Summary of the thesis

The selection of the method for quantification of the drug molecule depends on the objectives, type of matrix and analyte concentration. Determination of drug molecules *in vivo* requires highly sensitive and selective techniques while routine quality testing methods are concerned with the accuracy along with the cost per unit test. The requirement of expensive instrumentation restrains the resource limited countries from evaluating the quality of the drugs being sold in their markets. Sensitive and precise analytical methods for the quantification of drugs with added features of cost effectiveness, simplicity and ease of handling are requisites of many small scale industries and research laboratories. Hence novel approaches are required to raise sensitivity and selectivity of traditional low cost techniques for pharmaceutical and bioanalysis.

By the present work, we introduced various approaches to improve the performance of optical measurement techniques using metallic nanoparticles for determination of the drug molecule in different matrices. All chapters involve usage of some direct or indirect approaches for target analyte sensing which have been summarized in the following subsections.

**Chapter 2:**

Within Chapter 2, citrate ion modified silver nanoparticles (AgNPs) were synthesized using the conventional borohydride chemical reduction protocol. After adequate characterization of the AgNPs, they were found to have selective aggregation upon addition of a macrolide antibiotic namely azithromycin (AZT), which is usually silent in UV-Visible range of electromagnetic spectrum. The parameters affecting the selective aggregation were optimized. Possible mechanism is also provided in Figure 2.4. Under the optimized conditions, two different approaches were established to correlate the colorimetric response with the amount of AZT. Simple absorbance and ratiometric measurements enabled quantitation of AZT within the range of 0.2–20 μM, and 6.0–100 μM, respectively. High values of correlation coefficients along with other acceptable validation results indicated good applicability of the developed method.
Chapter 3:

Chapter 3 describes the post-synthesis functionalization of AgNPs studied in Chapter 2, and their use as colorimetric sensor for widely prescribed iron chelation drug i.e. deferiprone (DFP). The surface modification required incubation of AgNPs with tetrasodium pyrophosphate for only 10 min. A mechanistic approach, involving competitive binding, was studied, and Figure 3.3 described the basis for the development of the colorimetric sensor. Evaluation and optimization of the operating parameters yielded a linear response towards DFP concentration, which remained unaffected upon addition of different interfering substances. A brief comparison as presented in Table 3.1, indicated competitive usefulness of the present method. Lastly, the method was successfully applied for quantitation of DFP from two tablet formulations and spiked human plasma.

Chapter 4:

Apart from the colorimetric approach, as studied in the previous two chapters, this chapter presents supplementary fluorescence measurement mode for AgNPs based sensor. A simple fluorescence resonance energy transfer (FRET) mechanism based quenching was observed for Rhodamine-B (RB) when immobilized on the surface of AgNPs. Quenching constant in the order of $10^9 \text{ M}^{-1}$ specified high quenching efficiency of AgNPs. The surface bound RB molecules were found to have weaker surface binding ability and were easily displaced by the analyte of interest i.e. olanzapine (OLZ). The displacement of RB from the surface of AgNPs resulted in restoration of fluorescence intensity which, upon the optimization of operating conditions, was linear with OLZ concentration. Not only the sensor afforded fluorescence measurement up to sub-$\mu$M, but it also enabled extended detection up to 30 $\mu$M using colorimetric measurement (Table 4.1). The results of interference studies, as presented in Figure 4.9, indicated improved selectivity of RB immobilized AgNPs. Lastly, a good agreement between the found and labelled/spiked values indicate good applicability of both the approaches (Table 4.3).

Chapter 5:

Chapter 5 describes a first of its kind approach for the simultaneous detection of a drug-metabolite pair i.e. allopurinol (ALP) and oxypurinol (OXY)
respectively, using gold nanoparticles (AuNPs). For development of such probe, different functionalities on the surface of AuNPs were tested and their response for the analytes were measured. Upon suitable selection of capping reagent and further optimization of other experimental parameters, a suitable two step protocol was developed. The borohydride reduced and citrate capped AuNPs were having non-selective yet linear response for both the drug and metabolite (Figure 5.5). On the other hand, melamine modified AuNPs responded towards the presence of OXY only. Using a two-step measurement and subsequent mathematical conversion, it is possible to quantify co-existed ALP and OXY, which was evident from the results of laboratory simulated samples (Table 5.5).

**Chapter 6:**

This chapter describes a synergistic combination of zinc sulfide quantum dots (ZnS QDs) for sensitive detection of betahistine from different matrices. A good overlap between the emission spectra of ZnS QDs and the absorption spectra of AgNPs gave rise to inner filter effect. Even in the absence of QDs, the synthesized AgNPs showed aggregation with addition of betahistine (BTH). However the addition of ZnS QDs amplified the analytical response of the nanoparticle based sensor, and enabled the detection of BTH within the range from 0.2-9.0 µM, which was lower compared to colorimetric measurement alone (i.e. 2.0-16.0 µM). The good agreement between the found values and the claimed values of tablet samples, and recoveries point toward acceptable performance of the method (Table 6.2).