Iranian Journal of Pharmaceutical Sciences
Spring 2012: 8(2): 129-134
ijps.sums.ac.ir

Original Article

Determination of Sulfasalazine in Sulfasalazine Tablets Using Silver Nanoparticles

Zahra Ramezani[a,b,*], Najmeh Dibaee[c]

[a]*Nanotechnology Research center, Faculty of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran*
[b]*Department of Medicinal Chemistry, Faculty of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran*
[c]*Chemistry Department, Research and Science Center of Azad University, Ahvaz Branch, Ahvaz, Iran*

Abstract

Resonance light scattering (RLS) intensity of silver nanoparticles in the presence of sodium dodecylsulphate was quenched on addition of sulfasalzine. The quenching extent was shown to be proportional to the sulfasalazine concentration. Effect of different parameters such as pH, volume and kind of buffer, silver nanoparticles concentration, sodium dodecylsulphate (SDS) concentration, and waiting time on RLS quenching were investigated. Calibration curve was linear in the range of 10 to 70 μg ml^{-1}. Detection limit was determined as 0.012 μg.ml^{-1}. The method was successfully applied to the determination of sulfasalazine in different batch numbers of sulfasalazine tablets. The results were compared with those determined by USP method. Reasonable agreements were observed.

Keywords: Resonance light scattering; Silver nanoparticles; Sulfasalazine.
Received: December 5, 2011; *Accepted:* February 12, 2012

1. Introduction

Silver nanoparticles, because of their physical and chemical properties, are extensively used in many areas such as optics [1], microelectronics [2], clinical diagnosis [3], sensors and so forth. The surface Plasmon resonance of silver nanoparticles which is due to the surface plasmon oscillation modes of conduction electrons which are coupled via the surface to electromagnetic radiations [4], make it ideal for a vast variety of applications.

*Corresponding Author: Zahra Ramezani, Golestan Highway, Nanotechnology Research center, Jundishapur University of Medical Sciences, Ahvaz, Iran.
Tel: (+98)6113738380; Fax: (+98)6113738381
E-mail: zramezani@ajums.ac.ir

The use of these nanoparticles in determination and detection of clinical and environmentally important compounds is recently of concern. Recently, determination of trace chlorine dioxide based on decrease in surface Plasmon resonance of silver nanoparticles is reported [5]. In another study, resonance light scattering (RLS) quenching induced by adsorption of nucleic acid on the surface of silver nanoparticles becomes the basis for the detection and determination of RNA and DNA [6, 7]. However, nano-analytical chemistry using silver nano-particles is extensively seen in the literature [8-10]. Some of them are based on silver reduction by

the analyte. Others are based on quenching nAg RLS.

Sulfasalazine is a synthetic drug and a combination of antibiotic (sulfapyridine) and an anti-inflammatory agent (5-aminosalysilic acid) [11] which are extensively used in the treatment of inflammatory bowel diseases such as rheumatoid arthritis, Crohn's disease and ulcerative colitis [12-14]. Post marketing surveillance study (PMS) which is performed in every country to monitor the safety of pharmaceuticals their release on the market covers an important part of the pharmaceutical researches. Different methods of PMS study are reported in the literatures. In the pharmacopeia, a variety of methods are presented for different pharmaceutical forms of a certain drug. Although these methods are approved and addressed in the assay of sulfasalazine still new, accurate, and precise methods with higher number of analysis per time is required.

The objective of this study is to introduce a precise and accurate method for the assay of sulfasalazine contents of tablets based on nanotechnology. To our knowledge, it is the first report on the analysis of sulfasalazine contents of tablets using silver nanoparticles.

2. Material & methods

2.1. Reagents

All reagents used in this study were from analytical reagent grade. Phosphoric acid, sodium hydroxide, sodium citrate, silver nitrate, and methanol all were purchased from Merck (Germany). Pure sulphasalazine powder and sodium dodecylsulphate (SDS) were obtained from Fulka. Sulphasalazine tablets (Mehrdaru Company, Iran) with different batch numbers were prepared from local pharmacies. De-ionized water was used throughout the study.

2.2. Instruments

RLS and fluorescence spectra were recorded using a RF-5301PC Shimadzue spectroflourimeter (Japan). IKA-WERKE mechanical stirrer (Germany) and VS1902MF (Vision corporation) water Circulator (Korea) were used to control stirring speed and temperature during nano-silver preparation. Particle size of as prepared nano silver was determined using Scatteroscope1 nanoparticle size analyzer, Qudix Corporation (Seoul, Korea). All pH were recorded using 920-WTW pH meter (Germany).

2.3. Silver nanoparticles preparations

Silver nanoparticles were prepared via chemical reduction method previously described with slight modification [6]. To briefly describe, 0.0158 g silver nitrate was placed in a 250 ml beaker, 40 ml distilled water was added and incubated at 80 °C in a

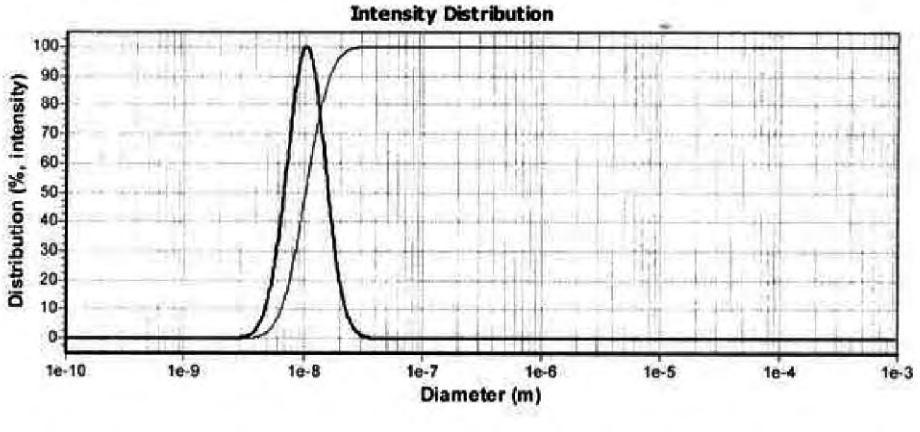

Figure 1. Intensity distribution view of PSA analysis of synthesized silver nano particles.

water circulator. Two ml of 1% sodium citrate solution was slowly added to the solution while stirring. The rate of citrate transfer was controlled so that its total volume was added to the beaker in about 30 min. The resulting suspension was transferred to a 50 ml volumetric flask and diluted to the mark with distilled water. This solution (2.0×10^{-4} g.ml^{-1} nano-silver) was stored in the refrigerator at 4 °C.

2.4. Sample preparation

Sulfasalazine Stock solution (250 µg.ml^{-1}): 0.0255 g of pure sulfasalazine powder were dissolved in 30 ml methanol and diluted to 100 ml using distilled water. Working solutions were prepared by step by step dilution of this stock solution in the desired ranges (0.01 to 100 µg.ml^{-1}).

Tablet preparation for sulfasalazine assay: tablet solutions were prepared as described in USP [15]. Shortly, twenty tablets from each batch number were completely grinded into the powder. About 0.17 g of this powder, equivalent to 150 mg sulfasalazine, was transferred to a 100 ml conical flask, and 50 ml of 0.1 N sodium hydroxide was added and completely mixed. The resulted solution was filtered and the first 20 ml of filtrate were discarded. The filtrate was used for the determination of sulfasalazine contents by both USP method [16] and the proposed method after dilution to appropriate concentrations with appropriate solvents of each method. Concentration of sulfasalazine in the filtrate is suggested to be 1500 µg.ml^{-1} according to the lable.

Table1. Method validation parameters.

Linear Range		**10-70 µg.ml^{-1}**	
Line equation(R^2)		**y= 9.363x+257.3(0.989)**	
D. L.		0.0127 µg mL-1	
CV			
	10[a]	1.35[b]	2.16[w]
	20[a]	2.06[b]	1.87[w]
	50[a]	1.36[b]	0.67[w]

[a]Concentration in µg.ml^{-1}; [b]between days; [w]within days.

2.5. Proposed method procedure

Three ml of 2×10^{-4} g.ml^{-1} of nano silver, 2 ml of phosphate buffer pH=8, 0.4 ml of SDS (8×10^{-7} M) and different amounts of standards and/or appropriate amounts of tablet solution (explained in section 2.4) were added to a 10 ml volumetric flask and the solution was made to volume using distilled water. RLS of these solutions were recorded using synchronous technique. Blank solutions were prepared in the same way except sulfasalazine was absent.

3. Results and discussion

3.1. Silver nano particles characterization

Silver naoparticles were characterized by observing the UV-Vis spectra in the range of 200-700 nm. As it is reported in the literatures [16, 17], surface Plasmon resonance band of nano silver is a good indicative of its particle size. So a maximum observed at 430 nm and

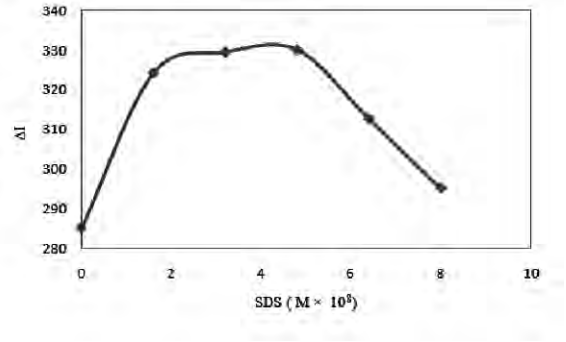

Figure 2. Effect of sodium dodecylsulphate (SDS) concentration on the change in SPR intensity of silver nano particles in the presence of 40 µg.ml^{-1} sulfasalazine.

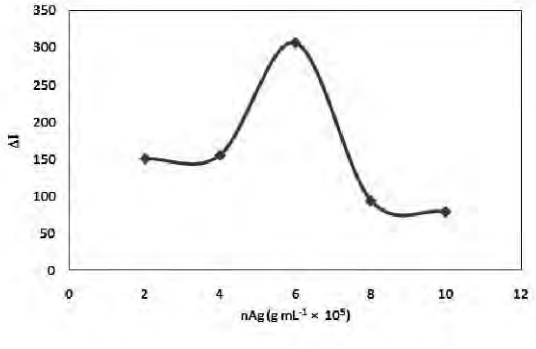

Figure 3. Effect of pH on the change in RLS intensity of silver nano particles in the presence of 40 µg.ml^{-1} sulfasalazine.

Table 2. Analysis of sulfasalazine content in the tablets with different batch numbers determined by both proposed and USP methods

Sample No.	USP	Proposed method	%Recovery
1	494.544±7.44	507.355±7.56	102.69
2	473.654±4.35	533.484±11.46	112.63
3	514.142±9.95	498.045±9.95	96.869

yellow color of the solution indicates that nano-silver was prepared. Particle sizes of the synthesized nanoparticle were also determined by observing intensity distribution data obtained by particle size analyser demonstrated in Figure 1, which indicates that the mean sizes of particles are around 30 nm.

The stability of refrigerated nAg was also evaluated by measuring the change in the absorbance of the nAg solution at 430 nm and the extent of wavelength shift with time. No shift in wavelength that is an indication of nAg aggregation and no absorbance changes within 3 months confirmed that particles are stable while keeping in the refrigerator at 4 °C.

3.2. Evaluation of the method

RLS of nAg in the presence of sodium dodecylsulphate was decreased on addition of sulfasalazine. However, this decrease was proven to be proportional to sulfasalazine concentration and can be the basis of its assay. In order to optimize the response, the effect of different parameters such as the amount of nanoparticles, pH, volume and kind of buffer, SDS concentration and time on nano-silver RLS quenching in the presence of sulfasalazine was investigated. At first it was shown that SDS increases RLS intensity of nAg compared to nAg alone. Adsorption of SDS on the surface of nAg causes more dispersion of the nanopartilces and decreased their aggregation. As the result changes in RLS intensity in the presence of sulfasalazine becomes higher. So, the effect of SDS concentration on the signal difference was investigated. As it is obvious in Figure 2, the best concentration of SDS is in the range of 1.6×10^{-8} to 4.8×10^{-8} M.

Figure 3 shows that the increase in pH increases the difference in RLS intensity till pH 8 while sulfasalazine is present. So, pH 8 was selected as the optimum pH. In this pH the anionic form of sulfasalazine is easily adsorbed on the surface of nAg. Different buffer systems, namely acetate, citrate, carbonate, borate, and phosphate were tested and among them phosphate buffer showed the most reliable results. Volume of phosphate buffer solution was also optimized and the 2 ml in 10 ml volumetric flasks was showed to be adequate.

Figure 4 shows that 6×10^{-5} g.ml^{-1} of silver nanoparticle produces the highest signal changes in the presence of sulfasalazine. It was indicated that after this concentration a slight decrease in △I is observed.

The effect of different waiting times after mixing all reagents was evaluated. It was concluded that signals must be recorded within 15 min. After that time, a slight decrease in the signal was observed. So the analysis time should not exceed 15 min.

Figure 5 shows the decrease in RLS intensity in the presence of different concentration of sulfasalazine prepared

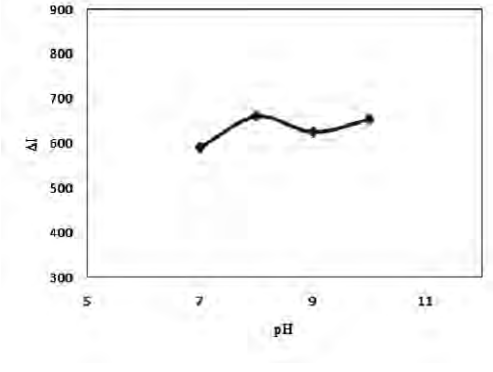

Figure 4. Effect of nano silver concentration on the change in RLS intensity of silver nano particles in the presence of 40 μg.ml^{-1} sulfasalazine.

according to procedure described in section 2.5. As it is demonstrated in Figure 5, sulfasalazine does not show any emission at working conditions but nAgs RLS intensity quenches more with the increase in sulfasalazine concentration. Adsorption of sulfasalazine on the surface prevents nAg electrons to interact with the incident light. So, this prevention is increased as the concentration of sulfasalazine increases. Table 1 demonstrates the figure of merit of the method as a quantitative technique for the analysis of the sulfasalazine. The table also indicates good precision.

The accuracy of the method was evaluated by determining sulfasalazine contents of tablets with different batch numbers by both proposed methods and those reported in USP. Table 2 illustrates that the proposed methods is reliable and can be successfully used for the assay of this pharmaceutical form of sulfasalazine.

It is to be mentioned that no interferences from tablet excipients was observed.

4. Conclusion

As the results indicated, silver nanoparticles can be used in the determination of sulfasalazine in sulfasalazine tablets with reasonable reliability. To our knowledge, it is the first report on use of nano silver in the assay of sulfasalazine tablets.

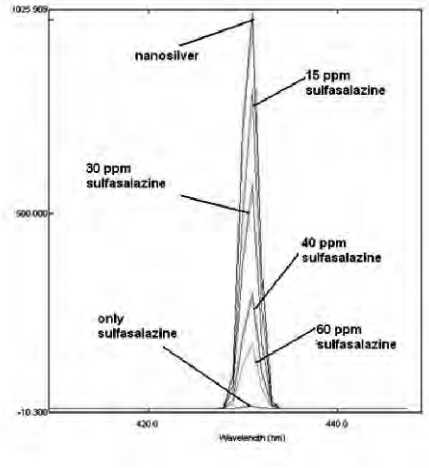

Figure 5. RLS spectra of silver nano particle+SDS and increasing concentration of sulfasalazine (15 to 60 $\mu g.ml^{-1}$).

Acknowledgements

We acknowledge Khozestan environmental protection agency, Ahvaz branch. Collaboration of their lab personnel especially Mr. Alanezhad and Miss Khaksar is gratefully acknowledged. Our thanks also go to nanotechnology research center of Jundishapur University of Medical Sciences.

References

[1] WU AG, Cheng WL, Li Z, Jiang JG, Wang E. Electrostatic-assembly metallized nanoparticles network by DNA template. *Talanta* 2006; 68: 693-9.

[2] Chau, Yuan-Fong; Tsai, Din Ping. Three dimentional analysis of silver nanoparticles doping effects on super resolution near field structure. *Optic Commun* 2007; 269: 389-94.

[3] Guo-Feng XUa, Hui LIa, Cui ZHANGa, Zi-Yin SUNb, Wen-Ying ZHONGa, Dan-Ke XUb, Hong-Yuan CHENb. Research and application of visual detection based on quantum dots coupled with silver enhancement. *Chinese J Anal Chem* 2010; 38: 1383-7.

[4] Stepanov AL. Quantitative analysis of surface Plasmon interaction with silver nanoparticles. *Optic Letters* 2005; 30: 1524-6.

[5] Cai Yan Kanga, b, Dan Li Xia, , Yuan-Yuan Chenb, Zhi Liang Jiang. Determination of trace chlorine dioxide based on the Plasmon resonance scattering of silver nanoparticles. *Talanta* 2008; 74: 867-70.

[6] Xu K, Georganopoulou DG, Hill HD, Mirkin CA. Homogeneous detection of nucleic acids based upon the light scattering properties of silver-coated nanoparticle probes. *Anal Chem* 2007; 79: 6650-4.

[7] Zheng J, Wu X, Wang M, Ran D, Xu W, Yang J. Study on the interaction between silver nanoparticles and nucleic acids in the presence of cethyltrimethylammonium bromide and its analytical application. *Talanta* 2008; 74: 526-32.

[8] Liang Y, Gong JL, Huang Y, Zheng Y, Jiang JH, Shen GL, Yu RQ. Biocompatible core-shell nanoparticle-based surface-enhanced Raman scattering probes for detection of DNA related to HIV gene using silica-coated magnetic nanoparticles as separation tools. *Talanta* 2007; 72: 443-9.

[9] Cao YC, Jin R, Thaxton CS, Mirkin CA. A two-color-change, nanoparticle-based method for DNA detection. *Talanta* 2005; 67: 449-55.
[10] Nazeeruddin MK, Angelis FD, Fantacci S, Selloni A, Viscardi G, Liska P, Ito S, Takeru B, Grätzel M. Combined experimental and DFT-TDDFT computational study of photoelectrochemical cell ruthenium sensitizers. *J Am Chem Soc* 2005; 127: 16835-47.
[11] Lay JD, Hong CC. Sulfasalazine suppresses drug resistance and invasiveness of lung adenocarcinoma cells expressing AXL. *Cancer Res* 2007; 67: 3878-.
[12] Buxbaum J. Sulfasalazine in arthritis: current practice and future directions. *Int Med Spec* 1990; 11: 79-90.
[13] Weiss JS, Gupta AK, Gupta JEAK, Ellis CN, Siegel MT. Oral ulcers and cobblestone plaques. Oral Crohn's disease (CD). *Arch Dermatol* 1991; 127: 889-92.
[14] Conaghan PG, Lehmann T, Brooks P. Disease-modifying antirheumatic drugs. *Curr Opin Reumatol* 1997; 9: 183-90.
[15] USP 2000, official Monographs, 1344:1629-30.
[16] Sileikaite A, Proscevas I, Puiso J, Juraitis A, Guobiene A. Analysis of silver nanoparticles produced by chemical reduction of silver salt solution. *Mater Sci* 2006; 12: 287-91.
[17] Fang Y. Optical absorption of nanoscale colloidal silver: aggregate band and adsorbate-silver surface band. *J Chem Phys* 1998; 108: 4315-8.