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Functionalized selenium nanoparticles for the targeted delivery of mesalamine

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ABSTRACT

The prime objective of this work was to formulate selenium nanoparticles, surface decorate them with phenyl alanine to impart colon targeting ability and load the particles with mesalamine. The colon targeted selenium nanoparticles were prepared by placing phenyl alanine on the surface of the selenium nanoparticles and then loading mesalamine on the surface of PA-d-SeNPs to produce PA-Se@Mes nanoparticles. The characteristic peaks of selenium nanoparticles (3555.92 for OH stretch, 3107.48 & 2943.50 for CH stretch, C-C stretch at 1465.96) were present in PA-d-SeNPs also. Additionally, a characteristic amide linkage (1712.86) was also visible confirming surface attachment of phenyl alanine. The colorimetric estimation revealed an attachment of 69.1% phenyl alanine on the surface of the nanoparticles. The yield of the nanoparticles was found to be in the range of 60-65%. The average drug loading was found to be 14.6 ± 0.21% whereas the average encapsulation efficiency was found to be 31.8 ± 0.37 %. The average particle size of selenium nanoparticles, PA-d-SeNPs and PA-Se@Mes was found to be 212.7, 140.6, 104.2 nm respectively. The zeta potential of the PA-Se@Mes was found to be -16.0 ± 3.70 mV. It was found that 85.19% mesalamine was released from PA-Se@Mes in presence of rat caecal content after 24 hours while only 69.31% was released in absence of rat caecal content, suggesting colon targeting.

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Introduction

An ample amount of attention is being focused on expanding the newer methods of delivery of active pharmaceutical ingredients (API) and is intended to minimize the limitations of the existing dosage form there by leading to optimization of the dosage regimen (Hirani et al., 2009). Despite extraordinary advancements in drug delivery, the oral drug delivery systems are still considered to be the most suitable for administrating the therapeutic agent (Yadav et al., 2009). The conventional oral drug delivery system permits only for a definite amount of API levels in plasma and does not leave a scope for any control over the delivery of drug. Development of site specific or targeted drug delivery system to any particular part or organ is aimed not only to improve the therapeutic efficacy by increasing drug concentration at the site of action, but also to trim down the side effects and cost of treatment by reducing the dose and dosing frequency (Krishnaiah et al., 1998).

During the last two decades, the interest of pharmaceutical scientists in development of colon targeted drug delivery systems (CTDS) has tremendously increased (Goindi et al., 2011). Colon targeting is done mainly to treat those ailments which are sensitive to circadian rhythm; the pathological state of colon; diseases like asthma, angina and rheumatoid arthritis and for delivery of drugs like proteins, peptides and steroids (Vemula et al., 2009 and Asghar et al., 2006). Colonic delivery offers several preferential benefits as the site of drug delivery (Ahmad et al., 2011; Ahuja et al., 2010).

Selenium is an essential micronutrient required for proper functioning of biological and metabolic mechanism within the human body. Deficiency of selenium leads to the generation of several harmful disorders such as cancer, neurological, muscular, immune, etc. Generally, selenium can be depicted within a very narrow concentration range due to its deficiency, physiological effect, and toxic doses. At optimal doses Se acts as an antioxidant, whereas at higher doses it shows pro-oxidant activity. To overcome this issue, a precisely controlled dosage of Se is generally suggested. Selenium has various health advantages because of its bioavailability in the form of selenoproteins as well as lower molecular weight. Various selenoproteins (Se1P, Se1F, Se1S, Se1M), thioredoxin reductases, and glutathione peroxidases show redox activity and control redox reactions in cells.

Mesalamine has been widely prescribed drug for management of ulcerative colitis. The targeting of drugs to colon has been widely investigated and selenium nanoparticles have been found improve targeting to various organs including colon.

Hence it was envisioned to formulate selenium nanoparticles, surface functionalize them with amino acid phenylalanine and load them with meselamine and study the colon targeting potential of the formulations.

Material and Methods

Preformulation Study (Ravi and Jain, 2021)

The color, odour and taste of the obtained drug sample were observed with the help of the sensory organs. Solubility was determined in different solvents like water, methanol, ethyl Alcohol, and DMSO. LOD was determined on IR moisture balance by heating a weighed amount of drug. Melting point was determined by open capillary method and is uncorrected. A small quantity of powder was placed into fusion tube and placed in the melting point apparatus. The temperature of the apparatus was gradually increased and the temperature at which the powder started to melt and the temperature at which all the powder got melted was recorded.

Calibration curve of mesalamine

An accurately weighed 10 mg of pure mesalamine was transferred to 10 mL volumetric flask and dissolved using 0.5M HCl and made upto 10 mL with 0.5M HCl (Moharana et al, 2010). Appropriate aliquots were withdrawn from the above solution and diluted up to 10 mL with 0.5M HCl to obtain working standard solutions of different concentrations (5,7.5,10,12.5 and 15 µg/mL). The solution was scanned in UV (200-400 nm) to obtain a absorption maxima of 304 nm and the absorbance of each dilution was observed at this wavelength. A calibration curve of absorbance against concentration was plotted.

Formulation of Selenium Nanoparticles

Selenium nanoparticles were prepared according to the method reported by Xia et al (2020). Briefly, 2 mL of 10 mM solution of ascorbic acid and 1 mL of 5 mM solution of sodium selenite in water were mixed to obtain a solution. The mixture was gently stirred at room temperature for about 30 min to prepare Selenium nanoparticles, indicated by formation of red color of the solution.

Surface decoration of Selenium nanoparticles

The surface of the selenium nanoparticles was

modified by interacting with a solution of amino acid phenyl alanine. Phenyl alanine was dissolved in water to obtain a 2 mM concentration and 1 mL of this solution was added dropwise into the solution of selenium nanoparticles under continual magnetic stirring. On completion of addition, the stirring was continued for additional 120 min to obtain the phenyl alanine decorated selenium nanoparticles (PA-d-SeNP).

Determination of amount of surface decoration

The amount of phenyl alanine attached to the surface of the selenium nanoparticles was determined using amine group titration method. Briefly, after decoration, the solution was centrifuged and the supernatant was removed to separate the unbound phenylalanine and was treated with 0.1M picric acid in DCM (1 mL) and allowing the reaction to proceed further for 1 h. The mixture was centrifuged thrice to remove unreacted picric acid. The bound picric acid was removed from the amino group by reaction with excess of 0.1M triethylamine (TEA) in DCM. The concentration of TEA-picrate was measured at 358 nm colorimetrically.

Loading of Mesalamine to PA-d-SeNP (PA-Se@Mes)

Mesalamine was loaded on the surface of the PA-d-SeNP by preparing as solution in DMSO. Briefly, 2 mg of Mesalamine was dissolved by sonication in 60 µL of DMSO and the solution was added dropwise with stirring to the PA-d-SeNP solution. The stirring was continued for 10-12 hours to ensure maximum loading of mesalamine on the nanoparticle (PA-Se@Mes).

Characterization of nanoparticles

Yield

The yield of the nanoparticles was calculated using the mass of the final product after lyophilization with respect to the initial total mass of drug, polymer and stabilizer used in preparing the nanoparticles.

Particle size, size distribution and Zeta potential

The average particle size, poly dispersity index (PDI) and Zeta potential of the nanoparticles was determined using the dynamic light scattering technique on a Malvern Zeta sizer. The nanoparticles were suspended in ultrapure water in a disposable sizing cuvette and analysis was performed at an angle of detection of 90°.

Encapsulation Efficiency (Mishra et al, 2013)

The percent drug encapsulated in the polymeric nanoparticles was assessed spectrophotometrically. Accurately weighed 25 mg of nanoparticles were dissolved in volumetrically measured 10 mL of DCM. FU was extracted from the solution thrice using 25 mL normal saline by shaking in a separating funnel for 15min and allowing the mixture to equilibrate for 10min before separating the aqueous portion. Absorbance of each aqueous extract was measured on UV spectrophotometer at 266 nm against normal saline as blank. Concentration of drug was calculated using the calibration curve. Encapsulation efficiency was calculated by the following formula:

$$\% EE = 100 * b/a$$

$$b = c * d/25$$

where a – theoretical drug loading in mg, b – actual drug loading in mg, c – amount of drug in 25mg of nanoparticles, and d – total weight of drug and polymer in mg taken for nanoparticle formation

***In vitro* release study**

In vitro dissolution studies for all matrix tablet formulations were performed by using USP dissolution test apparatus (Basket type, 37°C) at 100 rpm using phosphate buffer pH 7.4 as the dissolution medium (200 mL). The tablet was placed in the basket and the dissolution study was carried out for 12 h. At predetermined intervals, 1 mL of the dissolution media was pipetted out and its volume was made up to 10 mL using 0.5M HCl. Absorbance of the solution was recorded at 304 nm using UV visible spectrophotometer. 1 mL of fresh medium was added to the dissolution flask after each withdrawal.

***In vitro* release studies in presence of rat caecal content** (Atal and Kaushik, 2021)

To evaluate the ability of the nanoparticles to release drug in colonic environment, the release study was carried out in presence rat caecal content.

Preparation of rat caecal content

All the animal works were approved by the Institutional Animal Ethical Committee (IAEC) of the institute. The caecal contents were obtained from Wistar rats weighing 150-200 g, maintained on a normal diet were administered with 4 mL of 1% w/v of dispersion of Guar gum in water for 7 consecutive days. Thirty minutes before starting drug release studies, 3 rats were killed by spinal traction, after which abdomens were opened, dissected, and immediately transferred to pH 6.8 phosphate buffer previously bubbled with CO_2. The caecal bags were then opened; their contents were individually weighed, homogenized, and then suspended in pH 6.8 phosphate buffer to give the desired

concentration of 4% w/v of caecal content.

Release study in colonic environment

The drug release for all the formulations was carried out with 200 mL of phosphate buffer pH 7.4 with rat caecal content (4% w/v). At predetermined intervals, 1 mL of the dissolution media was pipetted out and its volume was made up to 10 mL using 0.5M HCl and the absorbance of the solution was recorded at 304 nm using UV visible spectrophotometer. 1 mL of fresh medium was added to the dissolution flask after each withdrawal. The experiment was continued for 24 h.

Results and Discussion

Preformulation Study

The physical characterization of the drug was performed according to the reported procedure (Table 1).

Calibration curve

The absorbance of mesalamine was measured at 304 nm using UV spectrophotometer against 0.5M HCl solution as blank. The plot of concentration vs. absorbance yielded the equation of calibration curve, which was used for analysis of the content of mesalamine in formulations and release samples (Figure 1).

Formulation of PA-Se@Mes

The colon targeted selenium nanoparticles were prepared by placing phenyl alanine on the surface of the selenium nanoparticles and then loading mesalamine on the surface of PA-d-SeNPs to produce PA-Se@Mes nanoparticles. Phenyl alanine utilizes L-amino acid transporter 1 (LAT1) which are overexpressed at colonic region for delivering the loaded drug. Nanoparticulate formulation enhances the internalization of the drug into the tissues. The surface decoration was studied with the help of the FT-IR spectrum of the selenium nanoparticles as well as PA-d-SeNPs. The characteristic peaks of selenium nanoparticles (3555.92 for OH stretch, 3107.48 & 2943.50 for CH stretch, C-C stretch at 1465.96) were present in PA-d-SeNPs also. Additionally, a characteristic amide linkage (1712.86) was also visible confirming surface attachment of phenyl alanine (Figure 2).

The amount of conjugation of phenyl alanine of the surface of selenium nanoparticles was studied by amine group titration. The colorimetric estimation revealed an attachment of 69.1% phenyl alanine on the surface of the nanoparticles.

Evaluation of PA-Se@Mes

Yield and drug loading

The yield of the nanoparticles was found to be in the range of 60-65%. The drug loading and encapsulation efficiency were calculated using the calibration curve equation. The average drug loading was found to be 14.6 ± 0.21% (n=5) whereas the average encapsulation efficiency was found to be 31.8 ± 0.37 % (n=5).

Particle size and zeta potential

The average particle size of selenium nanoparticles, PA-d-SeNPs and PA-Se@Mes was found to be 212.7, 140.6, 104.2 nm respectively (Figure 3). The polydispersity was found to be 0.259, 0.417 and 0.430 in the same order.

The zeta potential of the PA-Se@Mes was found to be -16.0 ± 3.70 mV. The negative zeta potential helps in maintaining electrostatic repulsion required for prevention agglomeration of the particles

In vitro release study

The *in vitro* release study was done for all the formulations to assess the time duration up to which the drug is released by the PA-SE@Mes and to prove a sustained release from the particles as well as to confirm the targeting efficiency of the surface decorated selenium nanoparticles (Table 2). The % cumulative release was plotted against time to obtain the release kinetics equation for the formulations (Figure 4).

As it can be witnessed from the results of release study that in absence of rat caecal content, the release of mesalamine was comparatively low at each time point with maximum release of 69.31% after 24 hours. On the other hand 85.19% mesalamine was released from PA -Se@Mes in presence of rat caecal content after 24 hours. This suggests the involvement of microbial erosion of the nanoparticles in the colon signifying a targeted delivery of the attached drug.

Conclusion

Selenium nanoparticles have been reported to be involved in colon targeted delivery of drugs. In the present investigation, selenium nanoparticles were surface decorated with phenyl alanine to promote colon targeting. The study was able to establish the involvement of LAT-1 transporters of phenyl alanine in helping the targeting of selenium nanoparticles to colon.

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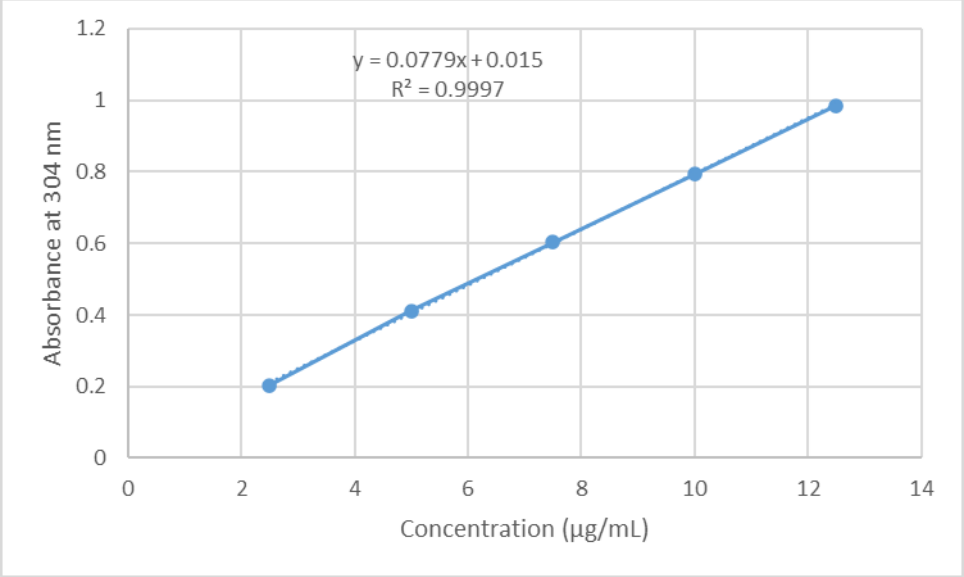

Figure 1 Calibration curve of mesalamine in 0.5M HCl

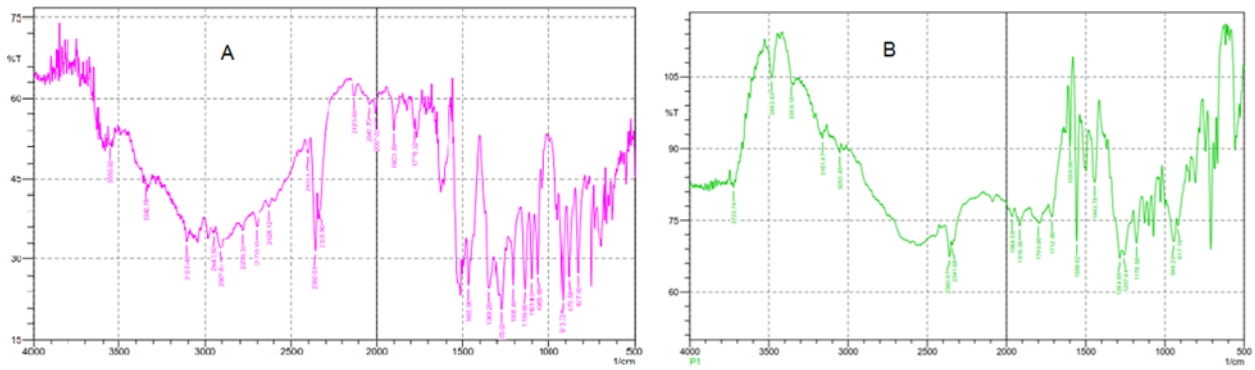

Figure 2 FTIR spectra of (A) **selenium nanoparticles (B) PA-d-SeNP**

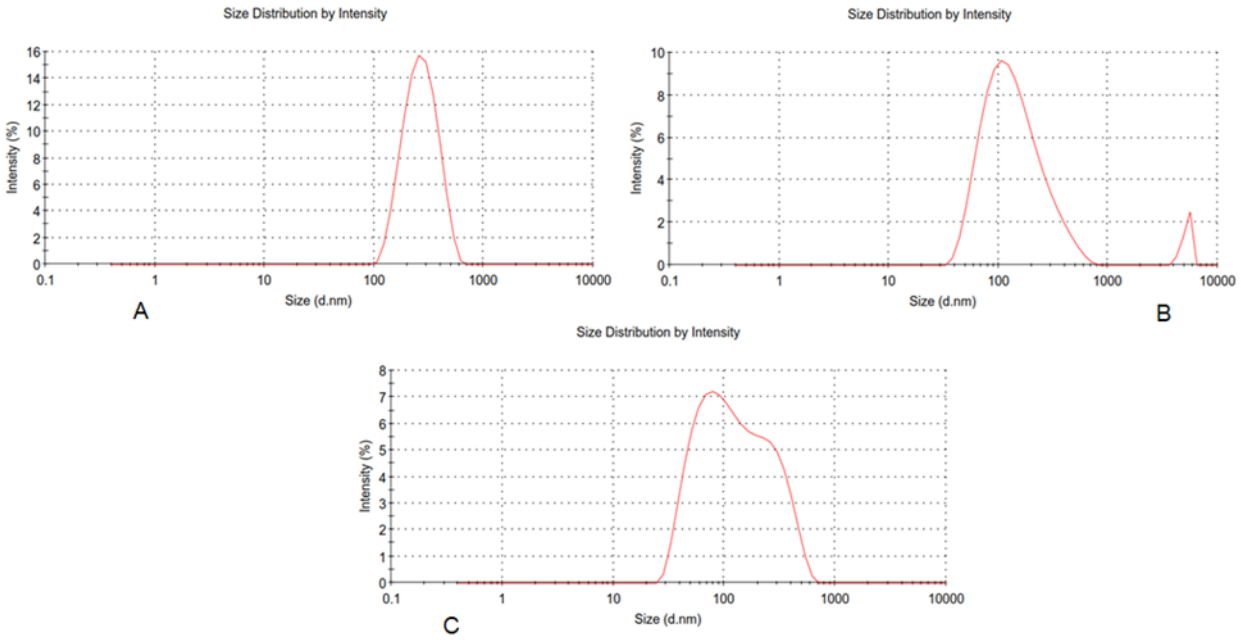

Figure 3 Particle size of (A) **selenium nanoparticles (B) PA-d-SeNPs (C) PA-Se@Mes**

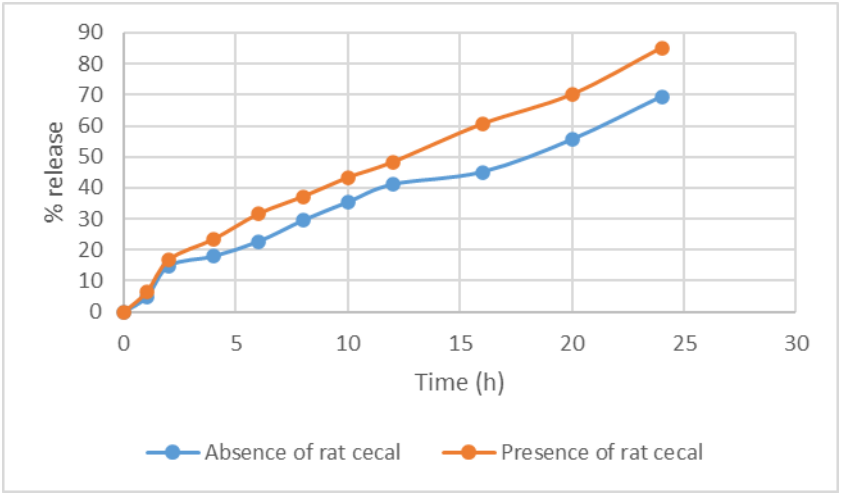

Figure 4 Percent of mesalamine released

Table 1 Preformulation characters of mesalamine

S. No	Parameter	Observation
1	Physical appearance	Off-white powder
2	Odour	Odourless
3	Melting Point	276-280°C
4	Taste	Bitter
5	Partition coefficient	1.8
6	LOD	0.32 %
7	Solubility	Insoluble in water, soluble in ethanol, DMSO

***In vitro* release of mesalamine from PA-Se@Mes**

Time (h)	% Cumulative release	
	Absence of rat caecal content	Presence of rat caecal content
0	0	0
1	4.81	6.48
2	14.82	16.88
4	17.88	23.49
6	22.62	31.63
8	29.49	37.23
10	35.36	43.36
12	41.03	48.37
16	45.03	60.64
20	55.5	70.18
24	69.31	85.19