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Nanoparticles based Therapeutics for the Management of Bacterial Infections

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Abstract: Dextran sulphate sodium nanoparticles with cephalexin were created, lyophilized (LCDNP), and then examined. The LCDNP has a zeta potential of -21.8 ± 8.36 mV, a PDI of 0.257, and a PDI percentage of 68.8. The size of a single particle in LCDNP was 260.4 ± 74.53 nm, and the conductivity of the nanoparticles in colloidal solution was 2.37 mS/cm. The zeta average nano size of LCDNP was 318.8 z. d.nm. Differential scanning calorimetry (DSC) has shown that LCDNP exhibits distinct endothermic peaks at 287.79 C. At 211.79 °C, the thermogravimetric analysis (TGA) revealed that LCDNP had lost 95% of its weight. When LCDNP was subjected to XRD analysis, it revealed distinct peaks at 2 as 10.7, 11.5, 12.5, 19.8, 21.4, 25.5, 29.3, 34.3, 39.8, and 41.5, indicating crystalline structure. The zero-order kinetics of the cephalexin release from LCDNP demonstrated a linear release with 38% of the medication released in 7 hours. When tested against tested human pathogenic bacteria, LCDNP's antibacterial impact shown broad-spectrum efficacy. The specified study showed LCDNP to be a promising antibacterial agent.

Keywords: Cephalexin, Bacterial Infections, Dextran sulfate sodium, Nanoparticles, etc.

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Introduction

Bacterial illnesses have been one of the main threats to public health since the dawn of humankind [1]. Due to improved public health, the creation of vaccines, and the development of antibiotics, there is less mortality from bacterial infections today; however, the emergence of antibiotic-resistant strains is a serious medical concern that has led to the failure of effective treatments against microbial infections. Furthermore, many illnesses do not respond favourably to immunisations made using conventional methods [2]. Due to the massive and indiscriminate use of antibiotics in recent decades, microbial resistance has risen. Antimicrobial resistance is ranked as the third biggest threat to world health by the World

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Health Organisation (WHO) [3]. A rise in the cost of treating infectious diseases, the failure of effective treatments, and finally an increase in infection-related morbidity and mortality are only a few of the detrimental implications of microbial resistance [4].

A first-generation cephalosporin derivative used orally is called cephalexin (CEP). It is marketed as a monohydrate and is a member of the -lactam class of antibiotics. It has the molecular weight of 365.41 g/mol and the chemical formula C16H17N3O4SH2O [5]. Treatment of infections caused by Staphylococci and Streptococci is successful [6]. Escherichia coli was also found to be resistant to it [7]. It is used as a backup medication for the successful treatment of chronic obstructive pulmonary disease (COPD), pharyngitis, skin infections, and soft tissue infections and is recognised as a key access antibiotic on the World Health Organization's essential medicine list [8]. For people with penicillin allergy, it is a suitable substitute [9]. A water-insoluble antibiotic with a half-life of 1 to 1.5 hours is cephalexin [5]. Around 80% of the absorbed medicine is eliminated unchanged in the urine, and it has a poor bioavailability of 35 % [10,11]. The frequent dosing and brief half-life make it vital to maintain the therapeutic level in order to demonstrate biological action. Additionally, short-half-life antibiotics must be kept at therapeutic blood levels to prevent resistance [12,13].

Nanotechnology, the most cutting-edge technology, can provide the right instruments for drug effectiveness, tailored distribution to cells, avoiding negative effects, and enhancing therapeutic compatibility. Delivering antibiotics to bacteria at the cellular level and overcoming these resistant routes have grown to be daunting challenges for researchers in the contemporary therapeutic age. These elements have driven pharmaceutical researchers to create novel drug delivery technologies, particularly to combat multiple drug resistance. Dextran sulphate sodium will be used in this project to design and construct a nanoparticle system for the delivery of Cephalexin.

Material and Method

Material

Cephalexin (CEP), Dextran sulfate (sodium salt, MW = 500,000) and sodium tripolyphosphate (MW = 367.86). Bacteriological media and other chemicals and solvents all the materials used in this research.

Formulation of Nanoparticles

Ionic gelation was used in a modified manner to create cephalexin-loaded dextran nanoparticles (CDNP) [14,15]. In a nutshell, 2% water-based dextran sodium sulphate solution was created. On a heated plate, the mixture was continuously swirled using a magnetic bead. For around 3 hours, the speed was maintained at 2000 rpm. After running for roughly 30 minutes, 1 mL of 1% (w/v) tripolyphosphate was produced in water and added as a chemical cross-linker. A reaction mixture (RM) is the name given to that reacting substance. Then, throughout the formulation process, tripolyphosphate was introduced in RM at predefined intervals following 1% (w/v). The RM was run for 15 minutes while 1 mL of 1% (w/v) Cephalexin was added dropwise at regular intervals. For the purpose of creating nanoparticles, sonication was used twice for three minutes each. The RM was then filtered using a 0.2 m membrane filter, and the filtrate was then put through a variety of tests.

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Lyophilization Process

A desktop freeze dryer model number BT85 from Millrock was used to carry out the lyophilization. In a glass flask, a reaction mixture was made by combining CDNP and 6% w/v mannitol in a 1:1 volume ratio. The mixture was placed in a deep freezer set at 80 C for 24 hours to perform freeze-drying. The CDNP was then placed in vacuum-controlled lyophilizing tubes after that. The vacuum pressure was held at 3000 pascals and the temperature was maintained at 84 C. After 24 hours, the lyophilized Cephalexin-loaded dextran nanoparticles (LCDNP) were eluted from the glass flask and pooled for later analysis.

Dynamic Light Scattering (DLS) Analysis

The zeta potential (ZP) in millivolts (mV), the conductivity in millisiemens per centimetre (mS/cm), the size in nanometers (d. nm and z. d nm), and the polydispersity index (PDI) were used to physically characterise the nanoparticles. In a nutshell, Zetasizer Nano NS from Malvern Instruments in Malvern, UK, was used to physically characterise the nanoparticles after they had been produced in water at a concentration of 5% w/v LCDNP and deposited in capillary cells [18].

Determination of Morphological Features

High-resolution scanning electron microscopy was used to examine the morphological characteristics and particle size of the synthesised nanoparticles. The morphological characteristics of LCDNP were examined using a VEGA3 TESCAN (Czech Republic) scanning electron microscope (SEM) with high resolution. By using Transmission electron microscopy (TEM), FEI, Morgagni 268, Brno, Czech Republic, the morphological characteristics of LCDNP were examined.

Energy Dispersive Spectroscopy (EDAX) Analysis

EDAX, or energy dispersive spectroscopy, is a helpful method for figuring out the elemental makeup of samples. Using EDAX APEX from AMETEK in the USA, the LCDNP EDAX spectrum was discovered. The data were recorded after 31 seconds with the acceleration voltage set to 5 keV.

Differential Scanning Calorimetry (DSC) Analysis

To determine the enthalpy changes that resulted from changes in the physical and chemical properties of the samples, LCDNP were examined using the DSC technique. The Shimadzu DSC 60 (Nakagyo-ku, Kyoto, Japan) performed the DSC analysis. LCDNP was placed in an aluminium pan that was not hermetically sealed, and the temperature was raised there at a rate of 10 degrees Celsius per minute while maintaining a 10 mL/min airflow.

Thermogravimetric Analysis (TGA)

A thermogravimetric analyser (Shimadzu thermo gravimetric analyser) was used to evaluate the thermal stability of the samples in an air atmosphere and to ascertain the thermal stability of LCDNP. The LCDNP (10 mg) was placed on an aluminium pan, and the test was conducted between the temperatures of 50°C and 300°C at a heating rate of 10°C per minute.

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X-ray Diffraction (XRD) Analysis

In order to study the crystalline structures of LCDNP, X-ray diffraction (XRD) was used. In Rigaku, Japan, the LCDNP was examined using X-ray diffraction (XRD). Cu K radiation from the incoming beam (= 1.5418) was used to create the XRD diffractograms at 2 in the range of 2-80 at 45 kV and 0.8 mA. The scanning range was set to 2 /, and the scanning speed was 10 min/1.

Loading and In Vitro Release Study

Preparation and Validation of Standard Cure

The working stock solution (1000 g/mL) of cephalexin was made by dissolving the powder in 10 mL of water. Then, working standard solutions in water with concentrations of 500, 250, 125, 62.5, 31, 25, 15.6, and 7.8 g/mL were made by serially diluting the stock standard solution. The calibration curve was made by measuring the absorbance of the made standard dilutions at four distinct wavelengths (250, 265, 290, 310, 380, and 405 nm) and contrasting them to a clear blank. The method's linearity was assessed at several wavelengths in order to validate it. The fact that the approach is linear at these wavelengths suggests that it follows Beer's law. Lambert's The development of the curve by plotting the maximum absorbance values against the levels of Cephalexin was possible.

Loading Study

By drawing a standard curve, the amount of cephalexin released from LCDNP was calculated. Cephalexin that had been entrapped was released after being suspended for 30 minutes in a solution of 0.1 N HCl and 5 g of LCDNP in 10 mL of 0.1 N HCl. The reaction mixture was centrifuged at 3000 RPM after which the supernatant was gathered and stored at 2 °C. From the standard curve, the cephalexin concentration was calculated. The drug loading (DL) was then determined using the following equations:

$$\text{DL (\%)} = \frac{\text{Total amount of Cephalexin extracted from the nanoparticles}}{\text{Total weight of Cephalexin -loaded nanoparticles}} \times 100$$

In Vitro Release Profile

With a magnetic bead rotating at 1000 rpm for 7 hours, 50 mL of phosphate buffer saline (pH 7.4/37 °C) was added to a dialysis bag containing 100 mg of LCDNP. After 30 minutes, the burst release phase was evaluated. Following that, 3 mL of the medium was drawn out of the tube once every hour. In Shimadzu, Japan, the samples were examined using UV/visible spectroscopy. By extrapolating the optical density against the cephalexin concentration, the release pattern was discovered.

In Vitro Antibacterial Study

Bacterial Strains Used and Standardization of Bacterial Cultures

Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Bacillus subtilis, Enterococcus facalis, Escherichia coli, Klebsiella pneumonia, Salmonella

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cholerasuis, Pseudomonas aeruginosa, and Proteus mirabilis were the bacterial strains employed in the study. In a nutshell, a 24-hour culture was made and standardised using nutrient broth and a gradient dilution from 10 to 1 to 10 7. Colony forming unit per mL (CFU/mL) analysis was used to determine the viability of bacterial cultures [15].

Determination of Minimum Inhibitory Concentration

According to the accepted approach created by the Clinical and Laboratory Standards Institute, USA [16], the minimum inhibitory concentration (MIC) of the LCDNP for the bacterial species that were investigated was estimated using the broth dilution method.

Determination of Antibacterial Susceptibility

Muller Hinton agar plates were quickly made in preparation for the antibacterial study [17]. From the stock culture, bacterial subcultures were created, and after 24 hours of incubation, the culture was tested for antibacterial properties. Both the standard ciprofloxacin disc (50 g/mL) and the sample analytes were analysed using the agar well diffusion method. A sterile cotton swab was used for the inoculation, which involved dipping it into a standardised (CFU/mL) culture that contained a variety of different organisms. The culture was then streaked on an MH agar plate by turning the petri dish to disseminate it equally. Approximately 10 minutes were given for the plates to dry before the sample analyte was administered. A conventional sterile stainless-steel borer was used to make holes on the inoculated MH agar plates for the agar well diffusion procedure. The corresponding wells were filled with LCDNP at a specified concentration (determined by the MIC test). The plates were incubated at 37 °C for 24 h, and the appearance of inhibitory zones after that time served to determine the antibacterial spectrum. The diameter of the inhibitory zones is inversely correlated with the spectrum of activity, which is tabulated.

Statistical Analysis

Each experiment was run three times (n = 3), and the data were subjected to a one-way analysis of variance (ANOVA). The $p < 0.05$ (Significant) level of statistical significance was used. Utilising the Prism 9 Graph Pad Instat software system, Boston, Massachusetts, USA, statistical analyses were carried out. Using Dunnet's post hoc analysis, values for the test samples were compared to those for the reference drug

Results and Discussion

Physical and Morphological Characterization

The physical characteristics of the free-flowing powder that was the LCDNP are displayed in Table 1.

Table 1: Dynamic light scattering analysis of Cephalexin loaded dextran sulfate sodium Nanoparticles

Zeta Potential (mV)	Zeta Average Size (z.d.nm)	Size (d.nm)	% Intensity	Y Intercept	PDI	% PDI	Pd (d.nm)	% Mass (d,nm)	Conductivity (mS/cm)
−21.8 ± 9.36	318.8	260.4 ± 74.53	91.3	0.855	0.257	68.8	216.3	58.64	3.37

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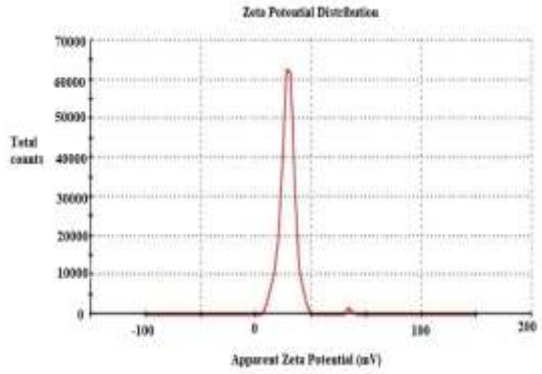
The ZP for the LCDNP was good, measuring -21.8 ± 9.36 mV with a distinct peak. The particles displayed strong electrostatic conduction and were electrostatically active due to the conductivity, which was 3.37 mS/cm (Figure 1A). With a PDI value of 0.257, the LCDNP was consistently produced and extremely homogenous in a single phase in the colloidal system (Figure 1B). Colloidal injectable LCDNP displayed 68.8 % PDI with 91.3% intensity. Zeta particle measurements showed an average size of 318.8 ± 74.53 nm. With a PD diameter of 216.3 d. nm, the observed particle diameter in radius was 260.4 ±74.53 d. nm.

The increased permeability and retention (EPR) effect, a crucial step in the process of targeting bacterial cells, is influenced by the size of the nanoparticle. The diameter of bacteria's pores varies. In a colloidal dispersion system, the size distribution fit was shown to be at about 95% in the cumulative fit analysis, while the linearity was better than 99.9% (Figure 1F,G). An essential tool for determining the signal-to-noise ratio of an instrument that measures the particle size intensity of samples and can be used to assess data quality is the Y-intercept value of the intensity peak. The best colloidal system has an optimum signal with a value larger than 0.9. The analysis's findings revealed that the 1% w/v LCDNP preparation had a good colloidal system with Y-intercept values of 0.855.

A)



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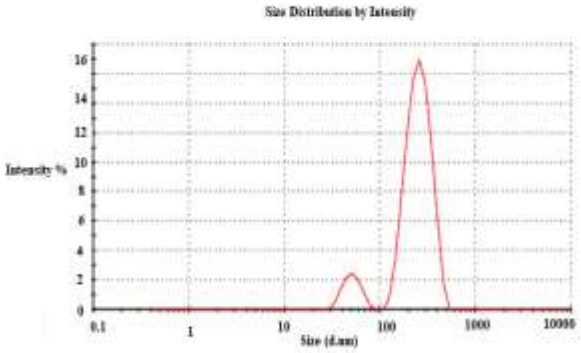

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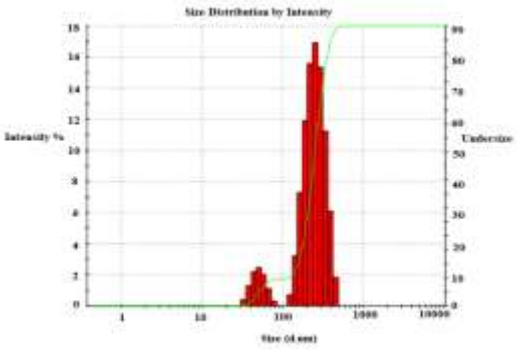

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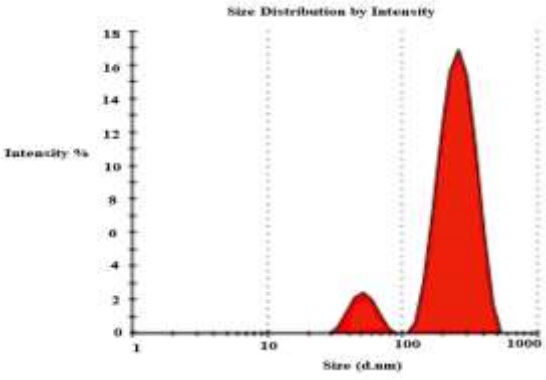

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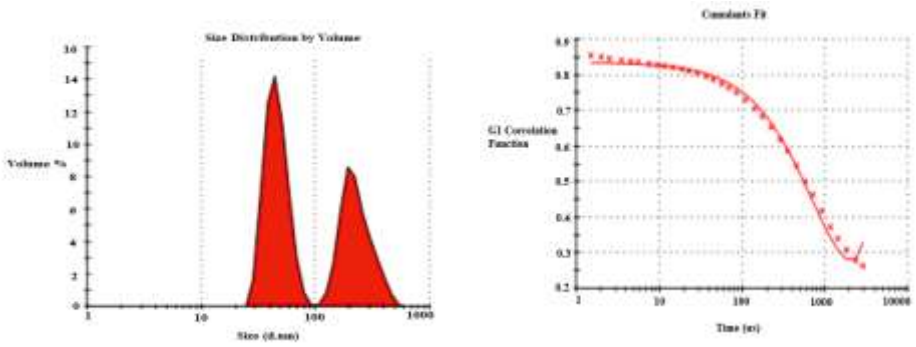

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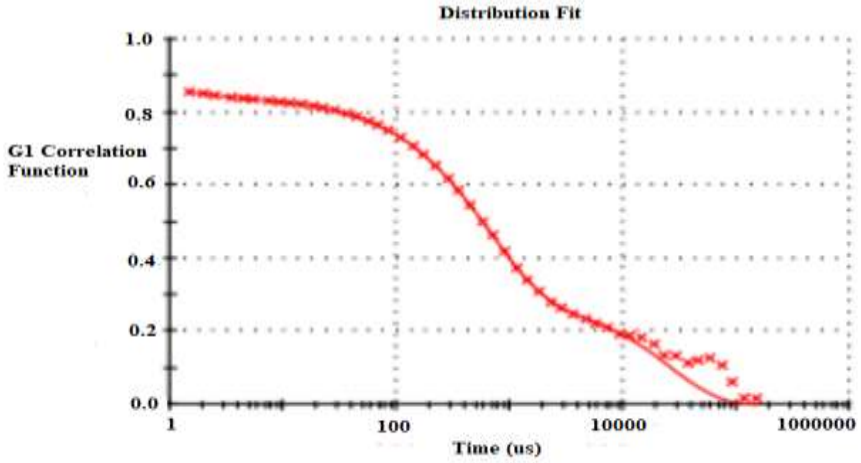

Fig. 1: Physical characterization of lyophilized Cephalexin loaded dextran nanoparticles. (A) Zeta potential analysis (B) Size distribution of nanoparticles through particle intensity. (C) Various particle size distribution analysis through the percent intensity (D) Particle Size distribution analysis through intensity (E) Particle Size distribution analysis through volume (F) Cumulative fit of particulate colloidal system (G) Particle distribution fit of the particulate colloidal system.

The SEM analysis of LCDNP is shown in Figure 2A. Less spherical particles are shown, and most of the particles are clumped together, which may be the outcome of the lyophilization procedure. It is important to stress that lyophilizing nanoparticles may cause the particles to aggregate.

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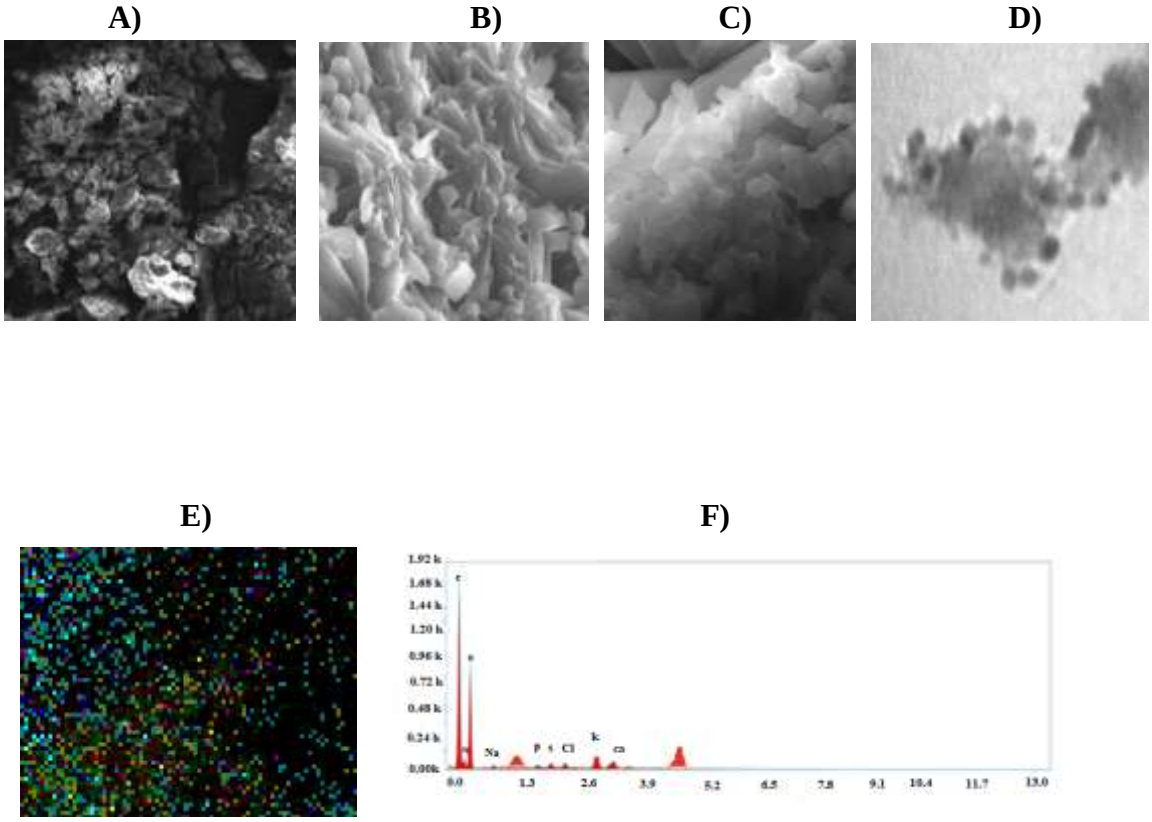

Fig. 2: Morphological analysis of lyophilized Cephalexin loaded dextran nanoparticles (LCDNP)/(A) Scanning electron micrograph of LCDNP (B,C) Crystalline structure of LCDNP captured using scanning electron microscope (D) Transmission electron micrograph of LCDNP (E) EDAX analysis of LCDNP, the area under scanning (F) EDAX graph of LCDNP

Some of the particles showed crystalline properties, as seen in Figures 2B and 2C. Figure 2D displays the LCDNP TEM results. The LCDNP had a spherical form, rough surfaces, and distinct particles. However, ruptured particles and crowded particles with spherical and elongated shapes were seen, along with non-uniformly sized particles. Dextran particles were seen to have similarly uneven in form, with spherical and elongated ends. Figures 2E,F depict the results of the energy dispersive X-ray spectrometry (EDAX) analysis of LCDNP. The analysis revealed that the particle had other trace elements like Na, K, S, Cl, and Ca as well as 52.33% carbon, 37.03% oxygen, and 11.69% nitrogen.

Thermal Analysis

Differential scanning calorimetry (DSC) is a method for detecting changes in nanoparticle thermodynamic parameters, including as enthalpy, entropy, and heat capacity, brought on by physical causes, chemical reactions, and phase transitions. Using DSC analysis, the thermal degradation property of LCDNP was investigated over the temperature range of 40-360 oC. In Figure 3A, it was noted that the glass transition (Tg) temperature ranged from 158.67 to 168.78 oC.

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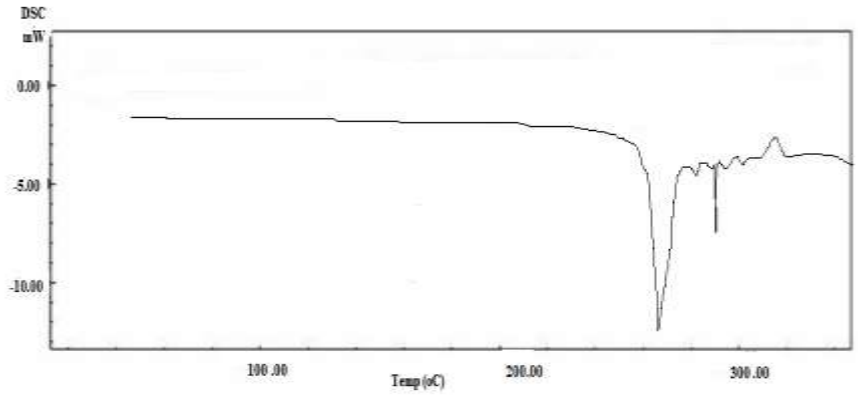
A)



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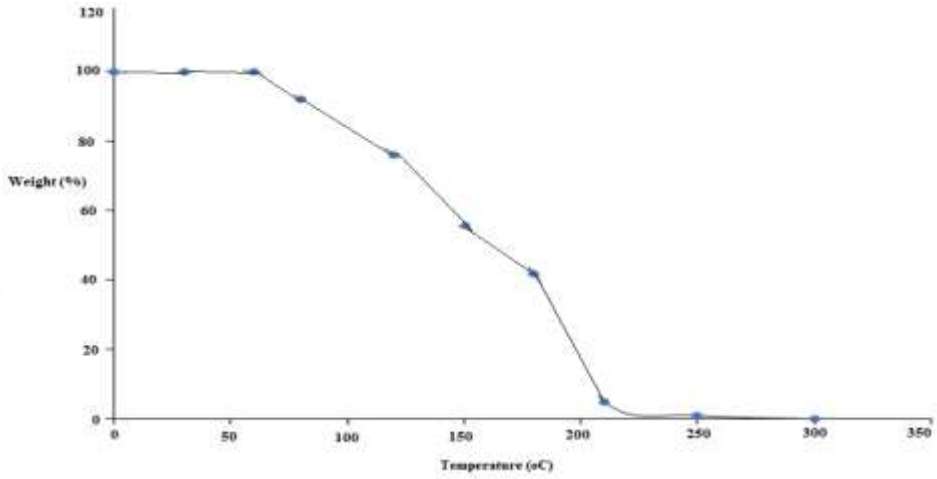

Fig. 3: Thermal analysis of lyophilized Cephalexin loaded dextran nanoparticles (LCDNP). A) Differential scanning calorimetry analysis of LCDNP B) Thermogravimetric analysis of LCDNP.

The endothermic peak for LCDNP in this investigation was at 287.79 oC. Using TGA analysis, the thermal stability of LCDNP in oxygen was determined. The LCDNP TGA analysis results are shown in Figure 3B, where a distinct peak consistent with a deterioration effect may be seen. Intriguingly, it can be deduced from the thermogram that the initial weight loss in LCDNP was noticed at roughly 80 oC. About 95% of the weight was lost at 212.85 oC, proving that LCDNP was thermally unstable at high temperatures. A weight loss of less than 7% is observed below 200 oC. The thermal study' findings showed LCDNP to have a high degree of thermostability.

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XRD Analysis

Utilising XRD data, the discrete crystalline nanoparticle structure is identified. Based on specific diffraction peaks at 10.7°, 11.5°, 12.5° and 19.8°, 21.4°, 25.5°, 29.3°, 34.3°, 39.8°, and 41.5° that confirmed the nanoparticles' distinctive design, XRD analysis at 2 in the current study indicated the presence of crystalline nanoparticles (Figure 4).

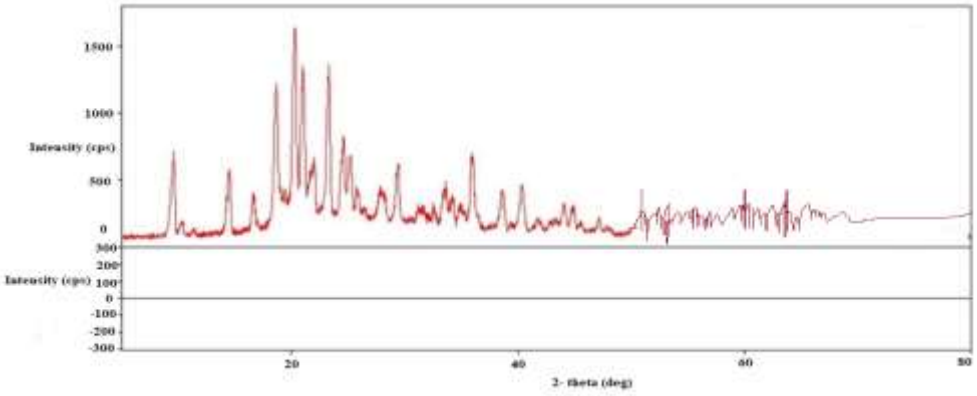

Fig. 4: XRD analysis of lyophilized Cephalexin loaded dextran nanoparticles (LCDNP). The diffractogram was obtained at 2 θ in the range 2◦–80◦

Loading and In-Vitro Release Profile

In this study, a brand-new method for standardising Cephalexin in the UV region is presented. Figure 5 shows the Cephalexin standard curve at various wavelengths.

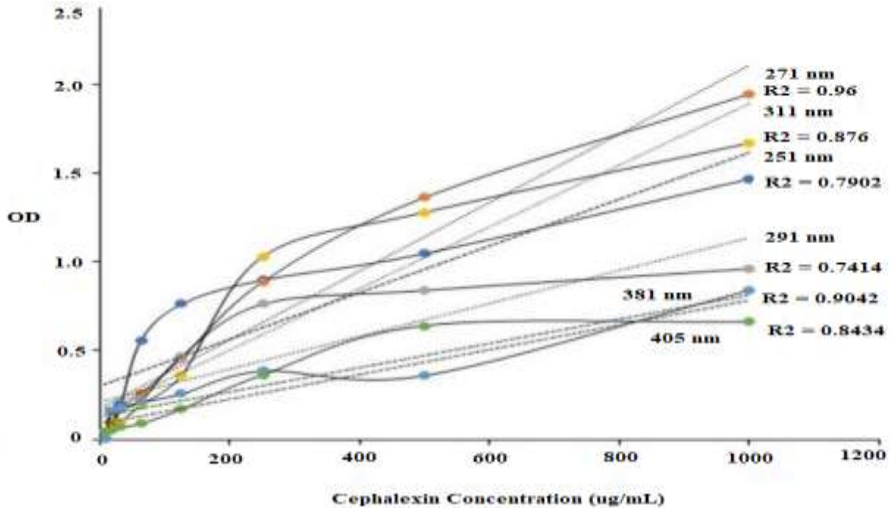

Fig. 5: Standardization of Cephalexin at various wavelengths

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The investigation found that the most successful wavelength for cephalexin determination was 271 nm, where its linearity (R^2) of 0.96 demonstrated compliance with Beers Lambert's law (Figure 6).

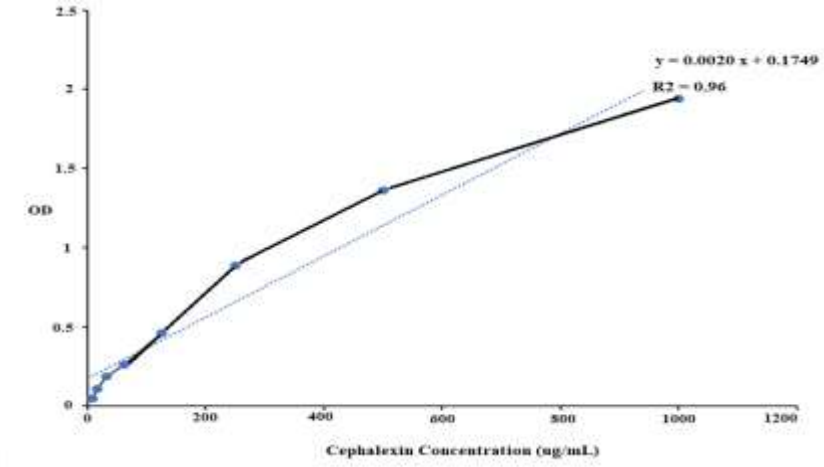

Fig. 6: The standard curve of Cephalexin was determined at 271 nm

According to the current investigation, Cephalexin was effectively loaded with a loading percentage of 92.6 ± 1.3%. Even while the cumulative percentage release of Cephalexin from LCDNP into the medium was quite sustained, it is abundantly obvious that there was no initial burst release of the drug. In 30 minutes, 0.5% of the cephalexin was discharged (Figure 7).

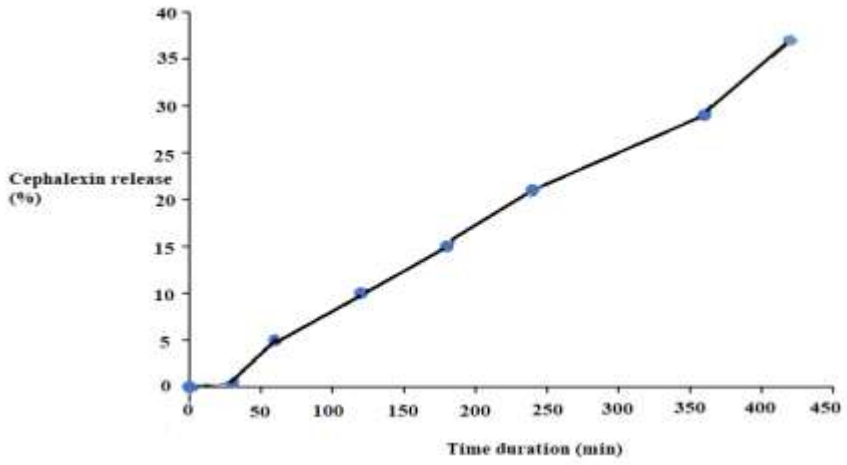

Fig. 7: In vitro dissolution profile

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Cephalexin did, however, release 6% in 60 minutes. Between 60 and 120 minutes, LCDNP's drug release was 6%. The release, however, was 6% between 120 and 180 minutes, and 6% between 180 and 240 minutes. The drug release was surprisingly 17% between 240 and 420 minutes. However, based on the results of their investigation, the current study's consistent Cephalexin release—which reached 38% in 7 hours—will make it more important to optimise the dosage to once daily because the frequency of the dosage varies depending on the pathological effects.

Antibacterial Study

Gram-positive and Gram-negative bacteria were both susceptible to the LCDNP's broad-spectrum effectiveness, which was encouraging (Table 2).

Table 2: Antibacterial study

Organisms	**Concentration CFU# /mL**	**MIC (µg/mL)**	**Zone of Inhibition (mm)**	
			LCDNP	**Ciprofloxacin (50 µg/mL)**
Bacillus subtilis	2×10^5	152.4 ± 2.6	26 ± 3	30.0 ± 3
Staphylococcus aureus	4×10^5	202 ± 2.5	23.68 ± 4	27.68 ± 1.6
Streptococcus pyogenes	4×10^3	261 ± 1.64	25.33 ± 1.6	26.68 ± 2.4
Escherichia coli	3×10^5	103.7 ± 5.3	31 ± 3	34.68 ± 1.6
Pseudomonas aruginosa	2×10^3	122 ± 3.0	26.4 ± 2.5	29.0 ± 3.0
Klebsiella pneumonia	2×10^4	124.4 ± 1.3	28.68 ± 2.6	31.0 ± 2
Proteus vulgaris	3×10^3	149 ± 1.7	26.34 ± 1.6	29.0 ± 3.0
Salmonella cholerasis	2×10^4	152.4 ± 3.5	25.0 ± 2	28.0 ± 2.8
Enterococcus facalis	4×10^3	123.4 ± 2.2	26.4 ± 2.6	28.68 ± 1.6

The study investigated LCDNP's possible antibacterial effects on the species it had screened. The MICs of LCDNP against the investigated bacterial species ranged from 103.7 ± 5.3 to 261 ± 1.64 g/mL depending on the organism. As a result, the antimicrobial spectrum tests in this investigation were conducted using 260 g/mL LCDNP. Because they affect how deeply nanoparticles penetrate bacterial cells, surface charge and particle size are essential factors when aiming for bacterial cells. The broad-spectrum effectiveness of LCDNP against different bacterial organisms was discovered in the current study. When compared to Staphylococcus aureus, the study found that LCDNP had the highest activity against

Escherichia coli, which is significant at the p <0.05 level. The action, however, is similarly efficient against the other bacterial species (Figure 8).

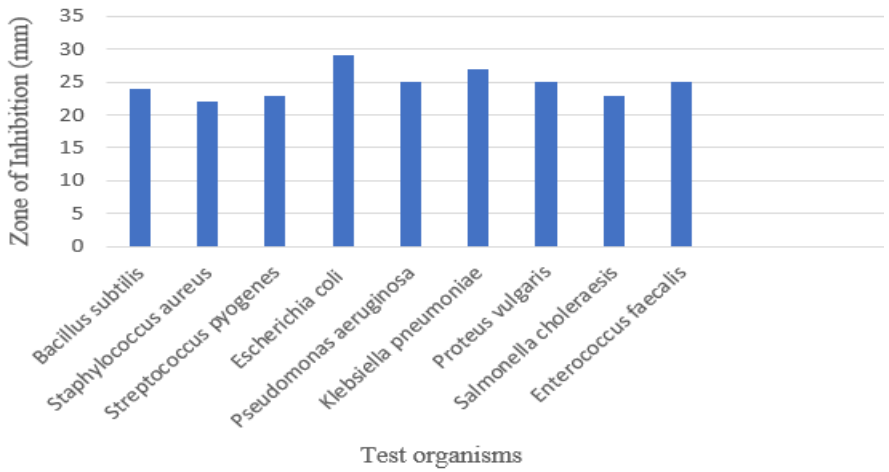
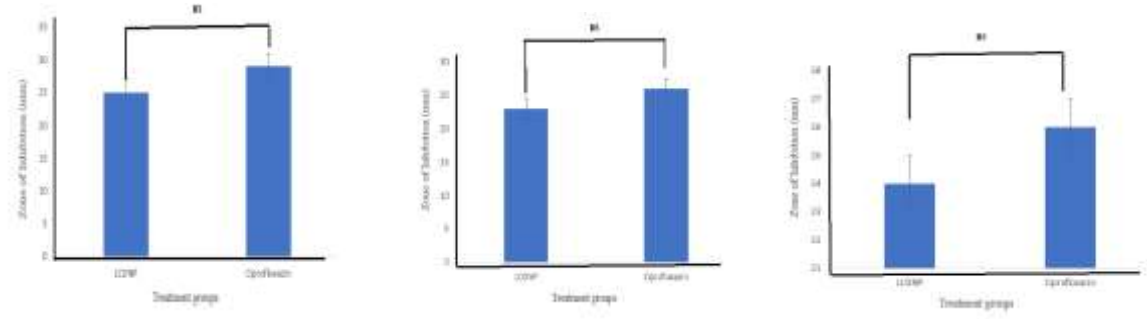


Fig. 8: Antibacterial spectral study of lyophilized Cephalexin dextran nanoparticles against various human pathogenic bacteria. Significant at p > 0.05

Gram-positive and Gram-negative bacteria were both susceptible to LCDNP in the current study (Table 2). The antibacterial activity spectrum of LCDNP was very equal to that of ciprofloxacin and non-significant at p< 0.005 (Figure 9).



A **B** **C**

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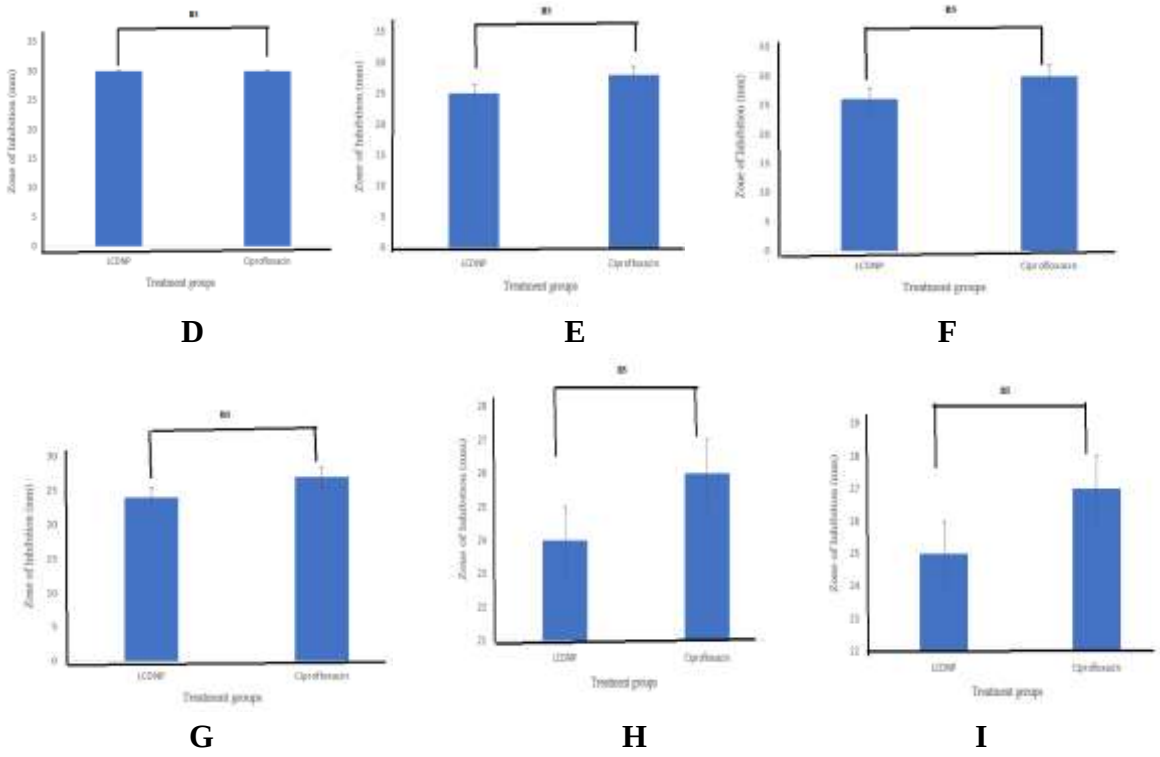

Fig. 9: Comparative antibacterial spectral study. Cephalexin dextran nanoparticles vs. Ciprofloxacin. (A) Against *Bacillus subtilis* at 2 × 10^5 CFU/mL concentration (B) Against *Staphylococcus aureus* at 4 × 10^5 CFU/mL concentration (C) Against *Streptococcus pyogenes* at 4 × 10^3 CFU/mL concentration (D) Against *Escherichia coli* at 3 × 10^5 CFU/mL concentration (E) Against *Pseudomonas aeruginosa* at 2 × 10^3 CFU/mL concentration (F) Against *Klebsiella pneumonia* at 2 × 10^4 CFU/mL concentration (G) Against *Proteus vulgaris* at 3 × 10^3 CFU/mL concentration (H) Against *Salmonella choleraesuis at* 2 × 10^4 CFU/mL concentration (I) Against *Enterococcus facalis* at 4 × 10^3 CFU/mL concentration. ns: non-significant.

Previous studies had demonstrated the value of nano formulations as potent antibacterial agents against bacterial pathogens such Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. As a result, there are potential advantages to using nanoparticles to treat bacterial infections. Targeting bacterial cells requires the enhanced permeability and retention effect, often known as the EPR effect. The ZP and size of the nanoparticles both influence this outcome. By considering both the ZP and the LCDNP particle size, one can acquire insight into the ease with which LCDNP can diffuse across bacterial membranes.

As a result, if the surface charges and nanoparticle size are optimised, LCDNP can diffuse into bacteria. It has been discovered that nanoparticles having a diameter of 50 to 200 nm can easily penetrate through bacterial membranes. The size of LCDNP ranged from 261 to 321 nm, which is an interesting finding and suggests a suitable particle size for bacterial cell

targeting. However, despite having an average zeta size of 318.8 z. d. nm, the LCDNP demonstrated exceptional antibacterial efficacy.

Conclusion

Ionic gelation was employed in this study to create dextran nanoparticles that were loaded with Cephalexin. The formulation achieved Cephalexin loading, sustained release from LCDNP, and a wide range of antibacterial activity, all of which were successful in terms of physicochemical properties. These findings suggest that injectable LCDNP is a new therapeutic formulation for the treatment of bacterial infections and a promising antibacterial agent. Dextran sulphate nanoparticles offer potential for use in a variety of antibiotics to treat infectious disorders, according to the results of recent research. To fill the gap, we discovered during the present study, additional research must be done on the formulation and evaluation process. As a result, the present study's long-term goal is formulation optimisation for LCDNP strength per injectable dosage form. After product development is optimised, speedy stability tests are crucial. In addition, it is necessary to test the LCDNP's potencies and toxicity using difficult techniques through in vivo analysis.

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