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A Novel UHPLC-MS/MS-Based Bioanalytical Method Developed for S-Allyl Cysteine in the Establishment of a Comparative Pharmacokinetic Study

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Abstract: A newly UHPLC-MS/MS method development and validation for S-Allyl Cysteine was used to evaluate the comparative pharmacokinetic parameters. SC PLGA NPs (S-Allyl Cysteine Poly (D,L-lactide-co-glycolic acid) Nanoparticles) were developed by the emulsion solvent evaporation method. SC PLGA NPs showed their drug loading and encapsulation efficiency to be 5.13 ± 0.10% and 82.36 ± 4.01%, respectively. SC PLGA NPs showed a spherical morphology of an average size (134.8 ± 4.61 nm), PDI: 0.277 ± 0.004, and −25.3 ± 1.03 mV Zeta-Potential (ZP), and is suitable for oral delivery. The development and validation of the UHPLC-MS/MS bioanalytical method were performed successfully for PK-parameter examinations with 1.219 min RT, MS (162.00/73.10), and a total run-time of 2.0 min. Additionally, 1.0–1000.0 ng/mL was a linear range with inter- and intra-day accuracy of 92.55–99.40%, followed by a precision of 1.88–4.23%. SC PLGA NP's oral bioavailability was significantly higher (** $p < 0.01$) in comparison to the SC-S treated groups' (iv and oral). The antimicrobial activity of SC PLGA NPs proved to be more effective than pure S-Allyl-L-Cysteine with significant results ($p < 0.01$) in comparison to SC-S. SC PLGA NPs showed fitted physicochemical and enhanced antimicrobial properties, which can be helpful for oral administration. Based on the proposed research results, SC PLGA NPs were used for the improvement in oral bioavailability with a sustained and controlled release of S-Allyl-L-Cysteine delivery.

Keywords: S-allyl cysteine; oral bioavailability; PLGA NPs; LC–MS/MS; pharmacokinetics

1. Introduction

S-Allyl-L-cysteine (SC) is a major constituent, containing a maximum bioactive organosulfur compound isolated from garlic, which contains various biological functions by several mechanisms [1]. S-Allyl-L-cysteine is a sulfur-containing amino acid isolated from bulbs

of garlic and onion, showing antioxidant activity [2,3], anti-cancer properties [4,5], anti-hepatopathic activity [6], and neurotrophic activity [7,8]. S-Allyl-L-cysteine is one of the important metabolites of allyl esters and allyl aldehydes [9]. In recent times, S-Allyl-L-cysteine is the one of the marker compounds that is used in clinical studies of garlic [10]. S-Allyl-L-cysteine also shows strong antimicrobial effects on Gram-positive bacteria, (a) *Bacillus cereus* and (b) *Staphylococcus aureus*, and Gram-negative bacteria: (a) *Escherichia coli*; (b) *Pseudomonas aeruginosa*: (c) *Salmonella typhimurium*; and (d) *Proteus mirabilis* [11–13]. Therefore, the oral bioavailability of SC is highly important for the biological therapeutic action of free S-Allyl-L-cysteine [14,15].

Patient compliance is one of the most important criteria for oral delivery. However, this can show poor bioavailability, which is a major concern to think tank institutions [16]. The therapeutic concentration is the most important criteria for oral drug delivery to maintain their enrichment by incremental dosage or the use of novel drug delivery, which will increase the pharmacokinetic parameters. If we were to enhance the dose or use multiple dosing, this would contribute to drug wastage, uneconomical practices, and would lead to enhancing the induction of adverse effects. At present, nanoformulation scientists are working within the same situations and finding ways to make their nano drug delivery solutions as cost-effective as possible [17].

Nanoformulation is an important tool for enhancing the bioavailability of drugs via their modification in dosage forms for water-soluble drugs [18]. Both polyglycolic acid (PGA) and polylactic acid (PLA) are copolymers responsible for the Poly (lactic-co-glycolic acid) (PLGA), which is already approved by the US-FDA for clinical application [19]. PLGA showed a maximum property of biocompatibility and degradation in physiological environments in the presence of lactic acid and glycolic acid, two biodegradation products that are natural metabolites. At present, scientists are using PLGA as a carrier for the delivery of drugs and food. Radix Ophiopogonis polysaccharide is a macromolecule that exhibited poor water solubility when encapsulated via PLGA, followed by PEGylation of PLGA, to improve their solubility and in vivo bioavailability [20]. The combination of glycyrrhetinic acid and PLGA biodegradable micelles was used for the encapsulation of Tanshinone IIA and the targeting of hepatocellular carcinoma [21].

Our proposed current research showed the enhancement of S-Allyl-L-cysteine's (SC) bioavailability via loaded PLGA NPs. The SC-loaded PLGA NPs were developed by emulsion solvent evaporation techniques, containing the lowest PDI with the help of high-pressure homogenization. The current drug showed maximum dissolubility in the basic pH of water than in distilled water. Therefore, an ammonia solution was chosen as the inner aqueous phase solvent. In this way, we enhanced the solubility, encapsulation, and drug loading in PLGA NPs of SC. All the characterization-related parameters were performed similar to an in vitro release, considering the physicochemical characteristics, particle size, PDI, Zeta Potential, etc. All the effects related to the nanoformulation parameters for SC PLGA-NPs were performed and examined.

Researchers have previously reported various methods for analyzing garlic extract samples or methods based on simultaneous analysis with more compounds [22–24]. Previously, two plasma analysis methods were published for S-Allyl-L-Cysteine: one of them was developed via μg per mL (0.10 to 100), and the other had a drawback run time of up to 12 min [1,25]. Additionally, we found no available, fast-acting method for nanogram level analysis in plasma SC-alone, alongside a sensitivity up to the picogram level with a speedier runtime, i.e., 2.0 min for the examination of our drug. Therefore, we have developed a novel LC-MS bioanalytical method for S-Allyl-L-Cysteine and validated it successfully for examining the pharmacokinetic parameters of S-Allyl-L-Cysteine PLGA NPs. The developed method showed many advantages, like high sensitivity, maximum efficiency, and a lower retention and runtime, for the examination of PK parameters in the plasma.

This is a first-time report to prepare SC PLGA nanoparticles used to increase the bioavailability of S-Allyl-L-Cysteine. S-Allyl-L-Cysteine's oral bioavailability was im-

proved via PLGA NPs, and maintained the drug's therapeutic level after oral delivery. SC PLGA NPs showed significant effective-controlled and sustained release. A comparative PK-study was performed for the SC-S and SC PLGA NPs with the help of PK examination (AUC_{0-t}, C_{max}, $K_{el,}$ $t_{1/2}$, etc.) and also comparative bioavailability based on the successfully developed and validated LC-MS/MS method. Finally, we also performed the most important antimicrobial study for SC-S and optimized-SC PLGA NPs against Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*) for the antibacterial activity with 100 µL of SC-S and SC PLGA NPs samples and the dose-dependent antibacterial activity with 30 µL; 50 µL and 100 µL of SC-S and SC PLGA NPs samples.

2. Materials and Methods

S-Allyl-L-Cysteine was purchased from Sigma. Poly (vinyl alcohol, MW 25,000) and PLGA were bought from Polysciences Inc., 400 Valley Road, Warrington, PA 18976. We used LC-MS grade solvents like Acetonitrile, Methanol, ammonium acetate, formic acid, and ammonium formate, which were bought from Sigma–Aldrich Corporation (St. Louis, MO, USA). Milli–Q–Water, i.e., Ultra-pure-water was used in the entire study. Other chemicals that were used were analytical grade and from various marketable sources. For antimicrobial activity, Mueller–Hinton agar medium and, for bacterial culture, Mueller–Hinton broth were purchased from Oxoid, UK.

2.1. Nanoparticles Preparations for S-Allyl-L-Cysteine

The emulsion solvent evaporation method was used in the development of S-Allyl-L-cysteine PLGA NPs [26]. PLGA (55 mg) was dissolved in 6.0 mL dichloromethane and, on the other hand, 30 mg S-Allyl-L-cysteine was dissolved in 1.5 mL ethanol. The experiment was performed over ice with the help of sonication for up to 2 min for the emulsification. An amount of 10 mL of 0.3% PVA was added drop by drop by walls of tubes in the mixture of PLGA solution. After that, more emulsification was performed at 0 °C with the help of a sonicator for an extra two minutes. The solution was stirred overnight until the evaporation of DCM at room temperature. The next day, the whole solution was centrifuged for forty minutes up to eighteen thousand rpm. After that, we collected the supernatant and then discarded it. The ppt was again dissolved into Milli-Q-water and obtained in the suspended form. These pellets were freeze-dried to obtain the SC-loaded PLGA NPs.

2.2. Morphology of S-Allyl-L-Cysteine

The determination of particle size is very important in studying NPs. The size of particles is going to be smaller, which means enhancing the surface area of drug absorption [27]. We have used the Zetasizer for the examination of particle sizes, PDI, and ZP. We diluted our preparation prior to determining the size of the particles, PDI, and ZP. This experiment was performed at 25.0 °C and the scattering angle was fixed at 90° [26].

2.3. TEM Study for SC PLGA NPs

The transmission electron microscope was used to determine the size, shape, and structure of the SC PLGA NPs. Dispersed nanosuspension was added into the TEM grids containing the carbon support film which had holes. The specimen inside the TEM grids were dried by air, followed by insertion for TEM imaging. We have taken the TEM images [27].

2.4. Other Characterization of SC PLGA NPs

The %EE and %LC of the optimized nanoparticles was examined with the help of the ultracentrifugation technique for thirty minutes at four °C at 15,000.0 rpm. We took the supernatant-free amount of S-allyl cysteine to determine the actual amount from our

in-house developed method LC-MS. We examined this three times and, after that, used the equation below to perform the %EE and %LC calculation of SC PLGA NPs [26]:

$$\text{EE (\%)} = [\text{SC entire amount} - \text{SC Free amount}]/\text{SC entire amount} \times 100$$

$$\text{LC (\%)} = [\text{SC entire amount} - \text{SC Free amount}]/\text{NPs-Weight} \times 100$$

%Process Yield was calculated based on the formula below:

$$\text{Process Yield (\%)} = [\text{W1 (Dried NPs Weight)}/\text{W2 (Total Dried Weight of Starting Materials)}] \times 100$$

2.5. The Release Amount of In Vitro for S-Allyl Cysteine

The release of SC (in vitro) was performed via a pre-treated dialysis membrane containing the pore size 2.4 nm and ~12.0–14.0 kD cut-off molecular weight [26]. NaCl (0.2% w/v) was prepared in the HCl (0.7% v/v) and maintained the pH:1.20 as a medium for the simulated gastric fluid and another one with pH:6.80 as simulated intestinal fluid not containing enzymes. The temperature was maintained at 37.0 ± 1.0 °C, continuously stirring for up to six hours at 100 rpm. Dialysis bags were checked thoroughly for any leakage and then SC PLGA NPs were kept in the dialysis bags of which SC:0.5 mg. We chose 30, 60, 120, 240, 360, 480, 720, and 1440 min for sampling time points to evaluate the release of S-allyl cysteine from SC-S and SC PLGA NPs. We withdraw a 1.0 mL sample for each sampling point. The withdrawn samples were filtered through a 0.22 µm syringe filter for the determination of S-allyl cysteine from our developed LC-MS/MS method.

2.6. Animal Examination

Ethical approval was given by the Animal Ethical Approval Committee, Imam Abdulrahman Bin Faisal University (IAU) for PK/PD studies. All rats were withdrawn from IAU Animal House and were 190 to 220 g in weight. They were separated and grouped, i.e., 5–6 in each cage, and kept in a natural light and dark cycle with free access to food and water. The temperature was maintained at 20–30 °C with 50–550% humidity and we followed the IAU laboratory-prescribed conditions.

2.7. Standard and Quality Control Samples Preparation

The stock was prepared in the water (1 mg/mL) with the help of a vortexer for five minutes. The dilution was performed further for the preparation of calibration curves with the help of 5% blank plasma spiking (950 µL plasma + 50 µL aqueous). We obtained the final standard plasma samples, i.e., 5.0, 10.0, 49.5, 214.4, 424.4, 640.0, 850.0, and 1000.0 ng/mL for the calibration curve. The QCs dilutions (5.01, 14.90, 445.00, and 840.00 ng/mL) were prepared in the spiking of plasma for the determination of inter- and intra-day precision and accuracy. All prepared aliquots were stored to freeze at −40.0 °C before analysis.

2.8. A Sample Preparation in Plasma

All plasma samples were stored in the deep freezer, before undergoing bioanalysis, at −80 °C. All plasma samples were withdrawn based on requirement and were allowed to thaw at room temperature for equilibration followed by vortexing. An amount of 550 µL of sample was transferred into the fresh test tube on the ice bath and 5.0% formic acid was added to the breaking of plasma protein with help of vortexing. Next, we added the Methanol (4 mL) and kept it on the shaker for shaking for up to fifteen minutes. The centrifugation was carried out for all samples for ten minutes at four thousand rpm at 4.0 °C. We dried, under N_2 Steam water bath, 2.5 mL of the supernatants which were placed into fresh test tubes. The mobile phase (750 µL) was transferred into dried test tubes and then vortexed for four minutes for reconstituting. We transferred reconstituted samples into the vials. Samples were injected (10 µL) into LC-MS for analysis.

2.9. LC-MS Bioanalytical Development and Their Validation for S-Allyl-L-Cysteine

Shimadzu LCMS-8050 containing ESI with triple quadrupole, with the help of Waters ACQUITY Column C-18 (100.0 mm; 1.70 µm), developed a novel method with highly sensitive and great resolution chromatogram used for bioanalysis and pharmacokinetic elution and separation by binary-solvent-manager Shimadzu UHPLC. We optimized our method with mobile phase (methanol: formic-acid (0.10%) :: 92:08), flow rate:0.30 mL/min, injection-volume (10 µL), and 2.0 min of total run time. Argon gas was used as collision gas, with scan time of 1 min, and inter scan (0.02 s) having 30,000 µ/s and 0.10 µ scan step. The +ve ion mode and collision energy (−13.0 eV) were used for the quantification of SC and their mass spectra are shown in Figure 1. SC quantity in plasma was determined by Lab Solution Software Version 5.93 (Kyoto, Japan). All the samples (CC and QC) were freshly prepared and plasma unknown samples were quantified with them (Figure 1). US-FDA guidelines were followed at the time of method validation and gave excellent results regarding their fit relationship of concentration–detector response. A regression equation ($1/x^2$) was used [26,27].

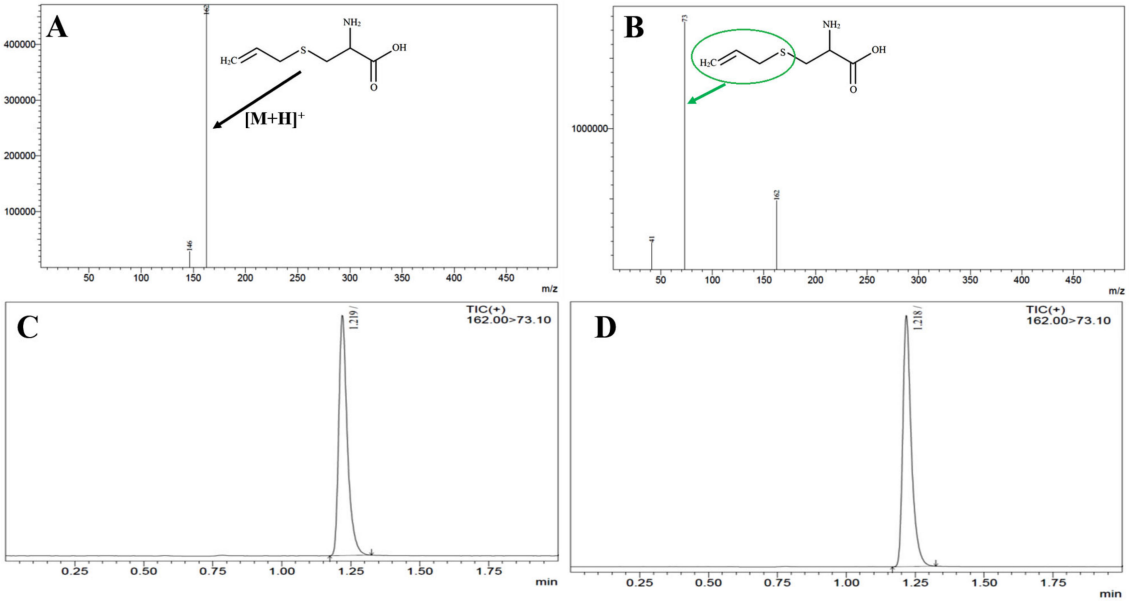

Figure 1. MS Scan (**A**), MSMS Scan (**B**), and chromatograms of extracted plasma (**C**,**D**) of S-Allyl Cysteine.

2.10. Pharmacokinetic Evaluation

We withdrew rats (18, 200–240 g) from the animal house and divided them into two groups for oral [SC-S or SC PLGA NPs] and two groups for iv [SC-S or SC PLGA NPs] with 15 mg/kg dose administration. The blood was collected in the heparinized tubes at various time intervals (0.0-, 0.5, 0.75, 1.0-, 1.5-, 2.0, 4.0, 6, 8, and 12 h). All collected blood samples tubes were centrifuged at 4000 rpm for 7 min to separate out the plasma. The plasma samples were stored at −80 °C [28]. All the parameters related to the PK study were analyzed, like half-life time ($t_{1/2}$), elimination rate constant (Ke), highest plasma quantity of SC (C_{max}), and the time of highest plasma quantity (T_{max}), etc.

2.11. A Comparative Study of SC PLGA NPs for Antimicrobial Activity by Agar Well Diffusion Method

2.11.1. Strains of Bacteria

All Gram +ve and Gram −ve bacteria clinical isolates were collected from Clinical Laboratory Sciences Department, Applied Medical Sciences College, King Saud University, Riyadh.

2.11.2. Agar Well Diffusion Method

The agar well diffusion method was used to examine the antimicrobial activity of SC-S and SC PLGA NPs. All Petri plates were prepared using Mueller–Hinton agar for six various microbial strains. We poured sterile Mueller–Hinton agar (18–20 mL) into all of the separated Petri plates. After solidification of media, sterile cotton buds were used to inoculate bacterial cultures (50 µL) prepared in Mueller–Hinton broth, and 6 mm wells were made in the plates with the help of a sterile cork borer according to Boyanova et al. and Feroze et al. with some modifications [29–31]. The activity was evaluated against two Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and four Gram-negative (*Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*) bacterial species. Two separate studies were performed: **1.** Antibacterial activity with 120 µL of SC-S and SC PLGA NPs samples; **2.** Dose (0.040 mg/mL)-dependent antibacterial activity with 30 µL; 50 µL and 100 µL of SC-S and SC PLGA NPs samples. All the plates were incubated at 37 °C for 18–24 h to measure the zone of inhibition in mm. All the tests were performed in triplicate.

2.11.3. Statistical Analysis

The Student's *t*-test was used to apply for statistics followed by one-way ANOVA and showed all the data in mean ± SD in which all values were statistically significant (** $p < 0.01$).

3. Results

3.1. NPs Preparation and Their Characterization

The solvent evaporation technique was used for the preparation of SC PLGA NPs. Figure 2 showed the PS, ZP, and PDI for the optimized NPs' characterization. The particle size was found to be, i.e., 134.8 ± 4.61 nm, dispersion (PDI: 0.277 ± 0.004), and zeta potential (−25.3 ± 1.03). %EE and % DL were determined, i.e., 82.36 ± 4.01% and 5.13 ± 0.10, respectively [32].

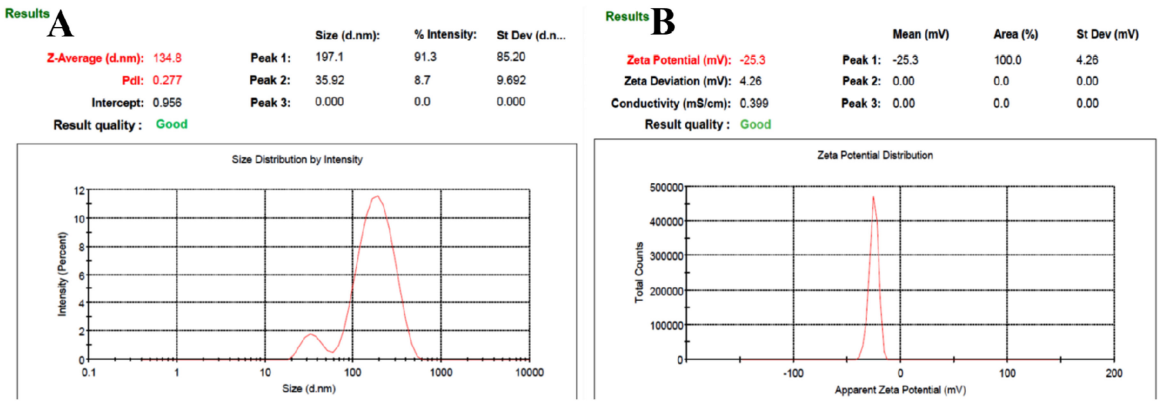

Figure 2. Particle size examined through diffraction light scattering (DLS) technique. (**A**): S-Allyl Cysteine PLGA NPs and Zeta Potential, (**B**): S-Allyl Cysteine PLGA NPs.

3.2. Nanoparticles Analysis by TEM

All the developed NPs were analyzed and showed spherical shape with a particle size of <200 nm, as shown in Figure 3.

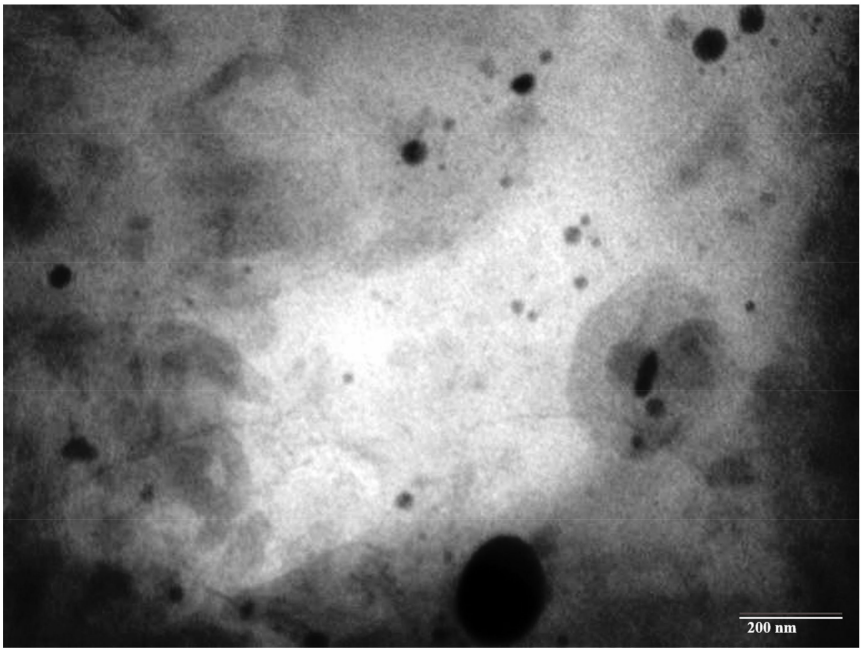

Figure 3. Transmission electron microscopy (TEM) images of SC PLGA NPs.

3.3. Release Kinetics (In Vitro)

S-Ally L-Cysteine was released from SC-PLGA-NPs in different environmental conditions like two different juices (gastric and intestinal). SC showed a fast release up to the first twelve hours in simulated gastric and intestinal fluids (Figure 4). This could be because the SC bonded loosely upon or near the NPs surface [32]. After that, it showed a very slow release up to 46.14 ± 4.93% in the artificial gastric fluids and sustained and delayed release up to 80.39 ±3.64% in the artificial intestinal fluids.

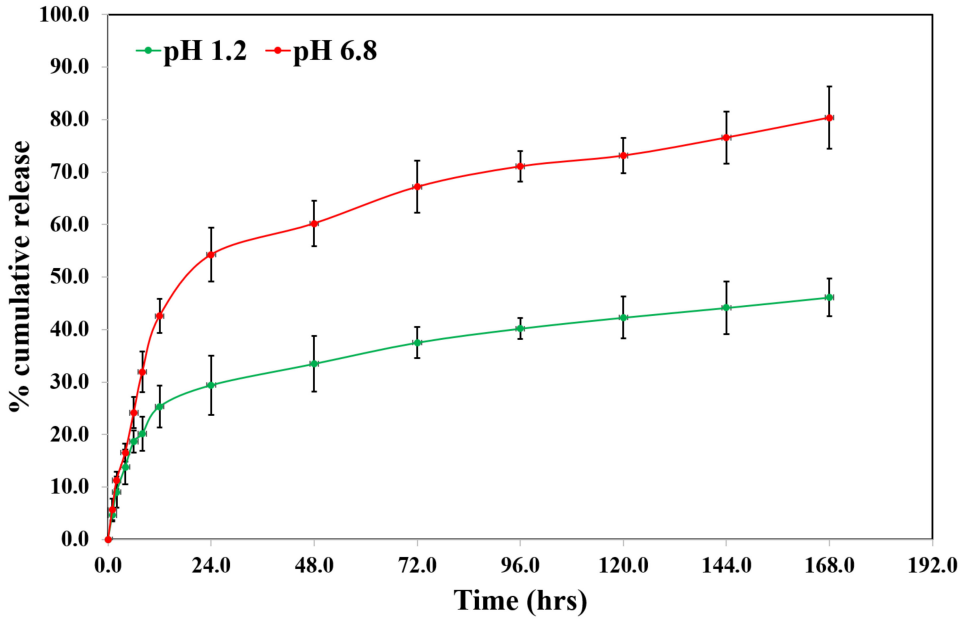

Figure 4. A comparative %release of SC from SC PLGA NPs in simulated gastric fluid at pH 1.2, and in simulated intestinal fluid at pH 6.8, for up to one week (mean ± SD, n = 3).

3.4. Bioanalytical Method Development and Validation

The method was optimized and validated in Figure 1A,B (MS and MS/MS scans of SC) and their chromatograms (Figure 1C,D). The recovery was found to be

>77.09 ± 5.07% for SC (n = 6) from extracted plasma. The linearity of the developed method was $r^2 > 0.993$ (1–1000 ng mL^{-1}). In terms of precision, intra-batch was 1.88–3.82% and inter-batch was 2.61–4.23% for all levels of QCs for SC. Accuracy (%) was 93.02 to 99.40% for intra-batch and 2.61 to 4.23% for all levels of QCs for SC, with % Recovery for all QCs being 77.09 ± 5.07% to 81.28 ± 3.96 (Table 1). All types of stability were validated for the developed bioanalytical method, which were shown to be under the acceptance limit of US FDA guidelines. Furthermore, the results regarding ex vivo stability data for S-Allyl Cysteine (SC) experiments are represented in Table 2 [33,34].

Table 1. Precision and accuracy data for S-Allyl Cysteine (SC).

Intra–Batch					Inter–Batch			
QC ID	**Theoretical Content (ng mL^{-1})**	**Mean Concentration Observed (ng mL^{-1})**	**Accuracy [a] (%)**	**CV [b] (%)**	**Mean Concentration Observed (ng mL^{-1})**	**Accuracy [a] (%)**	**CV [b] (%)**	**% Recovery**
LOQQC	5.01	4.98 ± 0.19	99.40	3.82	4.96 ± 0.21	99.00	4.23	77.09 ± 5.07
LQC	14.90	13.86 ± 0.26	93.02	1.88	13.79 ± 0.36	92.55	2.61	78.63 ± 3.37
MQC	445.00	433.81 ± 15.26	97.49	3.52	428.64 ± 16.38	96.32	3.82	80.51 ± 4.86
HQC	840.00	817.65 ± 23.09	97.34	2.82	808.91 ± 21.53	96.30	2.66	81.28 ± 3.96

Values (Mean ± SD) are derived from 6 replicates: [a] Accuracy (%) = Mean value of [(mean observed concentration)/(theoretical concentration)] × 100; [b] Precision (%): Coefficient of variance (percentage) = standard deviation divided by mean concentration found × 100; Theoretical contents; LOQQC: 5.01 ng mL^{-1}, LQC: 14.90 ng mL^{-1}; MQC: 445.0 ng mL^{-1}; and HQC: 840.0 ng mL^{-1}.

Table 2. Ex vivo stability data for S-Allyl Cysteine (SC).

Conditions	**LQC (14.90 ng mL^{-1})**	**HQC (840.0 ng mL^{-1})**
Long-term stability; recovery (ng) after storage (−80 °C)		
Previous day	14.88 ± 0.13	835.13 ± 21.29
30th Day	14.79 ± 0.15 (99.40%)	817.37 ± 20.67 (97.87%)
Freeze–thaw stress; recovery (ng) after freeze–thaw cycles (−40 °C to 25 °C)		
Pre-Cycle	14.87 ± 0.15	836.16 ± 21.17
First Cycle	14.83 ± 0.14 (99.73%)	821.38 ± 19.98 (98.23%)
Second Cycle	14.78 ± 0.19 (99.39%)	808.64 ± 21.11 (96.71%)
Third Cycle	14.74 ± 0.21 (99.13%)	795.09 ± 19.38 (95.09)
Heating–cooling stress; recovery (ng) after heating–cooling cycles (50 °C to 4 °C)		
Pre-Cycle	14.89 ± 0.14	836.88 ± 21.38
First Cycle	14.74 ± 0.25 (98.99%)	823.04 ± 20.06 (98.35%)
Second Cycle	14.65 ± 0.26 (98.39%)	807.37 ± 21.64 (96.47%)
Third Cycle	13.61 ± 0.36 (91.40%)	798.33 ± 21.11 (95.39%)
Bench top stability; recovery (ng) at room temperature (25 °C)		
0 h	14.85 ± 0.16	837.29 ± 20.18
24 h	13.99 ± 0.22 (94.21%)	824.69 ± 19.67 (98.50%)
Post processing stability; recovery (ng) after storage in the autosampler (4 °C)		
0 h	14.86 ± 0.17	835.66 ± 19.69
24 h	14.26 ± 0.29 (95.96%)	833.09 ± 21.09 (99.69%)

Values (Mean ± SD) are derived from six replicates. Theoretical contents; LQC: 14.90 ng mL^{-1} and HQC: 840.0 ng mL^{-1}.

3.5. In Vivo Bioavailability and Pharmacokinetics

S-Allyl-L-Cysteine shows the maximum plasma concentration time (for the iv and oral administration of SC-S and SC PLGA NPs), which is exhibited in Figure 5; the pharmacokinetic parameters are calculated and summarized in Table 3. The peak plasma concentrations of SC from SC-loaded NPs was found to be the highest in comparison to free SC-S. We also found the values of $t_{1/2}$ and t_{max} to be, i.e., 7.38 ± 0.316 and 1.00 h, respectively, which was greater than SC-S for the oral and iv administration. We found that SC PLGA NPs showed a greater plasma concentration, a smaller amount of clearance, and higher half-life in comparison to free SC-S in rats. SC-S showed smaller C_{max} which may be due to the lower amount of permeation, self-aggregation, and delayed metabolism facilitated through the P-glycoprotein (P-gp) efflux pump. On the other hand, the enhancement of C_{max} and AUC_{0-t} shown for SC PLGA NPs may be due to the reduction in the first-pass metabolism of SC PLGA NPs reaching the systemic circulation via gut-related lymphatic tissue [28]. SC PLGA NPs was also administered intravenously to examine the blood concentration of SC with others. SC PLGA NPs (iv) also showed a sustained release pattern and maintained the therapeutic concentration as with SC PLGA NPs (oral). SC PLGA NPs (iv) showed a better therapeutic concentration level with sustained release pattern compared to SC-S (oral) and SC-S (iv). SC PLGA NPs (iv) showed highly significant results compared to SC-S (oral) as mentioned in Table 3 and Figure 5. Based on the observation of SC PLGA NPs (iv), it can be used as SC PLGA NPs (oral) which is based on patient compliance and clinical studies in future.

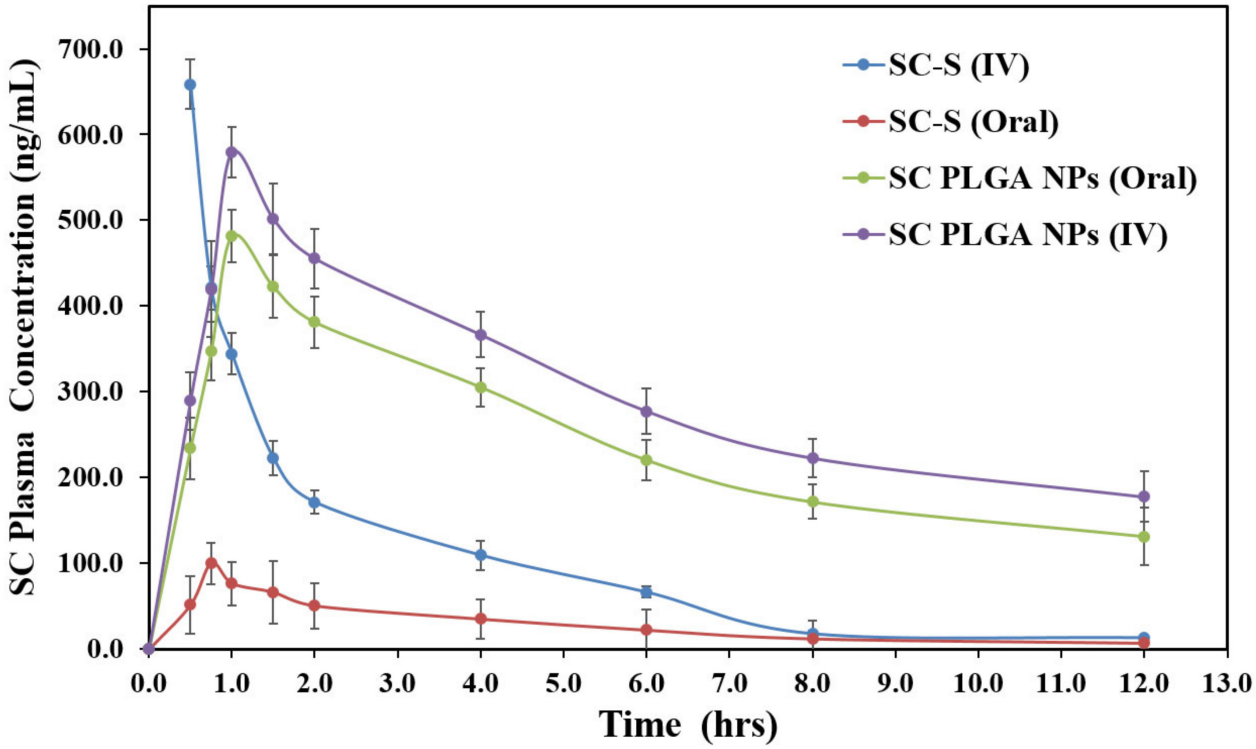

Figure 5. Pharmacokinetic study of S-Allyl Cysteine in rats' plasma for S-Allyl Cysteine PLGA NPs as compared with iv and oral routes of administration.

Table 3. Pharmacokinetic parameters of S-Allyl Cysteine (SC) after single iv dose of SC-S (5 mg/kg body weight), SC-S (Oral, 15 mg/kg body weight), SC PLGA NPs (Oral, 15 mg/kg body weight), and SC PLGA NPs (iv, 15 mg/kg body weight), and mean ± SD; n = 6.

Parameters	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$	K_{eli} (h^{-1})	AUC_{0-t} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)
SC-S (i.v.)	658.61 ± 43.67	0.50	2.08 ± 0.078	0.33273 ± 0.00011	1073.75 ± 53.98	1113.30 ± 59.87
SC-S (Oral)	99.68 ± 16.37	0.75	3.19 ± 0.056	0.21757 ± 0.00009	319.85 ± 12.94	352.07 ± 13.64
SC PLGA NPs (Oral)	481.64 ± 30.28 **	1.00	7.38 ± 0.316 **	0.09393 ± 0.00006	2813.50 ± 121.64 ***	4211.50 ± 171.68 ***
SC PLGA NPs (i.v.)	579.21 ± 36.13 ***	1.00	8.26 ± 0.485 **	0.08395 ± 0.00008	3487.91 ± 138.09 ***	5600.38 ± 190.35 ***

** $p < 0.01$; *** $p < 0.001$.

3.6. Antibacterial Activity

The antimicrobial potential (inhibition zone) of pure SC-S and SC PLGA NPs against different strains of Gram Positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*) bacterial species is shown in Table 4. All the antimicrobial potential effects of SC PLGA NPs are statistically significant ($p < 0.01$) against various strains of gram +ve and Gram −ve bacterial species in comparison to those treated with pure SC-S (Table 4, Figure 6). Our optimized polymeric nanoparticles of S-Allyl-L-cysteine (SC PLGA NPs) have the maximum effect against these pathogenic microbial strains [35].

Table 4. Antibacterial activity of pure SC-S and SC PLGA NPs and their minimum inhibitory concentration.

Bacterial Species	Diameter of Inhibition Zone DIZ (mm) for SC PLGA NPs	Diameter of Inhibition Zone DIZ (mm) for SC-S
Staphylococcus aureus	36.66 ± 0.57 ***	26.33 ± 0.61
Bacillus cereus	29.66 ± 1.52 *	23.0 ± 1.0
Escherichia coli	29.66 ± 1.53 **	20.33 ± 0.57
Salmonella typhi	23.33 ± 0.57 *	20.66 ± 1.15
Pseudomonas aeruginosa	24.0 ± 1.0 *	18.66 ± 0.57
Proteus mirabilis	20.66 ± 1.0 *	16.0 ± 1.0

* $p < 0.1$, ** $p < 0.01$; *** $p < 0.001$.

SC PLGA NPs showed great antibacterial activity against many species of bacterial strains. SC PLGA NPs for antibacterial activity were evaluated for many species and different concentrations of SC against gram +ve and gram −ve strains. Figure 7 shows the antibacterial activity for many concentrations (30 µL, 50 µL, and 100 µL) of SC PLGA NPs and SC-S. The zone of inhibition increases parallel to the enhancement of concentration, i.e., 30 to 50 to 100 mL. Sterile distilled water was used as the negative control and showed no inhibition on both strains (Figures 7 and 8). The SC PLGA NPs exhibited better antibacterial activity in comparison to free SC-S only, because SC encapsulated inside the PLGA NPs increased the stability and decreased the agglomeration (Figures 7 and 8; Table 5). Thus, the uniform distribution of NPs, i.e., PDI showed a very small value (0.277) which is also responsible for the enhancement of the antibacterial activity of SC-loaded PLGA NPs. A significant result was found for SC PLGA NPs based on dose in comparison to SC-S and also showed the dose-dependent effect for both preparations SC PLGA NPs and SC-S in which higher dose (100 µL) showed the maximum inhibition zone in comparison to 50 µL and 30 µL for both preparations [36]. Based on the concentration-dependent effect of SC-loaded PLGA NPs, an increased zone of inhibition was found in comparison to their free SC-S zone of inhibition (Figures 7 and 8; Table 5). Based on this data observation, the antibacterial effect of SC-loaded PLGA NPs was enhanced. It was also published

before that the SC-loaded PLGA NPs have antibacterial activity against gram +ve and gram −ve strains of bacteria [37]. At this point, we can say that the antibacterial effect of SC PLGA NPs is size- and dose-dependent and also supported by previously published reports [38,39], which have shown that SC-loaded PLGA NPs have more potential against gram −ve bacteria than gram +ve bacteria (Figure 7 and Table 5). We have also concluded that the enhancement of the size of NPs is parallel to the reduction in their antibacterial effects [40,41]. SC PLGA NPs showed great potential for antioxidant and antibacterial effects. We have also found that the antibacterial activity of SC PLGA NPs related to the S-Allyl-L-Cysteine concentration and the quantity of SC PLGA NPs in the medium [36,41].

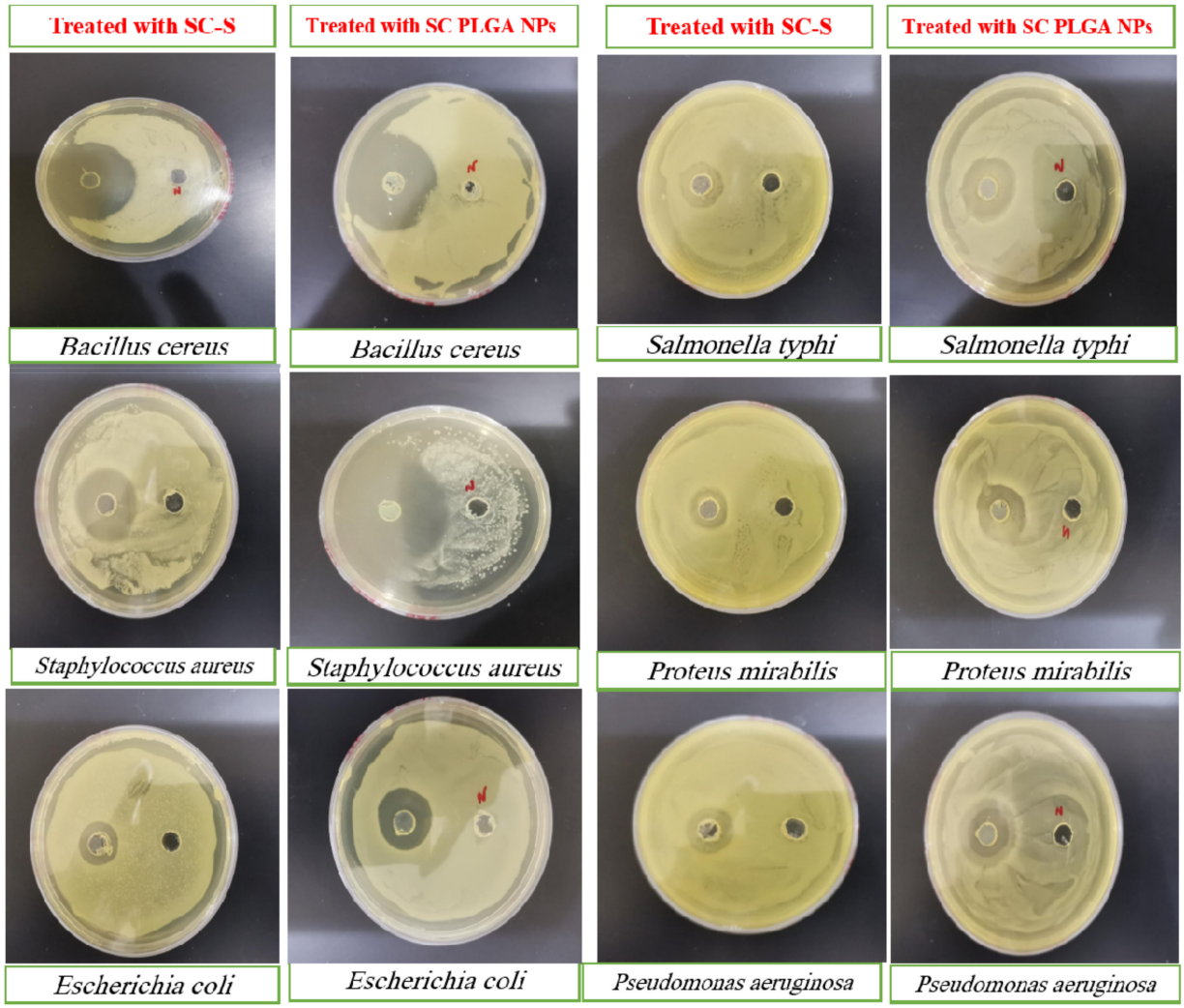

Figure 6. A comparison of inhibition zones of pure S-Allyl-L-cysteine simple suspension (pure SC-S) and S-Allyl-L-cysteine PLGA NPs (SC PLGA NPs) against various strains of Gram-positive and Gram-negative bacteria.

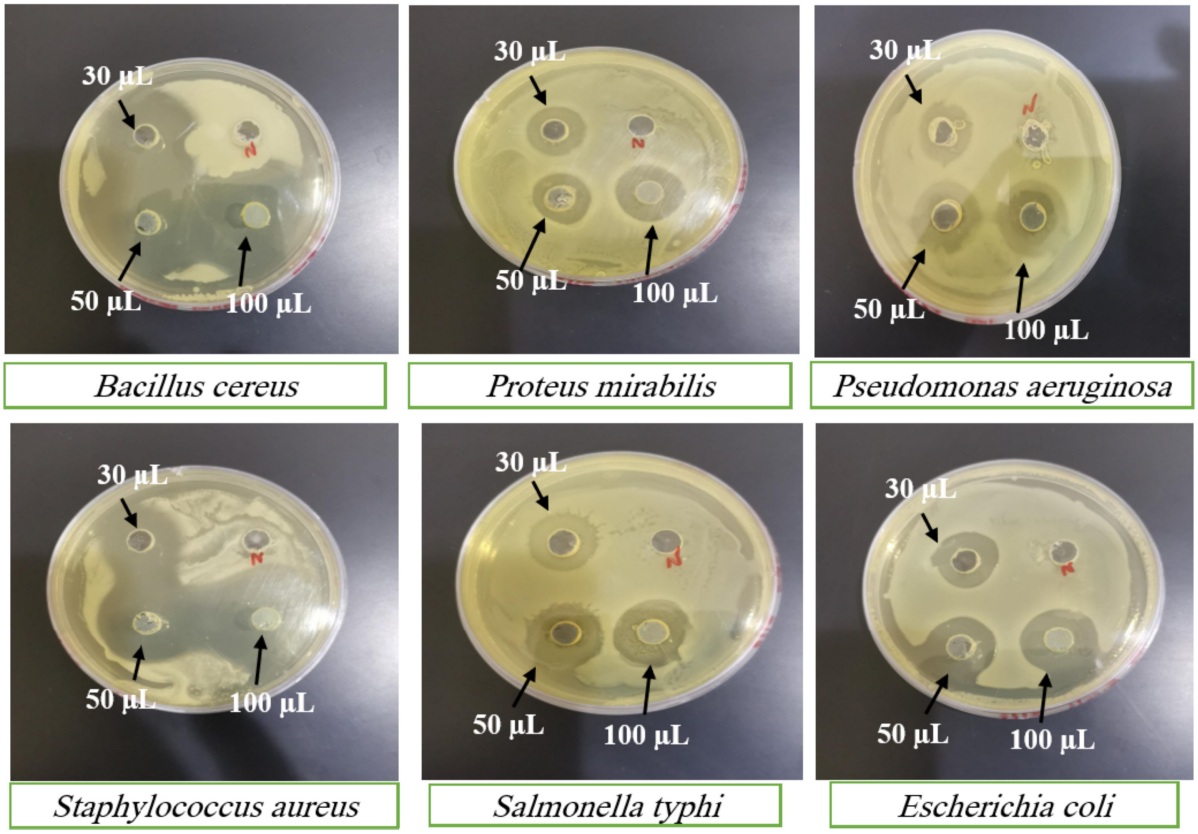

Figure 7. Dose-dependent antimicrobial activity of SC PLGA NPs against Gram-positive and Gram-negative bacteria.

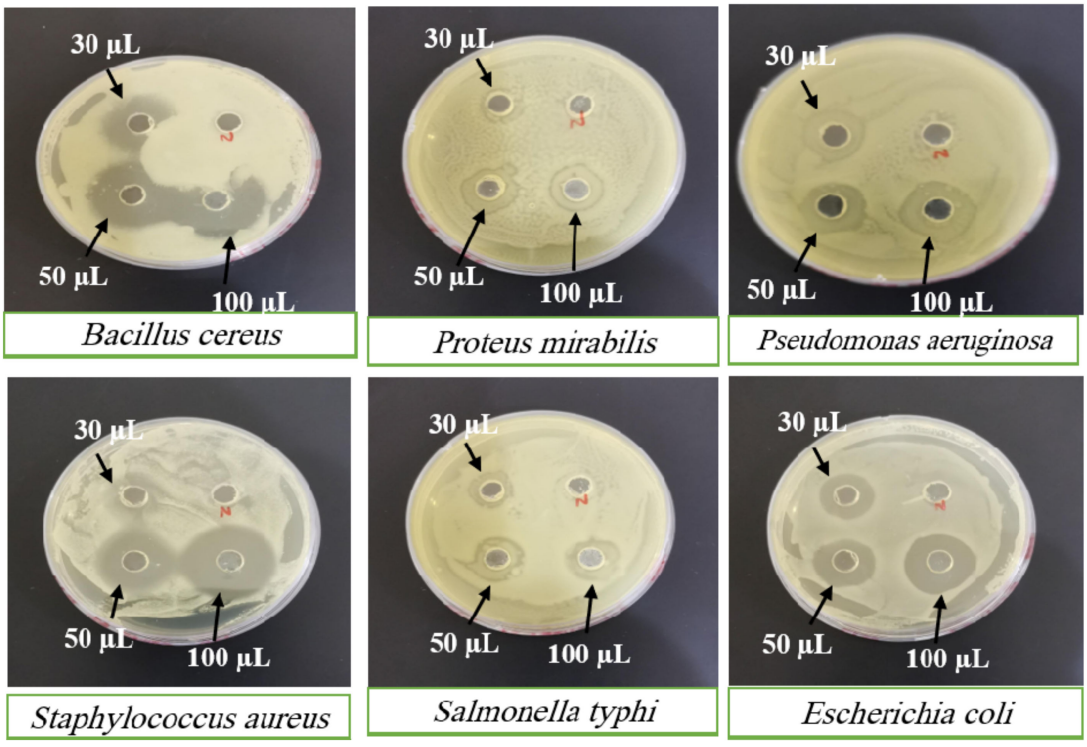

Figure 8. Dose-dependent antimicrobial activity of SC-S effect against Gram-positive and Gram-negative bacteria.

Table 5. Antibacterial activity of SC PLGA NPs and their minimum inhibitory concentration (30 µL, 50 µL, and 120 µL).

Bacterial Species	Diameter of Inhibition Zone DIZ (mm) for SC PLGA NPs			Diameter of Inhibition Zone DIZ (mm) for SC-S		
	30 µL	50 µL	100 µL	30 µL	50 µL	100 µL
Staphylococcus aureus	29.33 ± 0.57	31.33 ± 0.57	34.33 ± 0.57	20.33 ± 0.57	22.33 ± 0.57	25.33 ± 0.57
Bacillus cereus	24.33 ± 0.57	26.0 ± 1.0	28.0 ± 1.0	19.33 ± 0.57	21.0 ± 1.0	21.66 ± 0.57
Escherichia coli	19.66 ± 1.15	20.33 ± 0.57	22.0 ± 1.0	12.0 ± 1.0	16.0 ± 1.0	20.33 ± 0.57
Salmonella typhi	19.66 ± 0.57	20.66 ± 1.15	22.66 ± 1.15	15.0 ± 1.0	16.66 ± 0.57	19.66 ± 1.15
Pseudomonas aeruginosa	17.66 ± 1.15	19.33 ± 0.57	23.33 ± 0.57	13.00 ± 0.57	15 ± 1.0	18.33 ± 0.57
Proteus mirabilis	14.33 ± 0.57	16.0 ± 1.0	19.0 ± 1.0	10 ± 1.0	12.00 ± 0.57	15.0 ± 1.0

4. Discussions

The oral route, the most common route used in drug delivery, shows the excellent advantage of great patient compliance with the very small cost of dosage forms when compared to subcutaneous, intramuscular, and iv injection. The oral route of drug administration shows the drug's degradation and is very difficult to reach the minimum effective concentration (MEC) necessary to show their therapeutic effect. Here, nanoformulation (NPs) was used for oral drug delivery to enhance their solubility and protect them from digestive enzymes by changing their pH environment, which is very important for PLGA polymer given via the oral route. It is very important to the degradation of PLGA with biocompatibility in the presence of physiological environments, in the safety of products biodegradation, and in different grades of commercial availability. S-Ally L-Cysteine is an organosulfur compound that is water-soluble, extracted from garlic, and that contributes to various beneficial properties in aging and to many disease models. The scavenging properties of reactive oxygen species (ROS) of S-Ally L-Cysteine have given great protection with antidiabetic properties, etc., SC PLGA NPs can be used to treat inflammatory bowel diseases with the release of SC in optimum quantities, followed by the maintaining of the therapeutic concentration to treat the infections of inflammatory bowel diseases [32,42].

Therefore, we used solvent evaporation techniques for the preparation of SC PLGA NPs. We optimized the particle size, i.e., 134.8 ± 4.61 nm, dispersion (PDI: 0.277 ± 0.004), and zeta potential (−25.3 ± 1.03). %EE and % DL were determined, i.e., 82.36 ± 4.01% and 5.13 ± 0.10, respectively [32]. For the in vitro release study, PLGA showed protonation from their carboxyl groups in the presence of an acidic environment responsible for the NPs aggregation that converts the shapes into a stable structure up to a limit of SC release from NPs. We increased the pH of the artificial intestinal juice, stimulating the release of SC from the SC-PLGA-NPs; the time enhancement of the absorption of water stimulates the water penetration in the core of NPs. Based on the above observation, we concluded that a very small quantity of SC could be released from the PLGA NPs in the stomach when it was administered orally. On the other hand, the residues showed a sustained release and effective treatment. The most important part was that SC was released first exponentially, which is related to the surface of NPs, with a sustained and controlled release of SC, indicating the entrapped SC inside the core of NPs.

A highly sensitive bioanalytical method was optimized in the linearity range of 1–1000 ng mL^{-1} which was used for the pharmacokinetic study. An optimized nanoformulation was successfully used for antimicrobial activity against gram +ve and gram −ve strains.

5. Conclusions

S-Ally Cysteine PLGA NPs was prepared and optimized with the help of the emulsion solvent evaporation technique. SC PLGA NPs showed <134.8 ± 4.61 nm containing the spherical and homogeneous shape of NPs, which was confirmed by TEM examination

with 5.13 ± 0.10% loading capacity of SC. A sustained release profile of SC PLGA NPs showed the reproduction of the same digestion conditions found in gastric fluid (pH 1.2) and intestinal juice (pH 6.8) over 168.0 h. The highly sensitive LC-MS-based bioanalytical method was successfully developed and validated for a run-time of 2.0 min. The optimized LC-MS-based bioanalytical method was used effectively for the evaluation of plasma pharmacokinetic parameters of a newly developed SC PLGA NPs. SC PLGA NPs showed the higher oral bioavailability compared to conventional bioavailability. The oral bioavailability of SC PLGA NPs was significantly ($p < 0.01$) increased—4.83 times more in comparison to orally administered SC-S in rats. Moreover, the results also indicated that encapsulating S-Allyl-L-Cysteine in PLGA NPs enhanced the antibacterial effects of SC against all tested species of bacteria that are represented in the broad-spectrum antimicrobial effect of both Gram +ve and Gram −ve bacteria. Therefore, SC-loaded PLGA may be an excellent drug-active compound as an antibacterial agent. In the future, this method will be a great beneficial method for antimicrobial activity in the routine practice of life. Therefore, there is a need for more time to evaluate the proposed research on an industrial scale on the antimicrobial effects of the optimized nanoparticles (SC PLGA NPs).

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