Analytical Methods

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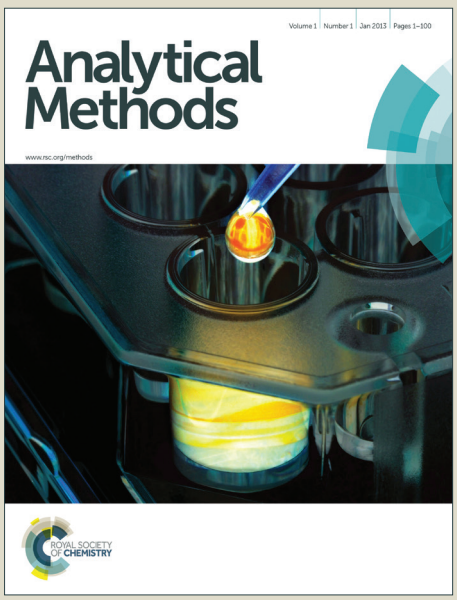

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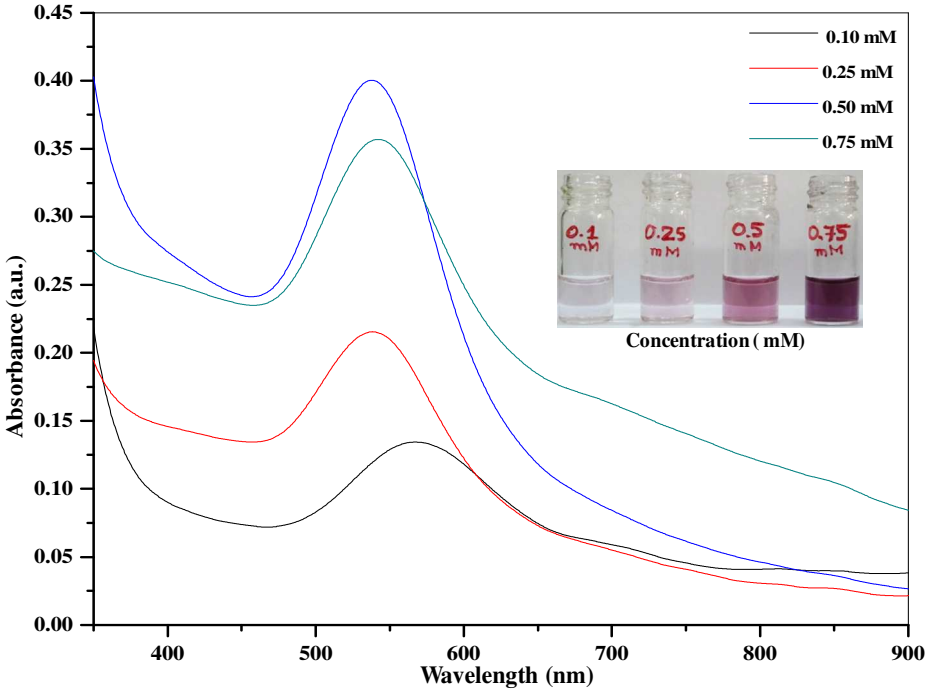
Graphical Abstract

This paper describes the use of 4-aminoantipyrine (AAP) as novel reagent for one-step synthesis of water dispersible gold nanoparticles (Au NPs) with surface plasmon resonance (SPR) band at 544 nm. The AAP-Au NPs aggregation induced by triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan) independently, resulting color change from pink to blue that can be observed with naked-eye.



UV-visible absorption spectra of Au NPs by using AAP as a reducing and capping agent at different concentrations from 0.1 - 0.75 mM.

One-pot synthesis of gold nanoparticles by using 4-aminoantipyrine as a novel reducing and capping agent for simultaneous colorimetric sensing of four triptan-family drugs

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Abstract

We report the use of 4-aminoantipyrine (AAP) as a novel reducing and capping reagent for one-step synthesis of gold nanoparticles (AuNPs) and their colorimetric sensing application for the analysis of four triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan) in pharmaceutical samples. Effecting parameters such as reagent concentration, temperature and reaction time were studied for synthesis of AAP-AuNPs. The synthesized AAP-AuNPs were characterized by using UV-visible spectrometry, dynamic light scattering (DLS) and transmission electron microscopic (TEM) techniques. The use of AAP is to reduce Au^{3+} and to provide large conjugate network on the surfaces of AuNPs which allows to act as a probe for colorimetric sensing of triptan-family drugs. The newly synthesized AAP-AuNPs are pink in color due to the intense surface plasmon resonance (SPR) band at 544 nm. On addition of triptan-family drugs, the pink color of AAP-AuNPs solutions turn to blue accompanying dramatic SPR band red-shift from 544 to 773, 667, 725 and 745 nm for naratriptan, sumatriptan, rizatriptan and zolmitriptan, respectively. It is presumed that the color change is a result of AAP-AuNPs aggregation induced by triptan-family drugs. The color change is used for naked eye and colorimetric determination of four triptan-family drugs in pharmaceutical formulations.

Keywords: AAP-AuNPs, UV-visible spectrometry, DLS, TEM and triptan-family drugs.

Introduction

In recent years, the development of AuNPs-based analytical approaches opens new opportunities to develop simple, effective and rapid miniaturized analytical methods for *in-situ* detection of a wide variety of molecules (biomolecules, drugs and inorganic species) with minimal resources.[1-2] Since, AuNPs exhibit distance-dependent optical properties that undergoes a red-shift in the resonance wavelength and show extremely high extinction coefficients at visible region.[3] As a result, AuNPs have received a great deal of attention in the development of visualizing sensors for chemical and bioassays with high sensitivity and selectivity.[1] Importantly, the colorimetric sensing ability of AuNPs is always dependent on their size (< 5 - 20 nm), SPR and surface chemistry, since the AuNPs aggregation is induced by target analytes, which yields a change in color from red to blue and a red-shift of SPR band towards longer wavelength. Therefore, the synthesis of water dispersible AuNPs with controlled size (<5 nm, SPR band at 520 – 550 nm) is very important in colorimetric assays. In this connection, researchers have widely reported on the use of sodium citrate as a reducing and capping agent for the preparation of AuNPs with controlled size (3.5 nm) and used as probes for colorimetric sensing of bio- and inorganic species.[1-2,4-7] To alter AuNPs optical properties, several inorganic and organic reagents such as sodium borohydrate,[8] tryptophan,[9] ascorbic acid,[10-11] lumanol,[12] octyldeciamine,[13] and *o*-aniside[14] have been used as reducing and capping agents for synthesis of AuNPs, and they have been extensively used as a probe for molecular recognizing events in complex samples. Furthermore, due to their reducing ability and molecular assembly, aromatic primary amine is used as a reducing and capping agent for the preparation of Au- and Ag- NPs.[15] Aslam *et al*. developed a one-step procedure for

synthesis of water-dispersible AuNPs by using oleylamine as a multifunctional molecule.[16] Similarly, Qi and co-workers described a simple method for synthesis and functionalization of AuNPs with carboxymethyl cellulose and used as a colorimetric sensor.[17] Zhou *et al*. developed wet-chemical approach for synthesis of water-dispersed AuNPs by using polyvinylpyrrolidone as a reducing agent and NaOH as an initiator.[18] The water dispersed AuNPs have exhibited unique analytical abilities for analytes assays in multidisciplinary research areas. These approaches inspired us to introduce AAP as a novel reagent for the preparation of AuNPs and for expanding the signal revealing mechanisms toward the design of a new colorimetric AuNPs visualization probe for simultaneous analysis of four triptan-family drugs.

Naratriptan, sumatriptan, rizatriptan and zolmitriptan are the class of triptan-family drugs and used as antimigraines in paroxysmal disorders such as headache, nausea, vomiting, photophobia, and malaise.[19-20] These drugs are very selective towards 5-hydroxy-tryptamine (5-HT, 1B/1D) receptor agonist with high affinity at the 5-HT (1B), 5-HT(1D) and 5-HT(1F) receptors.[21-22] It was reported that the triptan-drugs have shown very high oral bioavailability (63–74%) and exhibited a distinct clinical therapeutic profile.[23-24] Literature survey reveals that several analytical techniques such as UV-visible spectrometry,[25] voltammetry,[26-27] and high performance liquid chromatography [28-30] have been used for the determination of triptan-family drugs in pharmaceutical and biological samples. Furthermore, liquid chromatographic technique coupled with mass spectrometric (LC-MS) approach is widely used for the analysis of triptan-family drugs in pharmaceutical and biological matrices.[31] For example, Dulery's group developed a LC-MS method for the quantification of naratriptan and sumatriptan in

rabbit plasma.[32] Vishwanathan and co-workers described a LC coupled with electrospray ionization tandem mass spectrometric (ESI-MS/MS) method for rapid, sensitive, and selective detection of four triptan-family drugs (rizatriptan, zolmitriptan, naratriptan, and sumatriptan) in human serum.[33] Srivastav *et al*. developed a HPLC-ESI/MS/MS method for the determination of naratriptan in human plasma by using sumatriptan as internal standard.[34] Recently, Liu and Zhou described the utility of LC-MS method for simultaneous detection of zolmitriptan and its active metabolite (*n*-desmethy-zolmitriptan) in rat plasma.[35] Most of the methods rely on the use of liquid-liquid extraction and other reagents such as methyl-tert-butyl ether and dichloromethane. Even though, these approaches provided good sensitivity for the analysis of triptan-family drugs, unfortunately most of these methods are expensive, complicated, required specific internal standard and solvents, and difficulty for *in situ* detection of triptan-family drugs. Therefore, it is important to develop a simple, fast and inexpensive method for routine and simultaneous analysis of triptan-family drugs. Wide survey of literature reveals that the simultaneous analysis of triptan-family drugs by UV-visible spectrometry has not been reported. With advancements in nanoscience integrated UV-visible spectrometric methods, we report the use of AAP as a novel reagent for one-step synthesis of AuNPs for simultaneous colorimetric sensing of four triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan) in pharmaceutical samples. The four triptan-family drugs are induced the aggregation of AAP-AuNPS, yielding a color change from pink to blue, and these aggregations are confirmed by UV-visible spectrometry, DLS and TEM techniques.

Experimental

Chemicals and materials

All reagents used were of analytical reagent grade. Aurochloric acid ($HAuCl_4 \cdot xH_2O$), naratriptan, sumatriptan, rizatriptan, and zolmitriptan, tris(hydroxymethyl) aminomethane (Tris buffer), phosphate buffered saline (PBS buffer) were purchased from Sigma Aldrich, (St. Louis, MO). AAP (99%) was purchased from Across Organics. Ammonium acetate was procured from Labort Chemicals Ltd., India. Water used in the entire analysis was purified from Milli-Q water purification system. Stock solutions of naratriptan, sumatriptan and rizatriptan were prepared in water (1.0 mM). Zolmitriptan solution (1.0 mM) was prepared in a mixture of methanol and water (1:4). All the triptan-family drugs stock solutions were prepared afreshly. Suminat 50 (Sumatriptan – 50 mg, Sun Pharmaceutical Industries Ltd., India), RIZORA 5 (rizatriptan – 5.0 mg, INTAS Pharmaceuticals Ltd., Ahmadabad, India), Zolmist nasal spray (zolmitriptan – 5.0 mg, Cipla Ltd., India) were purchased from local medical store in Surat, Gujarat, India.

Synthesis of AAP-AuNPs

For synthesis of AAP-AuNPs, 4.5 mL of 2.5×10^{-4} M aurochloric acid was heated on water bath with constant stirring at 50 ^{o}C for 10 min. To this, 0.5 mL of freshly prepared AAP solution (5×10^{-4} M) was added into the above solution in single purge by using syringe. Then, the reaction mixture was stirred for 30 min at 400 rpm, resulting a change in color from yellow to dark pink, which confirms that the formation of AAP-AuNPs. The formed AAP-AuNPs solution is allowed to stand for 30 min at room

temperature and then stored at 4°C. Supporting Information of Scheme S1 shows the schematic representation for preparation of AuNPs by using AAP as a reducing and as a capping agent.

Instrumentation

UV–visible spectra were measured by using Maya Pro 2000 spectrophotometer (Ocean Optics, USA). Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer (FT-IR spectrum BX, Germany). DLS measurements were recorded by using Zetasizer Nano ZS90 (Malvern, UK). TEM images were measured by Phillips Tecnai F20.

Results and discussion

Effect of AAP concentration, reaction temperature and time.

In order to control the size and color of AAP-AuNPs, we studied the effect of AAP concentration, reaction temperature and time on the SPR band of AAP-AuNPs by using UV-visible spectrometry. Figure 1a shows the UV-visible absorption spectra of AuNPs by using AAP as a reducing and capping agent at different concentrations in the range of 0.1 mM to 0.75 mM (0.5 mL). As shown in Figure 1a, the absorbance of AAP-AuNPs is gradually increased with increasing AAP concentration up to 0.5 mM, after that there is drastic change in their SPR band, resulting the changes in the absorbance and color of the system. This is due to π-π interactions between inter-nanoparticles surfaces, causing self aggregation of AAP-AuNPs, which results the color change from pink to blue and red-shift with broad SPR peak. Therefore, we selected 0.5 mM of AAP

as the best concentration for synthesis of water dispersible AAP-AuNPs with controlled size.

Figure 1b shows the effect of reaction temperature on the reduction of Au^{3+} ions by using AAP as a reducing and as a capping agent. It can be noticed that the absorbance of AAP-AuNPs SPR band at 538±2 nm is gradually increasing with increasing temperate from 0 – 100^{o}C. However, the observed SPR band of AAP-AuNPs is very broad shape and the color of the AAP-AuNPs solution is turned into blue at reaction temperature 0, 25,75 and 100^{o}C, confirming that the color and SPR peak shape is not suitable for colorimetric sensing of triptan-family drugs. As shown Figure 1b, the SPR band of AAP-AuNPs at 538±2 nm was generated with good peak shape and the color of the solution is pink red, which ensures that a novel AAP-AuNPs-based colorimetric method can be developed for the analysis of triptan-family drugs. Therefore, we selected 50^{o}C as the optimum reaction temperature for preparation of AAP-AuNPs.

As shown in Supporting Information of Figure S1, the reduction (Au^{3+} ions) and oxidation (AAP) reactions are rapidly occurred, and the SPR band of AAP-AuNPs is generated at reaction time 5 min. In order to evaluate best reaction time, we measured the absorption spectra of AAP-AuNPs as function of reaction time from 0 to 65 min. As shown in Supporting Information of Figure S1, the SPR peak intensity of AAP-AuNPs is gradually increased with increasing reaction time up to 30 min, and the color of the solution is slightly changed from blue to pink. However, the SPR peak of AAP-AuNPs is slightly red-shift and the color of the solution was also changed after 30 min. Therefore, we selected 30 min as the best reaction time for the preparation of AuNPs with good SPR band intensity. Based on the above results, we confirm that the reagent concentration

(AAP 0.5 mM), reaction temperature (50ºC) and time play key role for the preparation of AAP-AuNPs with controlled size and color, which facilitates to use them as colorimetric probes for simultaneous detection of triptan-family drugs. Table 1 shows the best experimental conditions for the preparation of water dispersible AuNPs using AAP as a novel reagent.

Characterization of AAP-AuNPs

As illustrated in Supporting Information of Figure S1, AAP acted as a reducing agent to reduce Au^{3+} ions and the NH_2 group of AAP is preferentially linked with AuNPs by N–Au bond, resulting organic (AAP) network on the surfaces of AuNPs. To confirm this, we studied the FT-IR spectra of pure AAP and AAP-AuNPs (Supporting Information of Figure S2). It can be observed that the strong absorption band at 3325-3431 cm^{-1}, which is corresponded to free $–NH_2$ group of AAP. Similarly, the strong stretching band of amide carbonyl are appeared at 1689 cm^{-1}, and the bands between 900 – 670 cm^{-1} are corresponded to =C–H stretching bands of AAP. The band at 1290 cm^{-1} indicates to the stretching and vibrations of -C-N- groups of AAP. It can be noticed that the characteristic AAP-NH_2 group stretching peak at 3330 cm^{-1} is drastically reduced and appeared with very less intensity at 3329 cm^{-1}, confirming that the reduction of Au^{3+} ions by $–NH_2$ group of AAP. Furthermore, the peaks at 1641 and 1491 cm^{-1} represent in-plane aromatic (–C=C–) vibrations of AAP and the peak at 824 cm^{-1} shows the =C–H deformations of AAP on surfaces of AuNPs. These results indicate that AAP is effectively acted as a reducing and capping agent for the preparation of Au NPs with controlled size.

The hydrodynamic diameter and particle size of AAP-AuNPs were measured by DLS and TEM. Figures 5-6 show the DLS data and TEM images of AAP-AuNPs. As shown in Figure 5, AAP-AuNPs are well dispersed in water with hydrodynamic diameter ~13.9 nm and TEM image is also confirmed that the prepared AAP-AuNPs are well dispersed with an average size ~10 nm. Moreover, we also calculated average size of AAP-AuNPs based on the obtained SPR band of AAP-AuNPs at 544 nm, which is well described in the literature.[36] The size of AuNPs was estimated by using following formula

$$d = exp\left(B_1 \frac{A_{spr}}{A_{450}} - B_2\right)$$

Where *d* (nm) is the size of AAP-AuNPs, A_{spr} and A_{450} are the absorbencies of AAP-AuNPs surface plasma resonance peak at 538±2 nm (0.368) and at 450 nm (0.237), which is dependence of the logarithm of the particle diameter in the size range from 5 to 80 nm. The B_1 is the inverse of slope (m; 3.55) of theoretical data and B_2 is the B_0/m (3.11) where B_0 is the intercept. It was observed that the UV absorption spectra-based calculated size of AAP-AuNPs is found to be ~11.0 nm, which is closed to DLS and TEM data.

Mechanistic investigation of AAP-AuNPs for simultaneous colorimetric sensing of four triptan-family drugs

In order to establish AAP-AuNPs as a sensor for the direct colorimetric sensing of four triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan), several drugs (ampicillin, amoxicillin, cortisone, folic acid, tramadol, tropiramate, naratriptan, sumatriptan, rizatriptan and zolmitriptan, 25 µL, 1.0 mM) solutions were added

independently into 1.0 mL of AAP-AuNPs solutions. The sample vials were vortexed for 2 min and kept stand for 5 min. The color changes and their UV-visible spectra of the AAP-AuNPs solutions were shown in Figure 2a. As shown in Figure 2a, the color of AAP-AuNPs solution was clearly changed from pink to blue only by the additions of four triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan), resulting the distinctive SPR peak shift from 544 nm to 773, 667, 725 and 745 nm for naratriptan, sumatriptan, rizatriptan and zolmitriptan, while other drugs had no effect on the color of AAP-AuNPs. These results clearly indicated that four triptan-family drugs are strongly induced the aggregation of AAP-AuNPs, confirming that the AAP-AuNPs are more selective and sensitive to four triptan-family drugs, in comparison with the other drugs.

We also investigated the effect of PBS, ammonium acetate and Tris-HCl buffers pH (2.0 – 12.0) on the SPR intensities of AAP-AuNPs aggregation induced by triptan-family drugs (Figure 2b and Supporting Information of Figure S3-S5a). It can be noticed that the absorption ratios (A_{773}/A_{544}, A_{667}/A_{544}, A_{725}/A_{544} and A_{745}/A_{544}) are quite good by using PBS buffer and higher than that of ammonium acetate and Tris-HCl buffer systems. Furthermore, the spectrophotometric response of AAP-AuNPs aggregation induced by four triptan-family drugs to various PBS pH values was investigated by recording the absorption spectra over a pH range of 2.0 –12.0 (Figure 2b and Supporting Information of Figure S4-S5a). It can be observed that the maximum absorbance was observed at pH range between 2.0 and 6.0. As shown in Figure 2b, the SPR band of AAP-AuNPs is drastically changed at lower pH range from 2.0 – 4.0. Since, at acidic pH <4.0, the metallic NPs are tend to aggregate together, owing to their surface charge neutralization, resulting to change their SPR peak towards long wavelength without addition of target

analytes. At pH 6.0, the adsorption ratios (A_{773}/A_{544}, A_{667}/A_{544}, A_{725}/A_{544} and A_{745}/A_{544}) are quite good, conforming that the multiple interactions (electrostatic and π-π interactions) between AAP-AuNPs and triptan-family drugs, which allows effective triptan-family drugs-induced aggregation of AAP-AuNPs. Since, at pH 6.0, carbonyl group of AAP-AuNPs shows negative charge (pK_a = 4.94) and triptan-family drugs exhibit positive changes (9.56, 9.70, 9.54, and 9.55 for rizatriptan, naratriptan, sumatriptan, and zolmitriptan, respectively).[37-38] Meanwhile, AAP molecules can interact with benzene and heterocyclic rings of triptan-family *via* π-π interactions, which allows to form supramolecular structures on AuNPs. Therefore, pH 6.0 was chosen as the best pH for effective AAP-AuNPs aggregation induced by triptan drugs.

In order to investigate the influence of PBS buffer system on the absorption spectra of AAP-AuNPs, we studied the absorption spectra of AAP-NPs in the presence of PBS buffer pH in the range of 2.0 – 12.0 without triptan-family drugs (Supporting Information Figure S6a). It was confirmed that the PBS buffer did not induce the color change of AAP-AuNPs, and their absorption spectra are remained unchanged in PBS buffer pH range from 4.0 – 12.0, however the spectral change was observed at pH 2, owing to the leaching of AAP-AuNPs.

To verify the triptan-family drugs-induced aggregation of AAP-AuNPs, we measured the hydrodynamic diameters and sizes of AAP-AuNPs with triptan family drugs by using DLS and TEM. Figures 5-6 show the DLS data of four triptan-family drugs (rizatriptan, naratriptan, sumatriptan, and zolmitriptan) induced aggregation of AAP-AuNPs. It can be noticed that the average hydrodynamic diameter of AAP-AuNPs is ~13.9 nm, however the average hydrodynamic diameter of AAP-AuNPs was greatly

increased to ~ 55, ~207, ~554 and ~99 nm in the presence of naratriptan, sumatriptan, rizatriptan and zolmitriptan, confirming that the triptan-family drugs can induce the aggregation of AAP-AuNPs. Figure 6 shows the TEM images of AAP-AuNPs with four triptan-family drugs. As can be seen in Figure 5a, bare AAP-AuNPs shows good size-distribution, with a diameter of ~13.9 nm. However, the morphology and size-distribution of AAP-AuNPs were drastically changed by the addition of triptan-family drugs into AAP-AuNPs solutions, which confirm that triptan-family drugs can induce the aggregation of AAP-AuNPs. As a result, the sizes of AAP-AuNPs are increased to 55, 207, 554 and 99 nm for naratriptan, sumatriptan, rizatriptan and zolmitriptan, respectively. These results clearly demonstrated that the morphology and size of AAP-AuNPs were greatly changed by the addition of triptan-family drugs.

Before constructing calibration graph, we investigated the influence of the common surfactants (sodium dodecyl sulfate - SDS, cetyl trimethylammonium bromide - CTAB and tetradecyltrimethylammonium bromide - TTAB) on the absorption spectra of AAP-AuNPs without addition of triptan-family drugs. As shown in Supporting Information of Figure S6b, no spectral or color change was observed in the presence of common surfactant, which confirms that the color and the spectral change of AAP-AuNPs are only caused by triptan-family drugs and not interfered by any other matrices.

Analytical data

In order to establish calibration graph and to evaluate the sensitivity of the method, different concentrations of four triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan, 2.5 – 1250 μM) were added separately into the aqueous

dispersion of AAP-AuNPs containing PBS buffer pH at 6.0 to trigger the aggregation of AAP-Au NPs and further evoke the obvious changes in their dispersion, both in color (from pink to blue) and in their absorption ratios at A_{773}/A_{544}, A_{667}/A_{544}, A_{725}/A_{544} and A_{745}/A_{544} for naratriptan, sumatriptan, rizatriptan and zolmitriptan, respectively (Figures 3-4 and Supporting Information Figure S7). It can be observed that the absorbance of AAP-AuNPs at 544 nm is decreased dramatically accompanied with the appearance of a new absorption peaks at 773, 667, 725, 745 nm for naratriptan, sumatriptan, rizatriptan and zolmitriptan, and consequently the color of the solution is changed from pink to blue (Figures 3-4). Importantly, the absorbance ratios (A_{773}/A_{544}, A_{667}/A_{544}, A_{725}/A_{544} and A_{745}/A_{544}) showed good linearity with increasing concentration of four triptan-family drugs in the range of 2.5 – 1250 μM for naratriptan (A_{773}/A_{544} = -1.6 − -0.25 log C/μM, R^2 = 0.983), sumatriptan (A_{725}/A_{544} = -2.3 – 0 log C/μM, R^2 = 0.989), rizatriptan (A_{667}/A_{544} = -2.3 − -0.25 log C/μM, R^2 = 0.993) and zolmitriptan (A_{745}/A_{544} = -2.3 − -1log C/μM, R^2 = 0.988), respectively. The limits of detections (LODs) are estimated to be 0.031, 0.033, 0.043 and 0.019 μM for naratriptan, sumatriptan, rizatriptan and zolmitriptan, respectively. Table 2 shows the linear ranges, LODs and UV-visible spectral characteristics of AAP-AuNPs with four triptan-family drugs.

Selectivity of AAP-AuNPs

To investigate the selectivity of the method, we performed competitive experiments in the presence of four triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan, 0.5 mM) with the other drugs ampicillin, amoxicillin, folic acid, cortisone, tramadol, and tropiramate, at 0.5 mM in each. It is noticed that the similar

SPR absorption ratios at A_{773}/A_{544}, A_{667}/A_{544}, A_{725}/A_{544} and A_{745}/A_{544} are observed in the presence of other drugs and the color change is accompanied by a significant red shift in the absorption spectra (Figure 7a), indicative of triptan-family drugs-induced AAP-AuNPs aggregation. Other drugs had no obvious influence on the color and absorption spectra of AAP-AuNPs, indicating that the AAP-AuNPs probe shows good selectivity to four triptan-family drugs.

Analysis of triptan-family drug in pharmaceutical samples

To demonstrate its practicality in pharmaceutical analysis, this method is used to determine sumatriptan, rizatriptan and zolmitriptan in their pharmaceutical samples and spectra were shown in Figures 7b and 8. The experiments were carried out by a standard addition method. Briefly, tablets (Suminat 50 (sumatriptan 50 mg) and RIZORA 5 (rizatriptan 5.0)) were weighed and finely powdered using a mortar and pestle separately. The obtained powder was dissolved in deionized water and filtered by using a micron filter (0.45μm). For zolmitriptan, nasal spray (zolmitriptan 5.0 mg) solution was diluted (0.001 - 100 mg/mL) by using deionized water. From these, various concentrations of tablets solutions (0.0001 – 17.5 mg/mL) were taken separately into 4.0 mL sample vials those contained AAP-AuNPs and triptan-drugs concentration were quantified by using the above said procedure. The concentration of triptan drugs were calculated by comparing the obtained absorbance ratios with the standard drugs. Figures 7b and 8 show the UV-visible spectra and photographic images of sumatriptan, rizatriptan and zolmitriptan containing pharmaceutical sample solutions by using AAP-AuNPs as a colorimetric probe.

Table 3 shows the comparison of the present method with the reported analytical techniques (UV-visible spectrometry, voltammetry, HPLC, LC-MS) for the analysis of triptan-family drugs. These results indicate that the present method shows better ability for the simultaneous analysis of four triptan-family drugs with good selectivity and sensitivity, just like as chromatographic techniques (HPLC and GC). Based on these results, the present method would offer an effective way to directly monitoring triptan-family drugs concentration in pharmaceutical and biological samples.

Conclusions

In conclusion, we have successfully synthesized water dispersible AuNPs (~544 nm) by using AAP as a reducing and capping agent in single-step. The AAP molecules act as organic ligand for rational designing of AuNPs surfaces to tune strength of the multiple interactions (electrostatic and π-π interactions) with triptan-family drugs. The AAP-AuNPs acted as a colorimetric probe for simultaneous detection of four triptan-family drugs (naratriptan, sumatriptan, rizatriptan and zolmitriptan), which could be monitored by the color change from pink to blue. Through DLS and TEM data, we confirmed that the triptan-family drugs are induced the aggregation of AAP-AuNPs through electrostatic and π-π interactions between AAP and triptan-family drugs. The successful determination of triptan-family drugs in the pharmaceutical samples is envisaged to be applicable to monitor triptan-family drugs in biological samples. The obtained results demonstrated that this approach essentially paves the way to a new AuNPs-based miniaturized analytical platform to identify the triptan-family drugs in pharmaceutical and biological samples.

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Figure captions

Figure 1. (a) UV-visible absorption spectra of AuNPs by using AAP as a reducing and capping agent at different concentrations from 0.75 - 0.1 mM. (b) UV-visible absorption spectra of AuNPs by using AAP (0.5 mM) at different reaction temperature from 0 – 100^{o}C . Inset images are the corresponding photographs of AAP-AuNPs.

Figure 2. (a) UV-visible absorption spectra of AAP-AuNPs with the addition of 10 different drugs (ampicillin, amoxicillin, cortisone, folic acid, tramadol, tropiramate, naratriptan, sumatriptan, rizatriptan and zolmitriptan, 1 mM). (b) Effect of PBS buffer pH range from 2.0 to 12.0 on the absorption spectra of AAP-AuNPs aggregation induced by naratriptan. Inset images correspond for the color change of AAP-AuNPs.

Figure 3. UV-visible absorption spectra of AAP-AuNPs by adding different concentrations of (a) naratriptan (0.005 -1.00 mM) and (b) sumatriptan (0.0025 -1.00 mM) at PBS buffer pH 6. Inset images correspond to the change in color of AAP- AuNPs with varying concentrations of analytes.

Figure 4. UV-visible absorption spectra of AAP-AuNPs by adding different concentrations of (a) rizatriptan (0.005 -1.25 mM) and (b) zolmitriptan (0.005 -1.00 mM) at PBS buffer pH 6. Inset images corresponding to the change in color of AAP- AuNPs with varying concentrations of analytes.

Figure 5 . DLS graphs of (a) AAP-AuNPs and AAP-AuNPs aggregation induced by (b) naratriptan, (c) sumatriptan, (d) rizatriptan and (e) zolmitriptan, respectively.

Figure 6. TEM images of (a) AAP-AuNPs and AAP-AuNPs aggregation induced by (b) naratriptan, (c) sumatriptan, (d) rizatriptan and (e) zolmitriptan, respectively.

Figure 7. (a) UV-visible absorption spectra of AAP-AuNPs with triptan family drugs individually in the presence of other drugs (ampicillin, amoxicillin, cortisone, folic acid, tramadol, tropiramate, 0.5 mM) and also mixture of triptan drugs (0.5 mM). Inset images correspond to the color change of AAP-Au NPs with triptan-family drugs in the presence of other drugs. (b) UV-visible absorption spectra of sumatriptan containing tablet Suminat 50 (0.1 – 0.0001 mg/mL) upon the addition of AAP-AuNPs. Inset images correspond to the color change of AAP-AuNPs.

Figure 8. (a) UV-visible absorption spectra of (a) rizatriptan containing Rizora-5 tablet (7.5 – 0.01 mg/mL) and (b) zolmitriptan nasal spray upon the addition of AAP-AuNPs. Inset images correspond to the color change of AAP-Au NPs.

Table 1. Best optimal conditions for the preparation of AuNPs by using AAP as a reducing and capping agent.

Parameters		Color	λ_{max} (nm)	Size estimation by SPR band
AAP (mM)	0.1	Blue	565	22.52
	0.25	Light pink	535	12.90
	0.50	Pink	536	11.04
	0.75	Reddish violet	538	18.50
Temperature (°C)	0	Light blue	549	16.46
	25	Light pink	544	17.20
	50	Light red	538	13.79
	75	Violet	550	18.46
	100	Blue	572	16.24

Table 2. Analytical data of four triptan-family drugs by using AAP- Au NPs as colorimetric probes

Drug	Naratriptan	Sumatriptan	Rizatriptan	Zolmitriptan
Color with AAP-AuNPs	Blue	Blue	Blue	Blue
λ_{max} (nm)	773	667	725	745
Size (nm)	55	207	554	99
Linear range (M)	$2.5\times10^{-6} - 1\times10^{-4}$	$2.5\times10^{-6} - 1\times10^{-4}$	$5\times10^{-6} - 1.25\times10^{-4}$	$5\times10^{-6} - 1\times10^{-4}$
R^2	0.983	0.989	0.993	0.988
Slope	0.331	0.310	0.244	0.545
LOD (μM)	0.031	0.033	0.043	0.019

Table 3. Comparison of AAP-AuNPs-based UV-visible spectrophotometric method with the other reported analytical techniques in the literature for detection of triptan-family drugs.

Name of the triptan drug	Linear range	Limit of detection	Analytical technique	References
Zolmitriptan	10.0 – 250.0[a]	6.0[a]	UV-visible spectrophotometry	25
Zolmitriptan	0.6 - 8.0[b]	---	Voltammetry	26
Sumatriptan	0.008 – 100[b]	0.004[b]	Voltammetry	27
Zolmitriptan naproxen and propranolol	50 – 900[c]	20.79, 16.5 and 26.40[c]	HPLC	28
Zolmitriptan	150 - 1000[c]	50[c]	HPLC	29
Zolmitriptan	25 – 150[d]	1.5[d]	RP-LC	30
Zolmitriptan	1.0 – 5.0[b]	6.6 and 24.4[c]	LC-ESI-MS	31
Sumatriptan, naratriptan, zolmitriptan and rizatriptan	1.0 – 100[c]	250[e] for sumatriptan and 100[e] for others	LC-MS/MS	33
Naratriptan	103-20690[e]	103[e]	LC-ESI–MS/MS	34
Zolmitriptan	0.2 – 50[c]	0.2[c]	LC–MS/MS	35
Naratriptan	2.5 – 100[b]	0.031[b]	AAP-AuNPs-based UV-visible spectrometry	Present method
Sumatriptan	2.5 – 100[b]	0.033[b]		
Rizatriptan	5.0 – 125[b]	0.043[b]		
Zolmitriptan	5.0 – 100[b]	0.019[b]		

[a] μg mL^{-1}; [b]μM; [c]ng/mL; [d]μg/mL; [e]pg/mL.

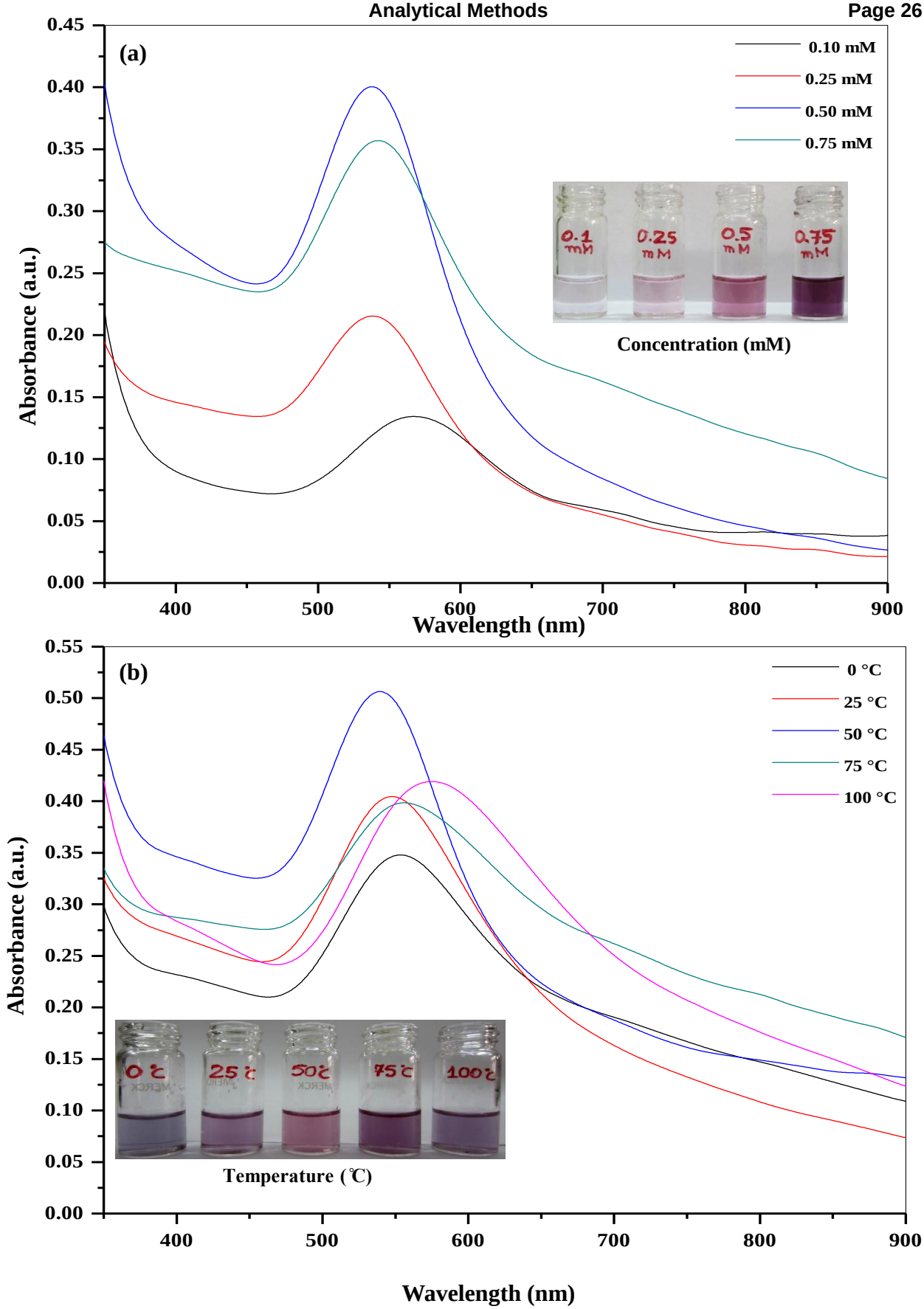


Figure 1.

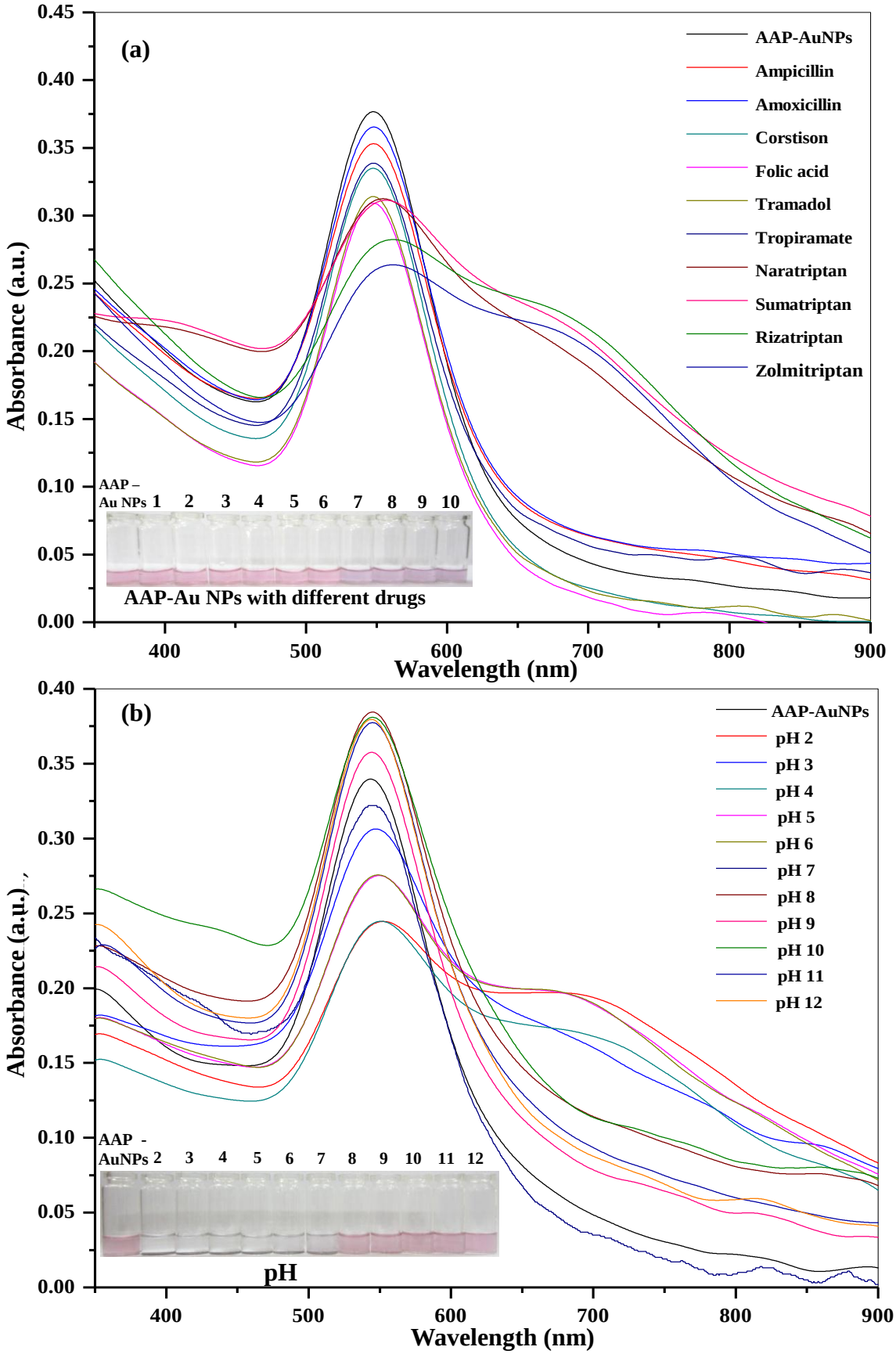


Figure 2.

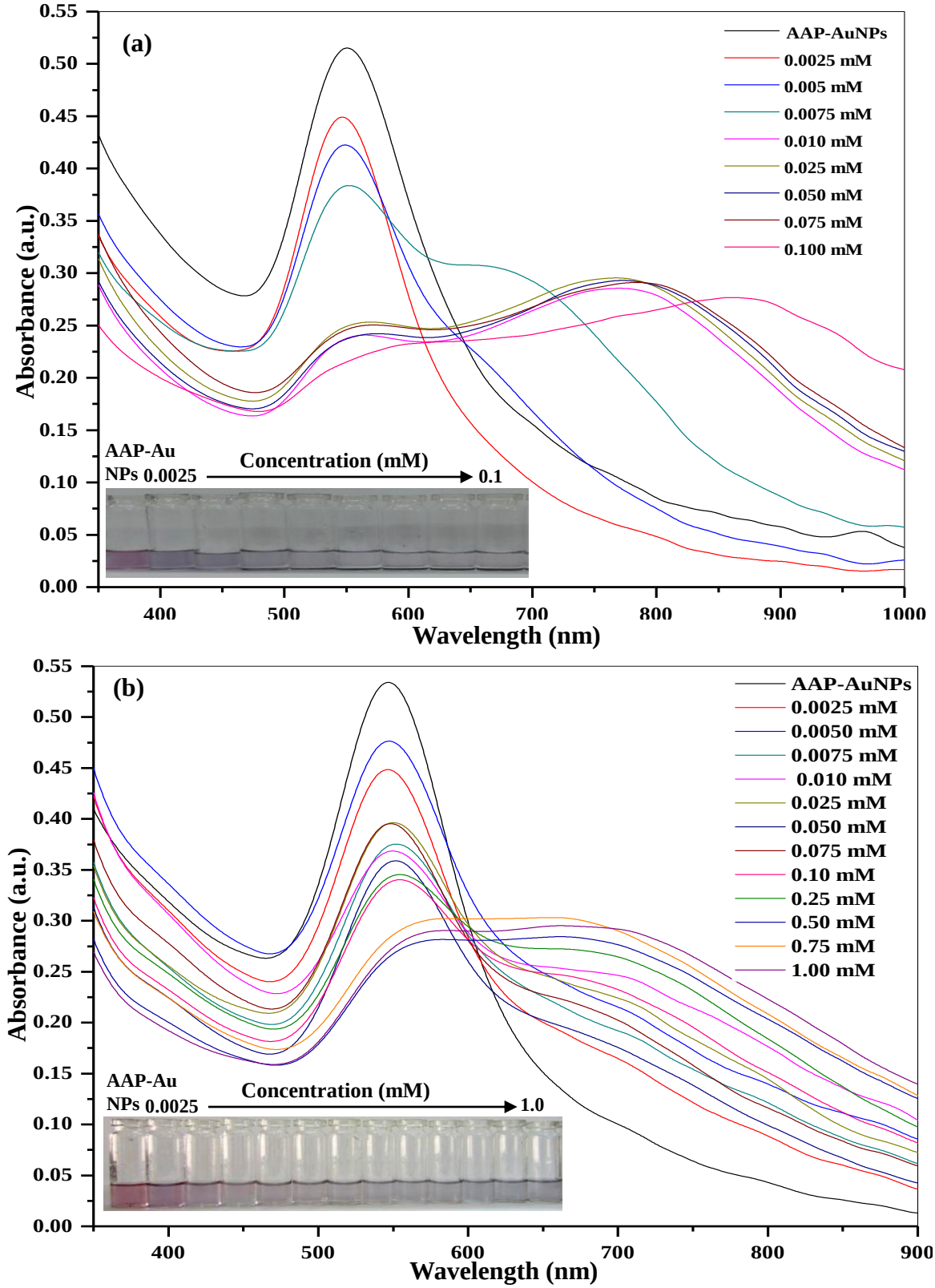
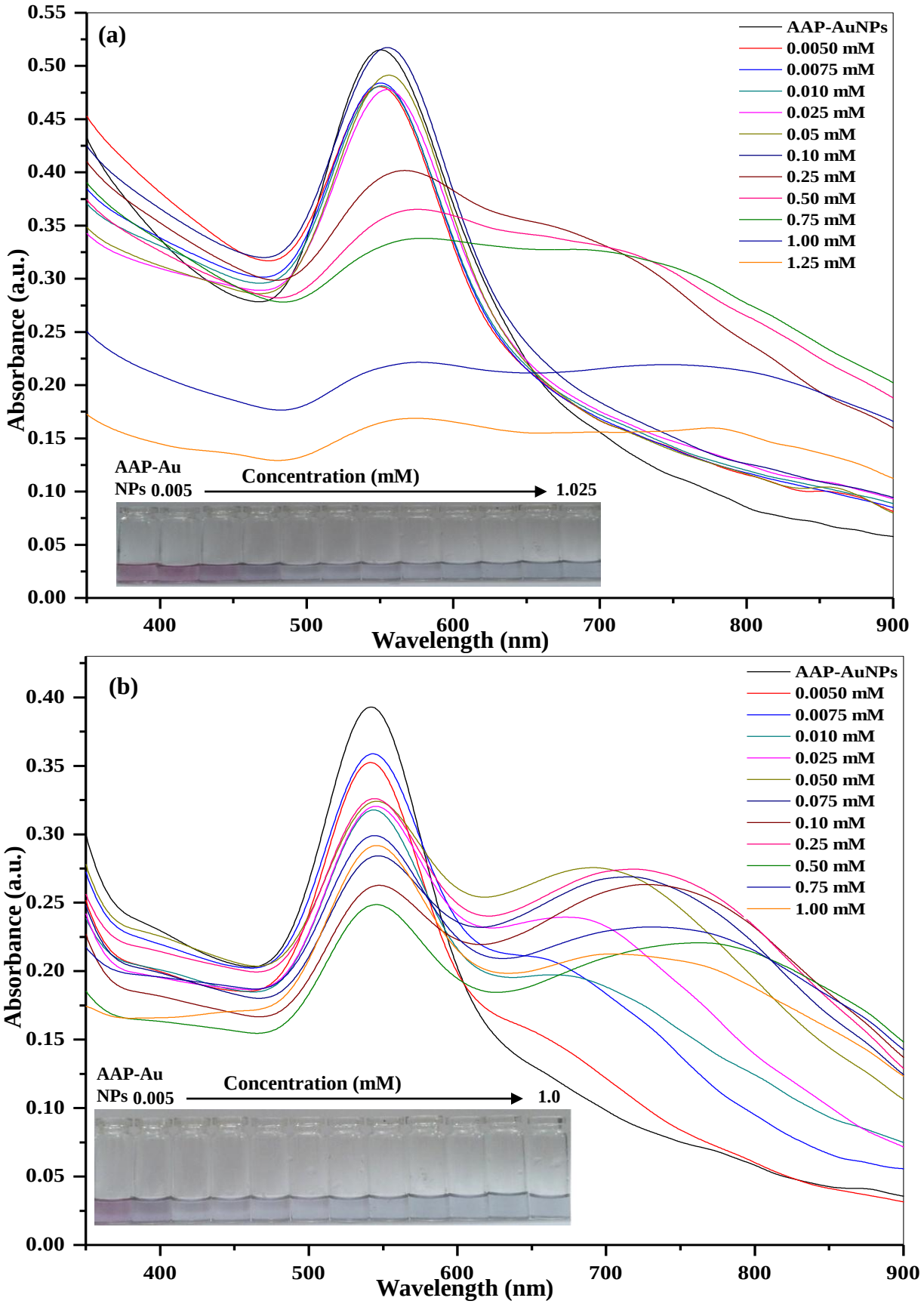


Figure 3



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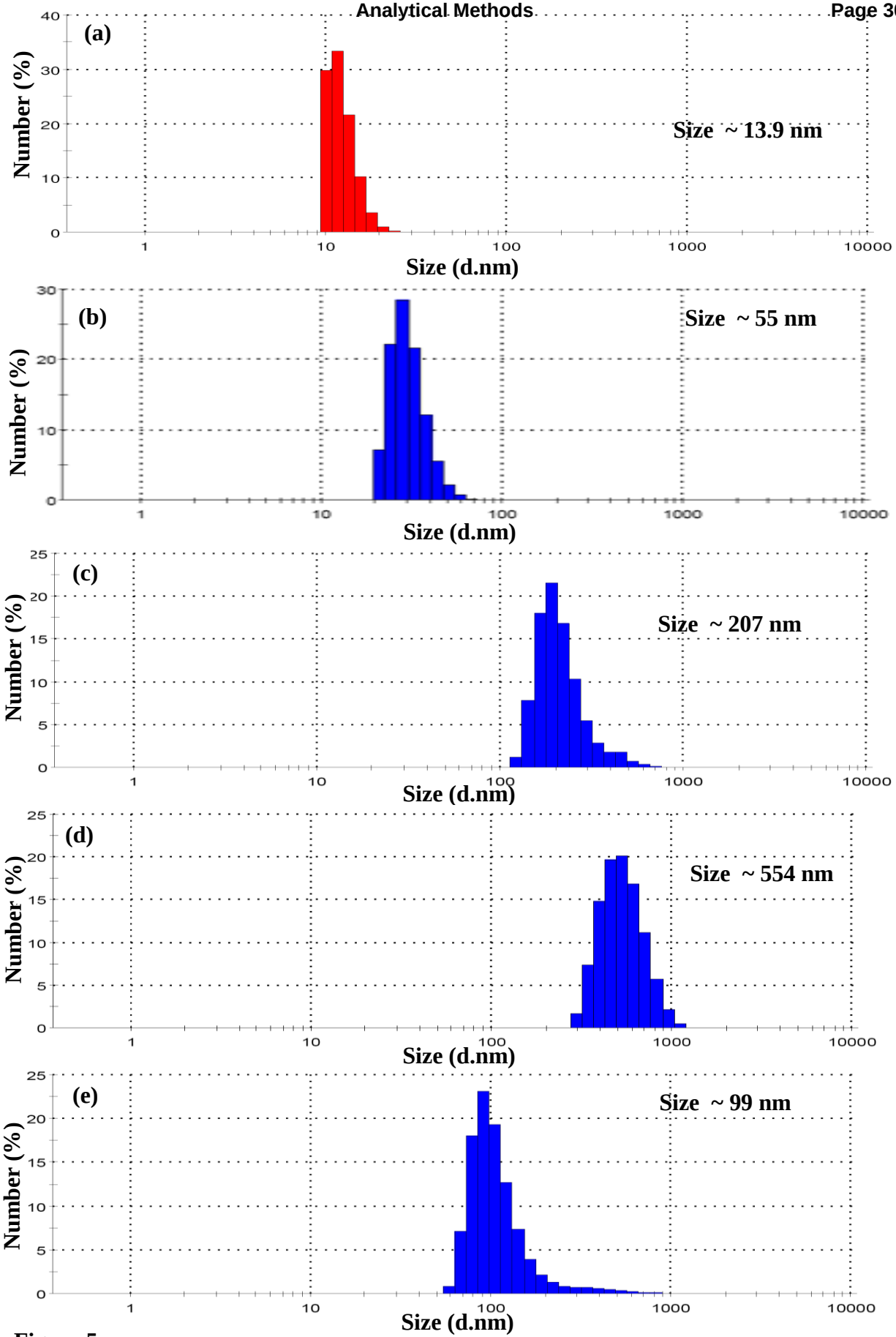
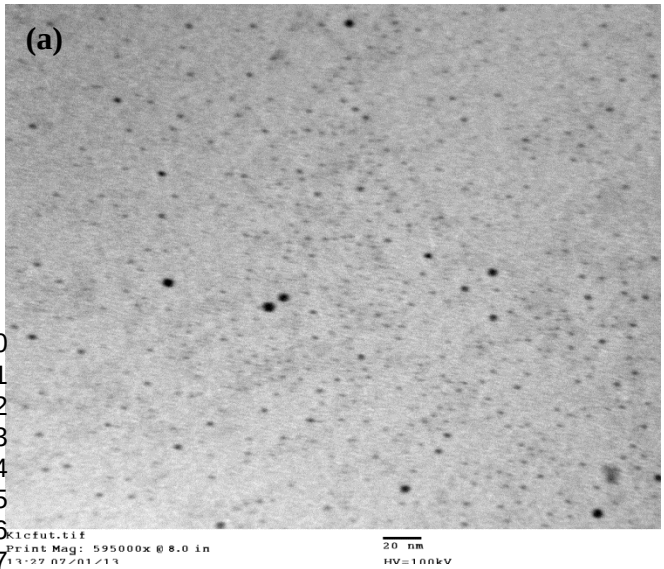
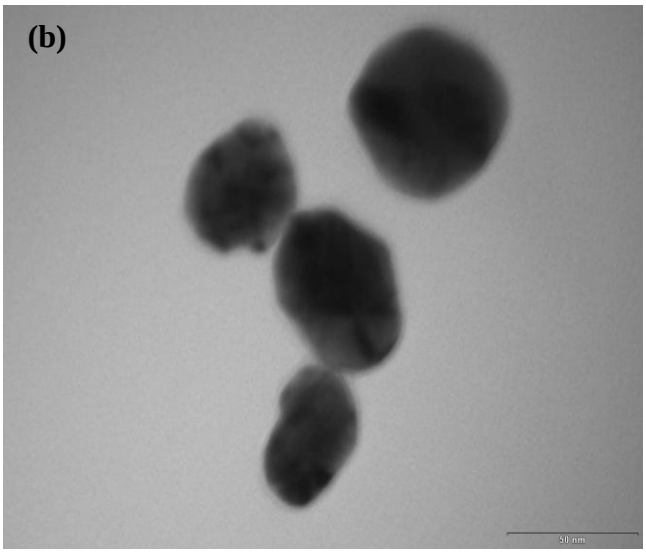
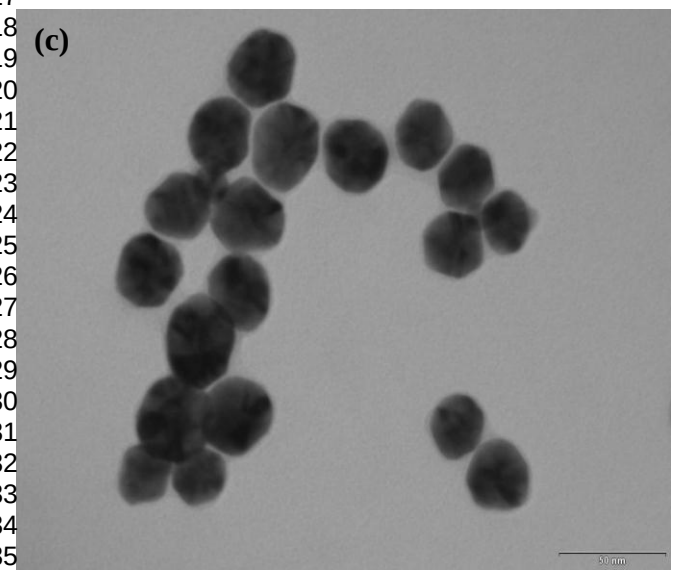
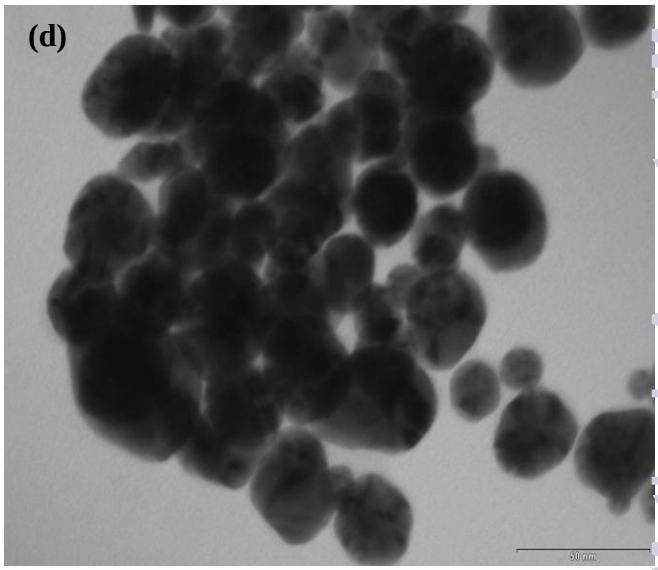


Figure 5.









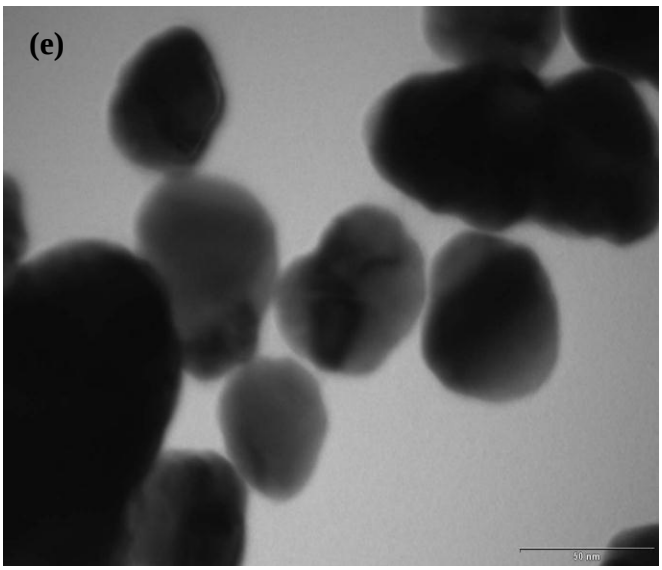
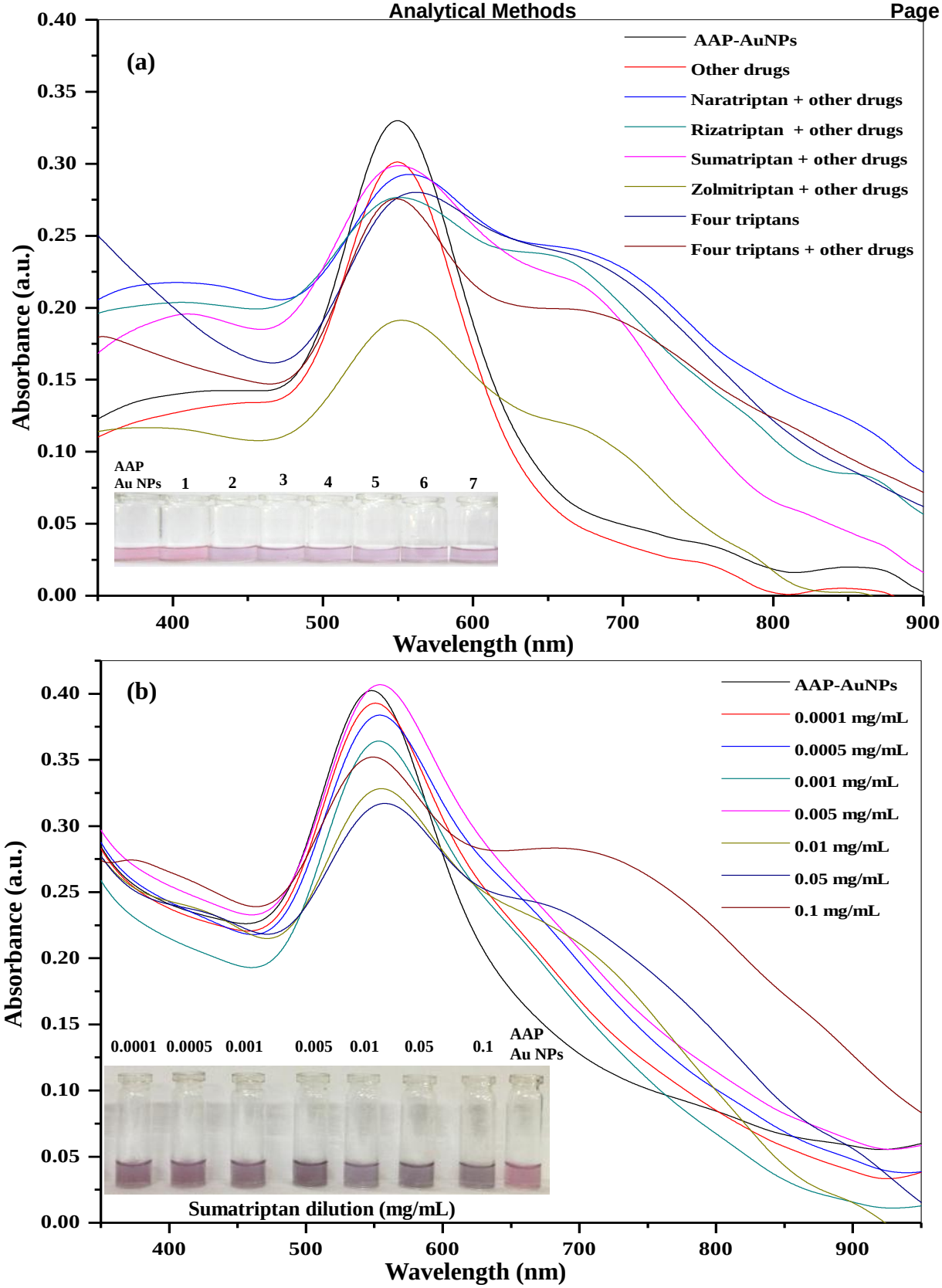


Figure 6.



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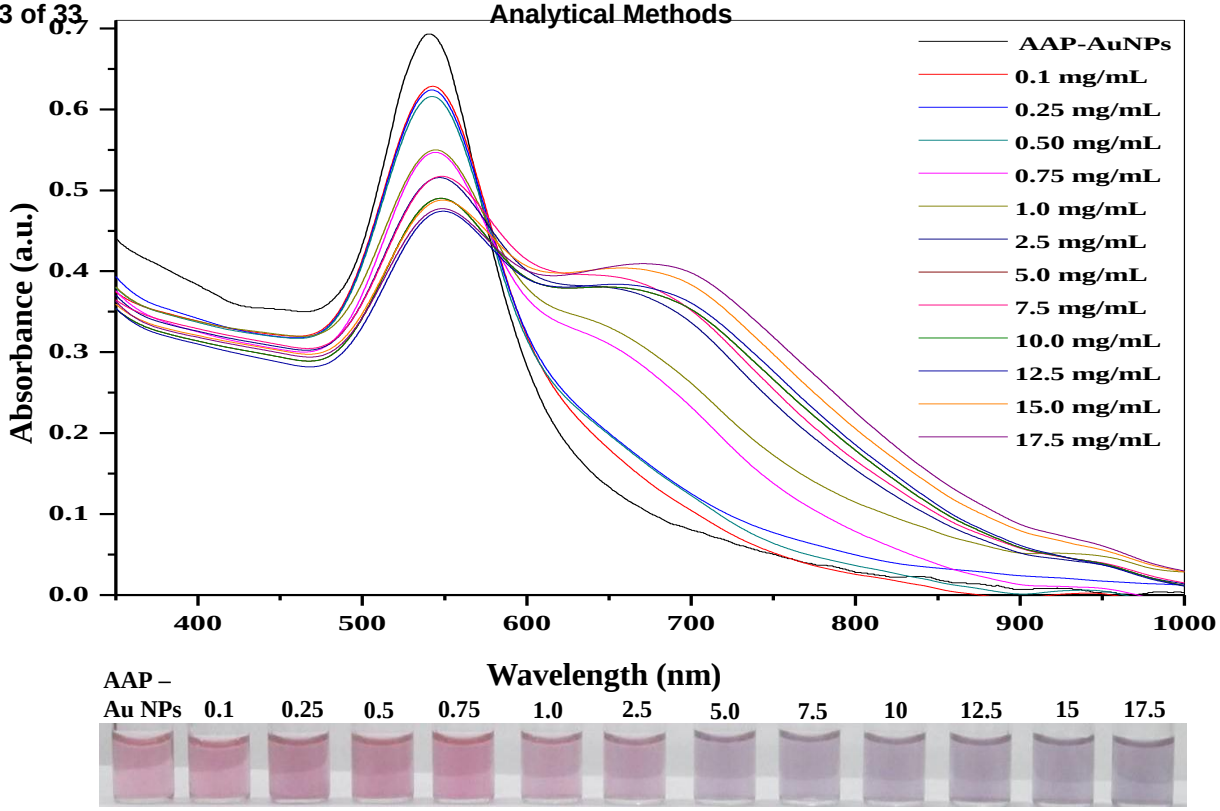

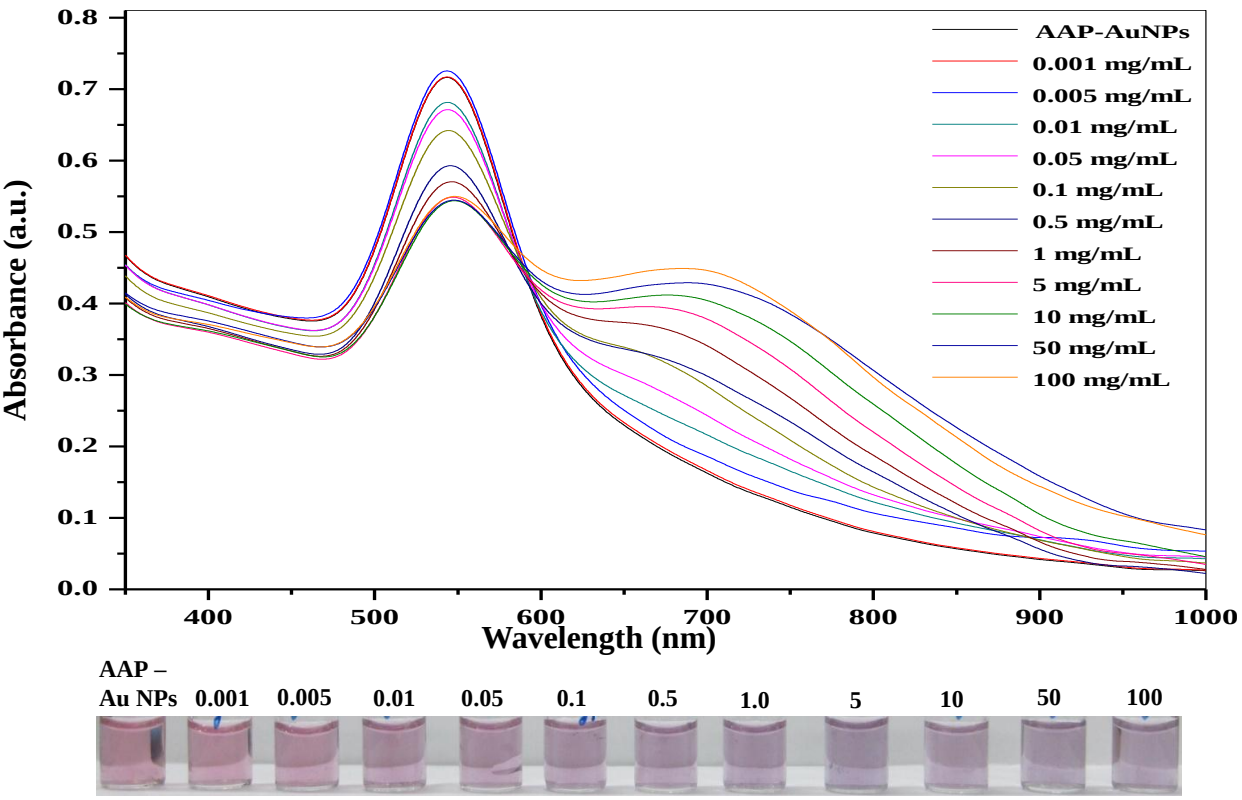

Figure 8.