Int. J.Nano Dimens. 6(1): 31-38, Winter 2015
ISSN: 2008-8868

Contents list available at **IJND**

International Journal of Nano Dimension

Journal homepage: *www.IJND.ir*

MCR of the quenching of the EEM of fluorescence of Aflatoxins (B1, G1) by Gold nanoparticles

A. Saidi*
M. Mirzaei

Department of Chemistry, Shahid Bahonar Universityof Kerman, 22 Bahman Boulevard, Kerman 76169, Iran.

Received 01 August 2013
Accepted 19 October 2013

ABSTRACT

In This research, gold nanoparticles were synthesized and functionalized by the antibody of aflatoxins. The quenching of the fluorescence of excitation emission matrices (EEM) of two type of aflatoxins (B1, G1), provoked by the gold nanoparticles, was studied by principal component analysis (PCA) and multivariate curve resolution with alternating least squares (MCR-ALS). These aflatoxins show quenching in the presence of the gold-labeled antibodies. Solutions containing a constant concentration of aflatoxins and variable concentrations of gold-labeled antibodies have been prepared and the fluorescence spectra have been recorded by scanning excitation and emission wavelengths. Excitation and emission spectra and quenching profiles were extracted from EEM using MCR-ALS. Applying suitable constraints lead to satisfactory results for the determination of aflatoxins in all samples.

Keywords: *Aflatoxins; Gold nanoparticle; Antibody; MCR-ALS; Chemometrics.*

INTRODUCTION

Aflatoxins are naturally occurring mycotoxins produced by many species of *Aspergillus*, most notably *A. flavus* and *A. parasiticus* during growth on foods and feeds [1]. They exhibit carcinogenic, teratogenic, mutagenic and immunosuppressive properties. To avoid human exposure to aflatoxins, serious programs have been established by various government agencies in regulating aflatoxin levels in foods and feeds [2]. Peanuts, nuts, spices, and cereals are often contaminated with this class of mycotoxins [3]. Many methods have been used to determine aflatoxins in food, such as Fourier transform near-infrared spectroscopy [4], HPLC [5], liquid chromatography–tandem mass spectrometry (LC–MS) [6] and enzyme-linked immunosorbent assay (ELISA) [7]. Among these methods, ELISA and HPLC are most generally used; however, they are usually time and money consuming and require special equipments.

* Corresponding author:
Asiye Saidi
Department of Chemistry, Shahid Bahonar Universityof Kerman, 22 Bahman Boulevard, Kerman 76169, Iran.
Tel +98 341 3222033
Fax +98 341 3222033
Email asiye_saidi@yahoo.com

Therefore, it is very important to create a sensitive, low cost, independent instrument or an easily performed assay to rapidly detect aflatoxins in food. One of the best detection techniques is fluorescence detection, which has achieved major developments in bioanalytical applications due to its wonderfully sensitivity and selectivity. In comparison with the usual fluorescence quenchers, gold nanoparticles (GNPs) have a very high ability for fluorescence quenching so, applying nanoparticles for the detection of biomolecules is extra interest [8]. GNP is a kind of promising nanomaterial, owing to their high extinction coefficients, strongly size-dependent surface plasmon resonance (SPR) and high surface to volume ratio [9]. It has acquired application as biological detection, biomarker, molecular recognition, catalysis, chemical sensor and nanoelectrode [10].

In addition, as super quenchers, GNPs have been served in fluorescence detection and fluorescence resonance energy transfer (FRET) assay [11]. Recent advances have led to the growth of functional nanoparticles linked to biological molecules like peptides, antibodies, nucleic acids and enzymes. Some of these functional nanoparticles that have been proved to be efficient and sensitive fluorescence quenchers have been hold for great promise for biosensors [12]. In this research, GNPs were synthesized and functionalized by the antibody of aflatoxins. Unlike fluorescence or enzyme-detection systems, gold-labeled antibodies are more stable and easy to use. There are no needs for fussy operations as incubation, washing and enzymatic reactions during signal generation, which surely shorten the detection time. Furthermore, nanoscale surfaces provided by GNPs could accelerate antibody–antigen reaction sufficiently, which supply an amplified signal [13]. The formation of gold-labeled antibodies was accomplished at optimal condition. Their formation processes were monitored by UV-visible (UV-vis) light measurements, whereas transmission electron microscopy (TEM) images were used to characterize the shape and particle size of the conjugates. Effect of GNP on the antibody in the conjugate system was studied by fluorescence spectroscopy. One of the best techniques to perform a global analysis on the effect of the gold nanoparticles-labeled antibodies on the fluorescence spectra of aflatoxins is excitation emission measurements (EEM). In EEM spectroscopy a total fluorescence spectrum is obtained by systematically varying the excitation and emission wavelengths and collecting the resulting i × j data matrix.

In order to extract all useful information from EEM data the chemometric methods must be employed. The good analytical potential of EEM coupled to chemometric methods has long ago been recognized. Multivariate curve resolution method based on alternating least squares (MCR-ALS) applies a more versatile model than the trilinear and, recently, has also shown to generate useful information from the analysis of EEM. MCR-ALS is a flexible method which can be used to analyze: (a) a single data matrix containing data recorded throughout an individual experiment, (b) a column-wise augmented data matrix containing data recorded over several experiments or techniques, (c) a row-wise data matrix, and (d) a simultaneous column and row-wise augmented data matrix. This method has some important features that differentiate it from other three-way data analysis methods. Firstly, it can be used for three-way data with different data structures: trilinear and not trilinear. Secondly, it has a simple algorithmic implementation based on matrix inversion. Thirdly, eigenvalue–eigenvector decomposition of the experimental data matrix is used to determine the number of independent contributions. And finally, it means that several constraints can be simply applied during the ALS optimization with increasing reliability of the solutions obtained.

This paper describes the application of MCR-ALS method on the EEM data obtained from some different mixtures of two kinds of aflatoxins (AFB1 and AFG1) acquired as function of the extent of quenching provoked by gold-labeled antibodies. The analysis of the spectroscopic data by MCR-ALS allows the determination of the pure spectra and concentration profiles of aflatoxins in the samples with several advantages, such as procedure simplicity, rapid operation and immediate results, low cost, and no requirement for skilled technicians or expensive equipment.

EXPERIMENTAL

Materials

Standard solutions of aflatoxins B1 and G1 (1000 ngL^{-1}) in methanol were purchased monthly from Marjaan Khatam (Training, Research & Q.C. Lab. Services, Tehran, Iran). These solutions were stored at -18 °C. All needed working solutions were prepared daily by diluting these standard solutions. Anti-aflatoxin (B1 and G1) mouse monoclonal antibody, Hydrogen tetrachloro Aurate(III) ($HAuCl_4.3H_2O$) and sodium citrate were obtained from Sigma-Aldrich (USA) used without further purification. Phosphate-buffered saline (PBS, pH 7.4, 0.01 M in 0.85% NaCl) was prepared. All other chemicals were of analytical grade and were used without further purification.

Instruments and software

UV–vis absorption spectra were carried out on a Spectrophotometer (VARIAN Cary 50). The transmission electron microscopy (TEM) images were taken with a Philips CM-10 instrument. The zeta potentials were carried out by a Zetasizer particle analyzer (Malvern Instruments, UK). All the fluorescence measurements were performed on an RF-5301PC spectrofluorimeter (Shimadzu Corporation, Japan). The software used in this work has been written by R. Tauler and can be downloaded from the group Web page (*http://www.ub.es/gesq/eq1_eng.htm*).

Synthesis and characterization of GNPs

An aqueous solution of tetrachloroauric acid (50 ml of 0.01% [w/v] $HAuCl_4.3H_2O$) was heated to boiling. To the boiling solution, 2 ml of 1% sodium citrate was immediately added under constant stirring. The color of the solution changed from yellow to red within 1 min. The solution was allowed to boil for another 10 min. After cooling, in dark, the solution volume made up to 50 ml with distilled water. The colloidal solution was stored in a dark bottle at 4 °C and was used in the preparation of the gold-labeled antibody. The transmission electron microscopy (TEM) indicated the image of GNPs.

Formation of gold-labeled antibody

Monoclonal antibody (1 mgL^{-1}, 1ml) prepared in pH 7.4 phosphate-buffer solution (0.01 M) was added to 1 ml of colloidal gold solution while stirring. The pH of the GNP solution was adjusted to 7.4 by addition of dilute 0.01 M Na_2CO_3 before adding the antibody. This was done in as much as the optimum stability of the conjugates at pH 7.4. The solution was stored for a period of 1.5 h at room temperature and centrifuged (5000 rpm at 4°C) in an Eppendorf centrifuge (Model 5804R, Germany) for 30 min to remove unconjugated antibody from the solution. The pellet obtained was dispersed in 2mL PBS at pH 7.4 and stored at 4 °C for further experiments. . The formation of gold-labeled antibodies was monitored by UV-visible light measurements, transmission electron microscopy (TEM) and fluorescence spectroscopy.

Fluorescence quenching by GNPs

Solutions containing a constant concentration of both of the aflatoxins equal to 1.6ngL^{-1} and a variable concentration of gold-labeled antibodies have been prepared. The range of concentration of the gold-labeled antibodies was about 0–0.22 mgL^{-1}. The fluorescence spectra have been recorded after the solution preparation. Excitation (Ex) wavelengths were set from 350 to 370 nm, and emission (Em) wavelengths were set from 400 to 570 nm. Ex and Em wavelength increments were 1 nm with a slit of 10mm. EEM data were measured using 1cm path length quartz cell. Each samples yield one data matrix which was analyzed by MCR-ALS method.

MCR-ALS of EEM

The presence of gold-labeled antibodies provokes quenching of the EEM due to the complexation of the fluorescent binding sites. The fluorescence spectra of both aflatoxins results from the contribution of two fluorophores, with somewhat different fluorescence properties, that are severely overlapped. Nevertheless, although this preliminary analysis of the EEM supported that they contain a lot of information about the aflatoxins samples and about the quenching process, chemometric methods must be used in order to extract useful information. The first objective in an aflatoxins EEM analysis is the calculation of the individual fluorophores that constitute the global EEM. Before the alternating least squares iterative process begins, the number of compounds in a particular experiment should be determined according to the principal component analysis (PCA) [14] by applying singular value

decomposition (SVD) or evolving factor analysis (EFA) methods [15]. It is assumed that the variance explained by chemical compounds is significantly larger than noise variance. In this study, SVD and EFA methods were applied for rank analysis.

The fluorescence intensity emitted by a fluorophore is related to the excitation and emission wavelength. An EEM contains all the steady state fluorescence features of a fluorophore. In excitation–emission fluorescence data, all the data matrices for the EEM spectra of each measured sample can be aligned to one another to acquire an augmented data matrix [16]. Both excitation-wise and emission-wise matrix augmentations are possible in fluorescence. In the excitation-wise augmentation used here, the matrices are put on top of one other and the common excitation wavelengths are kept in the same column. Using this resolution method, the excitation-wise (column wise) augmented data matrices are modeled with the equation:

$$D_{aug} = Y_{aug}X^{T} + E_{aug} \qquad (1)$$

Where D_{aug} is the excitation-wise augmented response data matrix, Y_{aug} the augmented matrix of excitation spectra, X^T the transposed matrix of emission spectra and E_{aug} is the matrix of residuals. The quantitative information is contained in the relative intensities of the excitation spectra Y_{aug}. It should be noted that this data arrangement presumes that the spectra of the common species, in the different matrices, are the same.

To initiate the iterative ALS procedure, an initial estimation is needed for the spectral or concentration profiles for each species. It is sensible to start with the best possible estimates available. Different methods are used for this purpose like evolving factor analysis [17] or the determination of the purest variables [18]. In this work, initial estimations based on purest variables were selected. Iterations continue until an optimal solution is obtained. The constraints applied to get physically meaningful solutions during the ALS optimization were non-negativity constraint, because either emission or excitation spectra must be always positive and equality constraints in pure spectral profiles, because the component spectra are known.

In order to evaluate the fitting error in the reproduction of the original matrix using the solutions either found by principal component analysis or by MCR-ALS, a lack of fit (LOF) value was calculated using the following equation:

$$LOF(\%) = 100\left(\sum_{i,j}(D_{i,j} - \hat{D}_{i,j})^2 / \sum_{i,j} D_{i,j}^2\right)^{0.5} \qquad (2)$$

Where $D_{i,j}$ and $\hat{D}_{i,j}$ represents the fluorescence intensity at spectra *i* and wavelength *j* in the experimental and calculated matrices, respectively.

RESULTS AND DISCUSSION

Conjugation studies of gold nanoparticles to aflatoxins' antibodies

For conjugation, antibody was directly adsorbed on the GNP surfaces, mediated mainly by London-van der Waals forces and hydrophobic interactions [19]. The colloidal gold was established in the solution by virtue of a balance between electrostatic repulsion and London-van der Waals attraction among the particles. However, on addition of ionic substance, the attracting force becomes greater than the counteraction, which leads to an aggregation accompanying a color change from red to blue [19]. Coating the colloidal surfaces with protein molecules, such as antibody, can prevent this instability.

- ***Optimization of the effective parameters on the conjugation process***

Optimal conditions of pH and antibody concentration for the coating can be determined by subtracting the absorption in the wavelength 520 and 580 nm (A_{520}–A_{580}). GNPs solutions adjusted to pH range of 5–9 were pipetted into a series of tubes. Antibody solutions (0.2–2mgL^{-1}, 1 ml) were added to each colloidal gold solution diluted in a series of concentrations. Each tube received 1 ml of 10% NaCl and was shaken for 5 min. Absorption of each tube at 520 and 580 nm was determined 10 min later. For antibody, the minimal concentration to stabilize colloidal gold was approximately at 1 mgL^{-1} and the pH of the gold-labeled antibody solution was determined to be 7.4.

Characterization of gold-labeled antibody conjugates

Figure 1 shows the UV-vis spectra of the colloidal gold nanoparticles (curve a) and their antibodies conjugates (curve b), prepared as described previously. A peak at ~519 nm in curve (a) is due to the surface plasmon resonance of GNPs. After addition of the antibody, the surface plasmon band broadened and red shifted due to the interaction of the antibody with colloidal gold nanoparticles.

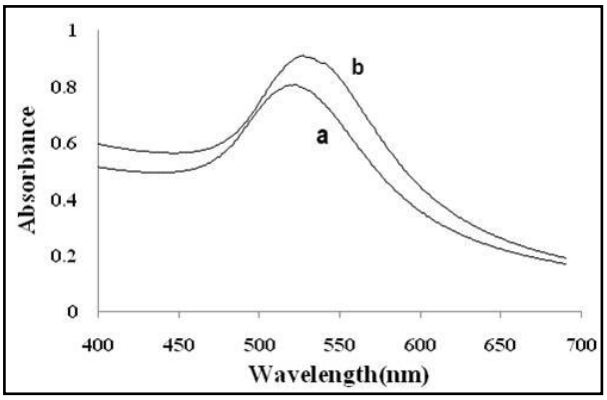

Fig. 1. UV-visible spectra of ofGNPs(a) and gold-labeled antibodies(b).

The immunoreactivity of antibody depends upon the tertiary structure of the antibody remaining unperturbed after formation of conjugates with GNP [20]. The tertiary structure of the antibody can be studied by fluorescence measurements, by exciting the sample at a particular wavelength and monitoring the fluorescence emission from the tryptophan or tyrosine residues in the antibody. Figure 2 shows the fluorescence spectrum of free antibodies to both aflatoxins (pH 7.4, 0.01 M PBS). The sample was excited at 275 nm, and the emission was monitored in the range of 300–400 nm. A broad band was observed at 336 nm and indicates intactness of the tertiary structure of antibody in solution. The curve of gold-labeled antibody showed the fluorescence spectrum recorded under the same emission conditions. The nature of the curve and peak position was quite similar to the free antibody indicating the intactness of the antibody after the conjugation. Comparing the tryptophan emission intensities of the gold-labeled antibody versus that of free antibody, a significant amount of fluorescence quenching of tryptophan residues in the antibody was observed.

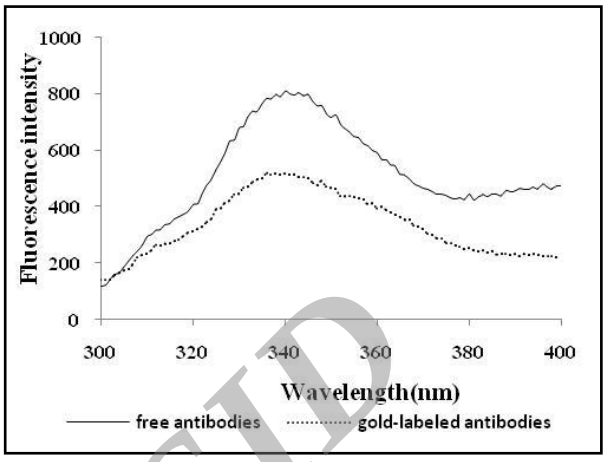

Fig. 2. Fluorescence spectra of free antibodies and gold-labeled antibodies.

Figure 3 shows the TEM images of the GNPs and the gold-labeled antibodies. The average diameter of GNPs was about 3 nm.

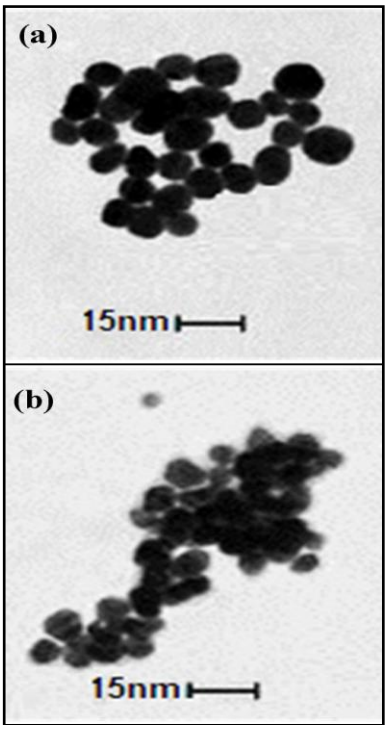

Fig. 3. TEM images of GNPs(a) and gold-labeled antibodies(b).

The interaction of aflatoxins with gold-labeled antibodies

GNP can be as a quencher to decrease the fluorescence intensity of fluorophores owing to FRET [21]. The dynamic quenching process included an instantaneous exciplex formed between excited fluorescent molecules and quenchers [22]. These exciplex could not emit fluorescence or become different from the original fluorescent molecules, which cause quenching happen. Similarly, this quenching process occurred when gold-labeled antibodies were mixed with aflatoxins in solution, the exciplex of GNPs and aflatoxins was formed, and then caused the decrease of fluorescent intensity of aflatoxins. In the process of resonance energy transfer, the efficiency depends on the overlap degree of emission spectra of donor and the absorption spectra of acceptor. The much more overlap, the higher efficiency [21]. Figure 4 shows the absorption spectrum of gold-labeled antibodies and the emission spectrum of aflatoxins. The prominent overlap between the absorption spectrum of gold-labeled antibodies and the emission spectrum of aflatoxins provides great probability of energy transfer from the excited aflatoxins to gold-labeled antibodies, and hence the intensively quenching of the fluorescence.

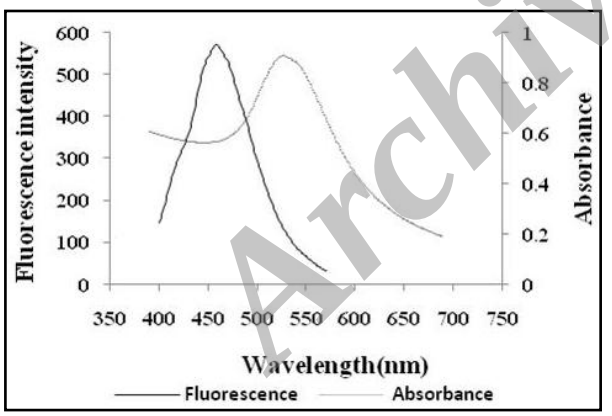

Fig. 4. Fluorescence emission spectra of aflatoxins and absorption spectra of gold-labeled antibodies.

Figure 5 exhibits the concentration influence of GNPs on the fluorescence of aflatoxins. In this experiment, a series of gold-labeled antibodies solutions with the final concentrations between 0-0.22 mgL^{-1} were prepared respectively. The as-prepared gold-labeled antibodies solutions were mixed with $2ngL^{-1}$ of aflatoxins solution, and then fluorescence spectra were examined. It can be found that all emission spectra were located at about 440 nm, but the fluorescence of aflatoxins was quenched obviously when mixed with gold-labeled antibodies. As expected, the fluorescence intensity of aflatoxins decreased with the increasing amount of the gold-labeled antibodies.

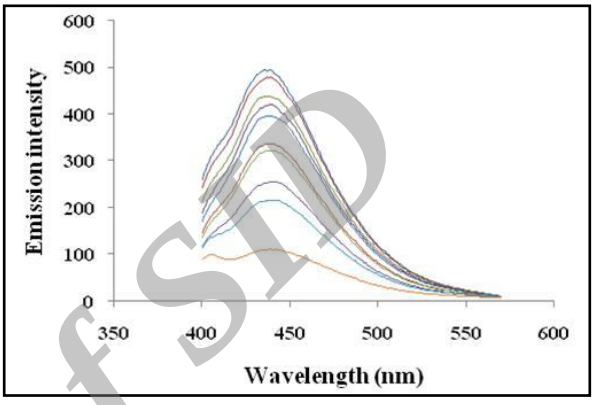

Fig. 5. Fluorescence emission spectra of aflatoxins added gold-labeled antibodies with different concentrations (from top to bottom 0–0.5 mg/L).

MCR-ALS Analysis of fluorescence data

In this work, data sets coming from fluorescence intensity of samples containing of AfB1 and AfG1 provided rank-deficient data sets, i.e., the number of distinguishable contributions is lower than the total number of chemical compounds in the data set. This may be the case of a process where two compounds have identical signals. As shown in Figure 6 the fluorescence spectra of both aflatoxins are severely overlapped. Rank-deficiency can be broken doing matrix augmentation of the mixture matrix with some standard aflatoxins matrices. Rules have been established proving that suitable full rank information appended to the rank-deficient matrix allows for the detection of all chemical contributions in the augmented data set [23].

The MCR-ALS procedure decomposes the augmented data matrices into two matrices: one matrix containing the excitation spectra as function of the concentration, and the second matrix containing the emission spectra common to all concentrations. Non-negativity constraint and equality constraint in spectral profiles were applied

in excitation and emission modes. In this particular case we used an excitation-wise augmented data matrix with emission spectra as initial estimates that we had obtained by individually analyzing standard matrices and choosing the most representative spectra of each analyte. The optimal solution with two components was obtained in the 20th iteration, with a fitting error (LOF) at the optimum of 0.129%. The percent of variance explained at the optimum (r^2) was 99.95%. Figure 6 shows typical results obtained by MCR-ALS for the augmented matrix of the aflatoxin mixtures and standard samples at different concentrations of gold-labeled antibodies.

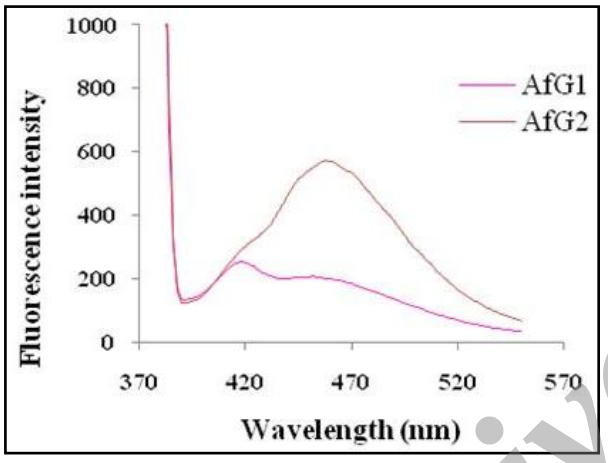

Fig. 6. Spectra profile of AFB1 and AFG1 from MCR-ALS.

CONCLUSIONS

The effect of gold nanoparticles on the fluorescence properties of aflatoxins B1 and G1 was studied in this paper. It was found that gold-labeled antibodies can quench the fluorescence of the aflatoxins owing to FRET. With the increasing of the concentration of GNPs, the fluorescence intensity of aflatoxins decreased correspondingly. As consequence of the relatively broad band that characterizes emission spectra EEM are usually characterized by several quite overlapped spectra that cannot be resolved by direct inspection of data. The strategy use in this study, constituted by MCR-ALS, provided to be easy to implement and to originate reliable results. MCR-ALS can be successfully applied to excitation–emission spectra matrices to simultaneous determination of AFB1 and AFG1.

REFERENCES

[1] Li P. W., Zhang Q., Zhang W., (2009), Immunoassays for aflatoxins. *Trends Anal. Chem*. 28: 1115–1126.

[2] Chu F. S., Mycotoxins. In: Food Borne Diseases, D. O. Cliver (Ed.), New York, *Academic Press*, 2002.

[3] Cervino C., Asam S., Knopp D., Rychlik M.,Niessner R., (2008), Use of isotope-labeled aflatoxins for LC-MS/MS stable isotope dilution analysis of foods. *J. Agric. Food Chem.* 56: 1873–1879.

[4] Tripathi S., Mishra H. N., (2009), A rapid FT-NIR method for estimation of aflatoxin B_1 in red chili powder. *Food Control*. 20: 840–846.

[5] Gomez-Catalan J., Pique E., Falco G., Borrego N., Rodamilans M., Llobet J. M., (2005), Determination of aflatoxins in medicinal herbs by HPLC. An efficient method for routine analysis. *Phytochem. Anal*. 16: 196–204.

[6] Edinboro L. E., Karnes H. T., (2005), Determination of aflatoxin B1 in sidestream cigarette smoke by immunoaffinity column extraction coupled with liquid chromatography/mass spectrometry. *J. Chromatogr. A.* 1083: 127–132.

[7] Lee N. A., Wang S., Allan R. D., Kennedy I. R., (2004), A rapid aflatoxin B_1 ELISA: Development and validation with reduced matrix effects for peanuts, corn, pistachio, and Soybeans. *J. Agric. Food Chem.* 52: 2746–2755.

[8] Zhao X., Hilliard L. R., John Mechery S., Wang Y., Bagwe R. P., Jin S., (2004), A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles. *PNAS.* 101: 15027–15032.

[9] Wang M., Chen S., Xia Y., Zhang Y., Huang W., Zheng J., Li Z., (2010), Nanoassemblies of colloidal gold

nanoparticles by oxygen-induced inorganic ligand replacement. *Langmuir* 26: 9351–9356.

[10] Kim Y. P., Oh E., Shon H. K., Moon D. W., Lee T. G., Kim H. S., (2008), Gold nanoparticle-enhanced secondary ion mass spectrometry and its bio-applications. *Appl. Surf. Sci*. 255: 1064-1067.

[11] Mayilo S., Kloster M. A., Wunderlich M., Lutich A., Klar T. A., Nichtl A., Kurzinger K., Stefani F. D., Feldmann J., (2009), Long-range fluorescence quenching by gold nanoparticles in a sandwich immunoassay for cardiac troponin T. *Nano Lett*. 9: 4558–4563.

[12] Kato N., Caruso F., (2005), A homogeneous, competitive fluorescence quenching immunoassay based on gold nanoparticle/polyelectrolyte-coated latex particles. *J. Phys. Chem. B.* 109: 19604-19612.

[13] Faulk W. P., Taylor G. M., (1971), Animmuno colloid method for the electron microscope. *Immunochem*. 8: 1081– 1083.

[14] Wold S., Esbensen K., Geladi P., (1987), Principal component analysis. *Chemom. Intell. Lab. Syst*. 2: 37-52.

[15] Massart D. L., Vandeginste B., Buydens L., Jong S., Lewi P., Smeyers-Verbeke J., In: Handbook of chemometrics and qualimetrics: *Part A, Elsevier, Amsterdam*, (1997).

[16] Saurina J., Leal C., Compa˜n´oR., Granados M., Tauler R., Prat M. D., (2000), Determination of triphenyl in seea-water by excitation-emission matrix fluorescence and multivariate curve resolution. *Anal. Chim. Acta*. 409: 237-245.

[17] Maeder M., Zilian A., (1988), Evolving factor analysis, a new multivariate technique in chromatography. *Chemom. Intell. Lab. Syst*. 3: 205-213.

[18] Windig W., Heckler C. E., Agblevor F. A., Evans R. J., (1992), Self-modeling mixture analysis of categorized pyrolysis mass spectral data with the SIMPLISMA approach. *Chemom. Intell. Lab. Syst*. 14: 195-206.

[19] Hermanson G. T., Mallia A. K.,Smith P. K., In: Immobilized affinity ligand techniques, San Diego. *Academic Press*, 1992.

[20] Johne B., Hansen K., Mark E., Holtlund J., (1995), Colloidal gold conjugated monoclonal antibodies, studied in the BIA core biosensor and in the nycocard immunoassay format. *J. Immunol. Methods*. 183:167–174.

[21] Gill R., Willner I., Shweky I., Banin U., (2005), Fluorescence resonance energy transfer in CdSe/ZnS−DNA conjugates: probing hybridization and DNA cleavage. *J. Phys. Chem. B.* 109: 23715-23719.

[22] Clegg R. M., (1995), Fluorescence resonance energy transfer. *Curr. Opin. Biotechnol*. 6: 103-110.

[23] Izquierdo-Ridorsa A., Saurina J., Herna´ndez-Cassou S., Tauler R., (1997), Second-order multivariate curve resolution applied to rank-deficient data obtained from acid-base spectrophotometric titrations of mixtures of nucleic bases. *Chemom. Intell. Lab. Syst*. 38: 183-196.

Cite this article as: A. Saidi *et al.*: MCR of the quenching of the EEM of fluorescence of Aflatoxins (B1, G1) by Gold nanoparticles.
Int. J.Nano Dimens. 6(1): 31-38, Winter 2015