



Azithromycin-based Albumin Nanoparticles Loaded Oral Fast-dissolving Films as Superheroes in the Fight against Strep Throat

Unnati Garg¹ · Shreya Kaul¹ · Rupali Verma¹ · Neha Jain¹ · Manisha Pandey² · Upendra Nagaich³

Received: 12 August 2025 / Accepted: 2 December 2025

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2025

Abstract

Streptococcal pharyngitis, strep throat, is a bacterial infection induced by group A *Streptococcus*. Azithromycin (AZI) is used as the second-line drug for its treatment. The study's goal was to formulate AZI-based albumin nanoparticles loaded on fast oral dissolving films, which would help reduce the dosage and dosing frequency of the drug by enhancing its local effect in the affected area. The desolvation process was utilized for the synthesis of albumin nanoparticles, while the solvent casting approach was applied for the formulation of the oral fast-dissolving films (OFDF). A 3-factor and 3-level CCD was implemented to ascertain the influence of critical material attributes on the critical quality attributes of the nanoparticles employing Design-Expert software. The optimized nanoparticles exhibited particle dimension, zeta potential, and entrapment efficiency of 250.6 ± 19.6 nm, -0.656 mV, and $98.67\% \pm 0.002$, respectively. The in vitro release of the optimized formulation demonstrated an initial burst release of $18.11\% \pm 0.0024$ within the first 2 hours, and subsequently, a sustained release profile was observed, with $96\% \pm 0.15$ of the drug over 36 h. The AZI-loaded albumin nanoparticles loaded oral fast-dissolving films demonstrated a drug release of $95\% \pm 0.659$ within 1 h, and the ex vivo permeation via goat buccal mucosa further revealed a flux of 45.36 mg/h. cm^2 . Moreover, the formulation was assessed for its antibacterial potency towards *Streptococcus aureus*, revealing a substantial zone of inhibition measuring 38 mm ± 0.152 compared to the marketed oral tablet. The findings collectively point to the formulation's efficacy in addressing strep throat, suggesting its potential as a viable treatment option for this condition. Further preclinical studies can be performed to ensure its successful translation.

Keywords Centre-composite design · Permeation · Controlled release · Streptococcal pharyngitis

1 Introduction

Pharyngitis arises from various infectious sources, and the key bacterial agent responsible for pharyngitis is group A *Streptococcus*, commonly recognized as strep throat. [1]. As

stated by the Centers for Disease Control and Prevention, as many as 3 in every 10 children and 1 out of 10 adults have strep throat [2, 3]. Mild cases of pharyngitis may manifest as irritation and a sore throat, while more severe instances involve elevated pain upon swallowing, cervical lymphadenopathy, and may or may not be accompanied by fever [4]. The clinical manifestations become apparent through the observation of an inflamed and swollen pharyngeal mucosa in a patient who reports indications including throat pain, difficulty in swallowing, elevated temperature exceeding 38.5 °C, headache, and sensitive front neck lymph nodes. Additionally, the occurrence of pus-filled discharge above the rear pharyngeal region, along with, tonsillar area, might be detected [5]. The transmission of pharyngitis infection occurs via inhalation or direct contact with secretions. The symptoms typically manifest after a brief incubation period ranging from 24 to 72 h; if left untreated, the infection typically persists for 7 to 10 days [6].

✉ Neha Jain
ngulati88@gmail.com; njain1@amity.edu

✉ Upendra Nagaich
Upendra_nagaich@hotmail.com

¹ Centre for Pharmaceutics, Amity Institute of Pharmacy, Amity University, Noida, U.P, India

² Department of Pharmaceutical Sciences, Central University of Haryana, Mahendergarh 123031, India

³ Center for Global Health Research, Saveetha Medical College, Saveetha Institute of Medical and Technical Science, Chennai, India

Different antibiotics have been recommended for the treatment of strep throat. These antibiotics shorten the duration of symptoms of the disease and prevent its transmission. Penicillin or amoxicillin are recommended for first-line therapy, but given that some individuals may exhibit allergic reactions to these drugs, azithromycin stands as a viable alternative for individuals with known allergies to penicillin or amoxicillin, providing a suitable treatment option that accommodates a more extensive patient population [7–9]. Moreover, the emergence of bacterial resistance, particularly in strains of bacteria like *Streptococcus pyogenes*, has been observed against penicillin and amoxicillin. [10]. Azithromycin demonstrates efficacy against strains resistant to penicillin and amoxicillin. These characteristics position azithromycin as a preferred choice, particularly in regions or cases where bacterial resistance is a prevailing concern [11]. The broader spectrum of antibacterial activity exhibited by azithromycin enhances its effectiveness against various bacterial species beyond the Group A β -hemolytic streptococcus. This broader coverage may be advantageous if there is uncertainty about the specific bacterial strain causing the infection or if there are concerns about potential mixed infections [12, 13].

Azithromycin dihydrate (AZI) is a bacteriostatic agent that inhibits the growth of bacteria by binding to the 50 S ribosomal unit of the 70 S ribosomal structure of bacteria, along with impeding the synthesis of proteins, thus helping in shortening the duration of the symptoms [14, 15]. The half-life of AZI is 68 h and is unstable at a low pH, i.e., pH ranging from 1 to 4 [16]; therefore, it has a very low oral bioavailability of 37% since the pH of the stomach is highly acidic [17, 18]. The existing dosage regimen of AZI involves an initial dose of 500 milligrams on day one, subsequently, a continuing daily dosage of 250 milligrams for the succeeding 4 days. Despite its extended half-life of 68 h, which would suggest a less frequent administration, the necessity for daily dosing arises due to its relatively low bioavailability [15, 19].

Albumin is the predominant plasma-derived protein and is biodegradable and biocompatible. It remains consistent over a broad spectrum of thermal conditions and pH levels and possesses a half-life of approximately 19 days in circulation [20, 21]. It also has multiple hydrophobic binding domains (Sudlow's sites I and II), which makes drug encapsulation easier. Azithromycin, being a lipophilic macrolide, can interact non-covalently (via hydrophobic interactions and van der Waals forces) with these sites. This effectively increases the apparent solubility of azithromycin by keeping it in a bound, dispersed form rather than precipitating out in water. Furthermore, the synthesis of albumin NPs is easy and can be done by various techniques

like desolvation, coacervation, or emulsion formation. Additionally, albumin tends to accumulate at an inflamed site [22], thus can be proved as a suitable candidate for strep throat infection. In strep throat infection, inflammation enhances that, in turn, increases vascular and mucosal permeability at the infection site. Albumin nanoparticles can extravasate and accumulate more at inflamed sites than in healthy tissue, increasing local drug exposure. In addition, Albumin also tends to be retained at sites with enhanced retention because of extracellular matrix changes and slower lymphatic clearance in inflamed tissue [23]. Albumin also carries a negative charge, mitigating the likelihood of nanoparticle aggregation [24].

The OFDFs are thin, water-soluble films that dissolve swiftly within the oral cavity without requiring liquid. Thus, OFDFs are an effective approach to enhance patient compliance for unconscious patients as well as for pediatrics and geriatrics. These films can be dissolved in under a minute when kept on the tongue or buccal mucosa, which results in quick absorption, enhancing the release of azithromycin-loaded albumin nanoparticles at the infection site. In addition, as albumin is mucoadhesive in nature, the release of nanoparticles will increase their adhesion to the inflamed mucous membranes and prolong the release the azithromycin at the local infection site. Therefore, combining the unique properties of albumin, such as enhanced permeation and retention effect, and the mucoadhesive nature, which can bind to inflamed mucosa, the formulation will allow the drug to act directly on the *Streptococcus pyogenes* colonies present in the oropharyngeal mucosa [25–27]. The incorporation of albumin NPs into OFDFs would enhance the oral absorption of the therapeutic agent across the buccal tissue and would prevent its first-pass metabolism.

The purpose of this preliminary current investigation was to develop AZI-based albumin NP-loaded OFDFs for the management of strep throat and evaluate them for physico-chemical characteristics, drug content, in vitro release, and ex vivo permeation studies.

2 Materials and methods

2.1 Materials

AZI was acquired from Medi Pharma Drug House, Mumbai, India. Egg albumin, glutaraldehyde, Tween 80, citric acid, menthol, sucralose, sodium starch glycolate, and glycerol were obtained from Central Drug House, New Delhi, India. Pectin was obtained from Maple Biotech Pvt. Ltd, Maharashtra, India. All remaining solvent substances and reagents utilized were of high purity.

2.2 Preformulation Studies

2.2.1 Drug-excipient Compatibility Studies

The compatibility assessment of the drug with various excipients was conducted employing Fourier transform infrared spectroscopy (FTIR) with a Bruker Alpha ECO-ATR spectrophotometer [28]. The spectral analysis encompassed AZI and egg albumin, both individually and in combination, i.e., physical mixture. The infrared absorption spectra of these samples were captured in the spectral range spanning 450–4000 cm^{-1} at of 4 cm^{-1} resolution. A comparative analysis was performed by examining the pellets and comparing the spectra with those of the polymer and pure drug reference [29, 30].

2.2.2 Selection of Formulation Variables

Before the formulation of albumin NPs, a preliminary optimization process was undertaken to identify the most suitable formulation variable. Two formulation variables, viz. cross-linking agent and desolvating agent, were systematically screened over a wide range of concentrations to facilitate the selection of the best formulation. Various cross-linking agents, including glucose, tannic acid, and glutaraldehyde, along with desolvating agents such as ethanol and acetone, were screened based on their ability to induce nanoparticle formation. This extensive screening process aimed to identify the most effective combination of these variables for subsequent albumin NP formulation [31].

2.3 Optimisation Via Quality by Design (QbD)

The fundamental principle that strengthens QbD is the notion that “the quality cannot be tested in the product but rather should be built into the product. QbD is an organized, customized, risk-based, pre-emptively driven approach that mitigates any potential for mistakes associated with the formulation [32, 33].

2.3.1 Quality Target Product Profile (QTPP)

The goal of the QTPP for oral films based on azithromycin albumin nanoparticles is to create a fast-dissolving, patient-friendly formulation that boosts azithromycin's antimicrobial properties and absorption while reducing gastrointestinal complications. As part of the QTPP, the ideal film thickness ($\leq 250 \mu\text{m}$), consistent drug content, quick disintegration time (less than 1 min), and crack-free, smooth layer should be achieved. To ensure consistent dispersion

inside the framework of the film and sustained drug release, the integrated albumin nanoparticles have been optimized to exhibit a low polydispersity index (< 0.3), a high drug entrapment ($> 85\%$), and particle dimensions under 300 nm. To ensure constant therapeutic efficiency and compliance among patients, the finished product should have proper folding endurance, durability for transportation, and storage stability [34, 35].

2.3.2 Critical Quality Attributes (CQAs)

The safety, effectiveness, and quality of azithromycin albumin nanoparticle-based oral films are directly impacted by the CQAs. The critical CQAs comprise attributes of nanoparticles that affect the release of azithromycin and stability, especially particle diameter, polydispersity index (PDI), zeta potential, and entrapment efficiency. Since these factors affect mechanical properties, convenient administration, and dissolving properties, the oral film's CQAs comprise the thickness of the film, drug content consistency, surface consistency, folding durability, elasticity, and disintegrating time [36–38].

2.3.3 Response-surface Method: Central Composite Design (CCD)

Response surface methodology (RSM) functions as a quantitative and statistical framework utilized for the construction of a series of experimental designs aimed at generating an optimal response. In the optimization of AZI-albumin NPs, a 3-factor and 3-level CCD design was implemented utilizing the Design-Expert[®] program, edition 12, developed by Stat-Ease, USA. This CCD design, occasionally known as Box-Wilson CCD, encompasses an integrated fractional or full factorial design featuring central points supplemented with axial points ($+\alpha$ and $-\alpha$), which gives extra edge to get the optimised formulation, facilitating the assessment of curvature in the experimental domain [39]. The independent variables analyzed were albumin concentration (%w/v), the concentration of glutaraldehyde (%v/v), and the amount of desolvating agent, i.e., ethanol (ml). The desired responses studied were particle size (PS), polydispersity Index (PDI), and entrapment efficiency (EE%), as shown in Table 1. Fifteen trials were conducted as illustrated in Table 2, with each test executed in triplicate to assess the reproducibility of the findings. AZI-albumin NPs were synthesized, and resultant measurements of parameters or responses were input through Design-Expert. Ultimately, to achieve the maximum desirability in the formulated product, constraints of substantial importance were implemented [40–42].

Table 1 Independent variables, dependent variables, and constraints for three-factor, three-level CCD

Levels						
Independent variables	Low (−1)	Medium (0)	High (+1)			
X ₁ =Albumin (% w/v)	4	5	6			
X ₂ =Glutaraldehyde (% v/v)	6	7	8			
X ₃ =Ethanol (ml)	8	9	10			
Constraints applied to dependent variables						
	Independent variables		Dependent variables			
	X ₁ =Albumin (% w/v)	X ₂ =Glutaraldehyde (% v/v)	X ₃ =Ethanol (ml)	Y ₁ =PS (nm)	Y ₂ =PDI	Y ₃ =EE (%)
Constraints	In range	Minimum	Minimum	In range	In range	Maximum
Importance	+++	+++	+++	+++	+++	+++

Table 2 Experimental runs of 3³ Central Composite design for the prepared nanoparticles

Formulation	Albumin (%w/v)	Glutaraldehyde (%v/v)	Ethanol (ml)
F1	5	7	9
F2	6	6	10
F3	5	7	7.58579
F4	4	8	10
F5	5	5.58579	9
F6	5	8.41421	9
F7	5	7	9
F8	6	8	8
F9	5	7	10.4142
F10	5	7	9
F11	6.41421	7	9
F12	3.58579	7	9
F13	5	7	9
F14	5	7	9
F15	4	6	8

2.4 Preparation of AZI Albumin Nanoparticles

AZI-loaded albumin NPs were synthesized via the desolvation technique. Firstly, the accurately weighed quantity of egg albumin was dispersed and swelled in distilled water, employing a magnetic stirrer set at 500 rpm, to develop an aqueous protein dispersion. Simultaneously, in a separate beaker, AZI was dissolved in ethanol and was subsequently introduced dropwise to the albumin mixture with ongoing magnetic agitation at 500 rpm to yield an opalescent drug-protein aggregated solution. The pH of the solution was continuously noted to maintain a range of 6.8–7.4. Secondly, a precisely prepared glutaraldehyde (%v/v) solution of the desired concentration was added dropwise into the solution to facilitate the cross-linking mechanism with constant stirring at 1100 rpm. The addition of PBS helps to control and maintain a pH neutral to mildly alkaline condition, overcoming the local acidification caused by glutaraldehyde addition. Along with glutaraldehyde, phosphate

buffer saline (PBS, pH 7.4) was simultaneously added and was constantly stirred at 1100 rpm for an hour to achieve the formation of nanoparticles.

After the formulation of azithromycin-loaded albumin nanoparticles, drug content confirmed that no significant chemical interaction or degradation of azithromycin occurred, indicating the process preserved drug stability [43–45].

2.5 Evaluation of AZI-loaded Albumin Nanoparticles

2.5.1 Shape and Surface Morphology

The transmission electron microscopy (TEM) snapshots were utilized to investigate the structural characteristics and morphology of AZI-albumin NPs. For assessment, Joel JEM-1400 (Jeol Ltd., Tokyo, Japan) was employed to measure TEM samples. To conduct TEM observations, the NPs underwent appropriate dilutions and were diluted at first with purified water. Subsequently, a droplet of the diluted NPs was applied onto a grid with a carbon film layer. After removing excess liquid with absorbent paper, the grid was allowed to dry at ambient temperature before performing scans at varying magnifications. The pictures were recorded at a magnification of 25,600 times with an acceleration voltage of 80 kV [33, 46, 47].

2.5.2 Assessment of Albumin Nanoparticle's Particle Size, Polydispersity Index, and Zeta Potential

Particle size distribution, PDI, along with ZP, was evaluated using the differential light scattering technique (Malvern Zetasizer, Worcestershire, UK). Before the examination, each sample underwent appropriate dilution with distilled water under sonication for 1 min. The duration of each measurement was 120 s and was conducted at a temperature of 25 °C, with a scattering angle of 90 °C. The entire study was replicated thrice for each sample [48].

2.5.3 Development of Optimized Formulation Via Desirability Model

Following the outcomes of the specified dependent variables (Y1, Y2, and Y3), an optimized formulation was designed to identify the influence of the independent parameters (X1, X2, and X3). The central objective of employing desirability was to integrate the responses about critical characteristics and present the probability of reliably projecting the optimal results for independent parameters. This assessment involved a thorough examination wherein the measured experimental values of the outcomes were contrasted against the anticipated values generated by the software. Through this evaluation, the aim was to ascertain the alignment between the observed and expected outcomes, ensuring the accuracy and reliability of the optimization process [49].

2.5.4 Entrapment Efficiency and Loading Capacity

For determining the amount of drug loaded into the nanoparticles, EE was assessed using a REMI/R-8 V cooling centrifuge (REMI, Maharashtra, India). The NPs were subjected to centrifugal separation at a speed of 10,000 rpm for 30 min. Following centrifugation, the supernatant, which had unbound drug, was cautiously discarded. Subsequently, the absorbance was measured for the free drug using a UV spectrophotometer (Spectronic 200 UV-Vis Spectrophotometer, Thermo Fisher Scientific, Massachusetts, United States) at 206 nm. The drug concentration was determined through the utilization of a calibration curve, which illustrated a linear formula expressed as $y = 0.0051x + 0.0089$ with an r^2 coefficient of 0.9878. The subsequent formulas were applied to compute the EE% and loading capacity of the nanoparticles [50, 51].

$$\% EE = \frac{\text{Initial weight of drug} - \text{Weight of free drug}}{\text{Initial weight of drug}} \times 100$$

$$\% \text{ Loading capacity} = \frac{\text{Initial weight of drug} - \text{Weight of free drug}}{\text{Total initial amount of drug and albumin}} \times 100$$

2.5.5 In Vitro Drug Release of AZI-loaded Albumin Nanoparticles

To assess the release of AZI from the optimized albumin NPs, an in vitro release analysis was conducted. A diffusion approach utilizing the dialysis bag method was utilized. A dialysis unit featuring a pore size of 2.4 nm with molecular weight cut-off spanning 12,000–14,000 Dalton (Spectrum Medical Inc., Los Angeles, CA, USA) was employed.

Before introducing the optimized AZI-albumin NPs (equivalent to 250 mg of Azithromycin).

The dialysis membrane underwent overnight immersion in phosphate buffer saline (PBS) at pH 6.8 and ethanol (50:50) to activate it. In this method, AZI-albumin NPs were placed in the dialysis bags, secured with clamps, and immersed in a dialysis beaker containing PBS served as a dispersion medium. The beaker was then capped and subjected to stirring on a magnetic stirrer operating at 200 rpm. The temperature was adjusted to 37 ± 0.5 °C throughout the entire study. 5 ml of an aliquot was extracted at time points of 5, 30, 60, 90, 180 min, and so forth. An additional 5 ml of fresh buffer solution was incorporated to sustain sink conditions. The concentration of AZI was quantified in each aliquot utilizing a UV spectrophotometer at 206 nm. All measurements were conducted in triplicate, and the mean was computed for accuracy and reliability [52, 53].

2.6 Preparation of OFDFs Loaded with Optimized AZI Albumin Nanoparticles

To fabricate OFDFs, the solvent casting technique was employed. The weighed quantity of polymer (pectin) was dissolved in 5 mL of purified water on a magnetic stirrer apparatus for 40 min at 500 rpm. After 1 h, 10 mg of Tween 80 was added and stirred for 5–7 min. Subsequently, 50 mg of sucralose, 10 mg of citric acid, 8% sodium starch glycolate, and 15 mg of menthol were introduced into it and stirred until a transparent solution was obtained. Then, 250 mg of plasticizer (glycerol) was incorporated into the mixture and stirred for one hour to achieve a clear solution. Ultimately, the prepared NPs (2.5 ml NP solution containing AZI equivalent to 250 mg of Azithromycin) were incorporated into this solution. Following agitation, the mixture was kept aside to get rid of the bubbles and then poured into a petriplate. The petriplate was subsequently positioned in a drying oven at 60 °C for 2 h. The final film was extracted from the petriplate and then wrapped in aluminium foil to avoid moisture absorption until the characterization parameters were performed [54–57].

2.7 Characterization of Optimized AZI Albumin Nanoparticles Loaded in OFDFs

2.7.1 Film Thickness and Folding Endurance

Ensuring uniformity in thickness is crucial, as it has a direct association with the accuracy of the film's dosage. Using digital Vernier callipers, the thickness of all the films was evaluated at five distinct points. The test was executed in three trials, and the average $\pm$ standard deviation (SD) was subsequently calculated. [58].

Subsequently, a folding endurance assessment was performed to assess the flexibility of all the films. A greater folding endurance signifies superior mechanical strength in the material. There is a correlation between mechanical robustness and folding durability. In this test, the film underwent repeated folding until it reached the point of rupture. The number of folds it took for the film to break was recorded and regarded as folding endurance. The folding endurance measurement and the film's flexibility were proportionate [59].

2.7.2 Content Uniformity

The content uniformity was conducted to verify the uniform dispersion of AZI in every OFDF. In this procedure, a random sample of 1 cm² OFDF was dissolved in 10 mL of distilled water in a 100 mL volumetric flask. The solution was then subjected to sonication for 15 min, and then it was filtered via a 0.22 µm filter to separate free drug from albumin. The solution was subsequently adjusted to 100 mL using distilled water. Subsequently, 2 mL aliquots were extracted from this solution and diluted with distilled water to reach a final volume of 10 mL. The prepared samples were subject to examination with a UV-visible spectroscopy at 206 nm. The subsequent equation was applied to assess the average content of the film [60]:

$$\text{Content uniformity} = \frac{\text{Actual AZI amount in OFDFs}}{\text{Theoretical AZI amount in OFDFs}} \times 100$$

2.7.3 Surface pH and Percent Moisture Loss

The pH measurement on the mucosal surface of the oral cavity falls within the limits of 5.5 to 7.4. OFDFs are uniquely crafted to dissolve quickly in the mouth once positioned on the tongue. Consequently, the pH values of OFDFs must align with the pH of the buccal environment, as formulations with excessively alkaline or acidic pHs can result in mucosal discomfort [61]. To assess the pH of the surface, a digital pH meter (Jenway-3510, Staffordshire, UK) was employed. For this evaluation, every film was contained in a petriplate that held 1 mL of purified water for 5 min at 25 °C. Subsequently, the pH meter electrode was positioned on the surface of the solution, and the measurement was documented after a 10-minute interval to enable the pH value to stabilize. The test was done in triplicate, mean ± SD [62].

This analysis was conducted to verify the integrity and physical robustness of the film samples. It was evaluated by precisely weighing the initial weight of the films on an electronic balance and then putting them in a desiccator for 72 h at room temperature. After this period, the films were retrieved, and the mass of the films was documented. The subsequent formula was applied to compute it [63]:

$$\text{Moisture loss \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.7.4 Mechanical Characteristics of Developed OFDFs

A texture analyser was used to measure the mechanical features of the OFDFs. Mechanical characteristics included tensile force, percent elongation (Strain), and Young's modulus.

Tensile Strength It represents the highest force necessary to rupture the film. It was done to determine the robustness and elasticity of the films. The following equation was used to estimate in terms of megapascal (MPa) [64].

$$\text{Tensile strength} = \frac{\text{Force at breakage (kg)}}{\text{Film thickness (mm)} \times \text{film width (mm)}} \times 100$$

Percent Elongation (Strain) It was measured to assess the durability and stretching of the developed OFDFs. The elongation and stretching of the film before breaking is known as the % elongation or strain. It is calculated using the following equation [65].

$$\% \text{ Elongation} = \frac{\text{Increase in length of OFDF}}{\text{Initial length of OFDF}} \times 100$$

Young's Modulus It is also referred to as elastic modulus; this metric was evaluated to determine the firmness or elasticity of the produced films. It was derived from the gradient of the linear section of the tensile stress-strain curve by applying the following formula [66].

$$\text{Young's modulus} = \frac{F/A}{dl/l} \times 100$$

2.7.5 In Vitro Disintegration time

Disintegration time refers to the duration from the contact of an oral film with water or saliva to the point where it begins to break apart. In the case of a fast-dissolving film, the disintegration time is expected to fall within the range of 5–30 s [67]. The produced OFDF was set in a petriplate containing 10 mL of Sorensen's PBS (pH 6.8) at 25 °C. The duration for the film to break down was documented. The readings were taken in triplicate [68].

2.7.6 In Vitro Dissolution Study

A USP Type II dissolution device (Labtronics LT-721 Microprocessor Dissolution Device, Haryana, India) was

utilized to measure the in vitro release of AZI from the OFDF. Sorensen's PBS served as the dissolution medium. The temperature was adjusted to 37 ± 0.5 °C, and the rotation rate was maintained at 50 rpm throughout the study. The films were set at the base of the dissolution container. 5 mL of an aliquot was extracted at time points of 5, 30, 60, 90, 180 min, and so forth. To preserve skin condition, an extra 5 mL of fresh buffer solution was incorporated into the vessel. The concentration of AZI was determined in each sample using a UV spectrophotometer at 206 nm. All readings were taken in triplicate, mean $\pm$ SD [52, 53].

2.7.7 Antibacterial Assessment

The antibacterial activities of AZI albumin nanoparticles OFDFs were assessed utilizing the well-diffusion method along with the cup-plate method and compared with the Azithral 250 mg oral tablet and pure drug on Mueller-Hinton agar (MHA). The direct comparative study between film and tablet aims to check the extent of the antibacterial effect that AZI may show after loading in a novel formulation, i.e., in nanoparticles and film. *S. aureus* (ATCC 25923) served as a standard for the antimicrobial evaluation. Bacterial cultures were subcultured using a broth medium and incubated at 37 °C for 24 h. Subsequently, overnight cultures were obtained, and MHA plates were seeded with bacterial isolates under sterile conditions. Wells (with a diameter of 6 mm) were charged with 50 μ L of the experimental solutions and cultured at 37 °C for 24 h. Following the inoculation phase, the width of the inhibition zones was recorded. The inhibiting regions were documented in millimetres (mm). Mean and SD were calculated based on triplicate testing [69, 70].

2.7.8 Ex Vivo Skin Permeation Study

The drug permeation of the OFDFs was analyzed on goat buccal mucosa utilizing a modified Franz diffusion (FD) cell. Buccal tissue from the goat was promptly obtained from a local slaughterhouse within 10 min of slaughter and preserved in Krebs buffer solution. Employing surgical technique, the buccal epithelium was cautiously isolated from the subepithelial connective tissues [71]. The isolated mucosal membrane was meticulously placed in the middle of the two sections of the FD-cell, with the epithelium oriented towards the donor chamber. Thereafter, the membrane was left to stabilize for nearly 60 min. OFDF was placed on top of the buccal membrane and hydrated with 1 ml of PBS. The receiver chamber was loaded with PBS pH 6.8. The full assembly was arranged on a magnetic stirrer rotating at 50 rpm, and the temperature was thermoregulated at 37 ± 0.5 °C [72, 73]. Subsequently, 2 mL of the sample was extracted at time points of 2, 5, 10, 15, 30, 60, 90, 120, and

240 min and supplemented with an equal volume of fresh buffer in the chamber to sustain sink conditions. The drug permeation through the buccal membrane was evaluated by quantifying the absorbance at 206 nm using a UV spectrophotometer. All measurements were performed in triplicate, and the mean was determined [66]. The flux (J) was calculated using the formula given below:

$$J = \frac{\text{Amount of AZI permeated from the optimized film}}{\text{Time} \times \text{Membrane area}}$$

The permeation enhancement ratio (ER) was calculated using the following formula:

$$ER = \frac{J \text{ of developed film}}{J \text{ of drug reference}} \times 100$$

3 Results and Discussion

AZI is categorized as BCS Class-II, showing limited absorption via the oral route at 37%, which contributes to an increase in its dosing frequency despite having a half-life of 68 h. Therefore, the use of nanoparticles and OFDF can facilitate evading the first-pass metabolism of the therapeutic agent, along with providing a sustained drug release via buccal absorption [19].

3.1 Preformulation Studies

To identify the pure drug and any possible interaction between AZI and egg albumin, FTIR analysis was done. AZI displayed sharp bands at 1377 cm^{-1} (C-H bending attributed to methyl groups), 1719 cm^{-1} (C=O axial stretching), and 1134 to 1221 cm^{-1} C-O axial stretching. Egg albumin showed peaks at 1653 cm^{-1} (C=O stretching associated with the amide I band), and 1533 cm^{-1} (C-N stretch combined with N-H bending linked to the amide II band). A combination of AZI and egg albumin showed absorbance ranging from 1134 to 1719 cm^{-1} . The FTIR spectrum distinctly indicated no evidence of interaction between the drug and the polymer. This spectrum confirms the compatibility between them.

3.2 Selection of Formulation Variables

Before creating albumin NPs, several process parameters, viz. albumin concentration, cross-linking agent along with its level, and desolvating agent concentration, were optimized over a wide spectrum to aid in choosing the optimal formulation. The cross-linking agent was selected based on the formation of nanoparticles; when glucose and tannic

acid were used as cross-linking agents, nanoparticles were not formed, while glutaraldehyde facilitated the formation of nanoparticles. This could be attributed to the fact that the aldehyde group of glutaraldehyde forms covalent bonds with the amino groups present on the albumin protein, resulting in the hardening of the nanoparticles [74]. The selection of the desolvating agent was also done based on the same criteria; when acetone was used, it evaporated too quickly, so nanoparticles could not be formed, while ethanol did not pose this problem [75]. The optimization of the concentration of the polymer, cross-linking agent, and desolvating agent was done based on PS and EE%.

3.3 Quality by Design

The QbD methodology was critical in ensuring that quality was built into the albumin nanoparticle-loaded OFDF formulation from the start of beginning to the end. By following the ICH Q8 Guidelines, our team constructed an organized and potential-based risk assessment at each level during the formulation of albumin nanoparticles loaded with OFDF that reduced potential failures and increased process efficiency [76]. This pre-emptive approach empowered us to carefully monitor essential components, handling, and processing aspects, resulting in substantial enhancements in formulation quality and effectiveness.

3.3.1 Quality Target Product Profiles

The QTPP for albumin nanoparticle-loaded OFDF was extremely important in optimizing quality, safety, stability, and therapeutic effectiveness by specifying important factors as mentioned in Table 3 [77]. In vitro investigations revealed that the modified formulation had significantly more drug release and bioavailability than other conventional formulations of azithromycin. Overall, the QTPP successfully linked product attributes with patient demands, demonstrating the promise of this non-invasive route of albumin nanoparticle-loaded oral rapid dissolving film administration systems for reduced dosage frequency encountered with other conventional azithromycin formulations and regulated drug administration, as well as paving the path for further research and clinical validation.

3.3.2 Critical Quality Attributes

It was fundamentally necessary to determine the CQAs to achieve optimal performance with albumin nanoparticle-loaded OFDF. According to the results, a reduced particle size resulted in a considerable improvement in permeation, low PDI, and Zeta potential, which signify the formulation's stability. These findings exhibited the significance of CQA in the process of manufacturing OFDF filled with albumin nanoparticles

Table 3 Quality target product profile of azithromycin-albumin nanoparticles-based oral fast-dissolving films

QTPP Parameters	Target specifications	Justification
Dosage Form	Oral Fast Dissolving Films	Mucoadhesion in the oral cavity bypasses the pre-systemic metabolism and follows sustained release to maintain therapeutic levels.
Dosage Type	Oral film	Self-administration and reduced dosage regime.
Route of Administration	Oral Cavity Buccal or Sublingual route of administration	Non-invasive route and ease in patient compliance.
Dosage Strength	500 mg Azithromycin	To reach within optimum therapeutic range for antibacterial activity.
Drug Release Profile	Immediate initially and sustained release further, $\geq 85\%$ drug release	Ensures rapid onset of action with sustained release.
Taste Masking	Palatable and optimum taste acceptance.	To improve patient compliance
Stability	At least 90 days at room temperature	Maintains the formulation integrity and prevents any therapeutic error.

of superior quality that are capable of efficiently satisfying the requirements of patients, as shown in Table 4 [78].

3.3.3 Central Composite Design

CCD was used to obtain the optimized formulation. The independent variables analyzed were albumin concentration (% w/v), the concentration of glutaraldehyde (% v/v), and the amount of the dissolving agent, i.e., ethanol (ml). The desired responses studied were PS, PDI, and EE%. Fifteen runs were generated, with each test executed in triplicate to assess the reproducibility of the findings, as shown in Table 5. The obtained results were analyzed through ANOVA, shown in Table 6, and the independent variable's coefficients and their associated probability (p)

Table 4 Critical quality attributes of azithromycin-albumin nanoparticles

CQAs Parameters	Target	Impact on Product Quality
Albumin Nanoparticles Particle Size	> 500 nm	Affects dissolution rate, bioavailability, and permeation
Polydispersity Index	< 0.300	Lower PDI indicates a more homogeneous droplet size within the formulation.
Zeta Potential	± 30 mV (stable dispersion)	Ensures the stability of the Albumin nanoparticles formulation
Encapsulation Efficiency	$\geq 85\%$	Affects drug loading and release profile

Table 5 Experimental runs of the design (CCD) for the prepared nanoparticles

Formulation code	Independent variables			Dependent variables						Drug loading (%)
	Coded Factors			Observed Response			Predicted Response			
	X1	X2	X3	Y1	Y2	Y3	Y1	Y2	Y3	
1	5	7	9	250.6±19.6	0.265	98.67±0.002	185.46	0.2809	98.48	55.19%±0.002
2	6	6	10	138.4±21.95	0.358	97.31±0.001	174.39	0.3977	96.84	49.65%±0.001
3	5	7	7.58	132.4±24.15	0.358	93.80±0.004	516.42	0.3183	94.27	53.78%±0.004
4	4	8	10	130.3±10.1	0.325	95.49±0.001	248.30	0.3647	88.02	60.59%±0.001
5	5	5.58	9	251.2±74.03	0.032	96.83±0.035	107.76	0.0077	97.3	53.80%±0.035
6	5	8.41	9	123.2±42.27	0.467	99.26±0.005	170.59	0.4273	99.73	55.15%±0.005
7	5	7	9	250.6±19.6	0.265	98.67±0.002	453.58	0.2809	98.48	55.19%±0.002
8	6	8	8	124.2±85.10	0.28	98.36±0.004	185.46	0.3197	97.89	49.74%±0.004
9	5	7	10.41	147.3±10.7	0.491	96.05±0.002	453.58	0.4513	98.52	53.37%±0.002
10	5	7	9	250.6±19.6	0.265	98.67±0.002	107.76	0.2809	98.48	55.19%±0.002
11	6.41	7	9	126.1±16.6	0.398	95.52±0.014	111.56	0.3583	95.99	45.35%±0.014
12	3.58	7	9	147.3±11.7	0.491	89.77±0.001	516.42	0.4513	86.24	63.32%±0.001
13	5	7	9	250.6±19.6	0.265	98.67±0.002	111.56	0.2809	98.48	55.19%±0.002
14	5	7	9	250.6±19.6	0.265	98.67±0.002	174.39	0.2809	98.48	55.19%±0.002
15	4	6	8	99.2±10.4	0.315	92.81±0.0048	248.30	0.3547	92.34	59.03%±0.004
Optimized Formulation	5	7	9	250.6±19.6	0.265	98.67±0.002	111.56	0.2809	98.48	55.19%±0.002

Table 6 Model fitting statistics and ANOVA for the responses

Response	Model fitting	R ²	Adjusted R ²	Predicted R ²	SD	Model F value	Model p-value	% CV
Y ₁	4FI	0.8692	0.8217	0.7234	67.76	18.28	0.0001	27.55
Y ₂	4FI	0.9517	0.9194	0.8472	0.0429	29.53	0.0001	8.65
Y ₃	4FI	0.8788	0.8182	0.6897	8.52	14.50	0.0003	123.55

value are shown in Table 7. Also, the 3D response surface graph, contour, and predicted vs. actual plots illustrating the impact of various process parameters on PS, PDI, and EE are shown in Figs. 1 and 2, and 3, respectively.

3.3.3.1 Influence of Independent Variables on Particle Size The results obtained for the particle sizes range between a minimum of 99.2±10.4 nm to a maximum of 251.2±74.03 nm. The particle size response was modelled using a polynomial equation derived from a CCD, considering the interaction between three independent variables: albumin (X1), glutaraldehyde (X2), and ethanol (X3). The coded polynomial equation is expressed as follows:

$$Y_1 = 248.15 - 7.50 \times_1 - 45.25 \times_2 + 5.27 \times_3 - 6.06 \times_1 \times_2 - 49.48 \times_1 \times_3 - 15.77 \times_2 \times_3 + -52.66 \times_1^2 - 27.51 \times_2^2 - 51.08 \times_3^2 \quad (1)$$

A coded polynomial Eq. 1 was obtained to determine the influence of the independent variable on PS. To generate projections for the response, the equation in coded terms can be utilized based on specified levels of the respective elements. The low levels of the elements are represented by -1, while the high levels are denoted by +1. This coded equation is a valuable tool for comparing the influence of various factors by analyzing their respective coefficients. The sign and magnitude of the coefficients provide insights into the nature and extent of their influence on the response. Positive coefficients suggest a synergistic effect, enhancing the response, whereas negative coefficients imply an antagonistic effect, reducing the response. The quantifiable effects of the independent variables (X₁, X₂, and X₃) and their interactions (e.g., X₁×₂, X₁×₃, and X₂×₃) on the Y1 response can be evaluated using this equation. The interaction terms reveal the interconnected effects among the variables. The equation for X₁ (albumin concentration) shows that higher albumin concentration leads to smaller particle

Table 7 Independent variable's coefficients and their associated probability (p) value

	Intercept	A	B	AC	A ²	B ²	C ²
Particle Size	248.14		-45.25	-49.4798	-52.6593	-27.5093	-51.0843
p-values			0.0002	0.0006	<0.0001	0.0004	<0.0001
PDI	0.280		0.153	0.170796	0.0619444		
p-values			0.003	0.0090	0.0320		
Entrapment Efficiency	98.29	2.032			-2.35917		
p-values		0.031			0.0050		

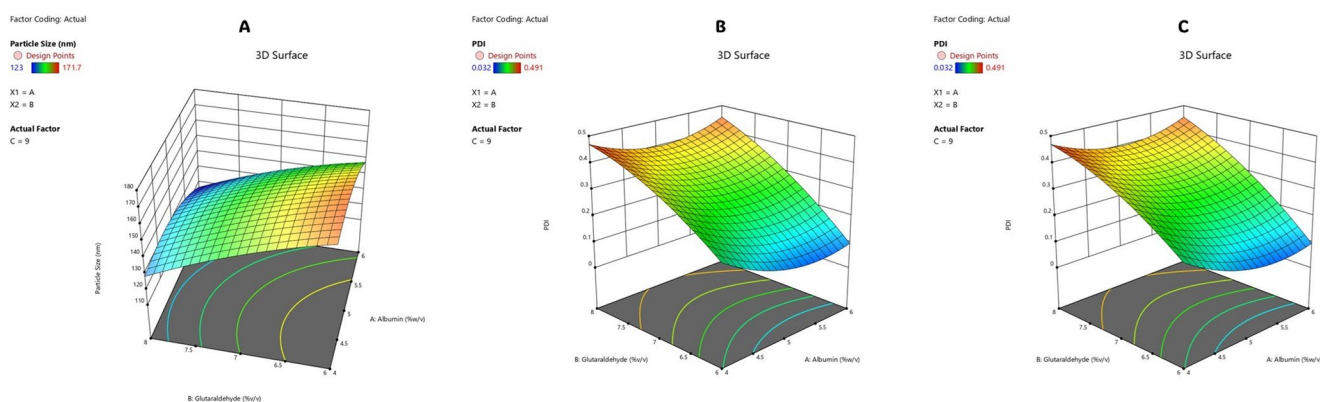


Fig. 1 3D surface response graph showing the effect of albumin and glutaraldehyde on (A) Particle Size, (B) PDI, (C) Entrapment Efficiency

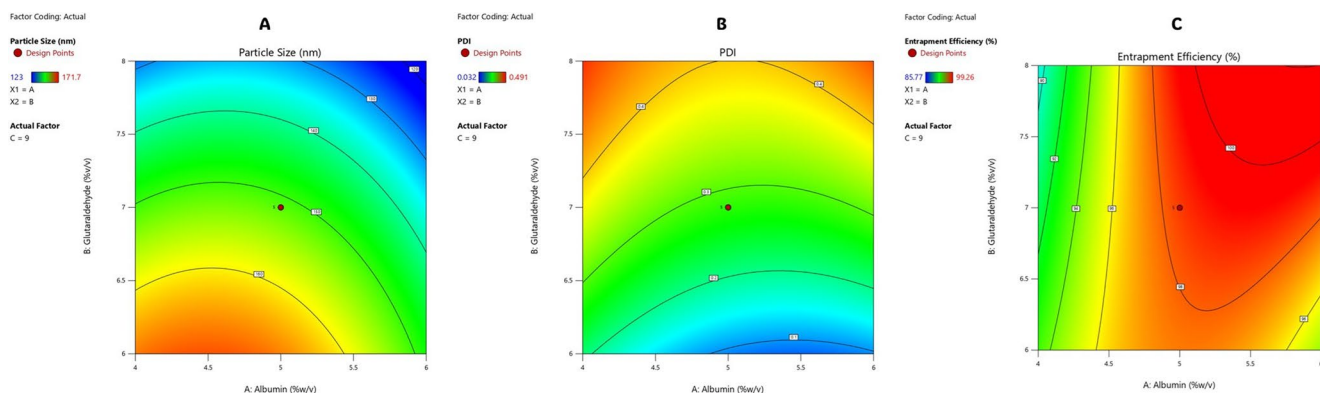


Fig. 2 Contour plot showing the effect of albumin and glutaraldehyde on (A) Particle Size, (B) PDI, (C) Entrapment Efficiency

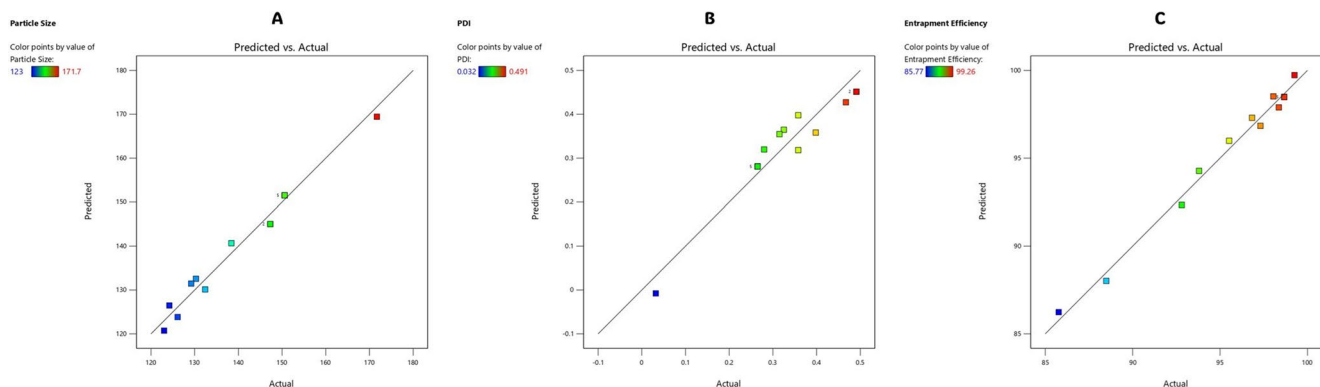


Fig. 3 Predicted vs. actual plot showing the effect of albumin and glutaraldehyde on (A) Particle Size, (B) PDI, (C) Entrapment Efficiency

sizes. This might be due to the increased surface area available for protein interaction, implying that greater protein concentrations promote nanoparticle formation and stability [79]. The equation shows that increasing glutaraldehyde concentration (X_2) results in a considerable decrease in particle size. This is further demonstrated by the significantly negative linear coefficient, indicating a considerable effect. Glutaraldehyde functions as a crosslinking agent, establishing the formation of more compact nanoparticles by tightening their structure. The positive correlation coefficient for X_3

(ethanol volume) indicates a small increase in particle size as ethanol concentration increases. This might be related to the influence of ethanol on the desolvation process, due to too much solvent can interfere with the formation of compact nanoparticles. The interaction between albumin and ethanol (X_1X_3) shows a considerable negative impact, suggesting that this combination helps counteract the size-increasing effect of ethanol. Similarly, the interaction between glutaraldehyde and ethanol (X_2X_3) reveals a negative coefficient, showing that they decrease particle size more efficiently

than individually. The model achieved an R^2 (0.9925) and p (<0.0001) that highlights the comparative impacts of the independent factors and their interactions, providing a comprehensive understanding of the response behaviour. The 3D response surface graphs (Fig. 1A) demonstrate the combined effect of albumin content (X_1) and glutaraldehyde (X_2) on particle size. A 3D response graph is seen, suggesting a quadratic relationship. The observed graph shows that as the albumin concentration is increased, particle size also increases, owing to elevated viscosity forming larger droplets. However, a moderate increase in particle size is observed with glutaraldehyde increased concentration, owing to more cross-linking. Figure 2A depicts a contour plot showing the effect of albumin and glutaraldehyde on PS. Figure 3A compares the predicted with the experimental particle size values, which shows data points are closely distributed around 45 degree line, highlighting the minimal deviation, good correlation, and predictive accuracy of the model. Also, a 3D response graph is seen, suggesting a quadratic relationship. Particle size initially reduces when albumin concentration increases, reaching its smallest size before increasing at higher levels. This suggests that optimal albumin concentrations facilitate nanoparticle formation by maintaining the protein matrix; however, high levels may lead to aggregation and an increase in particle size [80]. Similarly, the concentration of glutaraldehyde has a significant negative influence on particle size, as seen by the graph's descending slope. This happens due to its function as a crosslinking agent, which compacts nanoparticles by forming a denser network structure. The interaction of X_1 and X_2 also demonstrates that a balanced albumin-glutaraldehyde ratio results in the lowest particle sizes. The 2D contour plots also support these results with colour gradients: the core red zone represents the area with the lowest particle sizes, whereas the green-yellow zones are present where the particle size increases. The circular shape of the outlines indicates a strong interaction between X_1 and X_2 . The plots help visually determine the optimal range of both variables for minimizing particle size [81].

3.3.3.2 Influence of Independent Variables on PDI The results ranged from the minimum PDI (0.032) to the maximum PDI (0.491). Using CCD, a polynomial equation was developed to describe the PDI response (Y_2) as influenced by the interactions among three independent variables: albumin (X_1), glutaraldehyde (X_2), and ethanol (X_3). The resulting equation is:

$$Y_2 = 9.48 - 2.36 \times_1 + 0.81 \times_2 - 1.51 \times_3 + 0.02 \times_1 \times_2 + 0.17 \times_1 \times_3 - 0.03 \times_2 \times_3 + 0.06 \times_1^2 - 0.03 \times_2^2 + 0.05 \times_3^2 \quad (2)$$

The influence of independent variables on PDI was determined by an Eq. 2 that shows that increasing albumin content (X_1) reduces PDI, suggesting a more uniform particle size distribution. This might be due to enhanced nanoparticle stability at greater protein concentrations, which facilitates the creation of well-dispersed particles. However, the slight positive quadratic impact implies that excessively high or low albumin concentrations potentially slightly increase PDI. The equation for X_2 (glutaraldehyde concentration) reveals that higher concentrations lead to a slight increase in PDI, as evidenced by the positive linear coefficient. This might be related to excessive crosslinking, which could impact regularity in nanoparticle production [82]. The negative coefficient for X_3 (ethanol concentration) indicates that increasing ethanol content reduces PDI and promotes smaller particle size variations. Ethanol likely aids in the desolvation process, resulting in more uniform nanoparticles. The quadratic impact is modestly positive, implying that very high or low ethanol levels may slightly raise PDI. The interaction between albumin and ethanol (X_1X_3) has a positive coefficient, suggesting a slight rise in PDI when both are used together, possibly due to competition between protein binding and solvent effects. The interaction between glutaraldehyde and ethanol (X_2X_3) has a negative coefficient, indicating a more uniform particle size distribution by slightly lowering PDI. 3D response surface graphs demonstrate the effects of X_1 (albumin) and X_2 (glutaraldehyde) on the PDI. PDI is an important parameter that measures the homogeneity of particle size distribution; lower values suggest more uniformity and homogeneity of the particle sizes in the formulation. The 3D surface graphs show that the optimum albumin concentrations lead to the lowest PDI values. At low or extremely high concentrations, the PDI rises, most likely due to the formation of unstable or excessively dense particles. The impact of glutaraldehyde is less prominent, although it does contribute a slight change to PDI reduction. Moreover, in the contour plot, the green-blue gradient displayed lower PDI areas, but the yellow to red reflects more variability in particle distribution. The nearly parallel contour lines indicate that albumin has a stronger impact than glutaraldehyde. These plots help in determining the optimal formulation range for achieving a narrow size distribution of nanoparticles [48]. The model's reliability was confirmed with an R^2 value of 0.9097 and a p -value of 0.0362, highlighting the significance of the independent variables in predicting the PDI response. The equation shows that increasing albumin content (X_1) reduces PDI, suggesting a more uniform particle size distribution.

The interaction between albumin (X_1), glutaraldehyde (X_2), and ethanol (X_3) is depicted in 3D response surface graphs (Fig. 1B). Figure 3B shows the predicted vs. experimental PDI values, showing close clustering along

the diagonal line, with minimal deviation, good correlation, and accuracy. The low range of PDI signifies the uniform dispersibility of nanoparticles.

3.3.3.3 Influence of Independent Variables on EE% The results obtained the range of the EE% between the minimum EE% (89.77 ± 0.0099) to the maximum EE% (99.26 ± 0.0056). A polynomial equation was also generated using CCD to evaluate the effect of the independent variables on EE response (Y3). The equation for Y3, reflecting the interaction between albumin (X1), glutaraldehyde (X2), and ethanol (X3), is as follows:

$$Y_3 = 98.30 + 2.03 \times_1 + 0.85 \times_2 + 0.79 \times_3 + 0.38 \times_1 \times_2 - 0.07 \times_1 \times_3 + 0.19 \times_2 \times_3 + -2.36 \times_1^2 + 0.34 \times_2^2 - 1.22 \times_3^2 \quad (3)$$

The above equation shows the intercept value of 98.30, highlighting the entrapment efficiency when all independent variables are at their centre point, i.e., zero level. The main effect terms are X1, X2, and X3, showing a linear relationship with the entrapment efficiency. As per the coefficient values, albumin concentration has the strongest impact, while the stirring speed has the least. The combined effect terms, such as $X_1 \times_2$, $X_1 \times_3$, and $X_2 \times_3$, have different effects. When both albumin and glutaraldehyde increase, entrapment efficiency also increases, while there is a negative interaction between albumin and stirring speed, and a slight positive interaction between glutaraldehyde and stirring speed [83, 84].

3.4 Characterization of AZI-loaded Albumin Nanoparticles

3.4.1 Particle Shape and Surface Morphology

The surface characteristics of the particles were evaluated using TEM at a magnification of 25,600, which demonstrated the formation of well-defined, discrete nanoparticles. The particles exhibited a spherical to quasi-spherical shape, with smooth and uniform surfaces and consistency in Fig. 4. The size range was found to be within the nanoscale (about 50–150 nm), evident by the scale bars in the micrographs. TEM images revealed that the size distribution was appropriately homogenous, with a narrow distribution shown by the polydispersity index, indicating that the formulation's desolvation process was efficient. The strong contrast and electron-dense cores shown in the TEM pictures might indicate albumin's high protein density and probably drug entrapment beneath the nanoparticle matrix. Although some aggregation was seen in certain areas, however majorly part of the dispersion of particles remained individually

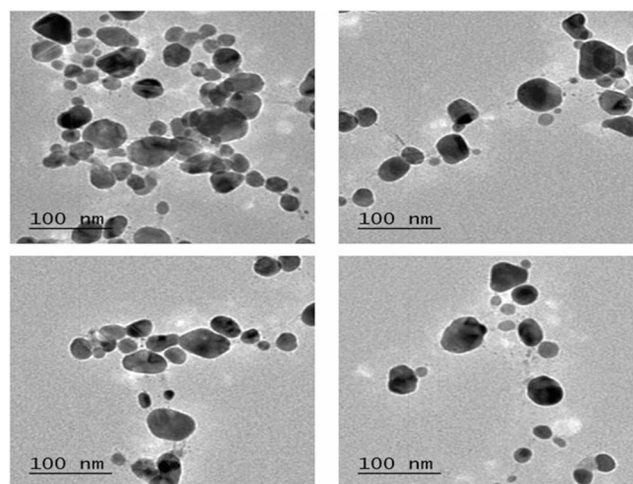


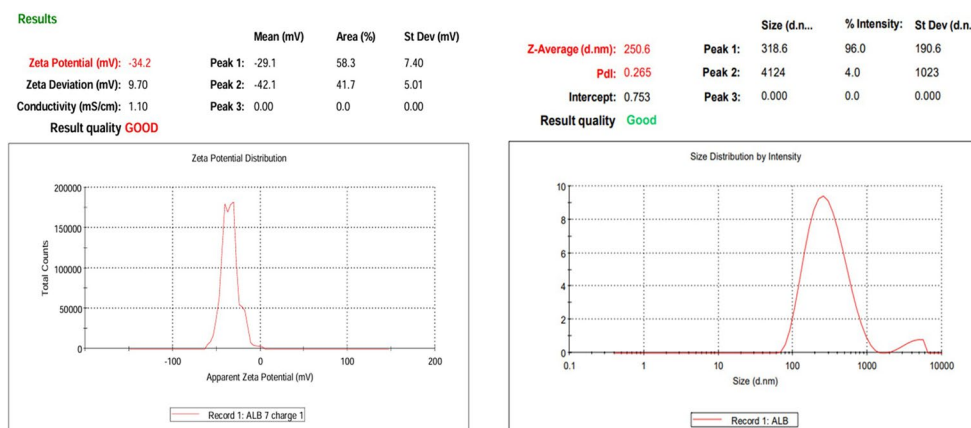
Fig. 4 TEM images of AZI-loaded albumin nanoparticles

recognized, indicating strong colloidal stability. The TEM evaluation demonstrates that the formulated albumin nanoparticles are structurally stable, well-formed, and prevent any de-stability conditions [85].

3.4.2 Assessment of Particle size, Polydispersity index, and Zeta Potential

The particle size of the optimized formulation was obtained of $250.6 \text{ nm} \pm 190.6$ with 96% intensity, which indicates that the major albumin protein nanoparticles were developed. However, a slight second peak was also observed, but with much less intensity; it might be due to the reason of protein coagulation. Moreover, the PDI obtained was 0.265, demonstrating that the developed formulation exhibited homogeneity and uniform distribution in the developed albumin nanoparticle formulation, as shown in Fig. 5B. Whereas, the observed zeta potential was $34.2 \text{ mV} \pm 7.40$, as shown in Fig. 5A. The small particle size obtained from the optimized formulation could be attributed to the concentration of albumin and glutaraldehyde combined. PDI demonstrated that the developed formulation exhibited homogeneity and uniform distribution in the developed albumin nanoparticle formulation [86]. Albumin is a protein that has a pH level where it carries no charge, around 4.7–5.0, so when the pH is higher than this, it has a negative charge. In this study, the nanoparticles were formulated in distilled water, and the albumin molecules became negatively charged, revealing carboxyl and other acidic parts on the surface of the particles. The use of ethanol as a desolvating agent did not alter the surface charge but facilitated the aggregation of albumin into nanoparticulate form. Glutaraldehyde was employed as a crosslinking agent to stabilize the nanoparticles by forming covalent bonds with free amine groups on the albumin.

Fig. 5 (A) Zeta potential curve of F1; (B) Particle size and PDI curve of optimized formulation of azithromycin-loaded albumin nanoparticles



This crosslinking process consumes some of the positively charged amino groups, thereby enhancing the net negative surface charge. The absence of cationic stabilizers or surfactants further ensured that the surface remained predominantly negatively charged. As a result of these combined effects, a zeta potential indicated good colloidal stability due to strong electrostatic repulsion between nanoparticles, which helps in preventing particle aggregation [87].

3.4.3 In Vitro Drug Release

The optimized azithromycin-loaded albumin nanoparticle exhibited overall drug release of 96% over 35 h, as shown in Fig. 6. To further understand the release kinetics, the data were fitted into many different types of kinetic models, including zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas. The Higuchi model showed the greatest fit and the highest correlation coefficient ($R^2 = 0.9789$), suggesting a diffusion-controlled release mechanism driven by Fickian diffusion. The zero-order model also showed a significant correlation ($R^2 = 0.9622$), indicating

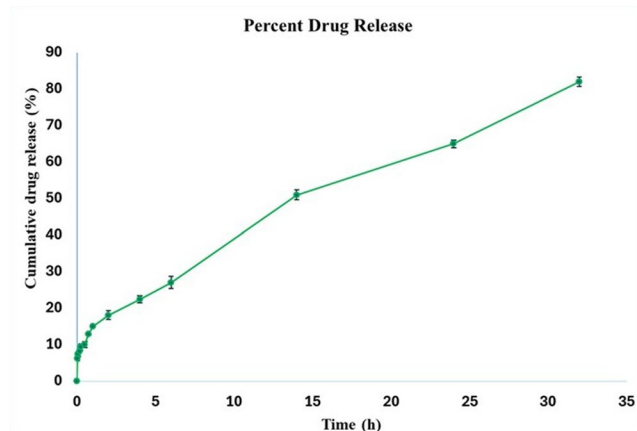


Fig. 6 In vitro drug release profile of the optimized formulation of azithromycin-loaded albumin nanoparticles

that the formulation maintained a near-constant drug release rate over a prolonged period. This is very beneficial for maintaining consistent therapeutic levels. Furthermore, the Korsmeyer-Peppas model revealed a release exponent ($n = 0.9029$), indicating anomalous (non-Fickian) transport, with diffusion and polymer breakdown contributing to the overall release process. The first-order model ($R^2 = 0.8919$) and Hixson-Crowell model ($R^2 = 0.9602$) confirmed the mixed kinetics of gradual erosion or dissolution of the nanoparticles' matrix. It was determined that the most optimal and appropriate formulation was the combination F1 formulation. The in vitro drug release profile of the optimized formulation was evaluated over 35 h to further understand the formulation's release kinetics and efficiency. The formulation exhibited a biphasic release pattern, which included an initial burst release followed by a sustained release period. During the first two hours of breakdown, approximately 18.11% of the drug was released. This quick release is due to the presence of a surface-bound or loosely entrapped drug inside the film matrix, which easily diffuses into the surrounding medium. This burst release is especially useful for buccal drug delivery systems, where a rapid action of therapeutic action is often required [88, 89]. Following this initial release, the formulation demonstrated a sustained and regulated drug release for up to 35 h, resulting in an overall drug release of over 96%. This prolonged release pattern indicates that azithromycin is well entrapped inside the albumin nanoparticle core, which acts as a persistent reservoir that controls the longer drug release [33].

3.5 Preparation and Selection of the optimized nanoparticles

By inputting the dependent variables—PS, PDI, and EE—into the Design Expert software, the optimized NPs formulation was generated and labelled as F1. The optimized nanoparticles were loaded into the OFDF.

3.6 Characterisation of nanoparticles loaded oral fast-dissolving films OFDF

3.6.1 Mechanical characteristics of OFDF

Mechanical properties such as tensile strength, percent elongation, and Young's modulus are vital indicators of the film's durability and flexibility (Table 8).

Tensile Strength The tensile strength of the developed OFDF was measured to be 6.75 ± 0.05 kg/mm. This result is within an acceptable range for buccal films and suggests that the film maintains suitable mechanical strength.

Percent Elongation The film's percent elongation was $31.25 \pm 0.08\%$, indicating remarkable flexibility. This amount of elongation is especially useful in buccal film applications, where the dosage form must conform to mucosal surfaces, retain contact for optimal drug absorption, and withstand mechanical stresses from mouth motions.

Young's Modulus The produced film has a Young's modulus of 21.6 ± 0.12 MPa, indicating an effective combination of flexibility and structural integrity.

In Vitro Disintegration time The disintegration duration for the formulated OFDF was recorded as 45 ± 2.05 s. This rapid disintegration indicates excellent film performance and patient convenience, particularly for individuals who may have difficulty swallowing conventional tablets, such as paediatric patients and the geriatrics. In vitro disintegration time exhibited that the developed OFDF disintegrated within 45 s, which demonstrates its exceptional performance as a film-based medication. This rapid disintegration time is particularly advantageous for patient convenience, especially for individuals who may struggle to swallow traditional tablets. Such patients include paediatric populations, who may find tablets challenging to consume, as

well as geriatric individuals, who often experience difficulties with swallowing due to various age-related conditions. Therefore, the optimized OFDF not only enhances medication adherence but also improves the overall experience for these specific groups of patients, making it a promising alternative to conventional oral dosage forms [68].

3.6.2 In Vitro Drug Release from OFDF

The in vitro drug release behaviour of the developed AZI-loaded oral OFDF was examined utilizing a USP Type II dissolution apparatus (Labtronics LT-721 Microprocessor Dissolution Device, Haryana, India). As shown in Fig. 7, the developed OFDF demonstrated a rapid and effective drug release of $69\% \pm 0.996$ in 30 min and $95\% \pm 0.659$ in 60 min. In vitro analysis demonstrated that the developed OFDF demonstrated a rapid and effective drug release profile, which is required for fast-acting buccal drug delivery systems. At 30 min, approximately $69\% \pm 0.996$ of the total AZI content was released, indicating an onset phase suitable for immediate therapeutic effect. This rapid release could be attributed to the OFDF's thin film matrix, increased surface area, and effective disintegration capabilities in an aqueous environment. After 60 min, almost $95\% \pm 0.659$ of the drugs had been released, indicating almost complete breakdown within an hour. This fast breakdown is crucial for increasing bioavailability in buccal distribution, where limited residence time is required for effective drug release [90]. The OFDF's rapid drug release implies that it benefits from the solubilization-enhancing action of albumin nanoparticles, which are anticipated to promote more rapid diffusion and dissolution of AZI via better wettability and decreased crystallinity. Furthermore, the polymeric composition and hydrophilic characteristics of the film matrix may have contributed to its fast disintegration and subsequent drug distribution into the dissolution media [91].

Table 8 Evaluation parameters of optimized Azithromycin hydrochloride albumin nanoparticles loaded OFDF

S.No.	Parameters	Results
1.	Film Thickness	0.22 ± 0.002 mm
2.	Folding endurance	200
3.	Content uniformity	Uniform throughout the film
4.	Surface pH	7.6 ± 0.002
5.	Moisture loss %	Negligible
6.	Tensile strength	6.75 ± 0.05 kg/mm
7.	Percent elongation (Strain)	$31.25 \pm 0.08\%$
8.	Young's Modulus	21.6 ± 0.12 MPa
9.	In vitro disintegration time	45 ± 2.05 s.

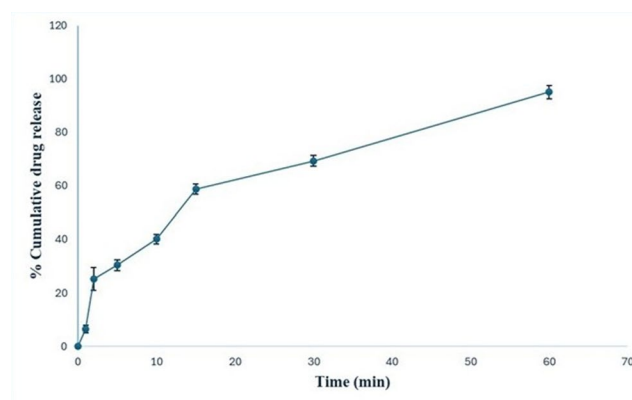


Fig. 7 In vitro drug release profile from oral fast-dissolving films

3.6.3 Antibacterial Study

The antibacterial activity of the optimized OFDF was evaluated and compared to that of the pure drug, a marketed formulation, and a negative control using the agar well diffusion technique. Figure 8 shows that the negative control (placebo film without drug) produced no measurable zone of inhibition, whereas the pure drug sample exhibited a zone of inhibition of $32 \text{ mm} \pm 0.17$. Both the new OFDF and the marketed formulation exhibited a significantly larger zone of inhibition ($38 \text{ mm} \pm 0.152$). The antibacterial activity exhibited that the negative control produced no measurable zone of inhibition, demonstrating the lack of intrinsic antibacterial characteristics in the excipients utilized to prepare the film. The pure drug sample exhibited moderate antibacterial activity in its natural state. On the other hand, both the new OFDF and the marketed formulation exhibited a significantly larger zone of inhibition of $38 \text{ mm} \pm 0.152$, indicating increased antibacterial efficacy. The OFDF and marketed formulation have identical inhibitory zones, indicating that the drug's therapeutic efficacy has been successfully preserved within the developed formulation. These data suggest that neither the albumin nanoparticle encapsulation process nor the OFDF formation approach produced a negative influence on the antibacterial activity of the active pharmaceutical ingredient AZI. The advantage of developed OFDF over marketed formulations is that they reduce the dose frequency and enhance patient compliance, as well as maintain the therapeutic antibacterial activity of AZI levels in the therapeutic range. Furthermore, the larger zone compared to the pure drug might suggest higher drug solubility and diffusion from the OFDF matrix. This highlights the developed OFDF's potential as a feasible and effective

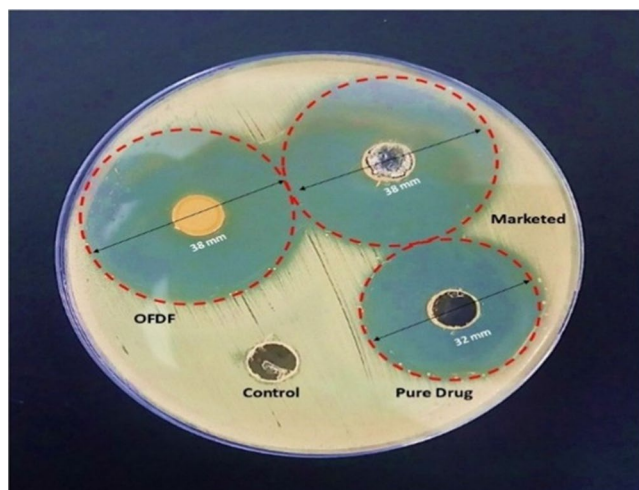


Fig. 8 Antibacterial assessment of zones of inhibition formed by different dosage forms

alternative to traditional dosage forms for systemic or localized distribution [92].

3.6.4 Ex Vivo Permeation Studies

The ex vivo permeation study of the optimized OFDF was examined on freshly excised goat buccal mucosa, which possesses a structure and permeability that resembles human buccal tissue. Figure 9 demonstrates that the quantity of drug penetrated (mg/cm^2) gradually increased over time, which indicates regulated release from the film matrix. The formulation's permeation flux (J) was estimated as $45.36 \text{ mg}/\text{h}\cdot\text{cm}^2$, indicating effective permeation across the buccal mucosa. The permeability coefficient (K_p) of $0.185 \text{ h}^{-1}\cdot\text{cm}^{-2}$ demonstrates an effective interaction between drug-loaded nanoparticles and the biological membrane. The ex vivo permeation analysis assessed the formulation's potential to enhance drug transport through the mucosal barrier, which is a major concern for buccal drug delivery systems. The amount of drug that penetrated the mucosal barrier showed a gradual increase over time, suggesting a controlled release mechanism from the film matrix. The initially observed phase exhibited a significant increase in drug permeation, followed by a steadier increase, indicating an initial burst release regulated probably caused by the surface-associated drug, followed by sustained release kinetics [92]. The formulation's observed permeation flux signifies effective drug permeation across the buccal mucosa. This indicates that the formulation is well-suited for delivering therapeutic agents through the oral cavity, where rapid absorption can lead to a quicker onset of action. Furthermore, K_p $0.185 \text{ h}^{-1}\cdot\text{cm}^{-2}$ highlights the favourable interaction between the drug-loaded nanoparticles and the biological membrane, suggesting that the formulation can efficiently traverse the mucosal barrier. This concentration-dependent permeation characteristic indicates that passive diffusion is the most prevalent transport mechanism, with the drug moving via a gradient-driven route

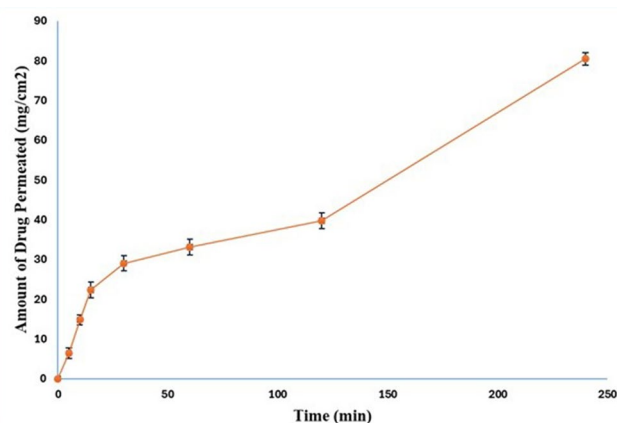


Fig. 9 Ex vivo permeation profile of oral fast-dissolving films via excised goat buccal mucosa

from the donor compartment to the lower concentration on the receptor side, driven by the concentration gradient. The higher permeability might be attributed to the nanoscale size of the albumin-based particles, which gives more surface area for interaction with the mucosal surface. The increased surface area not only facilitates mucoadhesion-allowing the formulation to adhere better to mucosal tissue-but also ensures that the drug is in close contact with the absorption site, thereby enhancing bioavailability. Additionally, the incorporation of ethanol possibly contributed to enhancing the drug solubilization and permeability. Ethanol can, to an extent, disturb the mucosal barrier, which allows for an efficient uptake of the drug. Moreover, the fast dissolution of the film contributes to the overall process because the drug becomes accessible for permeation promptly. Taken together, these factors make the formulation effective in providing the drugs through the buccal route, such that it can be an ideal administration route for patients who need rapid and efficient medication delivery [66].

4 Future Road Map: Transition from Lab To Clinical Settings

Several additional studies are suggested to advance this preliminary investigation. It is necessary to perform pre-clinical assessments like pharmacokinetic and pharmacodynamic profiling to validate the enhanced local availability, permeation, and therapeutic effectiveness of the developed formulation. Histopathological analysis of excised buccal tissues is one of the toxicological evaluations that must be considered to guarantee the safety of repetitive or extended treatment. To ascertain the formulation's shelf life and need for storage, stability tests as per ICH guidelines should also be carried out. Additionally, studies examining animal buccal models of mucoadhesion and residence time may bring more insight into the film's adherence and possibilities for sustained local release. Additionally, studies examining animal buccal models of mucoadhesion and residence time may bring more insight into the film's adherence and ability to achieve sustained local release. As a new, efficient, and comfortable treatment for streptococcal pharyngitis, these forthcoming studies may improve the scientific basis of the developed formulation and make it easier to transition it from laboratory testing to a clinical setting.

5 Conclusion

Oral fast-dissolving films containing azithromycin-loaded albumin nanoparticles have been effectively developed and tailored in this study for the management of strep throat. The solvent casting approach was used to prepare the film, and

the desolvation process was used to synthesize the nanoparticles. The use of a three-level, three-factor CCD was essential in meticulously evaluating how material attributes can affect significant formulation characteristics like particle dimensions, entrapment effectiveness, and zeta potential, highlighting good stability and drug-loading capacity. To provide increased local availability of azithromycin, the in vitro release and ex vivo permeation confirmed quick release of AZI-albumin nanoparticles from the oral films and effective penetration across excised goat buccal mucosa. Furthermore, the drug encapsulated in albumin nanoparticles successfully competed with the commercially available oral tablet in terms of its antimicrobial effectiveness against *Streptococcus aureus*. These findings represent that AZI-albumin nanoparticles loaded with OFDFs may provide a viable, patient-friendly substitute for the efficient treatment of strep throat. To validate the formulation's claim of enhanced local availability, permeation, and therapeutic efficacy, more research may involve preclinical studies. In addition, it is important to perform toxicity and biocompatibility tests to guarantee buccal application safety. Scale-up viability evaluations and stability tests under varied storage circumstances would also aid in establishing formulation consistency.

Acknowledgements The authors would like to thank all the administrative staff and laboratory staff for their support during the execution of this research. The authors have not received any funding to conduct this research.

Authors' Contributions Unnati Garg: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing Shreya Kaul: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing Rupali Verma: Formal analysis, Writing – original draft, Writing – review & editing Neha Jain: Conceptualization, Methodology, Supervision, Writing – review & editing Manisha Pandey: Methodology, Supervision, Writing – review & editing Upendra Nagaich: Project administration, Resources, Supervision, Writing – review & editing.

Data Availability The supplementary data can be made available to the editors on request by contacting the corresponding author.

Declarations

Competing Interests The authors declare no competing interests.

References

1. Strep Throat: All You Need to Know | CDC. (2022). <https://www.cdc.gov/groupastrep/diseases-public/strep-throat.html>. Accessed 5 Nov 2022.
2. Miller, K. M., Carapetis, J. R., van Beneden, C. A., Cadarette, D., Daw, J. N., Moore, H. C., Bloom D. E., Cannon, J. W. (2022). The global burden of sore throat and group A *Streptococcus* pharyngitis: A systematic review and meta-analysis. *EClinicalMedicine*, 48. <https://doi.org/10.1016/j.eclinm.2022.101458>

3. Mustafa, Z., & Ghaffari, M. (2020). Diagnostic methods, clinical guidelines, and antibiotic treatment for group A *Streptococcal* pharyngitis: A narrative review. *Frontiers in Cellular and Infection Microbiology*, 10, Article 644. <https://doi.org/10.3389/FCIMB.2020.563627>
4. Mustafa, Z., & Ghaffari, M. (2020). Diagnostic methods, clinical guidelines, and antibiotic treatment for group A *Streptococcal* pharyngitis: A narrative review. *Frontiers in Cellular and Infection Microbiology*. <https://doi.org/10.3389/FCIMB.2020.563627>
5. McIsaac, W. J., Goel, V., Slaughter, P. M., Parsons, G. W., Woolnough, K. V., Weir, P. T., Ennet, J. R. (1997). Reconsidering sore throats. Part 1: Problems with current clinical practice. *Canadian Family Physician*, 43, 485–493. <https://pubmed.ncbi.nlm.nih.gov/9116520/>
6. Somro, A., Akram, M., Ibrahim Khan, M., Asif, H.M., Sami, A., Ali Shah, S.M., Shahan, G., Ahmed, K., Rehman, R.U. (2011). Pharyngitis and sore throat: A review. *African Journal of Biotechnology*, 10(33):6190–6197. <https://doi.org/10.5897/AJB10.2663>
7. Pacor, M. L., Biasi, D., Maleknia, T., Lunardi, C. (1994). Safety of azithromycin in patients allergic to penicillin and cephalosporin. *La Clinica terapeutica*, 144(6):517–520. <https://pubmed.ncbi.nlm.nih.gov/8001336/>
8. Marcy, S. M. (2007). Treatment options for streptococcal pharyngitis. *Clinical Pediatrics*. <https://doi.org/10.1177/0009922807299720>
9. Pharyngitis (Strep Throat): Information For Clinicians | CDC. (n.d.). <https://www.cdc.gov/groupastrep/diseases-hcp/strep-throat.html>. Accessed 24 Nov 2023.
10. Dipanwita, R., Sukanta, S., Somnath, S., Sankar, K., Dutta, R. N., Sujata, B., Pal, N. K., Basudev, B. (2010). A preliminary sentinel surveillance report on antibiotic resistance trend of *Streptococcus pyogenes* in Kolkata region, India. *Al Ameen Journal of Medical Sciences*, 3(2):146–151.
11. Tarlow, M. J. (1997). Macrolides in the management of streptococcal pharyngitis/tonsillitis. *Pediatric Infectious Disease Journal*. <https://doi.org/10.1097/00006454-199704000-00028>
12. N. S. G., P. B. M. (2022). The Review on Azithromycin. *International Journal For Multidisciplinary Research*, 4. <https://doi.org/10.36948/ijfmr.2022.v04i06.947>
13. Usadadia, S. V., Patel, J. M., Vihol, P. D., & Urkude, A. P. (2020). Protective effect of Quercetin on Azithromycin induced hepatotoxicity and nephrotoxicity in Wistar rats. *International Journal of Current Microbiology and Applied Sciences*. <https://doi.org/10.20546/ijemas.2020.909.360>
14. Dinoo, G. P. (2017). The macrolide antibiotic renaissance. *British Journal of Pharmacology*, 174, 2967–2983. <https://doi.org/10.1111/BPH.13936>
15. Pharyngitis (Strep Throat): Information For Clinicians | CDC. (n.d.). <https://www.cdc.gov/groupastrep/diseases-hcp/strep-throat.html>. Accessed 3 Dec 2022.
16. Azithromycin: Uses, Interactions, Mechanism of Action | Drug-Bank Online. (2025). <https://go.drugbank.com/drugs/DB00207>. Accessed 14 Oct 2025.
17. Saita, M. G., Aleo, D., Melilli, B., Mangiafico, S., Cro, M., Sanfilippo, C., & Patti, A. (2018). pH-dependent stability of azithromycin in aqueous solution and structure identification of two new degradation products. *Journal of Pharmaceutical and Biomedical Analysis*, 158, 47–53. <https://doi.org/10.1016/J.JPBA.2018.05.042>
18. Fohner, A. E., Sparreboom, A., Altman, R. B., & Klein, T. E. (2017). PharmGKB summary: Macrolide antibiotic pathway, pharmacokinetics/pharmacodynamics. *Pharmacogenetics and Genomics*, 27, Article 164. <https://doi.org/10.1097/FPC.0000000000000270>
19. Azithromycin: Uses, Interactions, Mechanism of Action | Drug-Bank Online. (n.d.). <https://go.drugbank.com/drugs/DB00207>. Accessed 5 Nov 2022.
20. Hassanin, I., & Elzoghby, A. (2020). Albumin-based nanoparticles: A promising strategy to overcome cancer drug resistance. *Cancer Drug Resistance*, 3, Article 930. <https://doi.org/10.20517/CDR.2020.68>
21. Lee, E. S., & Youn, Y. S. (2016). Albumin-based potential drugs: Focus on half-life extension and nanoparticle preparation. *Journal of Pharmaceutical Investigation*, 2016(4), 305–315. <https://doi.org/10.1007/S40005-016-0250-3>
22. Kinoshita, R., Ishima, Y., Chuang, V. T. G., Nakamura, H., Fang, J., Watanabe, H., Shimizu, T., Okuhira, K., Ishida, T., Maeda, H., Otagiri, M., & Maruyama, T. (2017). Improved anticancer effects of albumin-bound paclitaxel nanoparticle via augmentation of EPR effect and albumin-protein interactions using S-nitrosated human serum albumin dimer. *Biomaterials*, 140, 162–169. <https://doi.org/10.1016/j.biomaterials.2017.06.021>
23. Wu, J. (2021). The enhanced permeability and retention (EPR) effect: The significance of the concept and methods to enhance its application. *Journal of Personalized Medicine*, 11, Article 771. <https://doi.org/10.3390/JPM11080771>
24. An, F. F., & Zhang, X. H. (2017). Strategies for preparing albumin-based nanoparticles for multifunctional bioimaging and drug delivery. *Theranostics*, 7, Article 3667. <https://doi.org/10.7150/T.HNO.19365>
25. El-Feky, Y. A., Mostafa, D. A., Al-Sawahli, M. M., El-Telbany, R. F. A., Zakaria, S., Fayed, A. M., Ahmed, K. A., Alolayan, E. M., & El-Telbany, D. F. A. (2020). Reduction of intraocular pressure using timolol orally dissolving strips in the treatment of induced primary open-angle glaucoma in rabbits. *Journal of Pharmacy and Pharmacology*, 72, 682–698. <https://doi.org/10.1111/JPHP.13239>
26. Lai, K. L., Fang, Y., Han, H., Li, Q., Zhang, S., Li, H. Y., Chow, S. F., Lam, T. N., & Lee, W. Y. T. (2018). Orally-dissolving film for sublingual and buccal delivery of ropinirole. *Colloids and Surfaces B: Biointerfaces*, 163, 9–18. <https://doi.org/10.1016/J.COLSURFB.2017.12.015>
27. Liu, C., Chang, D., Zhang, X., Sui, H., Kong, Y., Zhu, R., & Wang, W. (2017). Oral fast-dissolving films containing lutein nanocrystals for improved bioavailability: Formulation development, in vitro and in vivo evaluation. *AAPS PharmSciTech*, 2017(8), 2957–2964. <https://doi.org/10.1208/S12249-017-0777-2>
28. Singh, S., Kamal, S. S., Sharma, A., Kaur, D., Katual, M. K., & Kumar, R. (2017). Formulation and in-vitro evaluation of solid lipid nanoparticles containing Levosulpiride. *The Open Nanomedicine Journal*, 4, 17–29. <https://doi.org/10.2174/1875933501704010017>
29. León, A., Reuquen, P., Garín, C., Segura, R., Vargas, P., Zapata, P., & Orihuela, P. A. (2017). FTIR and raman characterization of TiO₂ nanoparticles coated with polyethylene glycol as carrier for 2-methoxyestradiol. *Applied Sciences*, 7(1), Article 49. <https://doi.org/10.3390/app7010049>
30. Eid, M. M. (2022). Characterization of Nanoparticles by FTIR and FTIR-Microscopy. *Handbook of Consumer Nanoproducts* (pp. 645–673). https://doi.org/10.1007/978-981-16-8698-6_89
31. Amighi, F., Emam-Djomeh, Z., & Labbafi-Mazraeh-Shahi, M. (2020). Effect of different cross-linking agents on the preparation of bovine serum albumin nanoparticles. *Journal of the Iranian Chemical Society*, 17, 1223–1235. <https://doi.org/10.1007/s13738-019-01850-9>
32. Alkahtani, M. E., Aodah, A. H., Abu Asab, O. A., Basit, A. W., Orlu, M., & Tawfik, E. A. (2021). Fabrication and characterization of fast-dissolving films containing escitalopram/quetiapine for the treatment of major depressive disorder. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics13060891>
33. Dawoud, M. H. S., Abdel-Daim, A., Nour, M. S., & Sweed, N. M. (2023). A quality by design paradigm for albumin-based

- nanoparticles: Formulation optimization and enhancement of the antitumor activity. *Journal of Pharmaceutical Innovation*. <https://doi.org/10.1007/s12247-022-09698-y>
34. Gupta, M. S., Kumar, T. P., Gowda, D. V., & Rosenholm, J. M. (2021). Orodispersible films: Conception to quality by design. *Advanced Drug Delivery Reviews*. <https://doi.org/10.1016/j.addr.2021.113983>
 35. Namjoshi, S., Dabbaghi, M., Roberts, M. S., Grice, J. E., & Mohammed, Y. (2020). Quality by design: Development of the quality target product profile (QTPP) for semisolid topical products. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics12030287>
 36. Dangre, P. V., Phad, R. D., Surana, S. J., & Chalikwar, S. S. (2019). Quality by design (QbD) assisted fabrication of fast dissolving buccal film for clonidine hydrochloride: Exploring the quality attributes. *Advances in Polymer Technology*. <https://doi.org/10.1155/2019/3682402>
 37. Abdel-Bar, H. M., Tulbah, A. S., Darwish, H. W., Salama, R., Naguib, I. A., Yassin, H. A., & Abo El-Enin, H. A. (2023). Quetiapine albumin nanoparticles as an efficacious platform for brain deposition and potentially improved antipsychotic activity. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics15071785>
 38. Katona, G., Sipos, B., & Csóka, I. (2022). Risk-assessment-based optimization favours the development of albumin nanoparticles with proper characteristics prior to drug loading. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics14102036>
 39. Mishra, R., Jain, N., Kaul, S., & Nagaich, U. (2023). Central composite design-based optimization, fabrication, and pharmacodynamic assessment of sulfasalazine-loaded lipoidal nanoparticle-based hydrogel for the management of rheumatoid arthritis. *Drug Delivery and Translational Research*. <https://doi.org/10.1007/s13346-022-01260-0>
 40. Asadbeigi, N., Givianrad, M. H., Azar, P. A., & Saber-Tehrani, M. (2023). Optimization by central composite design (CCD)-response surface methodology (RSM) of the highly selective molecularly imprinted Ni and F co-doped TiO₂ photocatalyst for photocatalytic degradation of bisoprolol. *New Journal of Chemistry*. <https://doi.org/10.1039/d3nj03149g>
 41. Karyab, H., Ghasemi, M., Ghotbinia, F., & Nazeri, N. (2023). Efficiency of chitosan nanoparticle with polyaluminum chloride in dye removal from aqueous solutions: Optimization through response surface methodology (RSM) and central composite design (CCD). *International Journal of Biological Macromolecules*. <https://doi.org/10.1016/j.ijbiomac.2023.125977>
 42. Laid, T. M., Abdelhamid, K., Eddine, L. S., & Abderrhmane, B. (2021). Optimizing the biosynthesis parameters of iron oxide nanoparticles using central composite design. *Journal of Molecular Structure*, 1229, Article 129497. <https://doi.org/10.1016/J.MOLSTRUC.2020.129497>
 43. Jithan, A., Madhavi, K., Madhavi, M., & Prabhakar, K. (2011). Preparation and characterization of albumin nanoparticles encapsulating curcumin intended for the treatment of breast cancer. *International Journal of Pharmaceutical Investigation*, 1, 119. <https://doi.org/10.4103/2230-973X.82432>
 44. Keshavarz, M., Ahmadi Nasab, N., Saeidifar, M., Nikoofal, S., & Safa, O. (2022). Curcumin-loaded albumin nanoparticles synthesis, characterization, and in-vitro evaluation of cytotoxic effects against U-87 MG cells. *Nanomedicine Research Journal*, 7, 245–253. <https://doi.org/10.22034/NMRJ.2022.03.004>
 45. Meng, R., Zhu, H., Wang, Z., Hao, S., & Wang, B. (2022). Preparation of drug-loaded albumin nanoparticles and its application in cancer therapy. *Journal of Nanomaterials*, 2022, Article 3052175. <https://doi.org/10.1155/2022/3052175>
 46. Younis, F. A., Saleh, S. R., El-Rahman, S. S. A., Newairy, A. S. A., El-Demellawy, M. A., & Ghareeb, D. A. (2022). Preparation, physicochemical characterization, and bioactivity evaluation of berberine-entrapped albumin nanoparticles. *Scientific Reports*. <https://doi.org/10.1038/s41598-022-21568-8>
 47. Ge, Z., Ma, R., Xu, G., Chen, Z., Zhang, D., Wang, Q., Hei, L., & Ma, W. (2018). Development and in vitro release of isoniazid and rifampicin-loaded bovine serum albumin nanoparticles. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research*, 24, 473. <https://doi.org/10.12659/MSM.905581>
 48. Galiyeva, A., Daribay, A., Zhumagaliyeva, T., Zhaparova, L., Sadyrbekov, D., & Tazhbayev, Y. (2023). Human serum albumin nanoparticles: Synthesis, optimization and immobilization with antituberculosis drugs. *Polymers*. <https://doi.org/10.3390/polym15132774>
 49. Varia, U., Joshi, D., Jadeja, M., Katariya, H., Detholia, K., & Soni, V. (2022). Development and evaluation of ultradeformable vesicles loaded transdermal film of boswellic acid. *Future Journal of Pharmaceutical Sciences*. <https://doi.org/10.1186/s43094-022-00428-2>
 50. Shaikh, F., Patel, M., Patel, V., Patel, A., Shinde, G., Shelke, S., & Pathan, I. (2022). Formulation and optimization of cilnidipine loaded nanosuspension for the enhancement of solubility, dissolution and bioavailability. *Journal of Drug Delivery Science and Technology*, 69, Article 103066. <https://doi.org/10.1016/J.JDDST.2021.103066>
 51. Solanki, R., Patel, K., & Patel, S. (2021). Bovine serum albumin nanoparticles for the efficient delivery of berberine: Preparation, characterization and in vitro biological studies. *Colloids and Surfaces, A: Physicochemical and Engineering Aspects*, 608, Article 125501. <https://doi.org/10.1016/J.COLSURFA.2020.125501>
 52. Al-Nemrawi, N. K., & Dave, R. H. (2014). Formulation and characterization of acetaminophen nanoparticles in orally disintegrating films. *Drug Delivery*, 23(2), 540–549. <https://doi.org/10.3109/10717544.2014.936987>
 53. Elsayed, I., El-Dahmy, R. M., El-Emam, S. Z., Elshafeey, A. H., el Gawad, N. A. A., & El-Gazayerly, O. N. (2020). Response surface optimization of biocompatible elastic nanovesicles loaded with rosuvastatin calcium: Enhanced bioavailability and anticancer efficacy. *Drug Delivery and Translational Research*, 10(5), 1459–1475. <https://doi.org/10.1007/S13346-020-00761-0>
 54. Elshafeey, A. H., & El-Dahmy, R. M. (2021). Formulation and development of oral fast-dissolving films loaded with nanosuspension to augment paroxetine bioavailability: In vitro characterization, ex vivo permeation, and pharmacokinetic evaluation in healthy human volunteers. *Pharmaceutics*. <https://doi.org/10.3390/pharmaceutics13111869>
 55. Salawi, A. (2022). An insight into preparatory methods and characterization of orodispersible film—A review. *Pharmaceutics*. <https://doi.org/10.3390/ph15070844>
 56. Farghaly, D. A., Afifi, S. A., Aboelwafa, A. A., & Mohamed, M. I. (2023). Oral dissolving film of Rivastigmine: Optimization using factorial design. *Journal of Pharmaceutical Innovation*. <https://doi.org/10.1007/s12247-023-09743-4>
 57. Irfan, M., Rabel, S., Bukhtar, Q., Qadir, M. I., Jabeen, F., & Khan, A. (2016). Orally disintegrating films: A modern expansion in drug delivery system. *Saudi Pharmaceutical Journal*. <https://doi.org/10.1016/j.jsps.2015.02.024>
 58. Shankar Raman, S., Narayanan, V. H. B., & Durai, R. (2021). Lamotrigine nanoparticle laden polymer composite oral dissolving films for improving therapeutic potential of the hydrophobic antiepileptic molecule. *Assay and Drug Development Technologies*, 19, 2–16. <https://doi.org/10.1089/ADT.2020.992>
 59. El-Nabarawi, M. A., Bendas, E. R., el Rehem, R. T. A., & Abary, M. Y. S. (2013). Transdermal drug delivery of paroxetine through lipid-vesicular formulation to augment its bioavailability. *International Journal of Pharmaceutics*, 443, 307–317. <https://doi.org/10.1016/J.IJPHARM.2013.01.016>

60. Kunte, S., & Tandale, P. (2010). Fast dissolving strips: A novel approach for the delivery of verapamil. *Journal of Pharmacy & Bioallied Sciences*, 2, Article 325. <https://doi.org/10.4103/0975-7406.72133>
61. Kunte, S., & Tandale, P. (2010). Fast dissolving strips: A novel approach for the delivery of verapamil. *Journal of Pharmacy & Bioallied Sciences*, 2, Article 325. <https://doi.org/10.4103/0975-7406.72133>
62. Chavan, D. U., Marques, S. M., Bhide, P. J., Kumar, L., & Shirodkar, R. K. (2020). Rapidly dissolving Felodipine nanoparticle strips -Formulation using design of experiment and characterisation. *Journal of Drug Delivery Science and Technology*, 60, Article 102053. <https://doi.org/10.1016/J.JDDST.2020.102053>
63. A, M.S., V, C. (2016). Formulation and evaluation of fast dissolving oral films of diazepam. *J Pharmacovigil*. <https://doi.org/10.4172/2329-6887.1000210>
64. Miles, K. B., Ball, R. L., & Matthew, H. W. T. (2016). Chitosan films with improved tensile strength and toughness from N-acetyl-cysteine mediated disulfide bonds. *Carbohydrate Polymers*, 139, 1–9. <https://doi.org/10.1016/J.CARBPOL.2015.11.052>
65. Londhe, V., & Shirsat, R. (2018). Formulation and characterization of fast-dissolving sublingual film of Iloperidone using Box–Behnken design for enhancement of oral bioavailability. *AAPS PharmSciTech* (3). <https://doi.org/10.1208/S12249-018-0954-Y>
66. Dahiya, M., Saha, S., & Shahiwala, A. (2009). A review on mouth dissolving films. *Current Drug Delivery*. <https://doi.org/10.2174/156720109789941713>
67. Bharti, K., Mittal, P., & Mishra, B. (2019). Formulation and characterization of fast dissolving oral films containing buspirone hydrochloride nanoparticles using design of experiment. *Journal of Drug Delivery Science and Technology*, 49, 420–432. <https://doi.org/10.1016/J.JDDST.2018.12.013>
68. Kaul, S., Nagaich, U., & Verma, N. (2021). Investigating nanostructured liquid crystalline particles as prospective ocular delivery vehicle for tobramycin sulfate: Ex vivo and in vivo studies. *Journal of Advanced Pharmaceutical Technology & Research*. https://doi.org/10.4103/japtr.japtr_188_21
69. Kaul, S., Nagaich, U., & Verma, N. (2021). Preclinical assessment of nanostructured liquid crystalline particles for the management of bacterial keratitis: In vivo and pharmacokinetics study. *Drug Delivery and Translational Research*. <https://doi.org/10.1007/s13346-021-01072-8>
70. Peddapalli, H., Bakshi, V., & Boggula, N. (2018). Formulation, in vitro and ex vivo characterization of mucoadhesive buccal tablets for antihypertensive drug. *Asian Journal of Pharmaceutical and Clinical Research*. <https://doi.org/10.22159/ajpcr.2018.v11i8.26126>
71. Gupta, V., & Trivedi, P. (2015). Ex vivo localization and permeation of cisplatin from novel topical formulations through excised pig, goat, and mice skin and in vitro characterization for effective management of skin-cited malignancies. *Artificial Cells, Nanomedicine, and Biotechnology*. <https://doi.org/10.3109/21691401.2014.893523>
72. Begum, M. Y., Alqahtani, A., Ghazwani, M., Ramakrishna, M. M., Hani, U., Atiya, A., & Rahamathulla, M. (2021). Preparation of Carbopol 934 based ketorolac tromethamine buccal mucoadhesive film: In vitro, ex vivo, and in vivo assessments. *International Journal of Polymer Science*. <https://doi.org/10.1155/2021/4786488>
73. Srivastava, A., & Prajapati, A. (2020). Albumin and functionalized albumin nanoparticles: Production strategies, characterization, and target indications. *Asian Biomedicine (Research Reviews & News)*, 14, Article 217. <https://doi.org/10.1515/ABM-2020-0032>
74. Mohammad-Beigi, H., Shojaosadati, S. A., Morshedi, D., Mirzazadeh, N., & Arpanaei, A. (2016). The effects of organic solvents on the physicochemical properties of human serum albumin nanoparticles. *Iranian Journal of Biotechnology*, 14(1), 45–50. <https://doi.org/10.15171/IJB.1168>
75. Q8(R2) Pharmaceutical Development | FDA. (n.d.). <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q8r2-pharmaceutical-development>. Accessed 1 May 2025.
76. Gupta, M. S., Kumar, T. P., Gowda, D. V., & Rosenholm, J. M. (2021). Orodispersible films: Conception to quality by design. *Advanced Drug Delivery Reviews*, 178, Article 113983. <https://doi.org/10.1016/J.ADDR.2021.113983>
77. Jacob, S., Boddu, S. H. S., Bhandare, R., Ahmad, S. S., & Nair, A. B. (2023). Orodispersible films: Current innovations and emerging trends. *Pharmaceutics*, 15, Article 2753. <https://doi.org/10.3390/PHARMACEUTICS15122753>
78. Luo, J., Wan, L., Zhang, Q., Cui, B., Li, C., Jiang, Y., Jiang, M., & Wang, K. K. (2023). Constructing a drug release model by central composite design to investigate the interaction between drugs and temperature-sensitive controlled release nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics*, 183, 24–32. <https://doi.org/10.1016/J.EJPB.2022.12.009>
79. Wang, Y., Chen, S., Yang, X., Zhang, S., & Cui, C. (2021). Preparation optimization of bovine serum albumin nanoparticles and its application for sirna delivery. *Drug Design, Development and Therapy*, 15, 1531–1547. <https://doi.org/10.2147/DDDT.S299479>
80. Sadhukhan, B., Mondal, N. K., & Chatteraj, S. (2016). Optimisation using central composite design (CCD) and the desirability function for sorption of methylene blue from aqueous solution onto *Lemna major*. *Karbala International Journal of Modern Science*, 2, 145–155. <https://doi.org/10.1016/J.KIJOMS.2016.03.005>
81. Madani, F., Esnaashari, S. S., Mujokoro, B., Dorkoosh, F., Khosravani, M., & Adabi, M. (2018). Investigation of effective parameters on size of Paclitaxel loaded PLGA nanoparticles. *Advanced Pharmaceutical Bulletin*, 8, Article 77. <https://doi.org/10.15171/APB.2018.010>
82. Abolhassani, H., & Shojaosadati, S. A. (2019). A comparative and systematic approach to desolvation and self-assembly methods for synthesis of piperine-loaded human serum albumin nanoparticles. *Colloids and Surfaces B: Biointerfaces*, 184, Article 110534. <https://doi.org/10.1016/J.COLSURFB.2019.110534>
83. Esim, O., Gedik, M. E., Dogan, A. L., Gunaydin, G., & Hascicek, C. (2021). Development of carboplatin loaded bovine serum albumin nanoparticles and evaluation of its effect on an ovarian cancer cell line. *Journal of Drug Delivery Science and Technology*, 64, Article 102655. <https://doi.org/10.1016/J.JDDST.2021.102655>
84. Kiarashi, M., & Yasamineh, S. (2024). Albumin nanoparticles are a promising drug delivery system in dentistry. *BioMedical Engineering OnLine*, 23, Article 122. <https://doi.org/10.1186/S12938-024-01318-9>
85. Syed, A., Devi, V. K., Karwa, P. (2020). Applying taguchi design for the synthesis of Albumin nanoparticles of methotrexate by desolvation technique. *RGUHS Journal of Pharmaceutical Sciences*, 10. <https://journalgrid.com/view/article/rjps/12433337>. Accessed 18 May 2025.
86. Bronze-Uhle, E. S., Costa, B. C., Ximenes, V. F., & Lisboa-Filho, P. N. (2017). Synthetic nanoparticles of bovine serum albumin with entrapped salicylic acid. *Nanotechnology Science and Applications*, 10, 11–21. <https://doi.org/10.2147/NSA.S117018>
87. Tayel, S. A., El Nabarawi, M. A., Amin, M. M., & Abou Ghaly, M. H. (2016). Sumatriptan succinate sublingual fast dissolving thin films: Formulation and in vitro/in vivo evaluation. *Pharmaceutical Development and Technology*, 21, 328–337. <https://doi.org/10.3109/10837450.2014.1003655>

88. Vedanayagam, S., Selvaraj, R., K. S., Kanniyappan, H., Mathew, M. T., Doble, M., & Perumal, G. (2024). Nanofibers loaded levosalbutamol sulfate fast dissolving oral films for sublingual delivery. *Journal of Drug Delivery Science and Technology*, 99, Article 105920. <https://doi.org/10.1016/J.JDDST.2024.105920>
89. Hassen Elshafeey, A., Moataz El-Dahmy, R., Khutoryanskiy, V., & Al-Obaidi, H. (2021). Formulation and development of oral fast-dissolving films loaded with nanosuspension to augment paroxetine bioavailability: In vitro characterization, ex vivo permeation, and pharmacokinetic evaluation in healthy human volunteers. *Pharmaceutics*, 13, Article 1869. <https://doi.org/10.3390/PHARMACEUTICS13111869>
90. El-Dahmy, R. M., Elsayed, I., Elshafeey, A. H., El Gawad, N. A. A., & El-Gazayerly, O. N. (2014). Optimization of long circulating mixed polymeric micelles containing vinpocetine using simple lattice mixture design, in vitro and in vivo characterization. *International Journal of Pharmaceutics*, 477, 39–46. <https://doi.org/10.1016/j.ijpharm.2014.10.003>
91. Kim, S., Kim, J. J., Jeong, J. H., Kim, Y., Park, J. H., Jeong, D. I., Kim, H. J., Hwang, C. R., Ahn, S. H., Ko, H. J., & Cho, H. J. (2021). Fast dissolving nanofiber mat for the local antimicrobial application of roxithromycin in oral cavity. *Materials Science and Engineering: C*, 131, Article 112537. <https://doi.org/10.1016/j.msec.2021.112537>
92. Giovino, C., Ayensu, I., Tetteh, J., & Boateng, J. S. (2013). An integrated buccal delivery system combining chitosan films impregnated with peptide loaded PEG-b-PLA nanoparticles. *Colloids and Surfaces B: Biointerfaces*, 112, 9–15. <https://doi.org/10.1016/J.COLSURFB.2013.07.019>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.