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The role of silver nanoparticles synthesized from *Brassica olerace* for determination of Aspirin in pharmaceutical samples by standard additions method

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In this study, silver nanoparticles (Ag NPs) were prepared from *Brassica oleracea* (BO) leaflet extract (BO-AgNPs) and applied for to determination of Aspirin in pharmaceutical samples through selective and sensitive spectrophotometric standard additions method. The measurements of UV-Visible, X-ray diffraction (XRD), Fourier transform infrared (FTIR) and scanning electron microscopy (SEM) have been used to confirm the formation of BO-AgNPs, revealing a prominent absorption band at 450 nm, crystallinity, and an agglomerated morphology having an average particle size of 29 nm. Aspirin in Acifein, Aspirin, Acylpyrin and Disprin tablets was determined through the complexation with BO-AgNPs, revealing a linear range of 1-10 mg/10 mL and limit of detection (LOD) as 0.09, 0.46, 0.69 and 0.99 mg/10 mL, respectively. An average percent recovery for four samples were found to be 90, 99, 101 and 99 % and relative standard deviation (RSD) as 1.6, 0.6, 0.3 and 1.4, respectively. The suggested method was successfully used for the quantitative analysis of pharmaceutical tablets, which opens up the possibility that Ag NPs could be used to determine biological molecules when interferences from real samples are present.

Keywords: Silver nanoparticles; Brassica oleracea; Aspirin.

1. INTRODUCTION

Aspirin, also referred to as acetylsalicylic acid, is one of the oldest medications that is still vital to contemporary therapeutics [1]. It is frequently used in pharmaceutical formulations to treat inflammation, fever, muscle pain, and headaches [2, 3]. The world uses this substance. Aspirin's effectiveness as an analgesic and an antipyretic was quickly acknowledged [4, 5]. It is used to treat acute conditions like arthralgia, headaches, myalgia, calm down bacterial or viral excitements, and in other mild analgesia cases [6, 7]. Gastrointestinal disturbances like nausea, dyspepsia, and vomiting are the most typical side effects of therapeutic doses of aspirin [8]. If the patient is suspected of taking more aspirin than recommended, repeated oral doses of activated charcoal may be administered [9, 10]. If the plasma aspirin concentration is higher than recommended, intravenous sodium bicarbonate may be administered to improve urinary salicylate excretion [11,12]. Due to its widespread consumption and both its therapeutic and toxic effects, this substance is a drug that is constantly being researched. and its antipyretic properties have been known for over ten years [13, 14, 15]. In order to effectively determine the presence of aspirin, a highly selective and cost-effective method must be used [16, 17].

Early in this decade, the study of nanoparticles became more popular due to their effective application in opto-electronic devices, clinical settings, and the medical field, particularly in the areas of diagnosis, optical switching, drug delivery, and cancer treatment [18, 19]. More specifically, silver-based nanomaterials made of noble metals have drawn an excessive amount of attention. Ag NPs' prominent surface plasmon resonance is the cause of this (SPR) [20, 21, 22]. The controlled and methodical changes in geometry of nanomaterial are made possible by the coupling of pharmaceutical advancements with SPR, which is a sensitive function of the nanocomposite structure [23, 24]. These properties enable the Ag NPs to be used in the development of novel, enzyme-free aspirin determination methods [25]. Additionally, the process used to create the silver nanoparticles is crucial [26]. As numerous physical and chemical preparation techniques that are frequently expensive and harmful to the environment have been reported [27, 28]. Researchers discovered a different method for producing silver nanoparticles for this purpose, specifically from plant extracts, which has been proven to be less expensive, effective, and safe [29]. Brassica oleracea (a medicinal and nutritional plant) was chosen in this study for the preparation of Ag NPs based on this supposition that the polyphenolic compounds in this plant would act as a reducing as well as stabilizing agent in nanoparticle formation and also would have the ability to interact with Aspirin molecules on the surface of BO-AgNPs [30, 31]. In this study, the spectrophotometric detection of Aspirin in pharmaceutical samples (Acifein, Aspirin, Acylpyrin, and Disprin) was carried out using a simpler, faster, and more reliable standard additions method using *Brassica oleracea* leaflet extract as the biosynthetic source of silver nanoparticles. The objective of the study was to develop an efficient and highly reliable approach along the utilization of Ag NPs for the Aspirin determination in pharmaceutical tablets. This work can add up further improvement in sensing of analytes in both laboratorial as well as clinical combinations.

2. EXPERIMENTAL

2.1. Chemicals and reagents

All the reagents used in our work were of high purity with requiring no additional purification. Silver nitrate ($AgNO_3$) and Aspirin were acquired from MERCK©, Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were obtained from Sigma-Aldrich©. Acifein, Aspirin, Acylpyrin and Disprin were obtained from local pharmaceutical company. Leaflets of *Brassica oleracea* plant were bought from the local plant farm of Mardan, Pakistan. Stock solutions were prepared in deionized (DI) water for 1 M silver nitrate and Aspirin, dilute 20% HCl and NaOH, each tablet per liter of Acifein, Aspirin, Acylpyrin and Disprin.

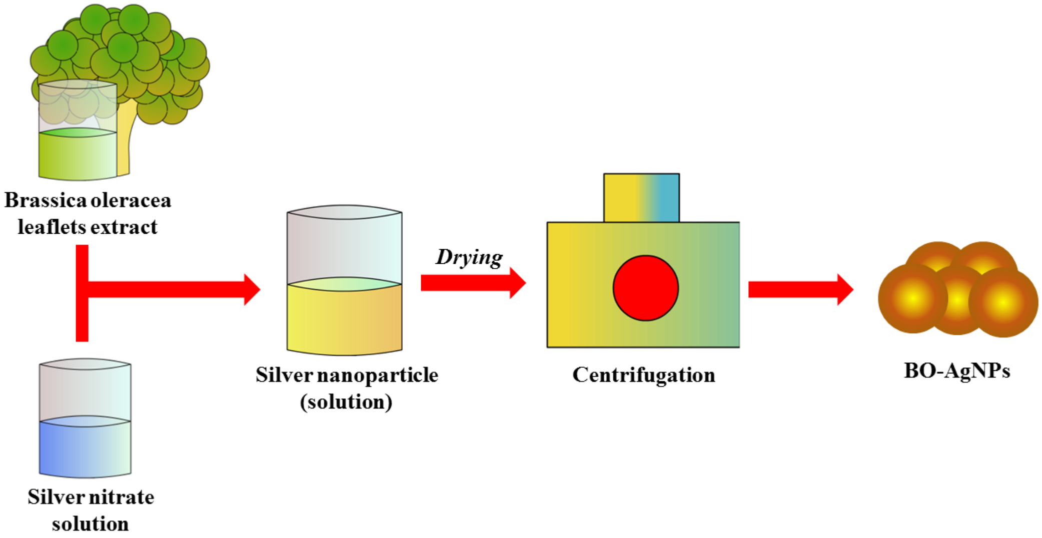
2.2. Instrumentation

The UV-Visible spectrophotometer 1800 from Shimadzu, Japan, was used to record all absorbance measurements. The FTIR thermo scientific nicolet 6700 spectrometer was used to examine the functional groups found in BO-AgNPs. The Bruker D8 advance X-ray diffractometer was used to examine the crystallinity of BO-AgNPs. Surface morphology and particle size of the BO-AgNPs was examined through JEOL JSM-7800F for SEM and JEM-3100F for TEM, respectively. The pH measurements were performed using a glass-calomel electrode and a digital Metrohm pH-meter.

2.3. Preparation of BO-AgNPs

Dry *Brassica oleracea* leaflets (50 g) were washed with deionized water before being powdered to obtain a leaflet extract. The powdered leaflets were then added to a beaker with 500 mL of deionized water and boiled for 20 minutes (min) to produce an extract with a greenish color. Filtration was used to separate the extract of the leaflets, which was then thoroughly purified and stored for later use.

In order to make BO-AgNPs, we stirred 50 mL of a 1 M silver nitrate solution at 300 rpm for 50 minutes on a heated magnetic stirring (80 to 100 ºC). The plant extract solution (1 mL) was dropwise added, stirred for 3 hours (h) at 600 rpm, and then left to rest for about 2 h. The solution turned out to be a dark golden brown, indicating that all of the Ag^+ had been converted to Ag^0. After being cleaned with deionized water and ethanol, the BO-AgNPs were desiccated with spinning for 30 minutes at 6000 rpm before being stored. The formation of BO-AgNPs from Brassica oleracea is depicted in Scheme 1.



Scheme 1 Schematic diagram for possible mechanism of BO-AgNPs preparation.

2.4. Stability study

Heating the sample, adjusting the pH, and monitoring the UV bands at regular times were all used to investigate how these factors affected the stability of BO-AgNPs. UV-Visible absorption spectra at 450 nm were taken before and after heating (refluxing) a BO-AgNPs solution at 60 ºC for 1 hour to examine the impact of heat. To examine the time period impact, UV-Visible absorption spectra of BO-AgNPs were obtained at 0, 2, 4, 6, 8, and 10 h. 100 mL of BO-AgNPs were split amongst six separate containers in order to analyze their response to varying pH levels. The pH was brought into close proximity to 2, 4, 6, 8, 10, and 12 by progressively introducing HCl and NaOH solution. Before recording UV-visible absorption bands at 450 nm, each solution was diluted to a 1:2 ratio.

2.5. Determination of Aspirin by BO-AgNPs

For the purpose of complexing Aspirin with BO-AgNPs (Aspirin-Ag NPs), 1 M of the freshly made AgNPs were combined with 1 M of Aspirin solution, and the mixture was stirred for 60 min at room temperature as monitored by UV–visible spectrophotometric scanning in the range of 300–800 nm against the reagent blank.

The pharmaceutical samples (Acifein, Aspirin, Acylpyrin, and Disprin) were combined separately with Ag NPs solution in a 1:1 ratio for the process of standard additions method. For each sample, 50 mL of these solutions were divided among seven different flasks (a total of 28 flasks). For each sample, aspirin solution containing 0, 1, 3, 5, 7, 9 and 10 mg/10 mL was added to the six flasks. At a wavelength of 450 nm, the UV-visible absorption spectra of each solution were measured. The Acifein in pharmaceutical tablets was determined by extrapolation points via regression equations using the plot of absorbance vs. concentration [32].

3. RESULTS AND DISCUSSION

3.1. Characterization of prepared BO-AgNPs

According to Figure 1, UV-Visible spectroscopy was used to analyze the absorbance spectra of BO-AgNPs and *Brassica oleracea* leaflets extract. The formation of BO-AgNPs was confirmed by an absorption peak in the BO-AgNPs at 450 nm, which is caused by the SPR of metallic Ag NPs. The absence of a plant's metallic SPR also prevented the observation of any absorption bands in pure *Brassica oleracea* leaflet extract at a particular wavelength range (300-800 nm).

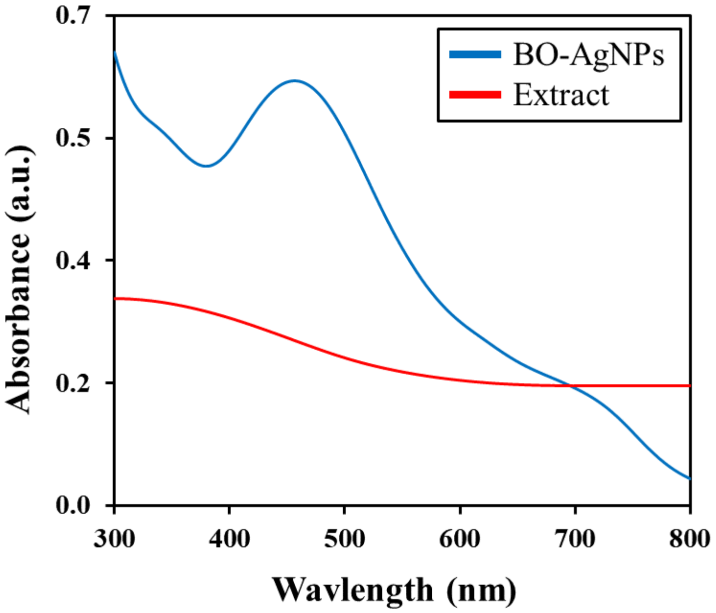


Figure 1 UV-Visible absorbance spectra of *Brassica oleracea* leaf extract and prepared silver nanoparticle

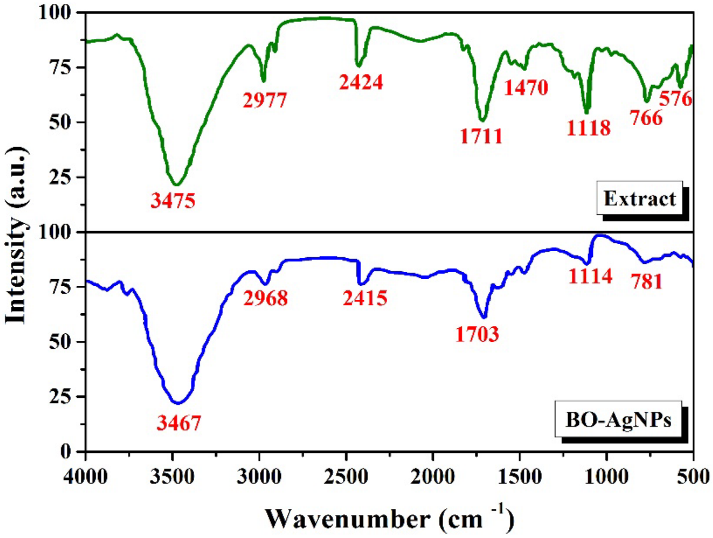


Figure 2 FTIR spectra of *Brassica oleracea* leaf extract and BO-AgNPs.

3.2. *FTIR analysis*

Figure 2 illustrates the FTIR absorption spectra results for the samples, which revealed similarities between the spectra before and after bio-reduction with some minor peak position shifts that amply demonstrated the presence of the extract residual as a reducing agent in the reaction mixture. Peak similarity suggested the presence of similar functional groups. Peaks at 3475 cm^{-1} are visible in the spectrum of the plant extract, indicating that the carboxyl group is being stretched O-H. The C-H stretching bonds of alkanes are also represented by the peaks seen at 2977 and 2424 cm^{-1}. The C=C aromatic conjugates and N-H deformation are represented by the peaks that were observed at 1711 and 1470 nm, respectively. Protein C-N stretching vibrations are attributed to the sharp peak at 1118 cm^{-1}. A thin band in the 700-600 cm^{-1} spectrum represents the C-S stretching. These bands' positions are very similar to those of native proteins, as reported. Contrarily, the FTIR-spectrum of biosynthesized Ag NPs from *Brassica oleracea* leaflet extract shows the presence of numerous fundamental groups involved in the transformation of silver ions into silver nanoparticles. The Ag NPs' IR spectrum shows that many fundamental groups are absent, along with peaks of lower intensity. Silver ion reduction is thought to be the cause of the bands' disappearance and decrease in intensity.

3.3. *XRD analysis*

Phase purity and BO-AgNPs crystalinity is confirmed by Figure 3. A comparison was made between the pattern and the silver standard powder diffraction card (JCPDS), file number 04-0783. The as-prepared Ag NPs display two values of diffraction peaks at 38.10, 44.28, 64.43, and 77.45 degrees, which are determined to be caused by silver metal and correspond to the hkl values (lattice planes) (111), (200), (220), and (311) of silver, in accordance with the experimental pattern. The resulting particles of the prepared sample are thus Ag NPs with face-centered cubic crystal structure, as confirmed by the XRDD study. The Debye-Scherrer equation states that the typical size of a nano crystallite is 28 nm. Two more peaks can be seen in the XRD pattern. These detected peaks could be the result of impurities and trace amounts of $AgNO_3$ that weren't reduced and ended up in the sample.

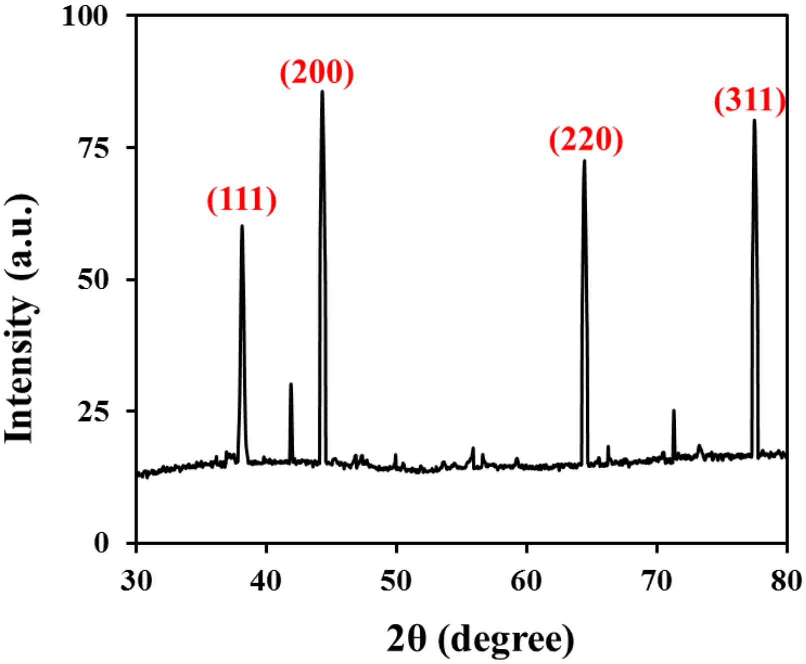


Figure 3 XRD pattern of Ag NPs.

3.4. Surface morphology analysis

Figure 4a displays the results of a SEM analysis of freshly prepared BO-AgNPs, which revealed the presence of very small nanoparticles. The SEM image clearly demonstrates the erratic distribution of grains with rough surfaces. In order to assess the nanoparticle size, the diameter of each nanoparticle was measured three different ways using ImageJ software. The average diameter of the particles was next established. Due to the uneven distribution of nanoparticles, the aspect ratio of the particles was calculated and a size distribution histogram was plotted. The micrographs in Figure 4b demonstrate an average particle size of 29 nm.

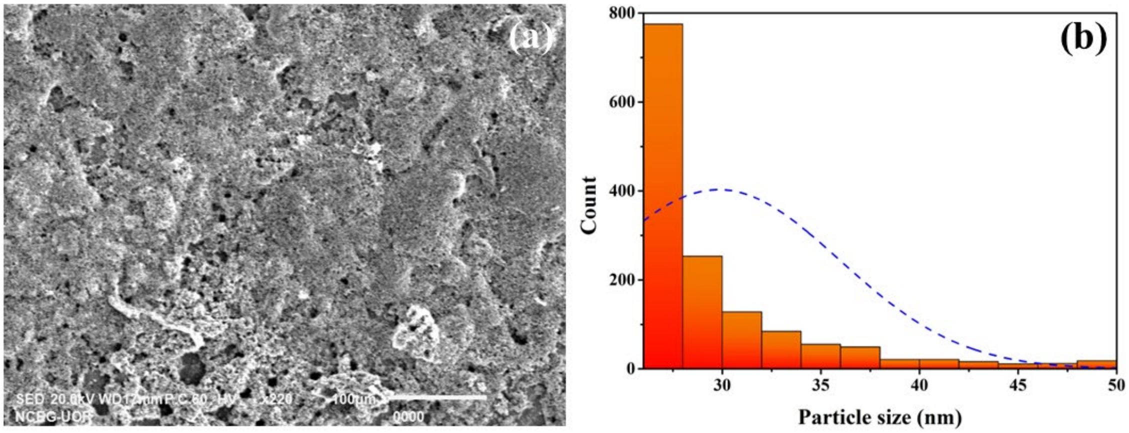


Figure 4 The SEM image (a) and particle size distribution (b) of BO-AgNPs.

3.5. Stability study

Heat effect is indicated in Figure 5a, showing increase in an absorbance after 1 h, but after 3 h, it decreased. This is might be as heat can distort the outer stabilizing molecular shell due to Ag NP shape and concentration variations after 3 h. Whereas, Figure 5b shows absorbance spectra collected over time. SPR bands show that BO-AgNPs' absorbance increased until 36 h and decreased afterward. This showed that plant extract reduced the silver ions completely in 36 h, but it distorts the stabilized shell around the Ag NPs afterwards, hence caused aggregation of silver atoms. In the last, a solution's pH affects Ag NP shape and size during preparation. Figure 5b shows the surface Plasmon peak changing with pH. When pH decreases, surface Plasmon peak absorbance values decrease. Thus, raising the solution's pH produces smaller Ag NPs, while lowering it produces larger ones.

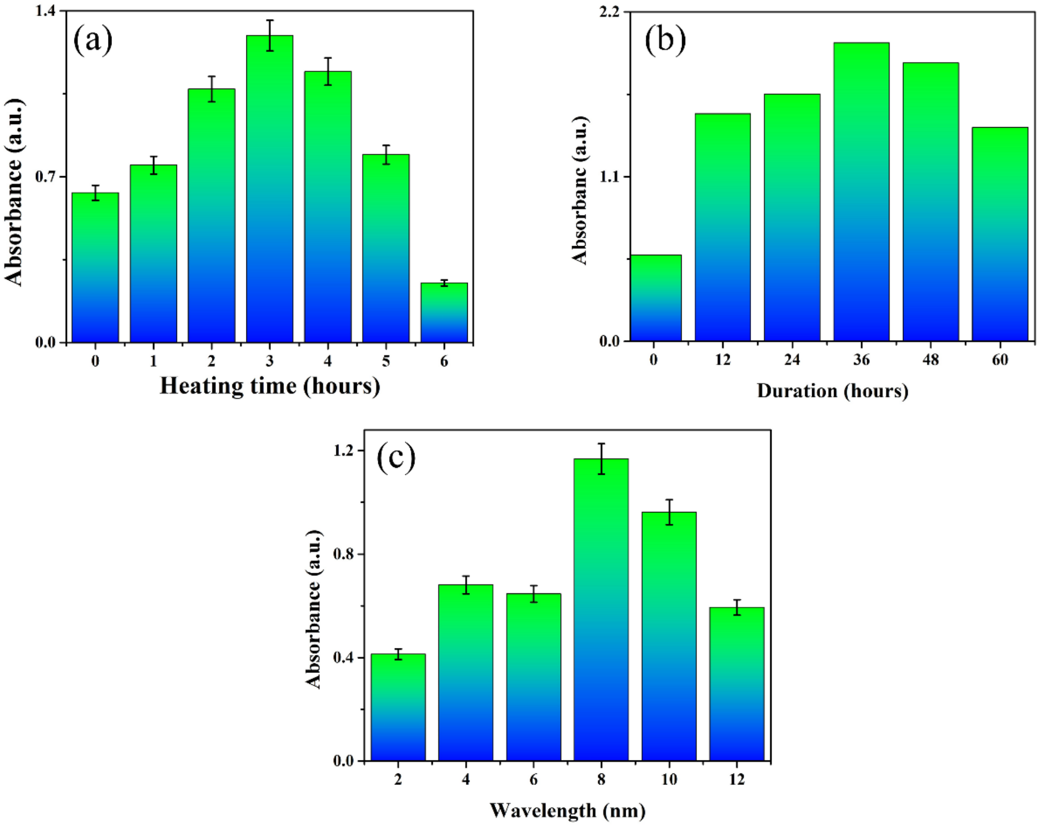


Figure 5 UV-Visible absorption of silver nanoparticle after (a) heating at various hours, (b) different durations (c) change in pH

3.6 Determination of Aspirin by prepared silver nanoparticle

By complexing Aspirin with BO-AgNPs, pharmaceutical samples' aspirin concentrations were determined. Aspirin molecules binding with Ag-NPs changes Plasmon resonance due to energy and electron transfer processes, possibly forming a new complex. Aspirin in pharmaceutical samples is determined by spectrophotometric standard additions using this complexation. Figure 6 shows Aspirin, BO-AgNPs, and Aspirin-AgNPs complex absorbance bands from 300-800 nm. The figure shows that all spectra have greater differences having no absorption sharp peak for Aspirin molecule has been observed in specific range. Spectral change from 430 to 450 nm confirms Aspirin-BO-AgNP complexation. The interaction of analytes with BO-AgNPs (surface coating) changes nanoparticle size, which changes dielectric constant.

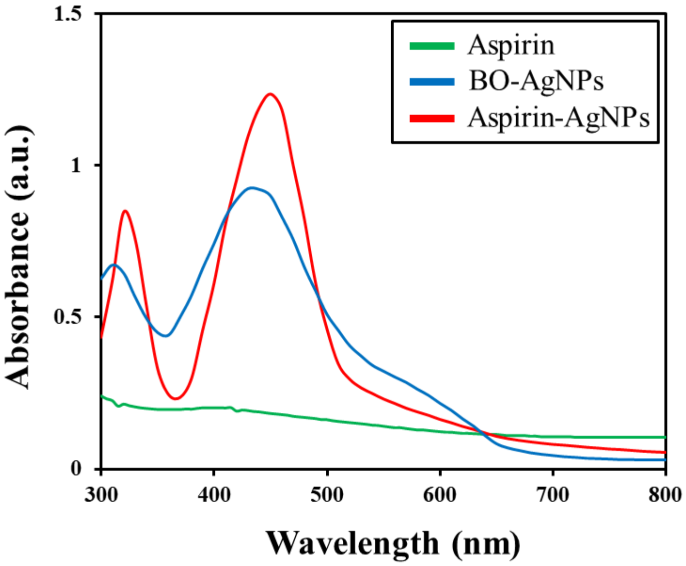


Figure 6 UV-Vis. spectra of Aspirin, BO-AgNPs and complex.

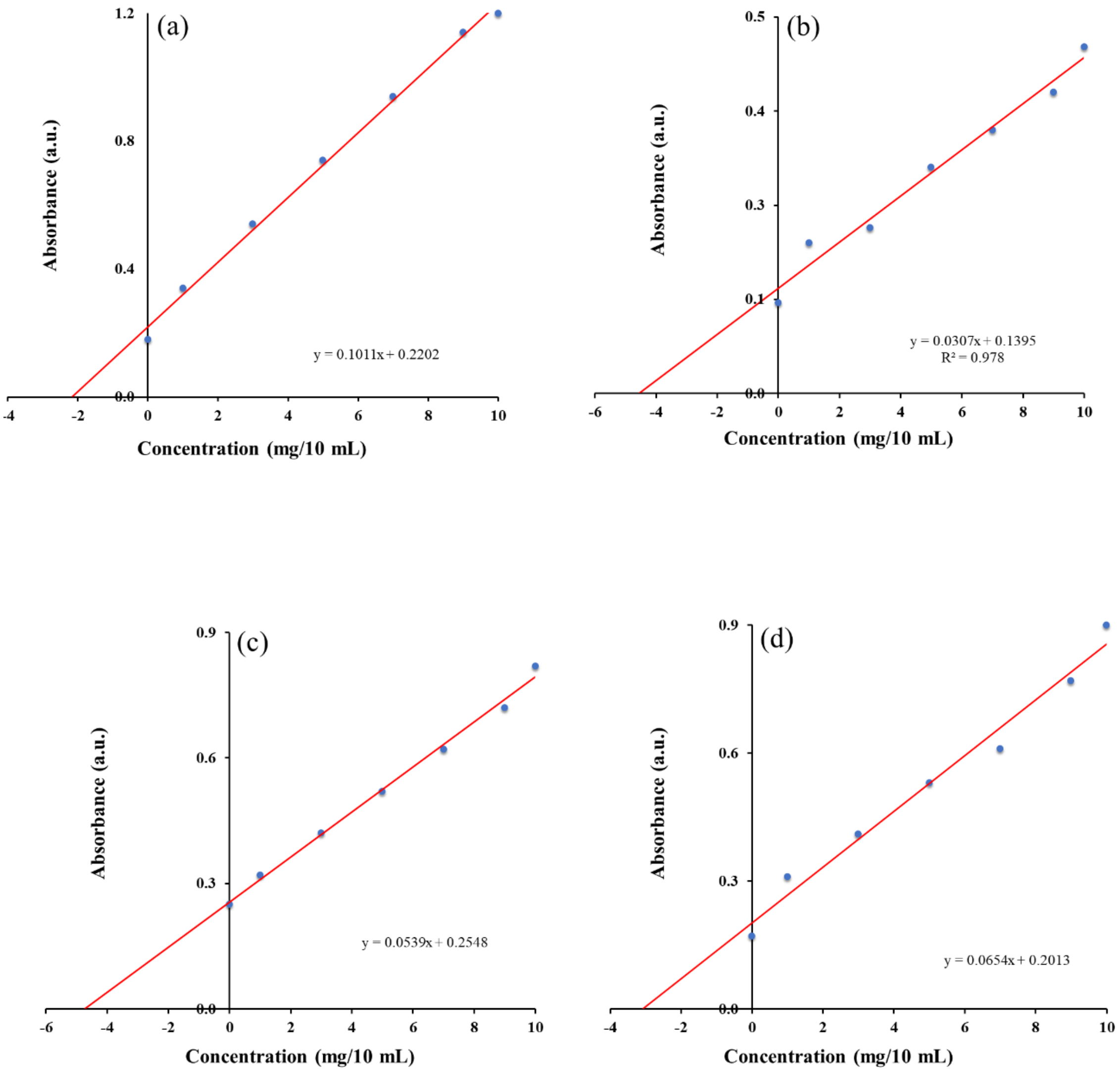


Figure 7 Plot for determination of Aspirin in (a) Acifein (b) Aspirin (c) Acylpyrin and (d) Disprin.

Table 1 Analysis of Aspirin in pharmaceutical samples (n=3).

Tablet	Amount present (mg/L)	Amount Found (mg/L)	Recovery (%)	RSD
Acifein	250	225	90	1.6
Aspirin	500	494	99	0.6
Acylpyrin	500	502	101	0.3
Disprin	300	296	99	1.4

Table 2 Analytical parameters for Aspirin in pharmaceutical samples.

Parameter	Acifein	Aspirin	Acylpyrin	Disprin
Linear range (mg/10 mL)	1–10	1–10	1–10	1–10
Limit of detection (mg/10 mL)	0.09	0.46	0.69	0.99
Limit of quantification (mg/10 mL)	0.28	1.40	2.10	3.00
Standard deviation (mg/10 mL)	0.01	0.02	0.01	0.02
Relative standard deviation (%)	0.24	0.14	0.24	0.39

Table 3. Accuracy and precision for Aspirin determination

S.No	Amount Taken (mg/10 mL)	Amount found (mg/10 mL)	% Recovery	Average % Recovery	RSD
1	1	0.98	98	101.67	9.9
	1	0.94	94		
	1	1.13	113		
2	3	3.01	100	99.11	5.7
	3	3.12	104		
	3	2.79	93		
3	5	5.10	102	96.93	10.0
	5	5.15	103		
	5	4.29	85		

The light scattering effect in turbid systems can have a significant impact on analyte determination because of change in absorption wavelengths due to interferences in chemical analysis, particularly in the medical or biological fields. When this occurs, a standard spectrophotometric method is created for the selective determination of aspirin even in the presence of background interferences. The absorption wavelength of 450 nm was used to determine the number of aspirin in the tablets Acifein, Aspirin, Acylpyrin, and Disprin. This wavelength is also used to plot the calibration curve between the number of aspirin in each pharmaceutical sample versus its concentration. Utilizing a regression equation to extrapolate points from the calibration curve for each individual pharmaceutical sample, as shown in Figure 7, the proposed method was used to determine the concentration of Aspirin. The summarized results for the tablets of Acifein, Aspirin, Acylpyrin, and Disprin were found and tabulated in Table 1 for the Aspirin determination, showing the percentage recoveries 90, 99, 101 and 99 %, respectively, while RSD values were 1.6 and 1.4, respectively. The RSD values are close to 10 and the percent recoveries are close to 100, indicating that the proposed method could be used to accurately determine the presence of aspirin in samples. Table 2 also displays the analytical parameters deduced from the calibration curves, and it shows that the linear range for the tablets of Acifein, Aspirin, Acylpyrin, and Disprin is 1–10 mg/10 mL. The sensitivity of the method is verified by the values of LOD, LOQ, and standard deviation (SD) less than 1, and values of RSD less than 10. Recovery study procedures were carried out to ensure that the suggested method was carefully examined. Table 3 displays the precision and accuracy determined using the proposed method. It shows that the average percent recovery for three aspirin samples was 101.67, 99.11, and 96.93%, respectively. The RSD was found to be 9.9, 5.7, and 10.0, respectively, which is less than 10, indicating nearly perfect accuracy results.

3 CONCLUSIONS

In this study, BO-AgNPs were successfully synthesized using an aqueous solution of Brassica oleracea leaf extract as a reducing and stabilising agent and $AgNO_3$ as a precursor. The main elements in the plant extract that contributed to the formation and stabilisation of BO-AgNPs were polyphenolic compounds. Multiple analyses, including FTIR, XRD, TEM, and UV-Visible spectrophotometer, show that BO-AgNPs were successfully formed. By combining spectrophotometry and the standard additions method, the synthesised BO-AgNPs efficiently and selectively determined the Aspirin in Acifein, Aspirin, Acylpyrin, and Disprin tablets, showing LOD 0.09, 0.46, 0.69, and 0.99, respectively. The aforementioned techniques were backed up by their lowest SD along with an excellent recovery value, making the proposed method suitable for analysis in blood, serums and clinical combinations, as well as biomarker for the diagnosis of diseases identified by change in Aspirin level in the living body. Our research can be of great assistance to analytical researchers for identifying biomolecules using Ag NPs through analytical as well as spectroscopic approach, predominantly in the real environment. This will help to advance the field of science and medicine's ability to identify biological molecules in future studies.

Ethics approval

The manuscript has not been submitted to more than one journal for simultaneous consideration.

Consent to participate

This study has not directly/indirectly involved human and/or animals.

Consent for publication

The manuscript was reviewed, and all authors consented to publish.

Competing interests

The authors declare no competing interests.

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Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

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