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Green-synthesized selenium nanoparticles using garlic extract and their application for rapid detection of salicylic acid in milk

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Abstract

The highly selective, sensitive, and quick interference green synthesis-based sensing of possible milk adulterants i.e., salicylic acid has been reported here. Salicylic acid interacts with nanoparticles through strong bonding interactions, hence resulting in an interruption within the formation of selenium nanoparticles which is confirmed by UV-VIS spectroscopy, scanning electron microscopy, and x-ray diffraction. This interaction in the synthesis of nanoparticles resulted in transmittance wavelength that decrease with the increasing amount of salicylic acid, showing strong binding of selenium nanoparticles with adulterant thereby permitting in-situ fast detection of salicylic acid from milk having a limit of detection at 10^{-3} mol and linear coefficient correlation of 0.9907.

Keywords: adulteration; green synthesis; selenium nanoparticles; salicylic acid; aggregation.

Practical Application: Milk adulteration is a global concern and the current study was plan to synthesize selenium nanoparticles by green method using plant extract of *Allium Sativum* commonly refered as garlic in English and Lehsan in Asia to characterize Selenium nanoparticles through different analytical techniques and to apply Selenium nanoparticles as fast and easy technique for the detection of salicylic acid in milk. Conclusively, it can be drawn, that colloidal selenium could be synthesize successfully by garlic extract in order to serve as a probe for fast and cheap testing of milk adulteration.

1 Introduction

Food adulteration is becoming significant distress in the past few years. Various awareness programs related to health welfare along with the prominence of bad health among the global population have vanguard the researchers to head-on their concentration on synthesizing portable detection techniques in order to monitor the quality of food (Bansal et al., 2017). Among these different concerns of present-day society, adulteration of milk is conceivably the most pervasive (Varun et al., 2017). Various types of adulterants are usually added to milk worldwide. In Asian countries, raw milk is disseminated via traditional method that includes middlemen called "Gawala". To maximize their benefit, these intermediaries (Gawala) practice to adulterate milk. Adulteration of milk is executed to increase its viscosity, by addition of reconstituted milk powders and starch. Often applied to maintain the shelf-life of foul milk by incorporating chemicals such as carbonates, hydrogen peroxide, bicarbonates, caustic soda, salicylic acid, antibiotics and even the extreme deadly substance formalin (Francis et al., 2020). Milk adulteration not only affects the quality but it can be hazardous for health if consumed (Gondim et al., 2017).

Nanotechnology is an emerging technological development and from the past decade, glance of its wide potential has known been witnessed in different fields like catalysis Banin et al. (2021), fuel cells development Choi et al. (2019), lithium-sulphur ion batteries Wang et al. (2017), etc. There is a lot of toxicants added in milk as adulteration by using nanotechnology the recent research is emphasizing on, salicylic acid (C_7H_6O) which is known to be added in milk as a preservative in order to enhance shelf life and is associated with gastric irritation, bleeding, diarrhea, renal disease and even death in mammals (Nagraik et al., 2021). Traditional detection methods of salicylic acid (SA) include high-end advanced instruments that are LCMS (Liquid Chromatography-Mass Spectrometry) (Protasiuk & Olejnik, 2018), Gas Chromatography-Mass Spectroscopy (Božić Luburić et al., 2022) TLC, and High-Performance Liquid Chromatography (Pyka-Pająk et al., 2018).

These methods need to demolish samples in the operation process, and most of them also require to regulate pretreatment of sample. The operation technique in the analysis is complicated, which escalates the detection complexity and is not applicable in large-scale practice (He et al., 2021). Additionally, due to their sheer dimensions and complex control, these methods and equipment are out of the reach of consumers and distributors. Due to these gaps, there is an urgent need to develop such in-

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situ technique that is time efficient, easy to operate, sensitive and rapid analysis of milk purity is required.

Moreover, nanotechnology taking up the foreground, there is a moderate yet significant formation in the synthesis of diminutive devices for recognition of melamine, hydrogen peroxide, urea, salicylic acid and various other milk adulterants utilizing different metallic and optical properties of nanoparticles that are gold (Au), silver (Ag) and ZrO_2 (zirconium dioxide) (Nataraj et al., 2021; Zhou et al., 2020; Wang et al., 2014). For instance, Taheri & Alizadeh (2020) studied electro-static interactions within negatively charged anionic salicylic acid and sorbent along with applied positive potentials during electro-sorption at the high surface coated rod sheet having LOD (Limit of Detection) and RSD% (relative standard deviation) at 5–100 nano-mol L^{-1}, 1.4 nano-mol L^{-1} and 4.9 (n = 5), correspondingly. Another study by Cao et al. (2019) utilizing gold nanoparticles doped graphene hydrogel nanocomposites (AuNPs-GHs) reported a detection of IAA (indole-3-acetic acid) and Salicylic acid utilizing chrono-amperometric quantification. The limit of detection (S/N = 3) was intended to be 0.22 µM for salicylic acid and 0.21 µM for IAA in spiked samples showing satisfactory consequences.

Recently, Anu et al. (2017) proposed a novel lateral flow strip composed of colloidal selenium synthesized by L-ascorbic acid for detection of adulterants in milk and milk powders. The limit of detection for test strip out reached to 150 µg/kg and 1,000 µg/kg, in milk and powder milk respectively having storage life of 1 year at ambient room temperature.

In above studies, the main postulate of sensing principally relies on pre-fabricated nanoparticles following by the functionalization, modification and aggregation of synthesized nanoparticles because of the presence of adulterant, which is a time-consuming procedure. The synthesis of low-cost, simple and rapid detection method for salicylic acid and other adulterants that is easily understood even by an unskilled or inexperienced person, by cognizance of a portable gadget is highly advisable.

By this study, a rapid alternate of detecting salicylic acid in milk by green synthesis of selenium nanoparticles using *Allium sativum* extract as a reducing agent is revealed. In this current study, a novel detecting methodology is reported for the first time via a solitary step by process of interference using garlic extract in the formation of Se nanoparticles. Garlic was selected because it is broadly represented for its remarkable therapeutic properties, since prehistoric duration. Its main compounds showed various properties like anti-hypertension, anti-hyperlipidemic (Amarakoon & Jayasekara, 2017), anti-microbial (Parvez, 2018), anti-glycemic (Ishak et al., 2018) anti-cancer (Li et al., 2018), anti-cardiovascular disease and anti-neurological disease (Netzel, 2020). Therefore, it is reasonable to conclude that the green synthesized selenium nanoparticles capping with extract metabolites of garlic can be a significant nanomaterial with latent therapeutic importance. Additionally, these sensors based on nanoparticles supply excellent sensitivity (≤0.1%), great precision (RSD < 6%) minimal cost (as no enzyme is requisite) and easy analysis, including milk samples handling (Seddaoui et al., 2022).

The novelty of this approach is that, it is sensitive enough to detect the analytes even at trace levels or it display acceptable linearity covering a wide dynamic range. These nano-based sensors are reproducible as well as accurate, meaning it give the selfsame results over time for same samples. These also exhibit fast response in time recovery on field applications. Real time rapid monitoring or minimal recovery time are together prerequisites for reusability and functionality of stable biosensors.

2 Materials and Methods

2.1 Materials

Salicylic acid (Vetec), sodium selenite 99.95% (sigma), Sodium hydroxide pellets (S D Fine-Chem Limited), hydrochloric acid (BASF), pottasium hexacynoferate (3.6% aquous), zinc sulfate (7.2% aquous) were utilized as received without any additional purification.

All of the stock and sub-stock solutions were stored under dark conditions at 4 ^{0}C in order to eliminate photochemical reactions and remained for minimum 10-15 days. Purified distilled water having conductivity of less than 0.10 µS cm^{-1} at 25 °C was utilized all over experiment.

2.2 Sample preparation

The milk samples were obtained from local market and stored in amber glass beakers at a temperature range of 4 to 5 °C. These milk samples were processed by removing fat and protein to eliminate any chance of interaction with synthesized nanoparticles. To remove this uncertainty, milk fat and protein was separated from test sample for accurate results by following (Inamuddin & Kanchi, 2020). For this purpose, 10 mL of raw milk was transferred into centrifuge tube along with 2.5 mL of Potassium hexacyanoferrate ($K_4Fe(CN)_{6.}H_2O$ and 2.5 mL of Zinc Sulphate Zn (SO_4). The complete solution was then subjected to mixing/shaking for 1-2 minutes as shown in Figure 1.

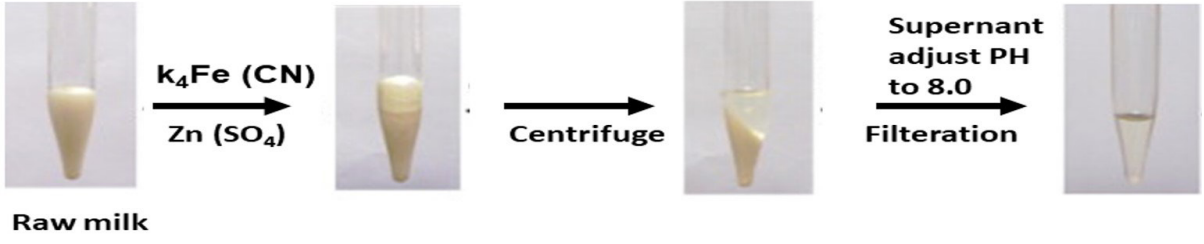


Figure 1. Preparation of milk sample.

The final milk solution obtained was centrifuged twice at 4500 rpm for 5 minutes and at 10500 rpm for 10 minutes. The centrifuged sample goes under filtration by the help of 0.22 µM filter paper and supernatant was separated by the help of syringe in a clean beaker.

A sample aliquot or standard salicylic acid along with 0.5 mL of Se nanoparticles were measured accurately into a 20 mL beaker. pH of solution was afterward adjusted to 8.0 using 0.1 M HCL/NaOH. After this, incubation was performed at 40 °C for 10 minutes. Finally, absorption spectra were recorded, respectively.

2.3 Preparation of selenium nanoparticles and its characterization

The selenium nanoparticles were synthesized by following the protocol of (Anu et al., 2017). A 10 mL of the garlic extract was admixture with 60 mL of 20 mM Sodium selenite powder (Na_2SeO_3) and was heated at 70 °C for 15 minutes using magnetic stirrer at speed of 150 rpm at specific alkaline pH of 8.0. After this, the solution was agitated on ambient room temperature for about 10 minutes until the color switched from pale yellow to brick red which confirms the synthesis of colloidal selenium nanoparticles.

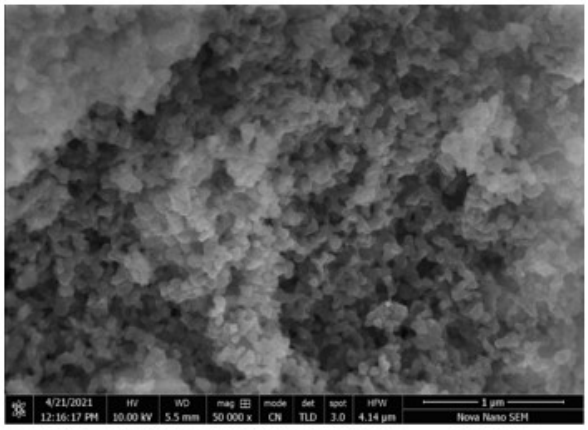
This characterization of Se nanoparticles was performed using UV-VIS Spectrophotometer (C-7200S), Bruker D2-Phaser X-Rays Diffraction (XRD) and The Nova Nano SEM 450 field-emission scanning electron microscope (FE-SEM). The salicylic acid was added according to the ordinary addition protocol followed by (Alagesan & Venugopal, 2019). The various concentrations of salicylic acid spiked milk were evaluated using DS5 dual beam UV-Visible spectrophotometer scan from 200 nm to 800 nm. The detection limit defined in the manuscript, is the lowest quantity of analyte that can be differentiated from the blank (sample containing no analyte).

3 Results and discussion

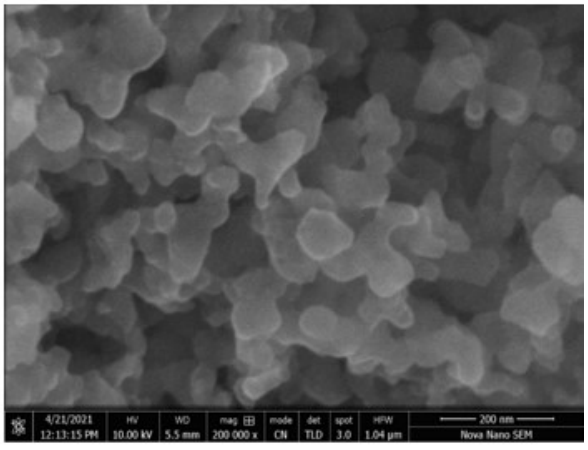
The synthesized or fabricated Se nanoparticles were identified visually by time-dependent color variation during mixture reaction. The solution changes color from orange to brick red. After the incubation time of 24 hours, no further color variation was observed. Change in color was observed because surface of the particles has exciting plasmon vibrations called as Surface Plasmon Resonance (SPR). SPR of Se nanoparticles gives specified spectroscopy signature that is easily readable on SEM (scanning electron microscopy), XRD (X-ray diffraction) and UV-VIS spectrophotometer. These methodologies have been utilized for the detection of diverse chemical and physical features of fabricated nanoparticles. The morphology characteristics of the nanoparticle is achieved by SEM which is of great impact as morphology always influences most of the nanoparticle properties. Characterization of structure is significant to study the nature and composition of bond materials. The XRD and EDX are also additionally done for the structural characterization of nanoparticles. UV-visible spectroscopy is used for the optical features of selenium nanoparticles.

3.1 Description of characterization of nanoparticles

SEM analysis is performed for the analysis of size and shape of synthesized nanoparticles as shown in Figure 2 reveals that the biosynthesized Se nanoparticles has interconnected lobes structures with particle diameter range of 200 nm, 500 nm and 1 µm. Shakibaie et al. (2015) also formed spherical Se nanoparticles having huge frequency of 120-140 nm within the range of 80-220 nm. The high magnification SEM analysis



(a)



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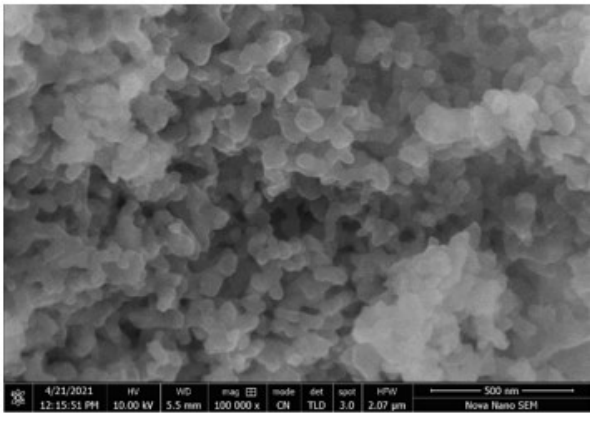

(c)

Figure 2. SEM images representing size and morphology of Se nanoparticles. (a) 1µm; (b) 200nm; (c) 500 nm.

Table 1. SEM–Energy Dispersive X-ray (EDX) showing the existence of K ratio and wt% of a strong peak from selenium element.

Element	Line Type	Apparent Concentration	k Ratio	wt.%	wt.% Sigma	Standard Label	Factory Standard
C	K series	13.68	0.1368	41.57	1.19	C Vit	Yes
O	K series	30.5	0.10262	30.92	0.69	SiO2	Yes
Na	K series	4.96	0.02095	3.12	0.12	Albite	Yes
Mg	K series	3.17	0.02102	2.54	0.11	MgO	Yes
P	K series	11.88	0.06646	7	0.18	GaP	Yes
K	K series	1.85	0.01566	1.47	0.07	KBr	Yes
Ca	K series	1.71	0.01529	1.4	0.08	Wollastonite	Yes
Cu	L series	0.05	0.0005	0.08	0.23	Cu	Yes
Se	L series	9.91	0.09907	11.89	0.31	Se	Yes
Total:				100			

reveals spherical lobes like aggregated nanostructures in a regular fashion. The homogeneity in SEM spectra describes single structure morphology of synthesized Se nanoparticles having average particle size of 50 nm. Similar results of SEM at 200 nm were found in (Salem et al., 2022). The nano structure with enhanced surface area are responsible for higher activity binding properties towards sensing (Liao et al., 2018).

Analysis by EDX was executed through same equipment to realize the elemental configuration of particles. The synthesis of elemental structure examination through SEM–EDX observed the presence of strong peak of selenium atom with wt. of 11.89%. Results presented in Figure 3 revealed that the structure of nanoparticles was composed of single Se atom. Other signals shown in Table 1 e.g., C, O, Na, Mg P, K, Cu and Ca peaks was also observed advising that they were varied precipitates Se salt that is generated by biomolecules present on Se nanoparticles surface. These configuration values are in accordance with Srivastava & Mukhopadhyay (2015).

Further confirmation was determined by XRD that shows the generation of Se°. XRD analysis is used to determine the phase distribution, crystalline structure and purity of the biosynthesized selenium nanoparticles. Figure 4 shows the XRD patterns of developed Se nanoparticles from the garlic extract. It clearly designates crystalline structure of synthesized Se nanoparticles. The sharp edged peaks were shown at 2Θ values are 24.03°, 30.3°, 44.2°, 52.2°, 54.5° and 56.6°. These values are in accordance with Anu Mary Ealia & Saravanakumar (2017) having reference number to the JCPDS No. 04-0783 that demonstrate that the Se nanoparticles were crystalline in nature having cubical shape with no such impurities.

The optical properties of Se nanoparticles shown in Figure 5 were determined by UV-VIS Spectrophotometer. After the addition of sodium selenite to the plant extract, the spectrum was taken in different intervals. The simplest and authentic technique as its role in analysis of absorbed or scattered light from a UV-VIS region by a substance under examination. The quantified light named as extinction (i.e., sum of scattered and absorbed light) give the characteristics of this technique. The results obtained by UV-VIS Spectroscopy helps in determining the accumulation in a sample through scattering. As scattering increases, accumulation of particles increases. Moreover, this technique is tool to check

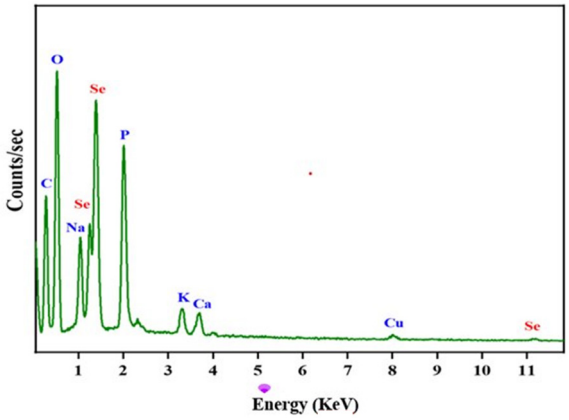

Figure 3. EDX analysis of different elements along with Se.

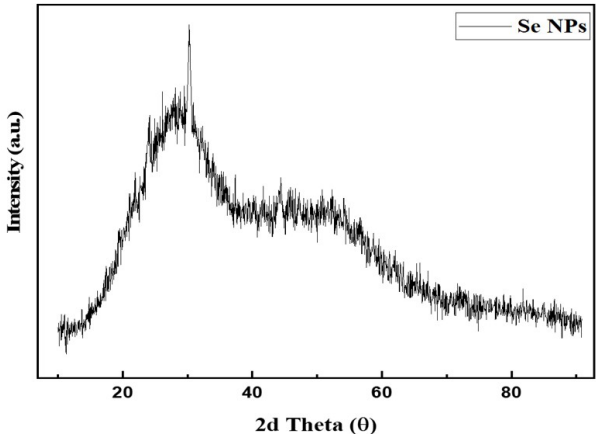

Figure 4. XRD analysis of Selenium Nanoparticles.

stability of nanostructure in various solutions and effect of aging on it. As the disperse particles agglomerates or destabilized in solutions the result can be attained from the extinction peak which may shorten due to destabilization of particles and more often the peak is either broaden or split into two at different wavelengths. The same effect can be seen for aging, in which

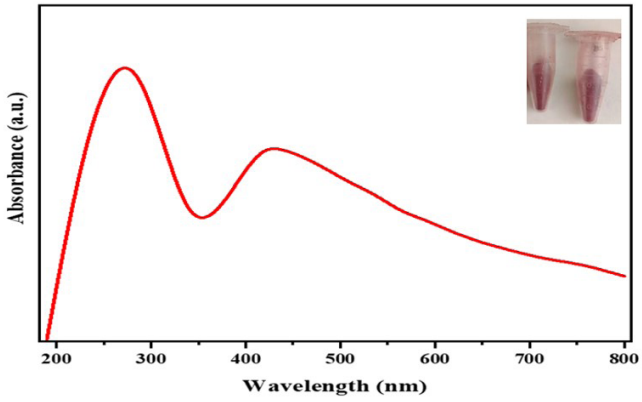

Figure 5. The UV visible spectra of Se nanoparticles.

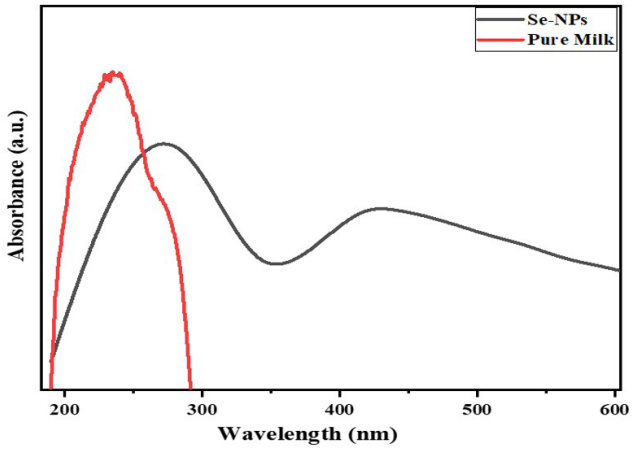

Figure 6. Comparative spectrum of Se nanoparticles and pure milk.

the extinction peak becomes short and broad which shows the disintegration of particles over-time (Fedenko et al., 2017).

The results of UV visible spectra of Se nanoparticles shows absorbance peaks at wavelength of 425 nm in visible region while at 265 nm in the UV region (i.e. λmax=425 nm). The obtained results of Se nanoparticles are in accordance with absorbance data in literature (Alagesan & Venugopal, 2019). This can be concluded from the absorbance spectra that biosynthesized nanoparticles are visible active and can be applied in various fields due to their characteristic sensor properties.

The Figure 6 shows a comparative spectrum of Se nanoparticles and pure milk without any adulterant addition. The pure milk has absorbance peak in UV region from 200-300 nm that is stated by Fohely & Suardi (2018) which explains no visible active species in milk.

3.2 Analysis of spiked milk samples by addition of Salicylic acid

After the pre-treatment of milk samples (i.e., removal of fat and protein) salicylic acid was intentionally introduced in order to confirm its presence through developed Se nanoparticles.

To validate the advanced method to precisely detect salicylic acid in raw milk, effect of different concentrations of salicylic acid was checked in raw milk samples. At first, various concentration of salicylic acid was added to the raw milk before pretreatment of the sample then the samples of raw milk were analyzed accordingly and depicted in Figure 7.

3.3 Analysis of adulterated milk using Se nanoparticles

For the detection of salicylic acid, UV–visible spectra of Se nanoparticles were observed with varying concentration of salicylic acid and percentage transmission results were recorded (Figure 8).

In this process, Nps (0.0025 g/ml) were added and sonicated for complete dispersion. After this, salicylic acid was added in different concentrations and were incubated at 40 ^{0}C for 10 minutes. The effect of the pH media (4.0 to 11.5) and time of reaction over the assay were also measured. The pH 8.0 was observed as suitable condition for execution of all experiment. It was also observed that the reaction accrued in less than few

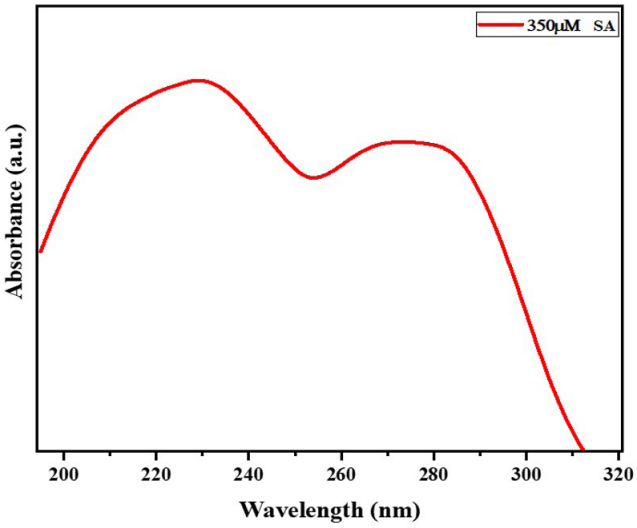

Figure 7. Spiked milk samples incorporated with salicylic acid.

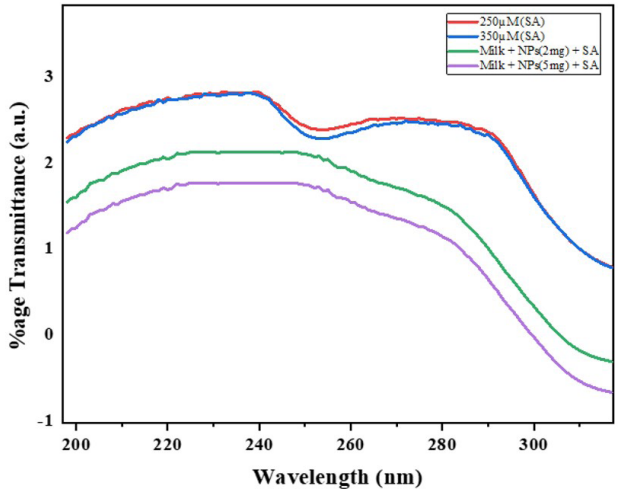

Figure 8. Analysis of salicylic acid in milk with Selenium Nanoparticles.

seconds and increased slightly after 10 minutes. Therefore, the incubation time for the following samples was set at 10 minutes in order to get the best results.

The UV spectrophotometer results presented in Figure 8 showed highly fast (time of absorbance < 1 minute) with increasing concentration of salicylic acid along with the decrease in the concentration of salicylic acid after addition of Se nanoparticles. The analysis revealed that there is a strong binding capacity of Se nanoparticles with salicylic acid. On addition of biosynthesized Se nanoparticles, the surface group present on Se nanoparticles selectively binds salicylic acid and forms a complex. Upon centrifugation, Se nanoparticles with salicylic acid settled down resulting in deconcentrating of salicylic acid in milk samples. The Figure 9 is showing reproducibility of Se nanoparticles in identification of adulterant.

3.4 Absorption mechanism of salicylic acid on Se nanoparticles

The Figure 10 exhibits a chemical structure of salicylic acid, that comprises carboxylic group (COOH) and hydroxy (OH) groups, having aromatic ring bonded with adjoining carbon atoms. The proposed adsorption procedure intricate bonding of bidentate chelating and bidentate bridging, involving outer sphere and inner bonding sphere. (Ata et al., 2014) Figure 11 represents chemical structures of the molecules applicable as dispersants. The negatively charged ion on the molecules are attributed to their COOH groups. The adsorption of numerous organic molecules on surface oxide in water suspension underline the significance of functionality groups, bonded to adjacent carbon atoms of the benzoic ring, like COOH and OH groups of salicylates, two atoms of OH in catecholates, and two atoms of COOH in phthalates. These groups make complex with chelation of metal atoms upon the surface of particle and provide adsorption of the adulterant as shown in Figure 12 depicting Fabrication of coordination structures by chemisorption of salicylic acid.

3.5 Limit of detection (LOD)

For the evaluation of LOD, increasing amount of SA was spiked in pre-prepared milk sample followed by incorporation of inducer (NaOH; 1 mM solution). We find the sensing method for amount dependent inhibition of Se nanoparticles aggregation for detection of salicylic acid by UV–vis spectroscopy. The biosynthesized Se nanoparticles shows enhanced binding properties with salicylic acid having detection limit of 10^{-3} mol which makes it suitable for bioactive sensitive material to detect different adulterants in milk samples. This study is further confirmed by (Hussain et al., 2020) that detects thiram and dicyandiamide in liquid milk using core shelled gold-silver nanoparticles.

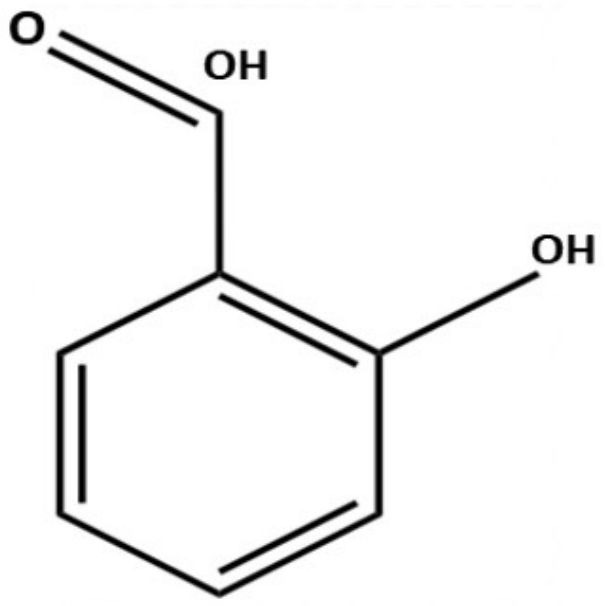

Figure 10. Chemical Structure of Salicylic Acid.|

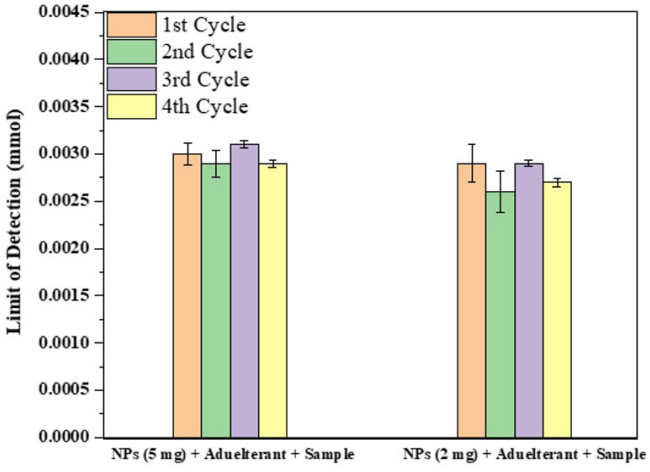

Figure 9. Reproducibility of Se nanoparticles in identification of adulterant.

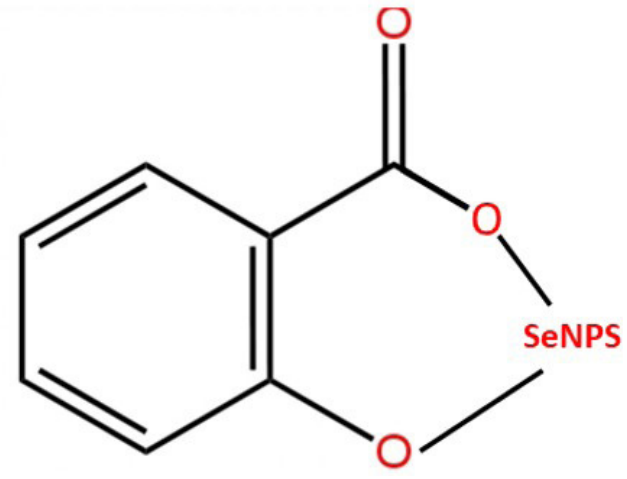

Figure 11. Chemisorption of Salicylic Acid with SeNps.

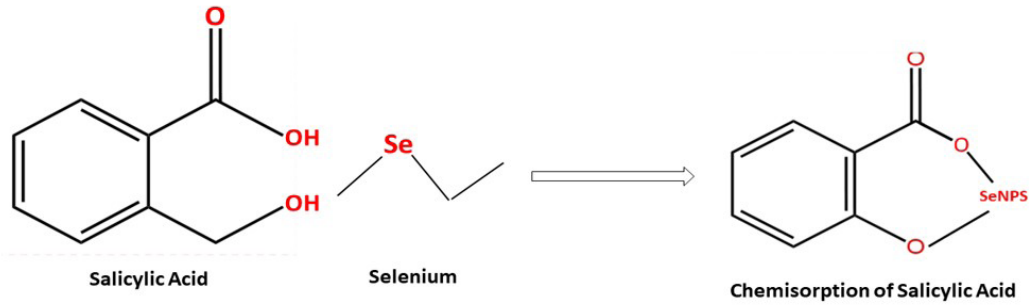

Figure 12. Fabrication of coordination structures by chemisorption of Salicylic Acid.

4 Conclusion

This current study was planned to synthesize the Se nanoparticles-based material for detection of adulterant (salicylic acid) in milk. The selenium nanoparticles were prepared through green approach which is considered as a safe, simple, cost-effective, non-toxic and eco-friendly as compared to the physical and chemical approaches. *Allium Sativum* was used as a base material which act as a reducing and stabilizing agent. The green synthesized Se nanoparticles were successfully characterized using different analytical techniques to verify their activity. The results after the characterization explicated that synthesized nanoparticles were in the range of an average size of 50 nm. The UV absorption peak was observed at λmax = 425 nm which clearly expressed the synthesis of Se nanoparticles. Furthermore, a concentration of 250 µm and 350 µm of salicylic acid in liquid milk was detected by the colloidal Se nanoparticles of 0.0025 g/mL indicating detection limit of 10^{-3} mol. This detection technique is rapid having linear dependent co-efficient of 0.9907 and could exhibit good selectivity against various other potential interfering analytes. Conclusively, it can be drawn hereafter, that colloidal selenium could be synthesized successfully by garlic extract in order to serve as a probe for fast and cheap testing of milk adulteration.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

Conflict of interest declaration and author agreement

The authors have no conflict of interest.

Author contributions

Conceptualization: Rashna Aftab. Validation: Adnan Khaliq. Experimental design: Muhammad Farhan Jahangir Chughtai. Laboratory work and data collection: Rashna Aftab. Manuscript writing: Atif Liaqat, Samreen Ahsan. Manuscript reviewing: Muhammad Adil Farooq, Muhammad Safdar, Muhmmad Nadeem. Software: Tariq Mehmood.

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