Synthesis Of Gold Nanoparticles Using Different Reducing Agents

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ABSTRACT
The main aim of the study is to conduct a preformulation research on methotrexate sodium in order to examine the drug's physicochemical characteristics and Creating, analyzing, and assessing PLGA-functionalized, methotrexate sodium loaded AuNPs (PLGA-MTX-AuNPs) for targeted and sustained administration and create, describe, and assess HA-MTX-AuNPs—methotrexate sodium loaded, hyaluronic acid functionalized AuNPs—for targeted and sustained administration and assess functionalized formulations utilizing flow cytometry and confocal imaging for cellular uptake and cytotoxicity studies, respectively, using MTT test.

KEYWORDS: AuNPs, MTX, synthesized, methotrexate sodium loaded

INTRODUCTION

Currently, there has been a notable progress in using inorganic compounds for the development of drug delivery systems. Certain specific inorganic nanoparticles exhibit little toxicity, adjustable surface characteristics, enhanced transport of drugs into cells, and extensive functioning. The most extensively studied inorganic nanomaterials are calcium carbonate, iron oxide, silica, gold, and calcium phosphate. At now, inorganic compounds are used for the administration of MTX to various illnesses.

Iron oxide, particularly Fe3O4 nanoparticles, have been investigated for their ability to transport MTX for very efficient anticancer therapy. The Fe3O4 nanoparticles, with an average size ranging from 10 to 20 nm, were coated with a molecularly imprinted polymer (MIP) that conferred thermal-responsive characteristics. These magnetised seeds can generate heat when exposed to an external magnetic field. Therefore, the magnetic seeds coated with MIP demonstrated a superparamagnetic characteristic, along with an 80% adsorption ratio of MTX. When subjected to a magnetic field, these nanocarriers released MTX by breaking hydrogen bonds and exhibited therapeutic efficacy in cancer cells. Thus, this notion has resulted in a distinctive thermosensitive approach for delivering drugs specifically to malignant cells, triggered by external stimuli. While polymeric nanostructures are now under investigation due to their biocompatibility, the incorporation of inorganic compounds is often used to enhance their compatibility with healthy cells and tissues. Integrating gold nanoparticles (Au NPs) into a polymeric shell enables imaging after exposure to near-infrared photothermal stimulation. In accordance with this methodology, MTX and AuNPs were amalgamated using the emulsion-diffusion-evaporation technique and incorporated into pegylated-poly (DL-lactic-co-glycolic acid) nanospheres.

LITREATURE REVIEW

Raut, Jiko & Sarkar, Olivia & Das, Tanmoy (2023) Methotrexate (MTX), a very effective chemotherapeutic agent, is used for the treatment of several cancers. Nevertheless, the breast cancer cell line MDA-MB-231 has acquired resistance to it because of diminished levels of the MTX transport protein and decreased folate carrier (RFC), hence diminishing its efficacy against these cancer cells. We have developed a straightforward approach to create amine-capped ZnO quantum dots that are biocompatible and non-toxic. These quantum dots were specifically created to address the issue of methotrexate (MTX) resistance in the MDA-MB-231 breast cancer cell line. The quantum dot (QD) was analysed using high-resolution transmission electron microscopy (HRTEM), dynamic light scattering (DLS), energy-dispersive X-ray spectroscopy (EDX), Fourier-transform infrared spectroscopy (FT-IR), ultraviolet-visible spectroscopy (UV-Vis), and fluorescence spectroscopy. Confirmation of MTX loading onto the QD was achieved using fluorescence and UV–Vis spectroscopy. Furthermore, thorough confocal microscopic examinations were conducted to ascertain the effective release of MTX on the MDA-MB-231 cell line. QD has been shown to be a more effective pH-responsive delivery method compared to earlier ones. It efficiently distributes MTX to MDA-MB-231 cells at a greater rate under acidic conditions than under healthy conditions. Quinone derivatives (QD) possess inherent antineoplastic properties and may independently eliminate malignant cells. The QD has these characteristics, which enable it to function as a very efficient pH-responsive delivery system, hence enhancing the effectiveness of drug in therapeutic diagnostics.

Verma, Rinki & Singh, Virendra (2023) In this study, we developed Methotrexate-loaded chitosan nanoparticles (Meth-Cs-NPs) via a single-step self-assembly process that included adopting the ionic-gelation approach. The cross-

linking of chitosan with Methotrexate was achieved using sodium tripolyphosphate (STPP). The nanoparticles obtained had a loading capacity of 49% Methotrexate, with a size of about 143 nm and a zeta potential of 34 ± 3 mV. The entrapment efficiency of Methotrexate in the nanoparticles was 87%. The effectiveness of nanoparticles was evaluated for the treatment of chemically induced breast cancer in the Sprague Dawley rats model. The Meth-Cs-NPs exhibited in-vitro release kinetics that conformed to the Korsmeyer-Peppas model. The effectiveness of nanoparticles on MDA-MB-231 triple-negative breast cancer cell lines was further assessed in vitro. The MTT test findings demonstrated that even little exposure to Meth-Cs-NPs (with an IC50 value of 15 µg/mL) resulted in a 50% reduction in cell viability within 24 hours. In addition, hemocompatibility experiments were conducted on Meth-Cs-NPs, revealing that they are biocompatible with a hemolysis rate of less than 2%. Further assessment of cellular uptake was conducted using confocal imaging. Furthermore, in-vivo pharmacokinetic research conducted on rats shown an elevation in the drug's plasma concentration and retention duration when administered in the form of nanoparticles. Additionally, there was a reduction in cellular clearance compared to the administration of free Methotrexate. Additionally, research on the effectiveness of nanoparticles against tumours shown a reduction in tumour volume from 1414 mm3 to 385 mm3, in comparison to free Methotrexate which only reduced the tumour volume from 1414 mm3 to 855 mm3. The present research demonstrates the potential of Meth-Cs-NPs as a feasible approach for treating breast cancer.

Bhattacharya, Sankha & Prajapati, Bhupendra (2023) Supercritical liquid technology was used to generate novel superparamagnetic iron oxide nanoparticles (SPIONs) of Methotrexate (MTX). These nanoparticles were optimised using a Box-Behnken design to evaluate their potential as a therapy option for breast cancer. The MTX-SPIONs, which were coated with poly(lactic-co-glycolic acid)-polyethylene glycol 400, had a collective size of 500 nm and an encapsulation efficiency of 46.8 ± 3.9%. The examination using Fourier-transformed infrared spectroscopy showed a displacement in the primary bands caused by intermolecular hydrogen bonding. Meanwhile, the analysis using differential scanning calorimetry indicated the lack of the MTX melting endotherm, suggesting the full encapsulation with oxide nanoparticles. The zeta potential measurements showed a value of 4.98 mV, whereas the in vitro release analysis demonstrated an initial rapid release followed by a significant release of 35.1 ± 2.78% after 12 hours. Flow cytometry was used to assess apoptosis in the control group, MTX, and MTX-SPIONs. The results showed that MTX-SPIONs induced a higher level of apoptosis compared to both the control group and MTX. Furthermore, the MTX-SPIONs suppressed cellular proliferation and disrupted cellular arrangement, resulting in a significant increase in the percentage of cells in the G1 and G2 phases compared to the control group. MTX-SPIONs demonstrated extended duration of anticancer activity against MCF-7 cell lines in comparison to MTX alone, suggesting that the delivery of chemotherapeutic agents via SPIONs may enhance their ability to cause cell death. The medicine demonstrated stability with little loss of encapsulated drug, indicating that the technique using supercritical liquid technology is a very promising approach for producing drug-polymer magnetic composite nanoparticles for the treatment of cancer.

Aslzad, Shaghayegh & Heydari, Parisa (2023) In this study, a chitosan/gelatin hybrid nanogel including gold nanoparticles (CS/AuNPs@Gel) was synthesised as a highly effective carrier capable of responding to enzymes for the administration of doxorubicin (DOX). The production of Nanogel included the use of ionic crosslinking of CS/AuNPs in the presence of gelatin as an enzyme-responsive component. The resulting compounds were analysed for their physicochemical characteristics using FT-IR, DLS, TEM, and UV-Vis spectroscopy. The average size of the synthesised CS/AuNPs and CS/AuNPs@Gel was around 83 nm and 119.3 nm, respectively. The zeta potential of CS/AuNPs was measured to be 83.9 mV, while the zeta potential of CS/AuNPs@Gel was found to be 31.9 mV. The loading efficiency of DOX in the CS/AuNPs@Gel nanogel was about 56%, and the release of DOX from the nanogel was sensitive to enzymes. The cytotoxic experiment conducted on the MCF-7 cells shown that the free nanogel is not harmful, whereas the drug-loaded nanogel (DN) and DN in the presence of an enzyme are effective. These results were compared with the toxicity of free DOX.

Ekinci, Meliha & Alencar, Luciana (2023) Millions of new instances of breast cancer are detected each year, making it a prominent cause of cancer-related deaths among women globally. To tackle the issue of breast cancer mortality, a thorough strategy is needed that encompasses timely identification, precise diagnosis, efficient treatment, and fair availability of healthcare facilities. Nano-radiopharmaceuticals have shown promise in improving breast cancer diagnostics by merging the advantages of nanoparticles with radiopharmaceutical agents. The use of nanoscale formulations may provide higher imaging capabilities, heightened targeted specificity, and improved sensitivity in the detection of breast cancer lesions. This research focuses on the creation and assessment of a new kind of radiopharmaceutical called [99mTc]-TRZ-MTX-HSA. It is made up of technetium-99m ([99mTc]) labelled trastuzumab (TRZ) adorned methotrexate (MTX) loaded human serum albumin (HSA) nanoparticles. The purpose of this radiopharmaceutical is to diagnose breast cancer. HSA and MTX-HSA nanoparticles were synthesised in this situation. The conjugation of MTX-HSA nanoparticles with TRZ was achieved using adsorption and covalent bonding techniques. The formulations that were developed underwent evaluation for several parameters including particle size, PDI value, zeta (ζ) potential, scanning electron microscopy analysis, encapsulation efficiency, loading capacity, and cytotoxicity on MCF-7, 4T1, and MCF-10A cells.

REESRACH METHODOLOGY

Selection of method

Chemical reduction techniques are widely used for synthesizing gold nanoparticles due to their simplicity, affordability, scalability, and vast application. Furthermore, these techniques provide a substantial amount of gold nanoparticles with

the desired dimensions and morphology. Therefore, the chemical reduction approach was used in the present study for the synthesis of gold nanoparticles.

Selection of reducing and stabilizing agent

Reducing agents are necessary to chemically reduce chloroauric acid in order to synthesize gold nanoparticles. Various stabilizing chemicals hinder the aggregation of nanoparticles by a capping mechanism. Various reducing and stabilizing substances have been tested and evaluated based on a thorough evaluation of the existing literature. The reducing agents used to manufacture gold nanoparticles were gelatine, guar gum, tannic acid, trisodium citrate, and xanthan gum. Certain chosen lowering agents possess a degree of stabilizing capability.

Preparation of stock dispersion of chloroauric acid

1 gram of chloroauric acid was obtained and added to 294 milliliters of deionized water to create a 10 millimolar stock solution. The solution was then transferred to an amber-colored container. The molecular weight of chloroauric acid is 339.785 grams per mole.

DATA ANALYSIS

The synthesis processes were carefully evaluated to ensure the high quality of the product. This was done by evaluating the attributes of the nanoparticles, namely their size, size distribution, and monodispersity. The current study included synthesizing AuNPs utilizing several reducing agents, including gelatine, guar gum, tannic acid, trisodium citrate, and xanthan gum. The preparation of AuNPs used a bottom-up strategy, where the synthesis of nanoparticles originated at the molecular level. The creation of gold nanoparticles involves a chemical reduction approach. In this process, the gold salt (Chloroauric acid) containing Au+3 is reduced by a reducing agent, resulting in the formation of neutral Au0. These gold nanoparticles have a distinctive ruby red color. In addition, chemically produced gold nanoparticles (AuNPs) were assessed for their ability to screen a reducing agent.

Physical observation

Gold nanoparticles are a kind of noble metal that exhibit optical features resulting from the surface plasmon oscillation of conduction electrons. As the size of the particles grows, their color may range from ruby red to brown to purple. The shift in color is contingent upon the shape and size of the nanoparticles. The synthesis of AuNPs resulted in a noticeable shift in color, transitioning from colorless to a vibrant ruby red hue. This change in color signifies the successful creation of gold nanoparticles with a tiny size, measuring less than 100 nm. The change in color of gold nanoparticles (AuNPs) produced using gelatine, guar gum, tannic acid, trisodium citrate, and xanthan gum was visually detected and is shown in Figure 1. Upon physical examination, it was seen that the trisodium citrate and gelatin resulted in the formation of stable gold nanoparticles with a tiny size. Nevertheless, the polydispersity index (PDI) of the gelatine-reduced gold nanoparticles (AuNPs) was discovered to be greater than that of the citrate-stabilized AuNPs.

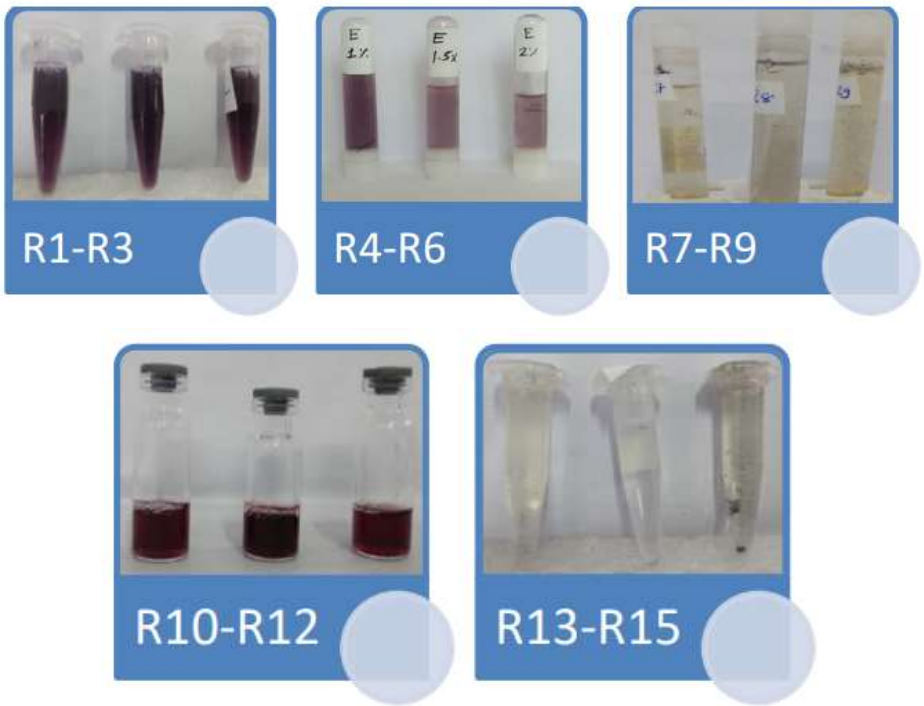


Figure 1 Colour change obtained at the end of the synthesis of AuNPs

Characterization

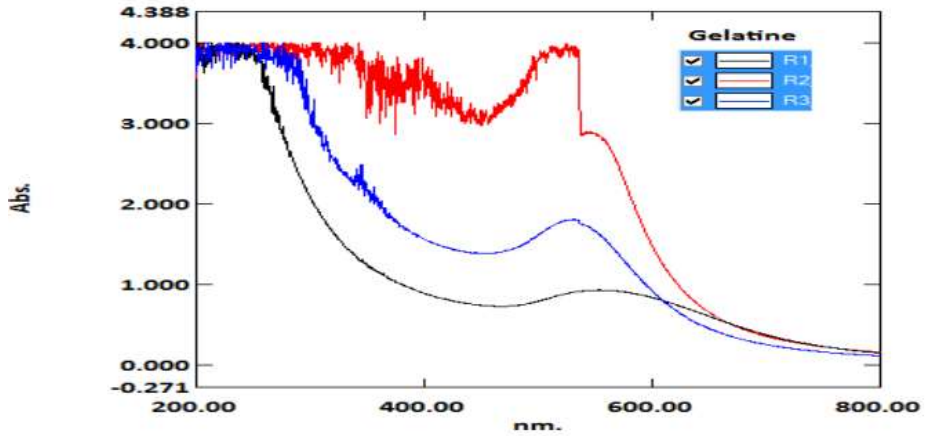
A reducing agent has been synthesized. AuNPs are assessed for their UV Visible absorption peak, particle size, polydispersity index (PDI), and zeta potential in order to evaluate the effectiveness of reducing agents. Table 1 displays the outcomes of batches R1 to R15.

Table 1 Screening of reducing agent

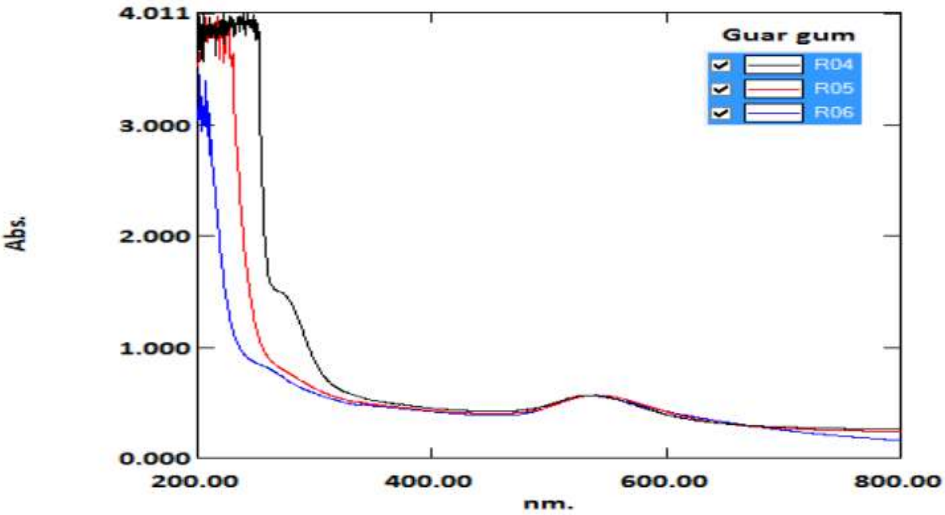
Batch No.	Reducing agent	Reducin g agent concentr ation (%)	Particle size* (nm)	PDI	Zeta potential* (mV)	UV Visible absorption peak* (SPR peak) (nm)
R1	Gelatine	0.2	243.26 ± 14.18	0.112	-03.17 ± 3.5	553.00 ± 1.0
R2		0.3	41.00 ± 08.52	0.563	-05.09 ± 2.1	524.00 ± 2.5
R3		0.4	94.95 ± 11.61	0.280	-05.56 ± 1.9	530.50 ± 2.0
R4	Guar gum	0.04	122.20 ± 14.18	1.000	-07.36 ± 3.5	560.00 ± 1.0
R5		0.08	111.50 ± 08.52	0.474	-12.2 ± 6.1	553.00 ± 2.5
R6		0.12	104.00 ± 11.61	0.395	-10.0 ± 4.9	552.50 ± 2.0
R7	Tannic acid	0.2	427.60 ± 12.31	0.749	-38.1 ± 5.7	363.00 ± 5.0
R8		0.3	460.90 ± 09.33	0.809	-37.8 ± 2.9	361.50 ± 1.5
R9		0.4	549.66 ± 04.33	0.958	-38.3 ± 4.7	358.00 ± 2.5
R10	Trisodium citrate	0.2	40.27 ± 03.39	0.318	-17.8 ± 3.4	521.00 ± 0.5
R11		0.3	58.97 ± 04.25	0.367	-14.4 ± 2.4	524.00 ± 1.5
R12		0.4	68.99 ± 03.22	0.375	-19.5 ± 1.3	525.00 ± 2.0
R13	Xanthan gum	0.04	285.00 ± 09.22	0.622	-25.7 ± 6.2	561.00 ± 3.5
R14		0.08	241.90 ± 10.36	0.507	-33.3 ± 8.6	552.00 ± 5.0
R15		0.12	168.30 ± 12.99	0.467	-32.6 ± 2.4	543.00 ± 2.0

UV Visible spectroscopy

The color shift was seen and tracked using UV-visible spectroscopy. The absorption peaks clearly exhibit a noticeable red shift for AuNPs. The absorbance maxima of colloidal AuNPs are contingent upon the dimensions and morphology of the nanoparticles. The spectra shown in Figure 2 illustrate the process of reducing HAuCl4 and the subsequent production of gold nanoparticles. It was discovered that small-sized particles have an SPR peak at around 520 nm, and this peak becomes more pronounced as the particle size rises. The change in color signified the commencement of gold reduction and the creation of AuNPs. The plasmon maximum absorption migrated towards longer wavelengths (red-shifted) when the concentration of trisodium citrate varied. The smallest particles exhibited a λmax of 521 nm, while the biggest particles had a λmax of 525 nm. The red shift is caused by the change in the dielectric constant of the surrounding medium surrounding the gold nanoparticles. The AuNPs capped with trisodium citrate exhibited a vivid ruby red color at completion of the process (Figure 1). In summary, based on the data shown in the result table, we have determined that AuNPs with a smaller particle size, namely those capped by trisodium citrate, will be selected for future formulation development. In addition, trisodium citrate-capped gold nanoparticles (AuNPs) were examined using transmission electron microscopy (TEM) to determine their size and form.



(a)


Guar gum
R04
R05
R06
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2.000
1.000
0.000
Abs.
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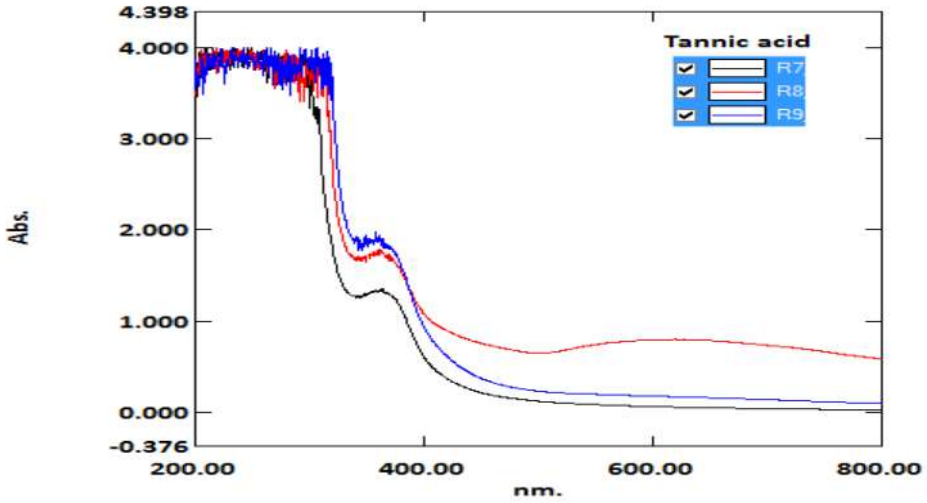
400.00
600.00
800.00
nm.

(b)


Tannic acid
R7
R8
R9
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4.000
3.000
2.000
1.000
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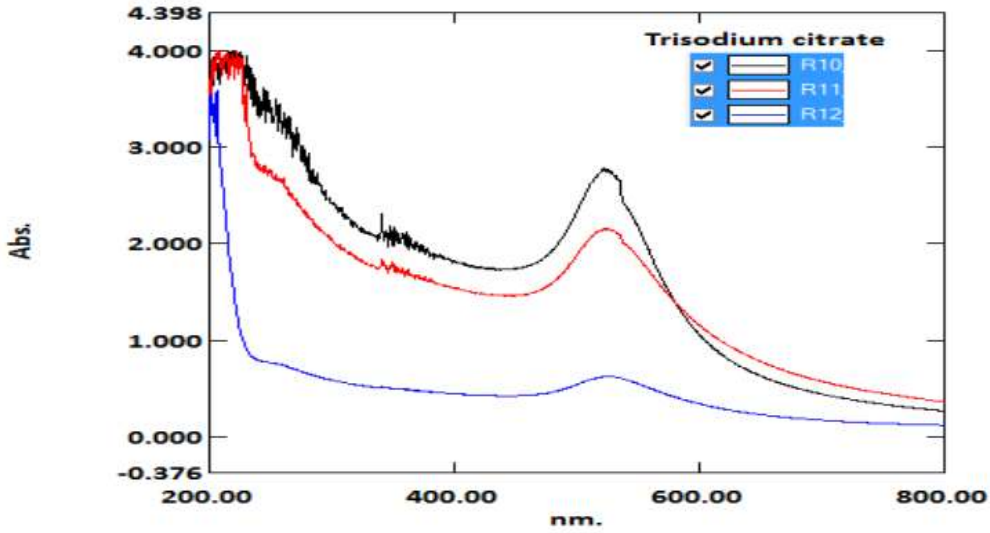
(c)


Trisodium citrate
R10
R11
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4.398
4.000
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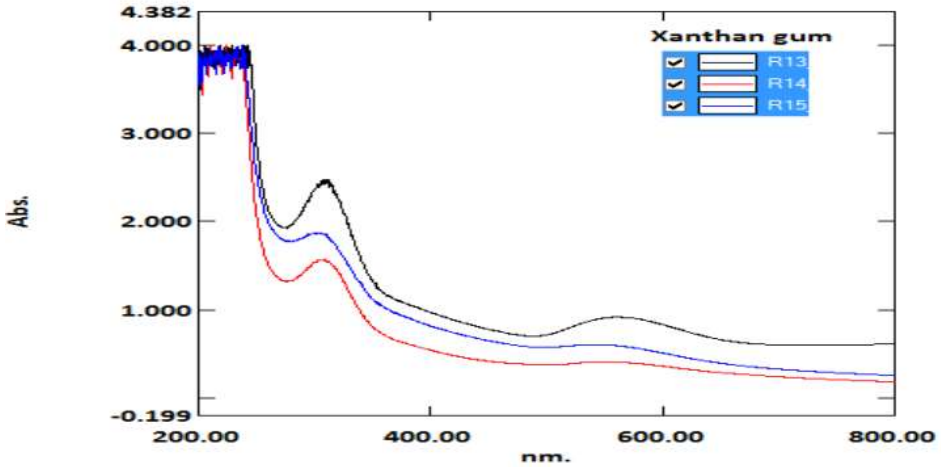
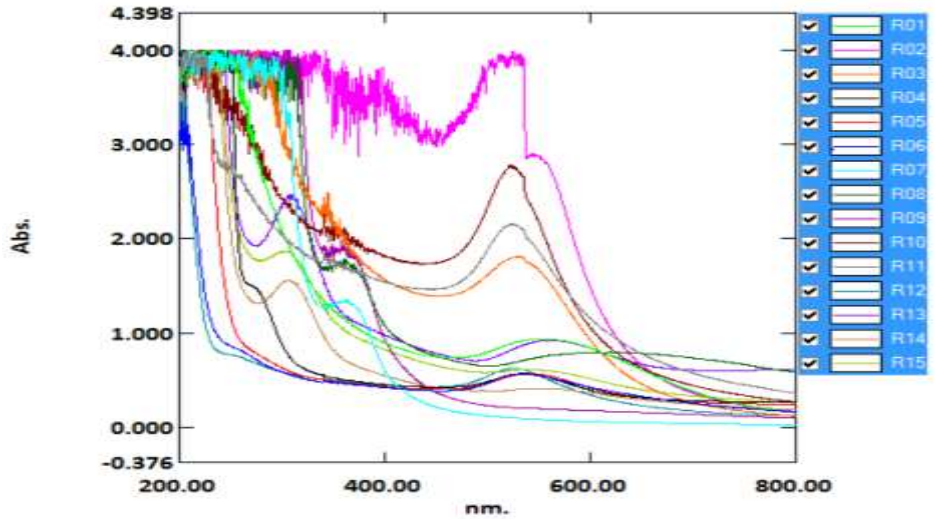
(d)



(e)



(f)

Figure 2 SPR peak of (a) Gelatine R1-R3 (b) Guar gum R4-R6 (c) Tannic acid R7- R9 (d) Trisodium citrate R10-R11 (e) Xanthan gum R12-R15, and (f) All reducing agents R1-R5

Particle size, size distribution, and PDI

The current investigation used various concentrations of reducing agents. Variations in the kind and concentration of the reducing agent directly impact the size of AuNPs. AuNPs with a small particle size were exclusively created using trisodium citrate, while other reducing agents resulted in bigger AuNPs. The kind and concentration of a reducing agent have a significant impact on the size and size distribution of AuNPs. The polydispersity index (PDI) was determined to assess the size distribution of the manufactured batches of gold nanoparticles (AuNPs)

Zeta potential

Zeta potential serves as a predictive method for assessing the stability of colloidal dispersions. The zeta potential of the produced batches ranged from -3.17 mV to -38.3 mV. The zeta potential value of xanthan gum and tannic acid fell within the stable range, but the zeta potential of other formulations ranged from -25 mV to +25 mV, indicating less stable dispersion. Nevertheless, the AuNPs generated using tannic acid and xanthan gum exhibited a greater size compared to the AuNPs capped with citrate.

Stability

The stability of gold nanoparticles (AuNPs) encapsulated with citrate was assessed for a duration of one week. Visual observation revealed alterations in color for gold nanoparticles capped with citrate. The AuNPs underwent aggregation after a week, resulting in a shift in color from ruby red to purple (Figure 3). Furthermore, conglomeration of particles was also detected near the base of the container.

Figure 3 Physical observation of citrate capped AuNPs after a week

Upon comparing the UV Visible spectrum of newly generated AuNPs with that of the AuNPs held for a week, it was observed that the absorbance peak of the AuNPs had undergone a change because of particle aggregation during the one-week storage period. The Appendices show the size and zeta potential graph of the F10-F12 batches after one week.

CONCLUSION

Our research indicates that gold nanoparticles (AuNPs) may be readily produced using a chemical reduction process, resulting in a distinct ruby red hue. Several reducing agents were used in the synthesis of small-sized AuNPs. However, the stability of the gold nanoparticles (AuNPs) was not accomplished only by applying a reducing agent. Therefore, to stabilize the gold nanoparticles (AuNPs), the non-ionic surfactant tween 80 was used as both a co-reducing agent and a stabilizing agent. In addition, to enhance the formulation and achieve nanoparticles with a stable tiny size, the concentration of trisodium citrate and tween 80 were manipulated as independent variables, and their impact on particle size and zeta potential was examined.

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