AuNPs-LAN and gold nanoparticles, considering the above results concerning the characterization of these compounds in the spectrophotometric method. Just mention the advantages of quantifying these nanostructures by capillary electrophoresis method as a key to progress in biomedical applications of this nanodrug considering the results previously reported in other publications [15, 16].

Moreover the formation of conjugated gold nanoparticles depends on the type of biomolecule and the reaction conditions to produce stable conjugates with small biomolecules (or synthetic analogues) to generate hybrid materials that may be used allowing the nanoparticles to interact specifically with biological systems [17]. The way in which the conjugation of peptide to gold nanoparticles are performed by covalently linking the ligand which bind to the gold surface of the core by using the tiols chemisorptions or disulfide groups [10]. The peptide Lanreotide (LAN) is an octapeptide [β-(2-naphthyl)-D-alanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-lysyl-L-valyl-L-cysteinyl-L-threoninamide, cyclic (2 → 7) disulfide] synthesized as an inhibitor of growth hormone. The LAN is constituted by eight amino acids which are held together by an internal disulfide crosslink (Cys-Cys). The somatostatin analogue peptide was used for peptide-mediated therapy receiver metastatic neuroendocrine tumors [18-20].

It has also been used in *in vitro* and *in vivo* studies of diagnosis and therapy for the inhibition of growth of the cell line of lung carcinoma (SCLC), NCI-H69 [13] because the majority of human tumors appear on to express one or more of the five known somatostatin receptors, whose hSSSTR are five known subtypes [21-23].

This has been considered as a valuable tool for the visualization of human endocrine tumors and their metastases in diagnostic studies. Likewise, the conjugate may AuNPs-LAN can maintain the unique electronic properties of absorption related resonance plasmon resonance of the gold nanoparticles related to physicochemical properties and binding capacity to specific receptors LAN octapeptide as somatostatin analogue specific or selective molecular recognition of target cells by vectorization is considered, this being an alternative for detection of cancer [23-25].

Furthermore, the selection of a suitable method to quantify the gold nanoparticles and conjugated AuNPs-LAN is important because adequate monitoring methodology would allow us to adequately quantify the amounts of each compound in specific biological systems.

Although different spectroscopic techniques reported [14, 15] to characterize other nanoparticles (for example spectrophotometry), capillary electrophoresis (CE) is a suitable analytical method that enables us to perform the separation of substances by considering the rate at which the ions migrate measuring the amount of load compared to the mass of the molecule and the size and allows us to quantify small amounts of samples as in the case of the nanoparticles, thus considering the electrophoretic movement of biomolecules and

nanostructured systems for the specific separation of complex molecules. Whereas capillary electrophoresis offers certain advantages such as high separation efficiency, analysis time, low cost and easy to implement we believe that this technique is suitable to quantify and characterize the gold nanoparticles and conjugated Au-LAN [26-28].

In addition there are reports since 2006 regarding the application of this CE method for the separation of nanosystems as it has proven to be a promising technique for testing complex biological compounds for clinical analysis and diagnostics [28, 29], including the use of functionalized AuNPs [28]. The real application of AuNPs for CE separation was reported in 2001 [29]. Currently the CE is a powerful analytical tool to quantify AuNPs bioconjugates [30] as well as the separation and characterization of complex biomolecules such as the determination of the concentration of deoxyribonucleic acid [31] or the molecular analysis certain substances among other applications, Oil Mexican Institute, 2012 [32-36].

In this paper, we develop an analytical method to characterize and quantify gold nanoparticles and AuNPs-LAN compound based on the principles of capillary electrophoresis (CE) and to confirm the usefulness of this technique also report details the methodology used to identify gold nanoparticles and conjugated AuNPs-LAN by capillary electrophoresis base on the spectrophotometric technique as reported, HRTEM (transmission electron microscopy and high resolution) and zeta potencial [14, 15].

MATERIALS AND METHODS:

Materials:

The tetrachloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), the sodium citrate tribasic ($Na_3C_6H_5O_7 \cdot 2H_2O$, 99%) and the LAN [β-(2-naphthyl) – D – alanyl – L – cysteinyl - L-tyrosyl-D-tryptophyl-L-lysyl-L - valyl - L - cysteinyl - L-threoninamide, cyclic (2→7)-disulfide] (99%), PEG (polyethilenglicol) were purchased from Sigma–Aldrich and were used as received. Polyvinyl alcohol and other chemicals for preparing buffers were also from Aldrich. For the aqueous solution preparation Milli-Q water was used.

Preparation of colloidal AuNPs:

AuNPs were synthesized by a variant of Turkevich method [37-39] according to a previously published procedure Molina-Trinidad et al. [14, 15]. Briefly, 95 mL 0.5mM $HAuCl_4 \cdot 3H_2O$ solution stirred vigorously was refluxed. To the boiling solution 5mL of 19mM sodium citrate tribasic ($Na_3C_6H_5O_7 \cdot 2H_2O$) was added. After approximately 15 minutes of reaction, the resulting solution was cooled to room temperature.

Preparation of AuNPs-LAN conjugates:

AuNPs-LAN conjugate was prepared according to a previously published procedure Molina-Trinidad et al. [14, 15]. Gold nanoparticles capped with LAN were obtaining stirring an aqueous mixture (water was added until complete 20 mL) of LAN (3.04 mL, 1 mM) and citrate-stabilized AuNPs (5 mL, 1.0 mM of gold atoms) for 20 min. The capped AuNPs formed were separated by repeated centrifugation at 35,000 rpm for 2 h.

CE procedure:

The new capillary was preconditioned by flushing 30 mM pH 6.5 citrate buffer in a polyvinyl alcohol capillary neutral range between 6 and 9. Subsequently, the capillary was washed with deionized water at 20 psi of pressure for 30 minutes and with citrate buffer for 15 minutes. Pressure of 5 psi for 15 minutes and voltage of 12 keV for 10-15 min were the conditions used for sample injections. The run of AuNPs-LAN sample was carried out in an Eppendorf tube to which were added 150 mL of 0.1 mM conjugate and 90 mL of citrate buffer to promote the bond between the peptide and AuNPs surface. The final volume was 250 µL. For the study of linearity, calibration curves were prepared by triplicate with standard solutions of AuNPs in the 0.2-3.0 µmol/mL interval of concentrations.

For the study of five solutions was prepared and linearity over a range of concentrations from a standard has gold nanoparticles from 0.5 to 3.5 micromolar, the study was performed in quintuplicate.

For the studies of reproducibility calibration curves for triplicate were carried out using two analysts

during three consecutive days within the same spectrophotometer of CE. For reproducibility and repeteability study of solutions of the compounds in a range from 0.5 to 3 micromolar to which the response was measured by area, considering different days (n=3) were prepared. Only five electropherograms were considered for each study.

Measurements:

The UV-Vis absorption spectra of the solutions were studied using a Thermo Scientific UV-Vis spectrophotometer, with 10 mm path length quartz cuvettes in the 190-900 nm wavelengths range. Zeta potential data was obtained by a Zetasizer DTS Nano (Malvern Instrument). The measurements were recorded at 25.0 ± 0.1 ºC. The size, morphology and crystallographic characterization of the nanoparticles were carried out using a JEOL JEM 2200 FS electron microscope transmission (TEM) operated at 200 keV of accelerating voltage. The sample for TEM measurement was prepared by dipping the TEM copper grid (400 meshes) in a dilute dispersion of nanoparticles in water. Capillary zone electrophoresis equipment (Beckman Coulter) was used to drive the electrophoresis. A neutral capillary (ECAP Beckham) was used for separation. A port-capillary cartridge with cuvettes was used to collect UV-Vis absorption spectra of the compound eluted out from the capillary separation.

RESULTS AND DISCUSSION:

Lanreotide peptide is a somatostatin analog that is recognized by specific receptors of the hormone. The encouraging clinical applications of labeled somatostatin analogues as therapeutic modality in somatostatin receptor-positive lesions have been reported. It has reduced the tumor growth by metastasis instudies where radiopharmaceuticals are used; a better quality of life by controling the symptoms and a larger survival of patients [19-28, 40-48]. Have been shown it is known that adenomas and various tumors of neuroendocrine origin are over expressed in somatostatin receptors. The lanreotide was created to suppress the hyper secretion and development of certain malignant tumors and for treating diseases such as acromegaly, Cushing's syndrome and other inflammatory disorders [32-44]. The strategy of using LAN for AuNPs functionalization took into account the presence of functional groups that have strong affinity for gold like disulfide bridges and the N-terminal primary amines or thiols groups [46]. The functionalization of the AuNPs by the LAN must be constrained by the stereochemical arrangement of the peptide ring [46-50].

Nevertheless, the high mobility of this macrocycle opens the possibility of disulfide bridge interaction with AuNPs. This interaction may be additive to that of the N-terminal primary amine (especially that from lysine), since amino groups are also known to have strong interaction with gold surfaces, the above and was already reported and explained in previous articles [14, 15]. It means that LAN was the limiting reactive, as a way to ensure the major possible conjugation. The AuNPs-LAN-PEG conjugate obtained was stable for 8 months at room temperature in aqueous solution [1, 2, 14, 15, 16, 37-39]. In the **Fig. 1** the UV–Vis spectrum of AuNPs-LAN-PEG shows a Plasmon absorption band at 530 nm. At 0.8 LAN/AuNPs ratio to 5 nm red shift and a decrease of the Plasmon band absorbance maximum of the AuNPs where a pink solution is observed (probably a weak aggregation process is produced, observing a plasmon resonante shift with respect to gold nanoparticles of 526 nm and 530 nm to identify the conjugate AuNPs-LAN).

Different solutions golden shown in the first figure: molecular solution obtained from a gold standard (yellow colour), gold nanoparticles (red wine) and gold-conjugated AuNPs-LAN-PEG (pale pink) where it was found that the presence of PEG stabilizes the complex AuNPs-LAN. Colloidal gold is a suspension of particles in an aqueous liquid (water). The formation of colloidal gold occurs by using citrate to reduce gold (III) to gold solid. The solution is red wine for particles smaller than 100 nm.

Thus colour refers to the size of the particles in the medium base don those reported by Turkevich, Frens, Kimling and Liz Marzán colloidal solution of gold nanoparticles represents the particle size base don their color as in this case in 5 nm confirmed by the micrograph obtained from a sample of nanoparticles by high-resolution microscopy, previously reported [1, 2, 14, 15, 37-39, 50-55].

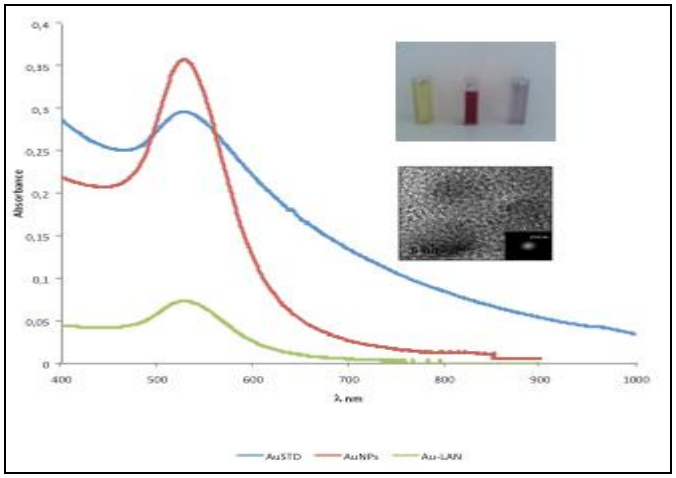

FIG.1: UV-VIS SPECTRUM OF GOLD STD (AuNPs, 5 nm), AuNPs SOLUTION (0.76 mm, 526 nm) AND AuNPs-LAN-PEG COMPLEX (0.31mM, 531nm). INDICATED HIGHER MAGNIFICATION SHOWS SMALL NANOPARTICLES IN THE ORDER OF 5 nm PROTECTED BY THE CITRATE MATRIX. COALESCENCE PROCESS IS DELAY BECAUSE OF THE PASSIVATE CITRATE ACTION.

Moreover, the wavelength band of maximum absorption spectrum is stable for several weeks, this is because the layer of peptide in the surface of gold nanoparticles decrease maintains stable nanoparticles aggregation; adding to the stability of the conjugate to add the biodegradable polymer polyethylene glycol (PEG) [51]. Studies confirmed by measuring the zeta potential on the conjugate stability study, results shown in **Table 1**. In **Fig.2** the spectrum of AuNPs-LAN-PEG are shown in the biological fluid (plasma), the absorbance maximum peak is observed at a wavelength of 328 nm, which explains the behavior detection depends gold conjugate as the physiological pH of blood pH is about 7.4 and the detection was performed in human blood plasma [1-8, 51-53].

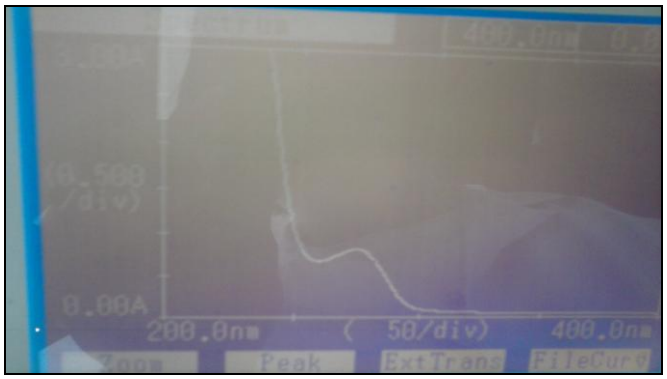

FIG. 2: UV-VIS SPECTRA FOR AuNPs SOLUTION (0.76 mM) USING A VARIAN CARY 50 CONC. UV-VIS SPECTROPHOTOMETER, WITH 10 mm PATH LENGTH QUARTZ CUVETTES IN THE 190-900 nm WAVELENGTHS RANGE. THE MAXIMUM PEAK IS OBSERVED AT A WAVELENGTH OF 328 nm. It is noteworthy that the pH changes in any chemical or biological system interfere with the detection of the biomolecule due to the physicochemical properties of each compound (pKa, partition coefficient and others factors).

Furthermore, the zeta potential indicates the degree of repulsion between adjacent and similar nanoparticles.

Gold nanoparticles with large absolute values of zeta potential have better stability and show no aggregation with respect to the values obtained in the presence of aggregation of particles [56]. The zeta potential value was obtained from the dispersion to the octapeptide with the gold nanoparticles in a ratio 0.8 of LAN / AuNPs, in the study was -18.4 ± 0.7 mV (mean of three measurements). The existence of a net negative charge on the nanoparticles in aqueous solution is demonstrated. Compared AuNPs colloid, this negative net charge is less than the above mentioned (see **Table 1**).

This fact can be explained as a consequence of the gradual replacement of citrate groups on the gold surface by molecules of LAN. This means that AuNPs-LAN has a higher tendency to aggregate and have a low stability. With this we argue about that the size gold nanoparticles are smaller than the forward-conjugated AuNPs-LAN, this is due to the size of the molecule octapeptide. Based on the above reports in the literature regarding the zeta potential of gold nanoparticles substances it is believed that maintaining a negative charge enhancing its stability due to repulsive electrostatic interactions [56, 57].

In a previous study in the IR and Raman spectra of this new conjugated gold nanoparticles capped peptide Lanreotide [14] the displacement of the groups of the AuNPs citrate LAN surface was confirmed. It seems that the formation of bioconjugate occurs through covalent interactions through internal amide groups and Cys-Cys disulfide interactions known biomolecule binding of thiol groups [58].

The size, morphology and structure of the nanocrystals of the compound obtained AuNPs-LAN were studied by transmission electron

microscopy as previously reported [14, 15]. In the **Fig. 1** show the image taken by high-resolution microscopy shows HRTEM nanoparticles AuNPs in the order of size of 5 nm, previously reported [14, 15].

TABLE 1: ZETA POTENTIAL (mV) of $AuNPs_{std}$, $AuNPS_{synthesis}$, LAN and AuNPs-LAN-PEG.

	Zeta potential (mV)			
	$AuNPS_{std}$	$AuNPS_{synthesis}$	LAN	AuNPs-LAN-PEG
1	-0.56	-35.6	-14.7	-18.9
2	-0.42	-27.9	-14.0	-17.9
3	-0.46	-27.1	-12.6	-18.4
mean	-0.5 ± 0.2	-30.2 ± 5	-5.4 ± 0.5	-18.4 ± 0.7
%CV	20	18	9	4

The above mentionated analysis in order to justify the use of capillary electrophoresis methods, which method is more accurate and allows us to quantify small amounts of analyte that could not be quantified by UV-vis spectrophotometry, this is because with biological samples such as blood or plasma of animals suchs as rats whose blood volumen is approximately 20mL is difficult to make an análisis by spectroscopic methods, specifically by UV-vis spectrophotometry. For this reason studies of pharmacokinetics and biodistribution in models is necessary to have a validated method that allows us to accutrately quantify the analytes under study.

In this study, the use of gold nanoparticle conjugate and AuNPs-LAN to identify and quantify these compounds by capillary electrophoresis is described when the nanoparticles are stabilized with citrate ions and were synthesized by the method of Turckevich-Frens and Kimling [37-39]. In previous studies it was demonstrated that the presence of citrate stabilized gold nanoparticles following a reaction of oxide reduction. **Fig. 3** shows electropherograms obtained gold nanoparticles AuNPs, lanreotide octapeptide and LAN-LAN AuNPs conjugate are shown (**Fig. 3, 4 and 5**) it can be seen that the presence of gold nanoparticles modified the electroosmotic mobility and mobility observed solutes.

These changes are manifested in the mobility selectivity of gold nanoparticles and AuNPs-LAN-PEG at low concentrations and the effect of the presence of the PEG polymer can stabilize the conjugate. Given the characteristics of the electrophoretic method to characterize and identify gold nanoparticles and LAN conjugate we can say that is specific and selective for these compounds and is an appropriate method to quantify the compounds under study, and that improves the accuracy of the analysis and increases the efficiency of separation of these compounds [57, 59].

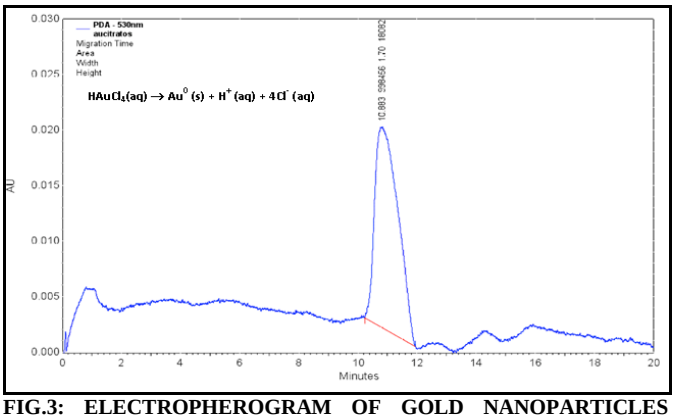

FIG.3: ELECTROPHEROGRAM OF GOLD NANOPARTICLES [AuNPs] OBSERVED AT 530 nm. IN THIS FIGURE ILLUSTRATES THE MIGRATIONN TIME, AREA, WIDTH AND HEIGHT. OXIDE REDUCTION REACTION PRESENTING AU REACTING WITH TRISODIUM CITRATE SOLUTION IS ALSO INDICATED. WE USED A FUSED SILICA CAPILLARY. THE INJECTION WAS CONDUCTED AT 12 kV FOR 15 MIN, P 20 psi AT 25 °C.

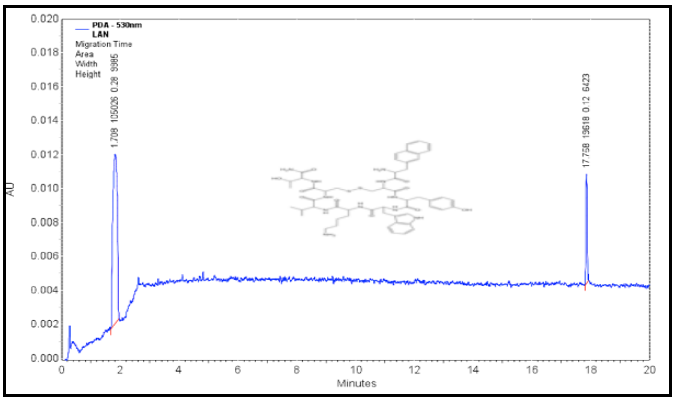

FIG. 4: ELECTROPHEROGRAM LANREOTIDE [LAN] OBSERVED AT 530 nm. IN THIS FIGURE ILLUSTRATES THE MIGRATIONN TIME, AREA, WIDTH AND HEIGHT. THE CHEMICAL STRUCTURE OF THE OCTAPEPTIDE IS PRESENTED. WE USED A FUSED SILICA CAPILLARY. THE INJECTION WAS CONDUCTED AT 12 kV FOR 15 MIN, P 20 psi AT 25 °C.

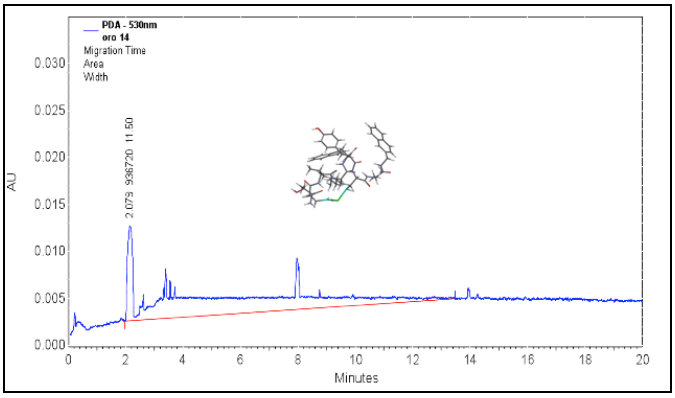

FIG.5: ELECTROPHEROGRAM AuNPs-LAN-PEG [Au-LAN] CONJUGATE. IN THIS FIGURE ILLUSTRATES THE MIGRATIONN TIME, AREA, WIDTH AND HEIGHT. WE USED A FUSED SILICA CAPILLARY. THE INJECTION WAS CONDUCTED AT 12 kV for 15 min, P 20 psi AT 25 °C.

In this paper, the aqueous colloidal dispersions of gold were prepared by citrate method (synthesis Turckevich-Frens and Kimling) reported in 1951 and 1973 well known that is based on reducing $AuCl_4^-$ by citrate ions [37-39]. In one step the preparation is done, the citrate ions were used to stabilize the nano-dispersion in the presence of the octapeptide lanreotide to form the conjugated AuNPs-LAN, likewise the conjugate was stabilized with PEG polymer [51]. Gold nanoparticles and conjugate AuNPs-LAN separated, identified and characterized by the method shown in capillary electrophoresis based on the results obtained that the separation is specific and precise as well as the identification of compounds, whereby efficiency analysis is aceptable [57, 59].

Furthermore, to optimize the conditions of a method is necessary to validate the technique or method used to quantify any analyte, in this case to quantify the gold nanoparticles and conjugated AuNPs-LAN. For us it is necessary to know the specifications and control in the analysis of analytical samples in order to verify that the quantification of our samples is reliable, for this reason we evaluate the parameters of linearity and precision (repeteability and reproducibility) shown in **Table 2** and **3** for the compounds under study in order selecting response variables in biological samples for further analysis in studies of pharmacokinetics and biodistribution in an animal model. We can say that studies have been reported in reference the gold nanoparticles, not for the conjugated AuNPs-LAN and as our interest is in this compound is important to know the conditions of analysis to quantify this nanostructure in biological fluids how delivery system [59].

TABLE 2: REGRESSION ANALYSIS OF CALIBRATION CURVE (AuNPs-Citrate)

Linearity parameters	Values
Absorbance maximum: resonance Plasmon (nm)	530
Linearity range (µmol/ml)	0.23 - 3.3
Correlation coefficient (R^2)	1
Regression equation	Y=10202 X – 3.73
Slope	10202
Intercept	-3.73
Limit of detection (µmol/ml)	0.08
Limit of quantitation (µmol/ml)	0.18

Based on the test results obtained following the methodology described by the electrophoretic method linearity test is reported, indicating that the response factors are similar and a coefficient of variation of the response factors are reported under 3.0%, indicating that based on the specifications to evaluate the linearity test meets this parameter, because the specification based on the coefficient of variation for this test should be in a range below 5%. (See **Fig. 6** where the calibration curve used for the analisis show linearity). **Table 2** shows the results obtained when evaluating the linearity parameter values where the relative standard deviation (Sb) was less than 2% is shown, demonstrating the linearity adjustment. We can say that the proportionality tests showed that the bias was small based on statistical criterion adjustment when confidence limits are close to zero.

In statistical significance testing of variance to assess the intercept (b) the "t" student, obtaining the value of the test "t" estudent 0.045, lower than the reported value test was used 2.13. Therefore these data indicate that reliable calibration curve used to quantify these compounds and that meets the linearity parameter specified as important for the validation of a method based on the indications suggested by the FDA [60] and test USP 29 [61].

The value obtained from the linear coefficient of determination was 1 and the coefficient of variation was less than 3.0%, as stated in the FDA and the U.S. Pharmacopeia [60, 61]. We also found that the relative standard deviations are less than the value

of 3.0%. By which meets FDA specifications.

We report the concentration of each analyte as follows: for AuNPs-LAN compound concentration of 92 μM, for AuNPs 98 μM and for octapeptide LAN 1.92 μM. With these results we found that this method is useful for capillary electrophoresis to quantify analytes in our *in vitro*, since the Studies continue to quantify these analytes *in vivo* for studies of pharmacokinetics and biodistribution.

TABLE 3: REPETEABILITY AND REPRODUCIBILITY (n=5) ANALYSIS OF AuNPs-CITRATE.

Analyte	Time (min)	Area	Repeteability (n=5) %CV		Reproducibility (n=5) %CV		N
			t_R	Area	t_R	Area	
AuNPs	20	997827±1188	0.6731	0.7206	0.5092	0.1190	102
AuNPs-LAN	20	940052±5653	0.5299	0.9138	0.2773	0.6014	2.9
LAN	20	19647±47 105162±163	0.3047 0.9161	0.8818 0.2104	0.2708 0.7203	(1) 0.2368 (2) 0.1545	(1) 97 (2) 2359

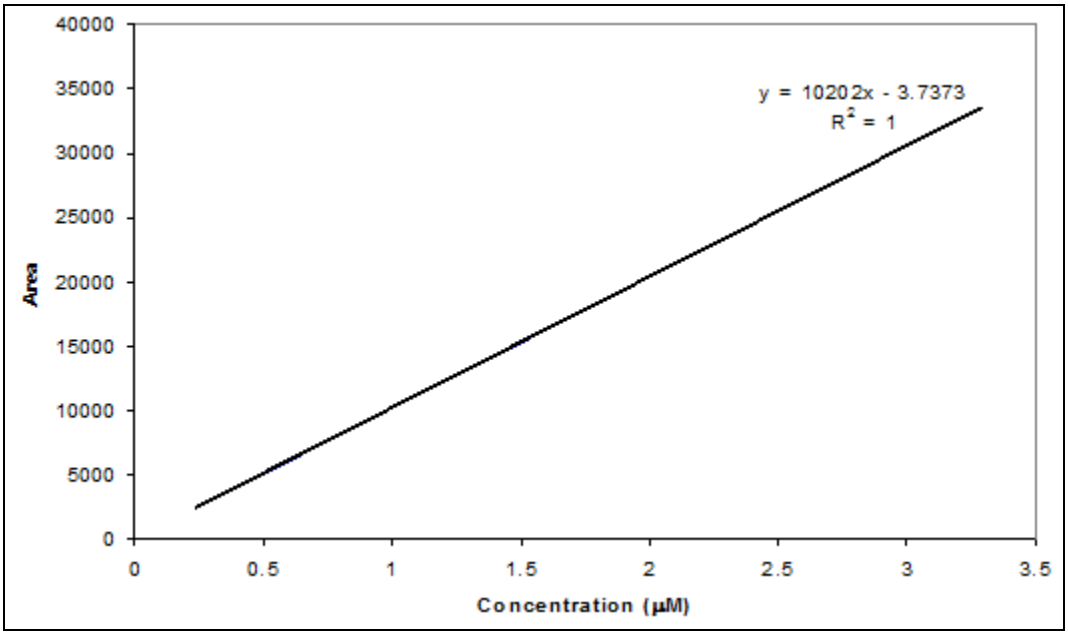

FIG. 6: CALIBRATION CURVE OF AuNPs-CITRATE STD BY THE PROPOSED ANALITYC METHOD, WHERE m = 10202, b = -3.7373, R = 1 and R^2 = 1

Finally, we think that this technique is suitable to quantify the conjugated AuNPs and AuNPs-LAN allowing us sampling of very small amounts picoliter level, as the minimum amount quantifiable to quantify gold nanoparticles was $18X10^4$ pmol/mL, and that would allow us to quantify isolated cells and subcellular structures, plus it is a technique to separate amino acids and neurotransmitters [57, 59].

CONCLUSION: The spectrophotometric analityc method use for identify the two compounds (AuNPs and AuNPs-LAN) is useful and reliable quantifying analytes microliters level or parts per million, but the Capillary Electrophoresis is more selective method useful for quantify the gold nanoparticles and nanodrug AuNPs-LAN. We were development and validate a method to characterizing, identify and quantify gold nanoparticles and AuNPs-LAN *in vitro* by Capillary Zone Electrophoresis (CZE). The results of AuNPs size show that nanoparticles may be produced and may be stable compounds with lanreotide peptide and this nanoestructures may be stable in biological fluids how in blood in the presence of polyethylene glycol.

We also found that the capillary electrophoresis technique allow us to quantify small amounts of analyte in biological samples accurately. This is an alternative for use in the diagnosis of diseases such as cancer and perhaps treatment of this disease in the near future. Also, this study will serve as

support for pharmacokinetic studies of these compounds *in vivo*.

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