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Original Research Article

Cytotoxic effect of Eudragit L-100 nanoparticle-based cisplatin on lung cancer

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Abstract

Purpose: *To develop Eudragit L-100 nanoparticle-based cisplatin (Cis) and determine its cytotoxicity effect against lung cancer.*
Methods: *Eudragit L-100 nanoparticles (NPs) were prepared using the double-emulsion solvent evaporation method. Morphology, size, polydispersity index, zeta potential, drug encapsulation efficiency, and in vitro drug release in phosphate-buffered saline (pH 7.4) were investigated.*
Results: *Cis-loaded NPs' cytotoxicity was higher than free drugs against lung cancer cells ($p < 0.05$). Moreover, cis-loaded nanocarriers inhibited NCI-H60 lung carcinoma cells using the MTT assay in a dose-dependent manner.*
Conclusion: *Eudragit L-100 NPs-based cisplatin exhibits a promising carrier system for effectively delivering chemotherapeutic agents targeting lung cancer. Further studies should focus on optimizing formulation parameters, conducting comprehensive pharmacokinetic and mechanistic studies, and evaluating therapeutic efficacy and safety in preclinical models.*

Keywords: *Cisplatin, Controlled delivery, Lung cancer, Eudragit L-100 nanoparticles, Cytotoxic*

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INTRODUCTION

Nanoparticles (NPs) are preferred to other drug delivery systems due to longer circulation time and are extremely effective for drug delivery [1]. Leaky tumor blood vessels have 100 – 600 nm between endothelial cells; therefore, NPs stay at the tumor site and release the drugs in a targeted manner [2]. The small and unique pathophysiological abnormality of tumor blood vessels makes it likely for NPs to leak out of blood vessels and release drugs at the tumor site. Most tumors have poor lymphatic drainage, making it easier for drugs to accumulate at the tumor site [3]. Polymeric NPs are extremely flexible nanocarriers with modifiable structures that may be customized for specific uses. As a result, polymeric NPs are the most desirable nanocarriers.

Nanoparticle-based oral formulations protect drugs from the gastrointestinal tract and selectively increase drug concentration within diseased lung cells, which improves therapeutic efficacy while reducing systemic toxicity [4]. A pH-dependent drug release is one of the most effective methods for targeted delivery to treat lung cancer [4]. For this purpose, the dosage

form is coated with an acid-resistant polymer to prevent its early release in the stomach and intestines [5]. When the polymer dissolves, the drug is released when it reaches the large intestine or colon, where the pH is close to neutral [6]. Therefore, Eudragit L-100 NP drug delivery system safely transports high doses of anticancer drugs to the lungs, increases the solubility and bioavailability of poorly water-soluble anticancer drugs, and reduces side effects of chemotherapy, including those on the liver and kidneys. Delivery of cisplatin to tumors is now more secure because it may be encapsulated in a polymer system [7].

In this study, a polymeric NP formulation of cisplatin was developed as a potential nanomedicine against lung cancer. The NPs were produced to specifically target the lungs after being administered orally. This could be achieved due to variation in the solubility of Eudragit® L-100 at different pH values along the gastrointestinal tract (GIT). We hypothesized that the pH sensitivity of the polymer would protect the drug as it passes through the GIT until it reaches the lungs. Once at the target site, the drug is released to produce its therapeutic effect while minimizing any potential side effects [7].

EXPERIMENTAL

Materials

Cisplatin powder was obtained from Sigma Aldrich and Pluronic (F-68) was obtained from Molekula, UK. Eudragit L-100 NP was purchased from Evonik, Germany and Polysorbate 80 was purchased from Eva Chem, USA. Potassium dihydrogen phosphate and absolute ethanol were sourced from Baker with ultrapure water made using a Milli-Q system, UK. NCI-H460 lung adenocarcinoma cell line was obtained from ATCC and MTT was obtained from HiMedia, India. Cell culture materials, including RPMI-1640 medium, fetal bovine serum, penicillin-streptomycin, sodium pyruvate, and methoxyethoxymethoxy (MEM) non-essential amino acids, were all purchased from GIBCO. All other chemicals and solvents used were of analytical grade.

Preparation of cis-NPs

Cisplatin-encapsulated polymeric NPs were prepared using the basic principles of double emulsion technique [8]. Approximately 0.75 mg of pure cisplatin was dissolved for each 1 mL of deionized water by heating it at 35 – 40 °C using ultrasonication for 2 min, and stirring for 5 – 10 min. This solution was then added to a 5.0-mL solution of Eudragit L-100 dissolved in absolute ethanol (C_2CH_5OH). The cisplatin/Eudragit L-100 solution was mixed for 1 min with a probe sonicator at 60 % voltage efficiency at 25 °C before adding to 0.5% of Pluronic F-68 (v/v). The produced double emulsion (W1/O/W2) was immediately stirred at 1000 rpm on a mechanical stirrer (Fisher Scientific, Germany) for 3 – 4 h to remove the excess ethanol, followed by mixing to allow ethanol to complete the evaporation of the organic solvent. The NPs were obtained by ultracentrifugation (Fisher Scientific, Germany) at 15000 rpm at 20 °C for 20 min, and the supernatant was analyzed for the free drug. Then, the pellets were washed twice with doubly distilled water to remove the unentrapped drug and pluronic was adsorbed on the NP surface; the suspension was then freeze-dried for 72 h (Labcono, Model: Free Zone 4.5 L) [9] by adding 5 % sucrose w/v (Bendosen, MW = 342.30 g/mol) for cryoprotection [10].

Physicochemical tests

Assessment of particle size, particle size distribution, and zeta potential

The average particle size and particle distribution index (PDI) of the formulation were determined using dynamic light scattering (DLS) based on photon correlation spectroscopy (PCS). Depending on drug concentration, the NP dispersions were diluted in redistilled water in a manner that made the signal sufficiently strong for the instrument. Zeta potential of NPs was measured in redistilled water using a Malvern Zetasizer Nanoseries (Malvern Instruments Ltd., Malvern, UK). Nanoparticle samples were transferred to a plastic cuvette where particle size and PDI were determined at 25 °C and a detection angle of 90° [11]. Measurements were made in triplicate [12].

Entrapment efficiency and drug content determination

Entrapment efficiency was estimated from the amount of unentrapped drug in the supernatant after centrifugation [13]. High-performance liquid chromatography-mass spectrophotometry (HPLC-MS) was used to determine the drug concentration (Spectrum Scientific). The standard and sample were absorbed at 210 nm, and at least three measurements were performed. Cisplatin content (C) and drug entrapment efficiency (CEE) were determined using Eq 1 and 2.

CEE (%) = (Wt-Wexp/Wt)100 ……..…. (1)

C (%) = (Wd/Wt(n))100 …… (2)

Drug–excipient interaction and polymorphism studies

Fourier transform infrared spectra

The chemical composition of pure cisplatin and Eudragit®L-100, the physical mixture of cisplatin and Eudragit®L-100, and the blank formulation of NPs were thoroughly characterized and compared using Fourier transform infrared (FTIR) spectroscopy (Nexus FT-IR Spectrometer, Thermo Nicolet). An FTIR spectrophotometer was used to analyze the samples. For this purpose, < 2 mg of air-dried samples were packed into KBr pellets for sample preparation. Samples were compressed to a thickness of 0.1 mm on a mini hand press and subjected to a pressure of 10 tons per square meter. The instrument's software was used to analyze the spectra obtained by scanning individual sample discs between 4000 and 400 cm^{-1}.

Polymorphism characterization using x-ray diffraction

For this experiment, the following operating conditions were used to obtain X-ray diffraction (XRD) patterns: a powder X-ray diffractometer (SEIFERT model JSODEBYEFLEX-2002) with a temperature range of −20 to 270 °C, a heating rate of 5 °C/min, a scanning rate of 0.5 °/min, a scanning step of 0.02 °/min, an exposure time of 3 s, and a measuring angle range of 10° – 70°. Each step took exactly 1 s to complete, and the step size was 0.048. Cisplatin-loaded NPs were the focus of this study. In addition to blank NPs, the stabilizer pluronic F-68, polymer Eudragit L-100, and physical mixture, x-ray determination was performed on cisplatin in a separate experiment.

***In vitro* cisplatin release studies**

The dialysis bag method was used to release cisplatin from loaded nanoparticles *in vitro*. Cis-NPs sealed in cellulose dialysis membranes (10,000-14,000 MW cutoff) (Spectrum Laboratories Inc., USA), that contained 0.75 mg equivalent of cisplatin were placed in a dialysis bag. The bag's ends were tied together to keep the nanoparticles inside. Furthermore, the dialysis bag was submerged in 15 mL of simulated gastric fluid (SGF) at pH 1.2 for the first two hours followed by 70 hours of phosphate-buffered saline (PBS, pH 6.8; PBS, pH 7.4). This action was taken to keep the sink in good condition, and it was placed inside a magnetic stirrer and heated to 37 °C. After 2 hours of sampling, the incubation medium was withdrawn and replaced with a new medium containing the same volume of fresh phosphate buffer saline (pH 6.8 or PBS pH 7.4) solution, while stirring at a rate of 100 rpm. To maintain sink conditions, 1 mL was withdrawn from the release medium and replaced with 1 mL of fresh medium. Quantitative drug release analysis was performed by comparing the UV absorbance at 210 nm in HPLC for each medium to a cisplatin standard curve. Throughout the study, percentages of the total amount released were plotted. This experiment was repeated three times, each with a different batch of cis-NP.

***In vitro* cytotoxicity (MTT assay) study**

The *in vitro* cytotoxic activity of cisplatin NP formulations and that of free cisplatin against non-small cell lung cancer cell line (NCI-H460) and normal human lung fibroblast cell line (CCD-19Lu) were conducted using 3-(4,5-dimethylthiazol-2-yl)2,5 diphenyltetrazolium bromide (MTT) assay [14]. Nineteen NCI-H460 cells were cultured in RPMI-1640 (Gibco, Invitrogen, Carlsbad, CA) containing 10 % fetal bovine serum (FBS) 0.08 mg/mL streptomycin (Gibo, Invitrogen, UK) and in a humidified atmosphere of 5 % CO_2 and 95 % air in sterile flasks. After two days, when the cell density reached 1.0×10^5 cells/cm^2, the cells were transferred to 96-well plates and incubated at 37 °C. When the cells became confluent, they were trypsinized and then diluted in the culture medium to achieve a total cell count of 5×10^4 cells/mL.

The cell suspension was subsequently transferred to a 96-well plate at a density of 5,000 cells per well and was left to attach overnight. After that, cells were treated with cisplatin NPs at different concentrations (0.031, 0.063, 0.125, 0.25, 0.5, and 1.0 mg/mL) in triplicates. The treated cells were incubated at 37 °C for 24 and 48 h in two sets of experiments, after which 1 mg/mL of MTT solution was added to each well and incubated at 37 °C for 2 h in a CO_2 incubator. Wells containing cell-free medium were used as blank, while cells without. Finally, the culture medium was removed from 200 µL of isopropanol and was added to each well to solubilize the purple formazan crystals formed. The plates were shaken, and absorbance was read at the nanometer scale using an ELISA reader (Bio-Tek Instruments, Winooski, VT) at a reference wavelength of an Ultrospec 1100 Pro UV/Vis spectrophotometer (Amersham Biosciences, Amersham, UK).

Statistical analysis

Analyses were performed using Microsoft Excel 2010. The assays were replicated three times, and results were expressed as mean ± standard deviation (SD).

RESULTS

Physicochemical properties

Surface charge, PDI, and particle size

Lyophilized cisplatin-loaded Eudragit L-100 NPs: The optimized NPs passively target tumors via enhanced permeability and retention with a mean particle size of 246.26 ± 10.48 nm after nanoprecipitation [2,15,16]. The zeta potential was determined to be −22.36 ± 1.45 mV and the PDI was 0.329 ± 0.035.

FTIR spectra

The FTIR spectra were obtained from pure cisplatin, Eudragit® L-100, a physical mixture of cisplatin and Eudragit®L-100, blank NPs, and cis-NPs formulation (Figure 1). Pristine cisplatin exhibited characteristic peaks, including those for amine stretching (3400 – 3200 cm^{-1}) [17]. Asymmetric amine bending (1625 – 1540 cm^{-1}), and symmetric amine bending (1300 – 1315 cm^{-1}) regions, as well as chloride stretching (796.58 cm^{-1}) [18]. Which is in agreement with a previous investigation [19].

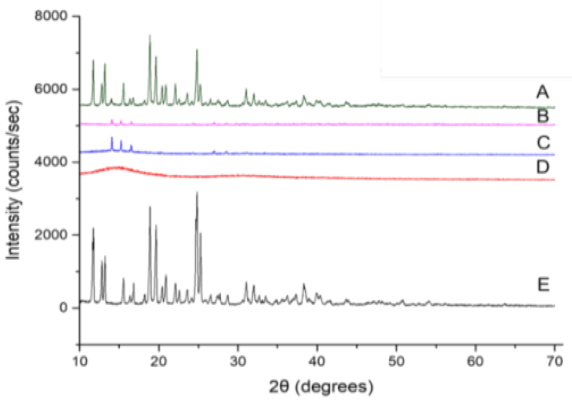

Figure 2: XRD patterns of **A**: blank NPs; B: Eudragit L-100; C: a physical mixture of cis-NPs/polymer; D: cisplatin (pure); and E: cisplatin-loaded NPs

Encapsulation of cisplatin into Eudragit L-100 NPs

Figure 2 shows the shapes of pure cisplatin and Eudragit® L-100, which is a physical mixture of cisplatin and cis-NPs. Five distinct, highly intense peaks appeared at 13.5°, 15°, 16.5°, 24°, and 26.5° due to the presence of Pt in the powder sample of pure cisplatin.

In vitro cisplatin release

Figure 3 reports the *in vitro* release profile of cisplatin from Eudragit L-100 NPs. Cisplatin solution released 100 % of its content within 2 h, while cis-NPs released 25 % of their drug content within 2 h in simulated gastric buffer. Cisplatin release in phosphate buffers 6.8 and 7.4 showed controlled release of cisplatin over 70 h of the experiment.

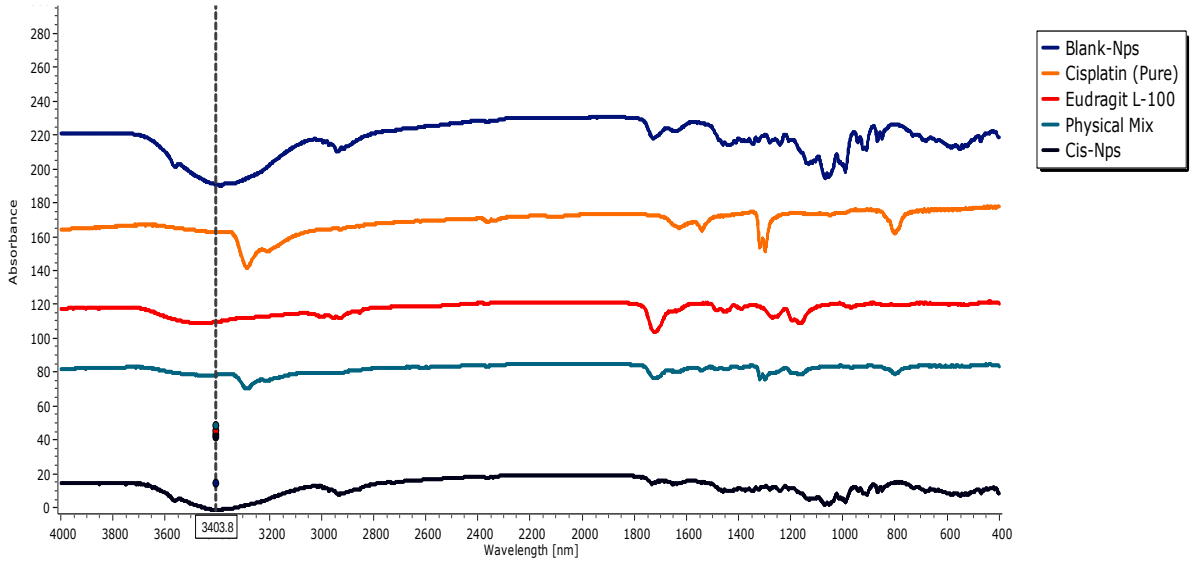

Figure 1: FTIR spectra of pure cisplatin, blank NP, Eudragit® L-100, physical mixture, and cisplatin-loaded NPs

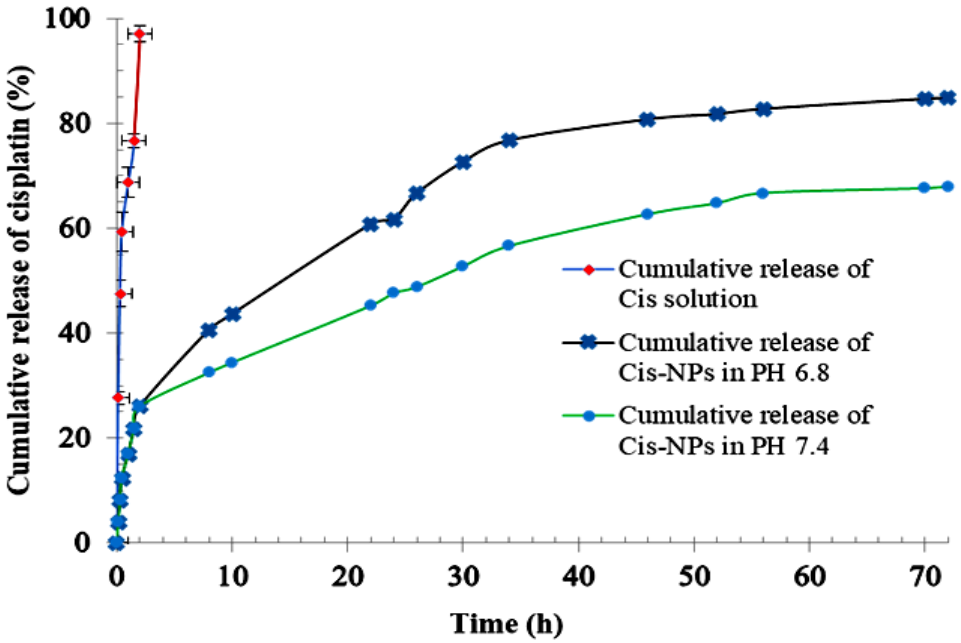

Figure 3: *In vitro release* profile of cis-loaded NPs. The NPs were initially exposed to gastric fluid SGF (pH=1.2) for two hours before being transferred to phosphate buffers (pH = 6.8 and 7.4) for 72 hours. Values are means (n=3)

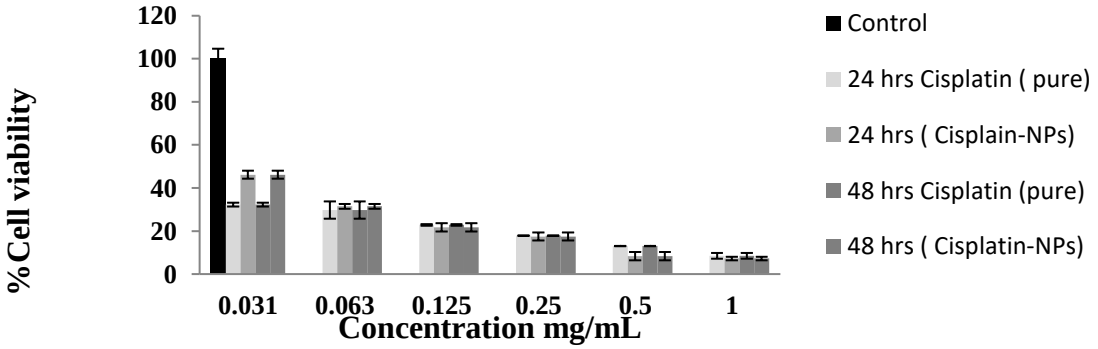

Figure 4: Cytotoxicity effect of Cis-NP iNCI-H640 cells after 24 and 48 h of incubation (n = 3)

Cell viability from MTT assay

The cytotoxicity of free cis, cis-loaded NPs, and empty NPs, which were evaluated in the NCI-H460 cancer cell line, demonstrated a dose-dependent decrease in cell viability. Results in Figure 4 demonstrate that blank NPs did not affect cell cytotoxicity, which indicates that the formulation was biocompatible.

DISCUSSION

Nanoparticles penetrate permeable tumors and stay in the inflammatory lung, increasing drug concentrations at the site of action. In tumors with decreased renal clearance, all formulations had particle sizes between 200 and 245 nm, which enabled EPR effect-based passive tumor targeting [16]. Negative zeta potential helped to improve drug delivery and circulation, passive targets increased permeability, and NP retention helped determine the tumor drug accumulation. Tumor vasculature and lymphatic drainage could get compromised and lead to the formation of tumor cells [14]. The usage of NPs with high stability and controlled systemic circulation is important for passive targeting [15].

The FTIR spectrum of Eudragit® L-100 polymer demonstrated bands corresponding to O-H stretching (3400 – 3700 cm^{-1}), sp^3 C-H stretching (2800 – 3100 cm^{-1}), and C=O stretching (1600 – 1800 cm^{-1}). The physical mixture of cisplatin and Eudragit®L-100 exhibited absorption bands associated with both compounds at 3285.9, 3203.3, 1718.7, 1539.5, and 896.4 cm^{-1}. The

FTIR spectrum after the encapsulation of cisplatin demonstrated two peaks at 2932.7 and 3387 cm^{-1} (related to asymmetric and symmetric stretching of the -NH amine group, respectively) and 1640 and 1344 cm^{-1} (related to the HNH asymmetric and symmetric bending, respectively). The major absorption bands were observed at 3390.3, 2932.1, 2362.9, 2341.2, and 1734.0 cm^{-1}. These spectra revealed certain peaks that were unique to the cis-NP formulation and some that were associated with either the pure components or the physical mixture of the components and blank NPs. There was also a broadening and decrease in the intensity of the O-H stretching peak (3000 – 3600 cm^{-1}) owing to the formation of intermolecular H-bonding interactions between cisplatin and Eudragit®L-100 during the formation of the cis-NPs. Interestingly, many studies have demonstrated that hydrogen bonding impacts the transition from the crystalline state of the drug to the amorphous state [20].

The crystalline structure of cisplatin was responsible for these peaks. Moreover, the spectra exhibited a typical broad XRD peak of Eudragit L-100 from 10° to 25° in the diffraction pattern owing to its amorphous nature, indicating a partial amorphous structure of the mixture. To prepare optimized cis-NPs, the amorphous state was changed into a crystalline state by using nanoprecipitation techniques [20,21]. In addition, because the hydrophilic surfactant was used in water instead of an organic solvent, the final product of NPs had a crystalline appearance [22]. The XRD peaks were observed at 13.5°, 15°, 16.5°, 24.09°, and 26.7° when cisplatin and Eudragit® L-100 were physically combined. The weak peaks were similar to those observed for pure cisplatin and Eudragit® L-100. Lower amounts of cisplatin in the mixture in addition to interference by Eudragit® L-100 molecules, could be responsible for the reduced intensity of cisplatin peaks in physical mixture. Many peaks at various angles were seen in the diffractogram of blank NP, with the largest ones occurring at 11.51°, 15.53°, 18.5°, 19.5°, 23.5°, and 28.5°. Finally, the preparation of cis-NPs via nonparticipation technique resulted in a diffraction pattern similar to that of pure Eudragit® L-100. This result is in agreement with those obtained using DSC analysis, showing that cisplatin is transformed into an amorphous state after being loaded into NPs. The cis-NPs exhibited multiple peaks at 11.56°, 12.11°, 15.56°, 18.59°, 19.48°, and 24.38°. Despite having low-intensity peaks similar to those of blank NPs, cis-NPs had 83.6 % crystallinity. This is attributed to the crystallization of cis-NPs, in which cisplatin fills the space in NPs and contributes to improvement in crystallinity [21]. This behavior may also explain the increased crystallinity of cisplatin-loaded NPs after the drug was entrapped in NPs. These results are in agreement with those obtained by previous studies.

Polymeric carriers do not contribute to cytotoxicity. After 24 h, cancer cell line NCI-H460 was more sensitive to free cisplatin compared with cisplatin-loaded NPs because the drug solution could easily enter the cell. Compared to cisplatin-loaded NPs, free cisplatin was cytotoxic to NCI-H60 lung cell line at all concentrations. Cisplatin-loaded NP formulation was more cytotoxic than cisplatin solution on NCI-H460 cancer cell line. This demonstrated that drug release from the formulation could be controlled and have a greater effect with time [7]. This behavior demonstrates that NPs that slowly released cisplatin were more toxic than the cisplatin solution after 48 h [7]. Therefore, the cytotoxic effect of cisplatin depended on both time and concentration. Previous studies have shown that most activity happens after 48 h [14]. Note that there was a 10 – 20 % increase in cytotoxicity in cisplatin-co-loaded polymeric NPs with more significant cytotoxicity compared to cisplatin-loaded polymeric NPs at all concentrations after 48 h compared to 24 h at the same dose.

Eudragit L-100 is a type of biodegradable polymer used in drug delivery systems. When loaded with cisplatin, these NPs offer a targeted approach to treating lung cancer. Herein, Eudragit L-100 was selected as a carrier for cisplatin because of its biocompatibility, ability to protect the drug from degradation, and controlled release properties. Cisplatin is a well-known chemotherapy drug used for treating various cancers, including lung cancer. It might involve specific targeting mechanisms such as ligands on nanoparticles that bind to receptors on cancer cells. The potential advantages of using Eudragit L-100 NPs for delivering cisplatin include reduced side effects, increased effectiveness, and the ability to overcome drug resistance in cancer cells. This study represents a convergence of pharmaceutical technology and cancer therapy, thus highlighting how advancements in material science enhance the effectiveness and specificity of cancer treatments.

CONCLUSION

Cisplatin-loaded polymeric NPs have been successfully developed and characterized for their physical and chemical parameters such as

particle size, entrapment efficiency, thermal behavior, compatibility of excipients, crystalline behavior, and *in vitro* drug release profile. Polymer matrix may be the rate-limiting step in drug absorption into systemic circulation. This is also indicated by the fact that Eudragit L-100 NPs possess these capabilities. Thus, further studies should focus on optimizing formulation parameters, conducting comprehensive pharmacokinetic and mechanistic studies, and evaluating therapeutic efficacy and safety in preclinical models.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Prof. Dr. Habibah A. Wahab and Dr. Waqas Ahmad supervised the data collection. Firas Al-Mamoori prepared the manuscript for publication. Habibah A. Wahab and Dr Waqas Ahmad contributed to this work.

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