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Fabrication of Nitrofurantoin Loaded pH Sensitive Solid Lipid Nanoparticles for Enhanced Bioavailability

by

Mariam Noor Khalid

A thesis submitted in partial fulfillment for the
degree of Master of Philosophy

in the

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Department of Pharmaceutics

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CERTIFICATE OF APPROVAL

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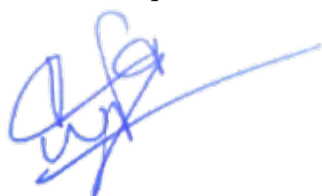
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(**Mariam Noor Khalid**)

Abstract

Nitrofurantoin (NFT) is a broad-spectrum antibacterial agent belongs to BCS class IV commonly used for the treatment of urinary tract infections (UTIs); however, its therapeutic effectiveness is limited by poor aqueous solubility, variable gastrointestinal absorption, and frequent dosing requirements. The present study aimed to develop and optimize pH-sensitive NFT-loaded SLNs coated with Eudragit® L100 to enhance oral bioavailability and achieve targeted intestinal drug release.

NFT-loaded SLNs were prepared using solvent evaporation method with slight modifications. Compritol® ATO 888 was employed as the lipid matrix, while Tween® 80 and Poloxamer 188 were used as stabilizing surfactants. Eudragit® L100 was incorporated as an enteric coating polymer to impart pH-responsive drug release characteristics at pH 6.8. A Box-Behnken Design (BBD) was utilized to optimize the formulation by evaluating the effects of lipid concentration, surfactant concentration, and polymer concentration on particle size, polydispersity index (PDI), and zeta potential. The optimized formulation was characterized for particle size using dynamic light scattering (DLS) technique, morphology using scanning electron microscopy (SEM), crystalline or amorphous nature of drug inside formulation using X-ray powder diffraction (XRPD), and for drug-excipient compatibility using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Further, in vitro drug release studies were performed in the simulated gastric fluid (pH 1.2) and phosphate buffer saline (pH 6.8), and in vivo oral bioavailability was assessed in the experimental rabbits.

The developed RP-HPLC method exhibited satisfactory linearity with a correlation coefficient (R^2) of 0.9914 and was successfully employed for quantitative analysis throughout the study. The optimized formulation exhibited a particle size of 228 nm, PDI of 0.189, and zeta potential of -31.8 mV. The entrapment efficiency of the optimized uncoated and Eu-NFT-SLNs was found to be 85.96 % and 79.20 %, respectively. The SEM analysis revealed predominantly spherical nanoparticles with smooth surfaces and minimal aggregation. XRPD studies

demonstrated a reduction in the crystallinity of NFT following encapsulation, indicating successful incorporation of the drug within the lipid-polymer matrix. The Eudragit® L100 coated formulation exhibited limited drug release, about 23.27 % in acidic medium of pH 1.2 and enhanced drug release of about 99.01 % at intestinal pH 6.8 in 24 hours, confirming the effectiveness of the Eudragit® L100 coating. In vivo studies demonstrated a $C_{\max}$ of 2.517 $\mu\text{g/mL}$, an AUC_{0-12} of 56.327 $\mu\text{g/mL}$, and a 11.1 folds enhancement in oral bioavailability of NFT-SLNs compared to the pure drug and 5-folds of Eu-NFT-SLNs compared to NFT-SLNs.

In conclusion, the developed Eudragit® L100-Eu-NFT-SLNs successfully encapsulated NFT and exhibited desirable physicochemical characteristics, pH - responsive drug release, and enhanced oral bioavailability. The formulation represents a promising oral drug delivery system for improving the therapeutic performance of NFT.

Keywords: Nitrofurantoin, Solid Lipid Nanoparticles, Eudragit ® L100, Oral Bioavailability, pH-Sensitive Drug Delivery, Controlled/Sustained Release.

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Abbreviations

%CDR	Percentage Cumulative Drug Release
%EE	Percentage Entrapment Efficiency
ANOVA	Analysis of Variance
API	Active Pharmaceutical Ingredient
ATR-FTIR	Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy
AUC	Area Under the Curve
BBD	Box–Behnken Design
BCS	Biopharmaceutics Classification System
CCD	Central Composite Design
CDR	Cumulative Drug Release
C_{max}	Maximum Plasma Concentration
DLS	Dynamic Light Scattering
DOE	Design of Experiments
DRAP	Drug Regulatory Authority of Pakistan
DSC	Differential Scanning Calorimetry
EE	Entrapment Efficiency
FTIR	Fourier Transform Infrared Spectroscopy
GIT	Gastrointestinal Tract
HLB	Hydrophilic–Lipophilic Balance
HPLC	High-Performance Liquid Chromatography
ICH	International Council for Harmonisation
IVIVC	In Vitro–In Vivo Correlation
KBr	Potassium Bromide

LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
NFT	Nitrofurantoin
NLCs	Nanostructured Lipid Carriers
PDI	Polydispersity Index
PK	Pharmacokinetic
PSD	Particle Size Distribution
QbD	Quality by Design
RPM	Revolutions Per Minute
RSM	Response Surface Methodology
R²	Coefficient of Determination
SD	Standard Deviation
SEM	Scanning Electron Microscopy
SLNs	Solid Lipid Nanoparticles
T_{max}	Time to Reach Maximum Plasma Concentration
UTI	Urinary Tract Infection
UV	Ultraviolet
UV-Vis	Ultraviolet–Visible Spectroscopy
w/v	Weight per Volume
w/w	Weight per Weight
WHO	World Health Organization
XRD	X-Ray Diffraction
ZP	Zeta Potential

Symbols

%	Percentage
<	Less Than
=	Equal To
>	Greater Than
C	Concentration
cm	Centimeter
cm ²	Square Centimeter
g	Gram
h	Hour
k	Release Rate Constant
kg	Kilogram
kV	Kilovolt
L	Liter
mg	Milligram
mg/kg	Milligram per Kilogram Body Weight
mg/mL	Milligram per Milliliter
min	Minute
mL	Milliliter
mm	Millimeter
mV	Millivolt
N	Total Number of Samples
n	Number of Replicates / Sample Size
nm	Nanometer
p	Probability Value

Q	Cumulative Drug Released
R ²	Coefficient of Determination
rpm	Revolutions Per Minute
sec	Second
t	Time
×	Multiplication
±	Plus or Minus
°	Degree
°C	Degree Celsius
≤	Less Than or Equal To
≥	Greater Than or Equal To
≈	Approximately Equal To
η	Entrapment Efficiency Parameter (if used)
ζ	Zeta Potential
λ	Wavelength
μ	Micro
μg	Microgram
μg/mL	Microgram per Milliliter
μL	Microliter
μm	Micrometer
ρ	Density
τ	Time Constant
Φ	Particle Diameter

Chapter 1

Introduction

1.1 Disease Burden and Clinical Importance of Urinary Tract Infections

Bacterial infections of the urinary tract are the most common infections in women, and research suggests that roughly 50% to 60% of women will have at least one episode in their lifetime [1]. The risk is especially high in women aged 55 to 75, of whom about 7% are affected each year. Among women who contract an infection, close to 53% experience another episode within the following twelve months [2]. UTIs also place a heavy financial burden on health systems. In 2012, treating and managing 22 million patients in the US cost approximately \$2.3 billion. While straightforward UTIs usually clear up fast with a short round of antibiotics, healthcare guidelines suggest stronger, proactive management plans for people who experience them frequently. These can include self-started treatments, ongoing preventative antibiotics, or other interventions [3]. To help stop infections before they start, options include vaginal estrogen, low-dose antibiotics taken daily or after sexual activity, cranberry products, probiotics, methenamine hippurate, and D-mannose [4].

1.2 Mechanism of Infection and Causative Organisms

A urinary tract infection occurs when pathogenic microbes overcome normal host defenses and attach to the urinary tract lining. The process typically begins as bacteria colonize the urethral region, subsequently ascending inside the bladder while in kidneys in severe presentations [5]. Basic bacterial triggers include *Escherichia coli*, *Klebsiella pneumoniae*, several species of *Staphylococcus* and *Streptococcus* and *Proteus* species [6]. Clinical signs may include fever, painful and burning urination, genital itching, and pelvic discomfort above the pubic bone. In some cases, sores or lesions also appear in the genital region [7].

Escherichia coli is the most common cause. Its fimbriae, adhesins, and biofilm-forming ability help it attach to and persist in the urinary tract [5].

1.3 Classification and Anatomical Forms of Urinary Tract Infections

While UTIs usually start small in the urethra or bladder, they can travel up to the ureters and kidneys, leading to serious health issues if they are ignored. Depending on where the infection settles, it is classified into different conditions [6, 8].

- i. Urethritis involves inflammation of the tube carrying urine out of the body, often triggered by chemicals, catheters, or sexually transmitted germs (split into gonococcal or non-gonococcal types based on the specific organism) [6, 8].
- ii. Cystitis is a very common bacterial infection inside the bladder and comes in various subtypes, including interstitial and hemorrhagic forms [6, 8].
- iii. Ureteritis happens when germs push further up into the ureters, frequently because faulty valves allow urine to flow backward [6, 8].

- iv. Pyelonephritis is a more dangerous kidney infection caused by bacteria moving upward or via urine reflux, which requires immediate medical care to prevent lasting harm to the kidneys [6, 8].

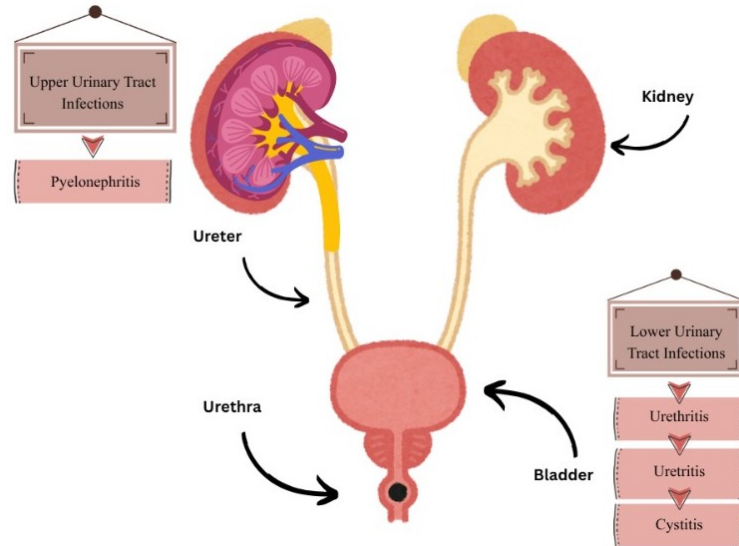


FIGURE 1.1: Urinary Tract Infections and its Types

1.4 Challenges Associated with the Long-Term Regulation of Recurrent Urinary Tract Infections

Treating recurrent UTIs is clinically demanding. The condition is generally identified when a patient has two or more infections within half a year, or three or more across twelve months. Women, the elderly, and people with conditions such as diabetes or malformations of the urinary system are affected most. Because therapy frequently depends on long or repeated antibiotic exposure, patients face more side effects, and the pressure on bacteria to develop resistance grows [15].

Another core obstacle is maintaining sufficient antibiotic levels inside the urinary tract. Ordinary oral formulations deliver unpredictable amounts of drug to the blood and urine, allowing some bacteria to survive and re-establish infection [16]. Frequent antibiotic use also damages the body's balanced microbial community, making future infections easier. As a result, drug carriers that release their cargo

steadily over an extended period are needed, so that patients can take fewer doses and achieve better clinical results [17].

1.5 Antimicrobial Resistance in Urinary Tract Infection Pathogens

Antimicrobial resistance (AMR) ranks among the most pressing health dangers of our time. Antibiotics once transformed medicine by curbing bacterial disease and saving countless lives, yet numerous microbes have since adapted to withstand them, eroding their usefulness. The main causes are the overprescription and wrongful use of these drugs, failure to follow treatment recommendations, and a thinning pipeline of newly discovered agents, which pharmaceutical companies have little financial or regulatory incentive to pursue [18].

Bacteria become resistant when genetic or biochemical changes allow them to survive exposure to drugs intended to inhibit or kill them. Examples include altered drug-binding sites, enzymes that break down or neutralize the antibiotic, a less permeable cell envelope, and pumps that eject the drug from inside the cell. Inappropriate antibiotic use hastens the process, because it removes susceptible bacteria and leaves resistant ones free to expand [19].

The spread of drug-resistant urinary pathogens is a serious worldwide problem that complicates UTI control. Common first-line drugs, including fluoroquinolones and trimethoprim-sulfamethoxazole, have lost effectiveness against many strains, which leaves clinicians with fewer therapeutic options [19].

1.6 Nitrofurantoin: Chemical Class and Physical and Chemical Characteristics

Nitrofurantoin (NFT) is a well-established oral antibacterial drug, commonly chosen for simple UTIs, especially those triggered by uropathogenic *Escherichia coli* (UPEC) [20]. Chemically, it falls within the nitrofuran family, whose members

carry a nitro group ($-\text{NO}_2$) on a furan ring. The drug is a yellow, crystalline solid that dissolves poorly in water, which markedly influences how well it dissolves in the gut and enters the body. Both its dissolving behavior and its stability change with pH, and it degrades more readily in highly acidic or highly alkaline surroundings [21, 22].

1.6.1 Mechanism of Antibacterial Action of Nitrofurantoin

Nitrofurans only become active drugs once their nitro group undergoes reduction, which transforms them into highly reactive species. Nitroreductase enzymes perform this conversion, and they are generally present in bacteria that carry working copies of the *nfsA*, *nfsB*, and *ribE* genes [23].

Unlike many standard antibiotics, NFT works through several routes at once. After it enters a bacterial cell, nitroreductases convert it into reactive electrophilic products [24]. These products damage a range of cellular materials, such as DNA, RNA, ribosomal proteins, and enzymes involved in metabolism, so that vital processes fail and the bacterium dies [25]. Since so many targets are affected simultaneously, a single mutation is unlikely to make a bacterium resistant. This multi-target action largely explains why the drug has remained useful for UTIs through many years of clinical practice [26].

1.6.2 Resistance Trends and Therapeutic Role of Nitrofurantoin

Despite being used widely for a long time, NFT has shown surprisingly little resistance relative to most competing antibiotics. Researchers link this to a few features: it reaches strong concentrations in urine almost immediately, it interferes with several bacterial targets so that one mutation rarely suffices, and it stays largely out of other tissues, which limits the chance of selecting resistant bacteria elsewhere in the body [27]. Resistance to NFT is possible, mainly via particular gene mutations, but it appears less often than for many other drugs used against

UTIs. Importantly, increased prescribing of NFT has not produced a comparable climb in clinical resistance, which reinforces its place in UTI treatment [28].

1.6.3 Pharmacokinetics of Nitrofurantoin

Once swallowed, NFT enters the body through the digestive tract, although uptake is partial and inconsistent because the drug dissolves poorly and its stability depends on pH [29]. The body breaks it down fast and removes it through the kidneys, which creates the high urine concentrations needed for it to work. Because the drug leaves the body quickly, patients must take it several times daily to keep urinary levels adequate [30]. Such pharmacokinetic restrictions can lead to swings in drug concentration, making insufficient exposure and treatment failure more likely [31].

1.6.4 Limitations of Conventional Nitrofurantoin Therapy

Although NFT retains clinical value, standard formulations come with significant disadvantages. They dissolve poorly in water, deliver unpredictable and usually modest amounts of drug into the bloodstream, are eliminated quickly, and require repeated daily dosing [32]. Side effects in the digestive system, including nausea and vomiting, may discourage patients from completing therapy, particularly over prolonged courses [33]. The drug's sensitivity to pH is a further complication, because shifting conditions in the gut can disturb both its stability and uptake. Together, these problems underline the need for newer delivery platforms that raise solubility, guard the drug against breakdown, and release it gradually [34].

1.7 Need for Novel Drug Delivery Systems for Nitrofurantoin

Given the physical, chemical, and pharmacokinetic weaknesses of NFT, designing improved delivery systems is well justified if its therapeutic potential is to be fully realized. Nanosized carriers, notably solid lipid nanoparticles, offer better

dissolution, higher bioavailability, shielding from degradation in the digestive tract, and regulated drug release [35]. Building in components that respond to pH could refine uptake further by making sure the drug is liberated at the desired site under appropriate physiological conditions [36]. Such strategies are expected to enhance efficacy, decrease how often doses must be taken, encourage patients to stay on treatment, and reduce the chance that resistance develops [37].

1.8 Overview of Nanotechnology in Drug Delivery

Nanotechnology has emerged as a significant breakthrough in pharmaceutical research, providing routes to upgrade drugs that suffer from unfavorable chemical, physical, or pharmacokinetic characteristics [38]. Carriers built at the nanoscale are engineered to boost solubility, stability, bioavailability, and site-specific delivery, while also lessening toxicity throughout the body. When drug particles are reduced to nanometer dimensions, their exposed surface expands and they interact more intensely with biological membranes, leading to better absorption and clinical benefit [39]. Among the many types of nanocarriers, those built from lipids have attracted notable interest thanks to their compatibility with the body, their ability to break down naturally, and their fitness for oral use [40].

1.9 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) function as sub-micron colloidal delivery systems typically falling within a 50 to 1000 nm size range composed of physiological lipids that remain solid at both ambient and physiological temperatures. These particulate structures are maintained in an aqueous suspension through the addition of appropriate stabilizing surfactants [41].

These particles were conceived as a substitute for polymeric nanoparticles, liposomes, and emulsions, aiming to avoid their problems with toxicity, drug escape, and physical instability [42, 43]. By merging advantages of lipid emulsions with

those of polymeric nanoparticles and trimming their downsides, SLNs offer a solid lipid framework that governs how the drug is released and protects the loaded substance from chemical and enzymatic attack [44]. Their flexibility and good tolerability have allowed use through oral, skin-applied, and injectable routes [45].

1.9.1 Composition and Characteristics of Solid Lipid Nanoparticles

An SLN is typically built from three main parts: a lipidic base (such as fatty acids, triglycerides, or waxes), stabilizing agents (for instance Poloxamer 188 or Tween[®] 80) that keep the suspension uniform, and the drug that is loaded inside. Which lipid and surfactant are selected has a major bearing on particle dimensions, crystal structure, and release pattern. The tightly packed lipid core defends the drug against degradation from its surroundings and also promotes prolonged release, so SLNs are well suited to drugs that dissolve poorly or break down easily [46].

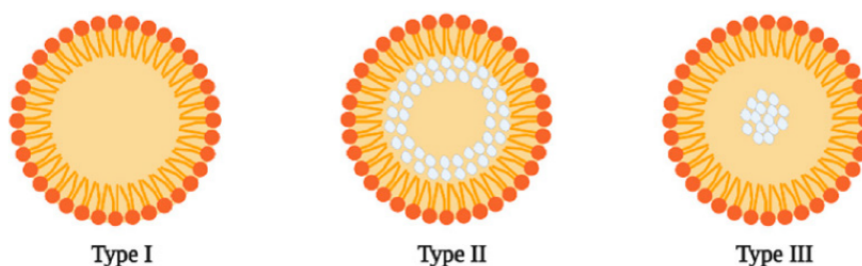


FIGURE 1.2: Types of solid lipid antiparticles [45]

1.9.2 Models of Drug Distribution in Solid Lipid Nanoparticles

SLNs can be sorted by the way the Active Pharmaceutical Ingredient (API) is arranged within the lipid matrix:

- i. Type I – Homogeneous Matrix Model: In this configuration, the active pharmaceutical ingredient is distributed uniformly throughout the entire solid

lipid framework, a design that promotes a slow and controlled release profile.

- ii. Type II – Drug-Enriched Shell Model: The drug gathers mainly in the particle’s outer region, producing an initial rapid out-burst followed by diffusion slowly.
- iii. Type III – Drug-Enriched Core Model: The drug is packed mainly in the particle’s center, resulting in regulated, long-lasting release with very little early burst [46–48].

1.9.3 Benefits of Solid Lipid Nanoparticles as Drug Carriers

SLNs have many strengths compared with conventional delivery approaches, which makes them appealing carriers for drugs. They can raise bioavailability by improving how a drug dissolves and by shielding it from chemical and enzymatic breakdown inside the body. They also release drugs in a controlled, prolonged manner, which keeps therapeutic concentrations in place for longer and makes treatment more effective. Because they are usually built from physiological lipids that the body tolerates well, they show excellent biocompatibility and little toxicity. Their production methods are generally scalable and affordable, so moving from laboratory research to industrial manufacturing is feasible. SLNs are especially helpful for drugs that dissolve poorly in water, are poorly absorbed, or need targeted delivery, since they can markedly improve delivery and therapeutic performance [38, 40, 43, 44, 49].

These qualities matter greatly for antibiotic delivery. SLNs can carry both water-loving and fat-loving drugs, guard them against degradation, and release them gradually, making them a promising platform for better antimicrobial therapy [50, 51]. By maintaining sustained therapeutic concentrations at the infection site, solid lipid nanoparticles can enhance clinical outcomes and help mitigate the emergence of microbial resistance [52].

For oral administration, SLNs hold considerable promise. They can protect drugs from breakdown in the gastrointestinal tract, increase absorption by improving solubility, and reduce the impact of first-pass metabolism [53]. This is especially useful for drugs like NFT, whose solubility depends on pH and whose bioavailability is limited in standard formulations [54].

Bioavailability is the fraction of an administered dose that reaches the systemic circulation in unchanged form and becomes available where the drug needs to act. For oral drugs, bioavailability is largely determined by solubility study, rate of dissolution, permeability across the intestine, first-pass metabolism, and stability in the GI tract.

Drugs that dissolve poorly in water or have unstable physicochemical properties tend to show erratic and low oral bioavailability, which can lead to treatment failure and force higher or more frequent dosing [54]. Low solubility is one of the leading barriers to bioavailability for many drugs [55].

Nanoscale increase the apparent solubility and dissolution of poorly water-soluble drugs through several mechanisms that work together. Their nanoscale size gives a much larger surface area, so the drug dissolves faster and appears more soluble. The lipid matrix also acts as a good reservoir for fat-soluble drug molecules, helping them dissolve and then cross the gastrointestinal membrane.

Encapsulation in the solid lipid carrier keeps the drug finely dispersed as it travels through the gut, which limits particle clumping and lowers the chance of the drug precipitating out. Overall, these features improve dissolution, raising both the speed and the extent of absorption and ultimately boosting oral bioavailability [51, 55–57]..

SLNs can interact with the intestinal lining in ways that promote drug movement through cells (transcellular) and between cells (paracellular). In the intestine, lipid nanoparticles may combine with bile salts to form mixed micelles, which improve drug permeation. Their small size allows close contact with the mucosal surface, which may increase uptake across cell membranes. Certain lipid carriers can also

temporarily loosen tight junctions between cells, further supporting paracellular transport.

Together, these mechanisms make membrane crossing more efficient and so improve oral bioavailability [54].

1.10 Rational

The rationale behind the study is to protect the drug from gastric degradation while enhancing its intestinal absorption and overall bioavailability, with the goal of achieving sustained and prolonged drug release.

1.11 Aim and Objectives

1.11.1 Aim

Fabrication and evaluation of NFT loaded pH sensitive SLNs (Eu-NFT-SLNs) to overcome its pharmacokinetic limitations, to achieve sustained drug release pattern and to enhance oral bioavailability.

1.11.2 Objectives

- i. To establish a high-performance liquid chromatography (HPLC) method for measuring NFT.
- ii. To prepare NTF-SLNs using appropriate lipids and surfactants.
- iii. Coating of the NFT-SLNs with the pH-responsive polymer Eudragit[®] L-100 to facilitate targeted release of the drug within the intestinal environment.
- iv. To comprehensively characterize the prepared solid lipid nanoparticles, evaluating particle size (PS), polydispersity index (ODI), zeta potential (ZP), entrapment efficiency (EE), attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR), X-ray powder diffraction (XRPD), and surface electron microscopy (SEM).

- v. To conduct *in vitro* release studies of drug at 1.2 and 6.8 pH, subsequently analyzing the release kinetics utilizing the DDSolver software program.
- vi. *In vivo* bioavailability evaluations and determine the key pharmacokinetic parameters.

Chapter 2

Literature Review

2.1 Patho-Physiology of Urinary Tract Infections and Therapeutic Role of Nitrofurantoin

UTI is among the most widespread infections worldwide, affecting roughly 150 million people each year. It is a serious public health issue that places a heavy economic load on health systems around the globe. Women are more frequently affected than men because of differences in anatomy, and roughly 50-60% of them will experience an infection at some point in their lives [58]. Uropathogenic *Escherichia coli* accounts for the vast majority of urinary tract infections, responsible for roughly 75% to 95% of cases. Nevertheless, infections can also stem from alternative Gram-negative and Gram-positive pathogens, including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus* [18]. Infection begins when gut-derived bacteria attach around the urethral opening and then travel up into the bladder. UPEC strains are highly virulent because they produce hair-like Type 1 and P fimbriae, which bind to uroplakin receptors on the bladder lining (uroepithelium). Once the bacteria invade the outer bladder cells, they multiply rapidly and form intracellular bacterial communities (IBCs) or biofilms. These structures shield the bacteria from both host immunity and antibiotics, which makes the infection persistent [59].

Historically, broad-spectrum drugs such as fluoroquinolones (for example ciprofloxacin), trimethoprim-sulfamethoxazole (TMP-SMX), and beta-lactams were regarded as the best treatments for UTI. However, excessive prescribing of these agents has fueled a global AMR crisis. The fast emergence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and multidrug - resistant (MDR) organisms has made these standard therapies unusable in many cases, so newer drugs or older alternatives are now recommended [18].

In light of this resistance problem, NFT, a synthetic nitrofurantoin antibiotic developed in the 1950s, has returned to prominence. Leading urology and infectious disease bodies worldwide advise it as empiric first-line treatment for uncomplicated lower UTIs. Its durable effectiveness is largely explained by its complex mode of action. After entering a bacterial cell, NFT is reduced by a bacterial enzyme called nitro reductase into reactive electrophilic intermediates. These are highly destructive and attack many bacterial macromolecules simultaneously: they block DNA and RNA synthesis, disrupt the citric acid cycle by inhibiting metabolic enzymes, and bind ribosomal proteins so that protein synthesis halts [60]. Since NFT disrupts so many vital bacterial processes at the same time, it is very unlikely that a single genetic mutation could produce resistance. Consequently, even after many decades of clinical use, resistance remains relatively uncommon, at under 5% [61].

2.2 Biopharmaceutical and Pharmacokinetic

Limitations of Nitrofurantoin

Although NFT is very effective and resists the development of resistance, its inherent biopharmaceutical and pharmacokinetic shortcomings seriously restrict its clinical usefulness.

The Biopharmaceutics Classification System (BCS) sorts drugs into four classes using the two main factors that govern oral bioavailability: aqueous solubility and intestinal permeability [57]. NFT is placed in BCS class II, and some of its derivatives fall in class IV. This implies that Nitrofurantoin exhibits severely restricted

aqueous solubility coupled with low intestinal permeability. Due to its highly crystalline and lipophilic nature, the drug dissolves at an extremely slow rate within the enzyme-dense, aqueous milieu of the gastrointestinal tract. Because complete dissolution is a fundamental prerequisite prior to membrane permeation, dissolution acts as the primary rate-limiting step governing nitrofurantoin systemic absorption [62, 63].

To resolve these challenges, nitrofurantoin is currently commercialized in two primary oral formulations: Furadantin (microcrystalline form) and Macrochantin (macrocrystalline form). The microcrystalline variant possesses a higher surface area, facilitating rapid dissolution and absorption within the stomach and upper gastrointestinal tract. Nevertheless, its abrupt dissolution in the highly acidic gastric environment provokes numerous gastrointestinal adverse effects—including nausea, emesis, dyspepsia, anorexia, and mucosal irritation—which frequently prompt patients to discontinue therapy [63–65].

The macrocrystalline form was created to reduce this GI toxicity. Its larger crystals dissolve more slowly and, in theory, irritate the stomach less. The cost of this slower dissolution is absorption that is erratic, incomplete, and highly inconsistent, which leaves systemic bioavailability uncertain [66].

Furthermore, once NFT enters the bloodstream, its biological half-life is very short, only 20 to 60 minutes. It is rapidly metabolized in tissues and quickly cleared by the kidneys through glomerular filtration and tubular secretion. This fast elimination gives high drug levels in urine, the site of infection, but plasma levels fall sharply [60].

The minimum inhibitory concentration (MIC) must be sustained in the urinary tract, so conventional NFT forms need frequent dosing: 50-100 mg four times daily for the microcrystalline form and twice daily for the macrocrystalline form. This regimen is inconvenient, leading to poor adherence and missed doses, and ultimately to failure to clear the infection [67].

Collectively, these limitations including inadequate aqueous solubility, restricted membrane permeability, significant gastric irritation, rapid elimination from the

body, and the burden of frequent dosing highlight the critical necessity for a sophisticated delivery platform capable of bypassing the stomach, enhancing water solubility, and delivering a prolonged release profile within the intestinal tract.

2.3 Evolution of Oral Nanocarriers in Modern Therapeutics

Oral administration remains the most favored, convenient, and patient-compliant method of drug delivery. Nevertheless, administering hydrophobic or unstable compounds via this route encounters substantial anatomical and physiological barriers.

The gastrointestinal tract presents a highly hostile environment characterized by broad pH fluctuations ranging from strongly acidic gastric conditions (pH 1.2-3.0) to neutral or alkaline intestinal regions (pH 6.0-7.5) abundant digestive enzymes (including pepsin, trypsin, and lipases), a protective mucosal lining, and selectively tight intercellular junctions between enterocytes. Furthermore, enterocytes express efflux transporters such as P-glycoprotein (P-gp), which actively expel lipophilic drug molecules back into the intestinal lumen [68].

Developments in nanotechnology and nanomedicine have offered new ways around these biological barriers. Nanocarriers are colloidal delivery systems made of particles from 10 nm to 1000 nm in size. Formulating a drug at the nanoscale greatly increases its specific surface area, and according to the Noyes-Whitney equation, this large surface area gain produces a dramatic rise in dissolution rate [67, 69].

Nanocarriers can be made from natural biopolymers, synthetic polymers, and lipids, using two basic strategies: [70]

- i. Top-Down Approach: Bulk material is broken into nanoscale pieces using high-energy processes. Ultrasonication, high-pressure homogenization (HPH), micro fluidization, and wet milling all use strong physical shear to fragment the material into nano-sized particles.

- ii. Bottom-Up Approach: Also called self-assembly or phase separation, this method builds nanoparticles up from the molecular level. Typical techniques include nanoprecipitation, solvent evaporation, microemulsion, and supercritical fluid technology.

Carrier types made this way include polymeric nanoparticles, liposomes, micelles, nanogels, and lipid nanoparticles. Nanoparticles can hold both water-loving and water-repelling drugs. Enclosing the API in the nanoparticle core protects it from enzymatic degradation, lessens the negative effect of food on absorption, helps it cross mucosal barriers by transcytosis, and permits precisely triggered, stimulus-based release. [70, 71].

2.4 Solid Lipid Nanoparticles as a Leading Oral Carrier System

Among the wide array of nanocarriers developed over recent decades, solid lipid nanoparticles are widely recognized as exceptionally promising vehicles for oral drug administration. Introduced in the early 1990s as the pioneer generation of lipidic nanoparticles, they were engineered to overcome the inherent limitations of traditional colloidal carriers such as liposomal, polymeric nanoparticles, and o/w emulsions while preserving their advantageous traits, ultimately designed to enhance therapeutic efficacy [72].

Solid lipid nanoparticles function as sub-micron colloidal systems (50 to 1000 nm) fabricated from physiological, biocompatible, and biodegradable lipids that maintain a solid state under both ambient and body temperatures. The inner solid lipid core may be composed of diverse lipids such as triglycerides (e.g., tristearin and tripalmitin), diglycerides, monoglycerides, fatty acids (e.g., stearic acid), waxes, and steroids. These particulate structures are stabilized within an aqueous continuous phase using multiple compatible surfactants i.e; Tween 80, Span 20, Poloxamer 188, or lecithin [46].

For oral delivery of lipophilic, poorly soluble drugs like NFT, SLNs bring several important biopharmaceutical advantages:

2.4.1 Better Solubilization and Higher Bioavailability

The lipophilic solid core lets the drug dissolve easily. After oral intake, gallbladder and pancreas tend to release bile salts, lipids and lipases respectively as a prompt from SLNs. This natural response leads to mixed micelles forming in the intestine, which solubilize the drug-lipid complex [73].

2.4.2 Lymphatic Uptake from the Intestine

A major benefit of lipid nanocarriers is that they can use the intestinal lymphatic system. Thanks to their high lipophilic nature and particle size, SLNs can readily be taken up by M-cells in the Peyer's patches, which are part of the gut-associated lymphoid tissue (GALT).

Within enterocytes, the lipids are packaged into chylomicrons, released into lymphatic capillaries, and then reach the systemic circulation directly via the thoracic duct. This route skips the portal vein and so avoids hepatic first-pass metabolism, which raises the drug's bioavailability [74].

2.4.3 Controlled and Prolonged Release Behavior

In a liquid-lipid nano emulsion, a drug can diffuse through the liquid droplets easily, whereas in SLNs the solid matrix restricts the movement of the loaded drug. Drug release from solid lipid nanoparticles typically follows one of three distinct patterns depending on the formulation design: a uniform matrix framework that yields a gradual, zero-order release profile; an active-rich outer shell model that produces a two-phase (biphasic) release, starting with an initial burst before shifting to sustained delivery; and an active-rich core configuration that achieves an exceptionally prolonged release duration over an extended period. This allows

therapeutic drug levels to be maintained for a long period and removes the need for repeated daily NFT doses [12].

2.4.4 Strong Biocompatibility and Minimal Toxicity

The SLN core matrix is made from physiological lipids, often classed as Generally Recognized as Safe (GRAS), which break down naturally through biological processes in the body. They are highly biocompatible and free of cytotoxicity and inflammatory reactions [55].

2.5 Function of Eudragit[®] L100 in pH - Triggered Enteric Drug Release

bioavailability, but strong stomach irritation remains a significant problem. If SLNs start releasing NFT while in the highly acidic stomach, mucosal toxicity, nausea, and poor patient adherence will result. To keep the drug out of the stomach entirely and deliver it only to the absorptive surfaces of the small intestine, pH-sensitive enteric polymers are required [37].

Eudragit[®] L100 functions as a sophisticated anionic synthetic copolymer extensively utilized within targeted pharmaceutical drug delivery systems. Chemically, it consists of a 1:1 molar copolymer comprising methacrylic acid and methyl methacrylate units. Its pH-dependent solubility profile is driven entirely by the presence of ionizable carboxylic acid functional groups (-COOH) [75]. Its mode of action depends on the ionization of the polymer in relation to the physiological pH of the GI tract. The polymer dissolves only above pH 6.0, particularly at pH 6.8 [76].

In the stomach: Gastric fluid typically exhibits a low pH between 1.2 and 3.0, which sits far below the pKa of methacrylic acid. Consequently, the carboxylic acid functional groups stay in a protonated, neutral state (-COOH) while passing through the stomach. In this state the polymer chains hydrogen-bond strongly and coil tightly, making the polymer insoluble in water. Consequently, when

Eudragit[®] L100 coats or is combined with SLNs, it forms an insoluble polymer layer that blocks gastric fluid from entering the formulation. This prevents early NFT release and fully protects the stomach lining from irritation by the drug, while also protecting the drug from the gastric environment [77].

In the small intestine: As the formulation leaves the stomach and moves in between the small intestinal region, the rise of 6 to 7.5 in pH occurs. At this pH the carboxylic acid groups on the Eudragit[®] L100 backbone ionize into negatively charged carboxylate ions ($-\text{COO}^-$). These charges cause electrostatic repulsion between polymer chains, so the network swells and then dissolves. Once the coating dissolves, the NFT-loaded SLNs are exposed to the intestinal environment, where lipase activity hydrolyzes the particles and provides sustained NFT release [77]. The combination of SLNs and Eudragit[®] L100 therefore creates a sophisticated dual-action nanocarrier. Eudragit[®] L100 provides spatial control (where the drug is released), while the SLN core provides temporal control (how fast it is released) [14].

TABLE 2.1: Summary of Literature Review of Drug-SLNs formulations in last decade

Active Agent	Lipid-Based Vehicle	Intended Use	Key Outcomes	Ref
Diclofenac Sodium	Solid lipid base	Prolonged drug delivery	SLNs made by the emulsion-solvent evaporation method achieved a high degree of drug entrapment, particle sizes in the nano range, and extended drug release.	[12]
Alprazolam	Compritrol [®] ATO 888	Prolonged oral administration	SLNs loaded with alprazolam gave regulated drug release, strong encapsulation efficiency, and better stability.	[12]
Curcumin	Solid lipid nanoparticles	Treatment of lung cancer	Curcumin-loaded SLNs released the drug steadily and showed stronger cytotoxic effects and greater cell uptake than the free compound.	[79]

Table 2.1 – continued from previous page

Active Agent	Lipid-Based Vehicle	Intended Use	Key Outcomes	Ref
Bimatoprost	Solid lipid nanoparticles	Delivery to the eye	SLNs increased penetration through the cornea, kept the drug in place longer, and supported better glaucoma control.	[80]
Fucoxanthin	Enteric-coated solid lipid nanoparticles	Raising oral bioavailability	The formulation made fucoxanthin more stable and bioavailable, and shielded it from breakdown in the gastrointestinal tract.	[81]
NFT	Lipid-based nanocarrier	Better oral bioavailability	The lipid nanocarriers promoted oral absorption and lowered the variability in NFT bioavailability.	[35]
Curcumin	Lipid nanoparticles	Antibacterial treatment	Lipid nanoparticles containing curcumin displayed notable antibacterial action against <i>Enterococcus faecalis</i> .	[82]
Curcumin	Solid lipid nanoparticles	Optimization of the formulation	A design-of-experiments approach effectively tuned particle size, EE, and stability.	[83]
<i>trans</i> -Resveratrol	Lipidic nanoparticles	Delivery through the skin (topical)	SLNs made resveratrol more stable and better suited for application on the skin.	[84]

Chapter 3

Methodology

3.1 Chemicals and Reagents

Eudragit[®] L100 was obtained from Evonik, Germany. Merck was the source of sodium chloride, and disodium hydrogen phosphate, whereas sigma-Aldrich was the source of Tween[®]80. Ethanol, methanol & acetone were sourced from Glitz Pharma, which also supplied the lipids evaluated during screening (stearic acid, soy lecithin, Compritol[®] ATO 888 [glyceryl behenate], and glyceryl monostearate). Glitz Pharma, Pakistan, was likewise the origin of the NFT (API). Distilled water was produced with the system of Purification at the CUST (Capital University of Science & Technology, Islamabad). All materials were used in the form as received, with no additional purification.

3.2 Ethical Clearance for Animal Studies

The Research Ethics Committee from the Faculty of Pharmacy, CUST, Islamabad, approved all study procedures (**Approval No: CUST/DERC/PH/ERF/PG/2026/021**), which complied with national and institutional rules on the care of laboratory animals. Healthy, home-bred domestic rabbits (*Oryctolagus cuniculus*) were chosen because laboratory-bred rabbits were not readily available. All animals came from the same stock and were kept in the same conditions of housing

and feed to limit multiple variation. They weighed 1.0-1.5 kg and had unrestricted food and water access.

3.3 Methodology

3.3.1 Preliminary Pre-Formulation Investigations

3.3.1.1 Development of the High-Performance Liquid Chromatography Method

A RP-HPLC method was developed and optimized to measure NFT in nanoparticle formulations and plasma samples, following the principles of the United States Pharmacopeia (USP), using a Shimadzu gradient HPLC system (SCL-10AV) [85, 86]. The system was fitted with an ultraviolet (UV) detector set at 254 nm, and separation was achieved under isocratic conditions [86]. A column made up of stainless-steel with dimensions 30 cm $\times$ 3.9 mm, loaded with stationary phase (L1), was used.

The given mobile phase was combined of phosphate buffer (7.0 pH) and acetonitrile at a ratio of 12 ratio 88. Potassium dihydrogen phosphate (6.8 g) was dissolved in distilled water to make the buffer, and 1.0 N sodium hydroxide solution was then added until the pH reached 7.0. Distilled water was added to make up a final volume of 1000 mL. A flow rate of 1 mL/min was selected, and under it, NFT eluted after roughly 2.5 minutes. 1 mg/mL acetanilide solution served as the internal standard, and injection volumes of 5 to 10 μ L were used.

Before analyzing samples, system suitability was checked to confirm satisfactory chromatographic performance. The system was accepted when the resolution between the NFT and acetanilide peaks exceeded 3.0. In addition, Precision and reproducibility of the method were verified by requiring that the RSD of the peak area ratio across replicate injections not exceed 2% [85, 87, 88].

3.3.1.2 Construction of the Calibration Curve

Standard solutions for the NFT calibration curve were made from USP NFT reference material. Specifically, 50 mg of the reference substance was taken up in 40 mL of DMF, and 50 mL of the internal standard solution (acetanilide, 1 mg/mL) was then added. A series of dilutions covering 80%-120% of the working concentration was subsequently prepared to span the needed range.

Every standard was injected three times under the tuned HPLC settings. For each injection, the NFT peak area was divided by the acetanilide peak area, and the resulting ratios were graphed against the NFT concentrations. The link between concentration and detector signal was assessed by linear regression, and the equation obtained was applied to calculate NFT content in later samples [89].

3.3.1.3 Linearity Assessment and Regression Parameters

The HPLC method's linearity was tested using standard NFT solutions spanning 0.4 mg/mL to 0.6 mg/mL. Least-squares linear regression was applied to the calibration data, and the correlation coefficient (R^2), slope, and intercept were examined.

$$y = mx + c$$

The method was regarded as linear when the calibration curve gave an $R^2 \geq 0.99$. Its suitability for quantifying NFT in the developed formulations and in biological samples was further assessed according to USP and International Council for Harmonization (ICH) guidelines [85, 88].

3.3.2 Fabrication of Solid Lipid Nanoparticles

3.3.2.1 Techniques Available for Producing Solid Lipid Nanoparticles

Many modern techniques exist for making SLNs. Frequently used ones include Homogenization under high pressure, performed with either hot or cold phase, microemulsion approaches, solvent emulsion under evaporation, and ultrasonication. Recent work concentrates on refining these methods so that particles are

uniform in size, drug loading is high, and release is consistent [90]. Microfluidic technologies have also been adopted in more advanced formulations to improve reproducibility and scalability [91].

NFT-loaded SLNs were prepared by slightly modifying previously reported methods, using melt emulsification followed by hot homogenization [13, 14, 92–94].

3.3.2.2 Preparation of Drug-Loaded Solid Lipid Nanoparticles

The lipid phase was prepared by keeping 200 mg of Compritol® ATO 888 on a hot plate at 80 °C, which is past its melting point, until a transparent melt formed. NFT (1 mg) was first taken up in 1 mL of ethanol, and the solution was sonicated for 1–2 minutes so that the drug dissolved fully, after which it was blended into the molten lipid. In parallel, an aqueous phase was made from 10 mL of distilled water carrying 1% Tween® 80 and 0.5% Poloxamer 188 as surfactants, and it was warmed to the same 80 °C.

This hot aqueous solution was then dripped slowly into the lipid-drug mixture while a magnetic stirrer kept it agitated, which yielded a transparent microemulsion [95]. High-speed homogenization (15000 rpm, 15 minutes) was next applied to the microemulsion, and the resulting dispersion was ultracentrifuged at 10,000 rpm for 15–30 minutes, which sedimented the purified SLN pellets. 10 mL of distilled water were used to resuspend the collected pellet, and freeze-drying this suspension gave the SLNs as a dry powder [13].

3.3.2.3 Application of the Enteric Polymer Coating

Eudragit® L100 (1 mg) was dissolved in 1 mL of acetone. This pre-dissolved solution was added dropwise to the redispersed NFT-SLN pellets and continuously stirred at 1000 rpm for 2 hours, allowing the polymer to coat the particles while the solvent evaporated [96]. The suspension formed in result will then be sonicated for 10 to 12 mins to get a evenly distributed suspension of Eu-NTF-SLNs.

3.3.2.4 Freeze-Drying of Eudragit-Coated Nitrofurantoin-Loaded Solid Lipid Nanoparticles

To finish, the suspension will be centrifuged a second time at 10,000 rpm for half an hour and then held at -20 °C overnight. Freeze-drying will follow, lasting 48 hours at -80 °C in a lyophilizer, which will convert the frozen material into a dry powder of Eudragit[®] L100-coated SLNs loaded with NFT (Eu-NFT-SLNs) [96].

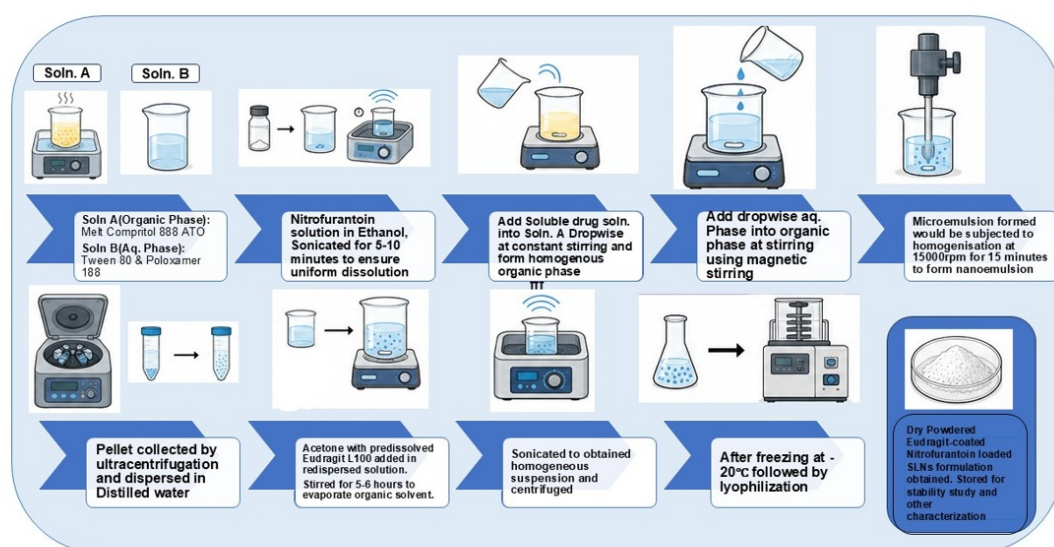


FIGURE 3.1: Flow chart of the formulation steps of Eu-NFT-SLNs

3.3.3 Formulation Optimization by Statistical Experimental Design

Formulation optimization was performed using Design-Expert[®] version 13.0 and a Box-Behnken Design (BBD) to investigate variables affecting the physicochemical properties of Eu-NFT-SLNs. Based on preliminary screening, three formulation factors were selected: Compritol[®] ATO 888 concentration from 100 to 300 mg (X1), surfactant conc. consisting of Poloxamer 188 and Tween[®] 80 from 1% - 2% (X2), and Eudragit[®] L-100 concentration from 0.5 - 1.5 mg (X3) [97, 98].

The BBD comprised 15 experimental runs. The formulation factors were evaluated against the measured responses designated as particle size (Y1), PDI (Y2), and zeta potential (Y3) in the design description [97].

Each formulation generated by the software design matrix was prepared and evaluated for the selected responses. Response Surface Methodology (RSM) was then used to examine the individual influence of each formulation factor as well as interactions between factors.

Numerical optimization was subsequently applied to identify an optimized formulation meeting the specified response criteria.

3.3.4 Analytical Characterization of Solid Lipid Nanoparticles

3.3.4.1 Measurement of Particle Size, Polydispersity and Zeta Potential

Each batch was run on a Zetasizer Nano ZS (Malvern Instruments, United Kingdom), a DLS instrument, to obtain the average particle size, PDI, and ZP. Samples were first thinned with distilled water, a step that limits multiple scattering and makes the readings more dependable [13].

3.3.4.2 Determination of Drug Entrapment

NFT EE within the SLNs was determined indirectly by measuring the amount of unentrapped drug remaining in the supernatant after separation of the nanoparticles.

Freshly fabricated NTF- SLNs were centrifuged at 10,000 to 12,000 rpm for about 15 to 20 minutes. The supernatant formed, was collected and the concentration of free NFT was quantified using the developed HPLC method [13].

%EE and %DL were calculated by given equations:

$$\text{EE (\%)} = \frac{(\text{Total Drug} - \text{Free Drug})}{\text{Total Drug}} \times 100 \quad (3.1)$$

$$\text{DL (\%)} = \frac{\text{Weight of NFT in SLNs}}{\text{Total weight of SLNs}} \times 100 \quad (3.2)$$

Here, the initial amount of NFT added is mentioned as “Total Drug”, while the amount found in the supernatant is “Free Drug”.

3.4 Surface Morphology by Scanning Electron Microscopy

SEM was used to study the morphology of the optimized formulation, including particle form and surface appearance. Freeze-dried powder was fixed onto aluminum stubs with double-sided carbon adhesive tape, and a thin gold film was deposited on it by sputtering before the analysis [94].

Images were recorded on a Tescan VEGATM SEM (Vega 3, Tescan Orsay Holding, Brno, Czech Republic) at several magnifications, which allowed the shape, surface texture, and degree of clumping of the nanoparticles to be inspected.

3.5 Crystallinity Analysis by X-Ray Powder Diffraction

XRPD was used to examine how ordered the solid-state structure was in pure NFT, Compritol[®] ATO 888, their physical mixtures, and the optimized SLNs. Every sample was scanned across a suitable 2θ interval with routine instrument parameters.

Comparing the resulting diffraction patterns revealed any shift in crystallinity and showed whether NFT stayed crystalline within the lipid carrier or had turned disordered and amorphous [99].

3.6 Spectroscopic Compatibility Study by Infrared Analysis

Possible interactions between NFT and the formulation excipients were looked for by ATR-FTIR analysis [100].

Infrared spectra of NTF, lipid (Compritol[®] ATO 888), and the final optimized nanoparticles were collected across the 4000-400 cm⁻¹ window on a Lambda Scientific M6RL2703 FTIR spectrometer. The positions of the characteristic absorption bands were then compared between samples, so that any sign of incompatibility between drug and excipients, or of structural alteration caused by the preparation process, could be detected.

3.7 *In vitro* Drug Release Assessment

The dialysis-bag diffusion technique was selected to follow NFT release in vitro from Eu-NFT-SLNs and from the uncoated NFT-SLNs [101]. The optimized coated product and its uncoated counterpart were each examined in triplicate, in release media of pH 1.2 and pH 6.8.

The dialysis membrane was first soaked in distilled water until fully hydrated. A measured volume of nanoparticle suspension holding the required drug dose was then loaded into the bag, which was tightly closed and lowered into the release medium [102].

Testing began in the pH 1.2 medium to check whether the Eudragit[®] L100 coating could withstand gastric conditions, because this polymer does not dissolve in acid. Release was then studied in phosphate buffer at pH 6.8, a pH at which Eudragit[®] L100 is known to dissolve [103]. The setup was held at 37 ± 0.5 °C and stirred continuously.

In the pH 1.2 medium, aliquots were taken every half hour for the first 2 hours, a window chosen to mimic typical stomach residence. In the pH 6.8 stage, aliquots

were drawn every 2 hours from the 2nd to the 12th hour, with two further samples at 24 and 48 hours. After every sampling, the same volume of fresh medium was returned to keep sink conditions [104, 105].

The aliquots were assayed by the HPLC method established earlier, with detection at 254 nm, and percentage cumulative drug release (% CDR) was computed at each time point.

The drug release statistics were fitted to zero-order kinetics, first-order kinetics, Higuchi model, Hixson-Crowell model, and Korsmeyer-Peppas model. Each model was ranked on its coefficient of determination (R^2), mean squared error (MSE), root mean square error (RMSE), Akaike Information Criterion (AIC), and Model Selection Criterion (MSC). The best-suited model was the one combining a relatively high R^2 and MSC with low MSE, RMSE, and AIC.

For the release analysis, whichever model gave the greatest R^2 was taken as the most faithful description of how NFT leaves the developed SLNs [104].

3.8 *In vivo* Bioavailability Assessment in Animals

Healthy rabbits (*Oryctolagus cuniculus*) were used to measure how well Eu-NFT-SLNs are absorbed when given by mouth, and the work started only once ethical clearance had been granted (Approval No: CUST/DERC/PH/ERF/PG/2026 / 021). The 3Rs principles (Replacement, Reduction, and Refinement) were observed throughout the *in vivo* work.

Replacement: Non-animal alternatives were considered wherever they were scientifically possible, but an animal model was still necessary to assess the pharmacokinetics and oral bioavailability of the formulation.

Reduction: The smallest number of animals needed to produce scientifically meaningful pharmacokinetic data was used. Suitable study design and statistical analysis were applied to get the maximum information from each animal.

Refinement: Animal welfare was a priority throughout, with proper housing, feeding, handling, and care, and with stress, discomfort, and distress kept to a minimum during procedures [143].

Twelve rabbits weighing approximately 1.0-1.5 kg were allocated to the experimental groups, with $n = 4$ animals reported for each group.

Group I: Animals receiving pure NFT.

Group II: Animals receiving NFT-SLNs.

Group III: Animals receiving the Eu-NFT-SLN formulation.

The treatments were given orally by gavage at an 10 mg/kg dose of NFT [106, 107].

At 0, 2, 6, 12, and 24 hours after the oral dose, blood was drawn from the marginal ear vein of each rabbit. The samples went into tubes holding EDTA and were spun in a centrifuge so that plasma could be separated. To extract the drug, a suitable amount of extraction solvent was mixed into the plasma, and vigorous vortexing caused the proteins to precipitate. A second round of centrifugation followed, and the transparent supernatant, now free of protein, was collected and run through the HPLC method developed earlier to find the NFT concentration in plasma.

From these concentrations, plasma concentration-versus-time curves were drawn for the sampling points, and the pharmacokinetic evaluation used non - compartmental analysis in PKSolver.

The highest plasma concentration observed (C_{max}) and the time it was reached (T_{max}) were read straight from the concentration-time data. Systemic exposure over the first 24hrs (AUC_{0-24}) was obtained with the PKSolver using linear trapezoidal rule. Further parameters, namely the elimination rate constant (K_{el}), elimination half-life ($t_{1/2}$), mean residence time (MRT), and area under the first moment curve (AUMC), were also derived in PKSolver to describe how NFT behaves in the body after oral dosing.

The optimized SLNs were calculated for their relative bioavailability by dividing its AUC by that of the pure NFT suspension, using the equation that follows.

$$\text{Relative Bioavailability } F (\%) = \frac{\text{AUC (Eu-NFT-SLNs)}}{\text{AUC (NFT-SLNs)}} \times 100 \quad (3.3)$$

If the nanoparticle formulation gave a larger AUC and C_{max} than the plain drug suspension, this was taken to show that the lipid-based, pH-responsive carrier had improved oral bioavailability.

3.9 Storage Stability Evaluation

Every one of the 15 batches generated by the BBD was put through stability evaluation according to ICH recommendations.

The formulations was sealed in an airtight container and held for a minimum of 3 months under three sets of storage conditions, long-term (25°C ± 2°C with 60% relative humidity (RH) ± 5%), accelerated (40°C ± 2°C with 75% RH ± 5%), and was tested at predetermined time points [108]. At each point, particle size, PDI, ZP, and visual appearance were checked to track any change.

The purpose of this work was to judge whether the formulations hold on to their physical and chemical properties during storage, and to catch early signs of aggregation, phase separation, drug leakage, or polymer breakdown [109].

3.10 Data Analysis and Statistical Methods

Statistical evaluation of the experimental data was carried out with Microsoft Excel, PK Solver, DDSolver, and Design-Expert® software, all of which are commonly applied for scientific data handling, graphical presentation, and bio-statistical work. Findings from each experimental group are expressed as mean ± standard deviation (mean ± SD), a format that reflects the dispersion of individual values around the mean and thus the consistency and dependability of the measurements. Differences among group means were assessed through analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons, and results with a p-value under 0.05 were regarded as statistically significant.

Chapter 4

Results and Discussion

4.1 Analytical Method Validation of Nitrofurantoin

The chromatographic method for nitrofurantoin (NFT) was validated following ICH Q2(R1) guidelines. The parameters evaluated were specificity, linearity, range, accuracy, precision, LOD, LOQ, robustness and system suitability. The target concentration was 50 $\mu\text{g/mL}$, which is 100% of the working level.

4.1.1 System Suitability

Before validation, three replicate injections of the standard solution were made. The peak areas were 1,798,515, 1,784,941 and 1,826,177, with a mean of 1,803,211 and a standard deviation of 21,015. This gives an RSD of 1.17%, below the usual 2.0% limit. The system therefore gave consistent responses and was suitable for the validation work.

4.1.2 Specificity

Specificity was assessed by comparing the chromatograms of the blank, the placebo (blank SLNs and excipients: Compritol[®] 888 ATO, surfactants, Eudragit[®] L-100) and the standard nitrofurantoin solution. Because the method is applied to nitrofurantoin-loaded solid lipid nanoparticles, the absence of interference from the lipid matrix and polymer is what makes the method suitable for later entrapment, release and *in vivo* studies.

4.1.3 Linearity

Five concentration levels from 40 to 60 $\mu\text{g/mL}$ (80–120% of the target) were analysed. The peak area rose steadily with concentration.

TABLE 4.1: Calibration data for linearity assessment

Level (%)	Conc. ($\mu\text{g/mL}$)	Peak area
80	40	1,433,543
90	45	1,646,401
100	50	1,833,908
110	55	2,070,349
120	60	2,186,807

Least-squares regression gave $y = 38,609.52x - 96,274.4$, with a correlation coefficient (r) of 0.9957 and a coefficient of determination (R^2) of 0.9914. The response is therefore linear over the studied interval. The same equation was used later to quantify free drug in the entrapment efficiency study and drug in the release samples.

4.1.4 Range

The method showed acceptable linearity, accuracy and precision from 40 to 60 $\mu\text{g/mL}$, i.e. 80–120% of the 50 $\mu\text{g/mL}$ test concentration. This range meets the ICH recommendation for assay methods and covers the concentrations expected in the formulation samples.

4.1.5 Accuracy

Accuracy was tested at 80%, 100% and 120% of the target concentration. Recovery was 99.79% at the 80% level, 101.70% at 100% and 101.06% at 120%. The mean recovery was about 100.85%, within the accepted 98–102% window. As a cross-check, back-calculating each sample from the regression line gave recoveries of 99.45%, 99.98% and 98.55%, which also fall inside the limits. The method therefore measures the true amount of nitrofurantoin without meaningful bias.

4.1.6 Precision

Repeatability was assessed from five replicate determinations, which gave peak areas of 5,712,261, 5,611,638, 5,633,006, 5,662,273 and 5,746,448. The mean was 5,673,125 and the standard deviation was 55,713, giving an RSD of 0.98%. This is below the 2% limit and shows good repeatability.

4.1.7 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated as $3.3\alpha/S$ and $10\alpha/S$, where α is the residual standard deviation of the regression line (32,756) and S is the slope (38,609.52). The LOD was 2.80 $\mu\text{g/mL}$ and the LOQ was 8.48 $\mu\text{g/mL}$. Both are well below the lowest calibration level (40 $\mu\text{g/mL}$), so the method can measure nitrofurantoin at concentrations far below the working range. This is useful for the low free-drug levels seen in entrapment and early-time release samples.

4.1.8 Robustness

Robustness was evaluated by making small deliberate changes to the method conditions, namely [flow rate ± 0.1 mL/min, mobile phase ratio $\pm 2\%$, detection wavelength ± 2 nm, column temperature ± 2 °C]. If system suitability criteria are still met after these changes, the method can be considered reliable under routine laboratory variation.

4.1.9 Overall Discussion

Taken together, the validation shows that the method is linear, accurate, precise and sensitive for nitrofurantoin over 40–60 $\mu\text{g/mL}$.

The system suitability, repeatability and recovery results all fall within accepted limits. The method is therefore suitable for the drug analysis in this work; entrapment efficiency of NTF-SLNs and Eu-NTF-SLNs, in vitro drug release at acidic pH 1.2 and intestinal pH 6.8, and plasma samples from the bioavailability study [110–112].

4.2 Construction of the Calibration Curve and Linearity Assessment

Standard solutions of NTF spanning 0.04 to 0.06 mg/mL were used to build a calibration curve. To judge the linearity of the newly developed method, the HPLC peak areas were graphed against their respective concentrations. The resulting graph is shown in Figure 4.1.

The fitted regression equation to the calibration data was [85, 86]:

$$y = 38,609,520x - 96,274.4$$

where the peak area is represented by y and x is the NTF concentration (mg / mL). The fitted line had a 0.9914 of R^2 (correlation coefficient), showing that detector response is strongly linear with drug concentration over the selected range [111].

Peak area grew consistently as concentration rose, which confirms that the HPLC detector responds in proportion to NTF. Linearity of this kind is a prerequisite for reliable quantification of the drug in pharmaceutical formulations as well as biological samples.

The R^2 value met the limits normally accepted in analytical practice, indicating that the method is suitable for quantitative analysis [112].

The validated calibration model was subsequently used to determine NFT concentrations in the EE studies, the in-vitro drug release kinetics, and the pharmacokinetic evaluation.

Since the peak area versus concentration plot was linear and fitted well, the method can be regarded as dependable, yielding accurate and reproducible results across the concentration range studied [110].

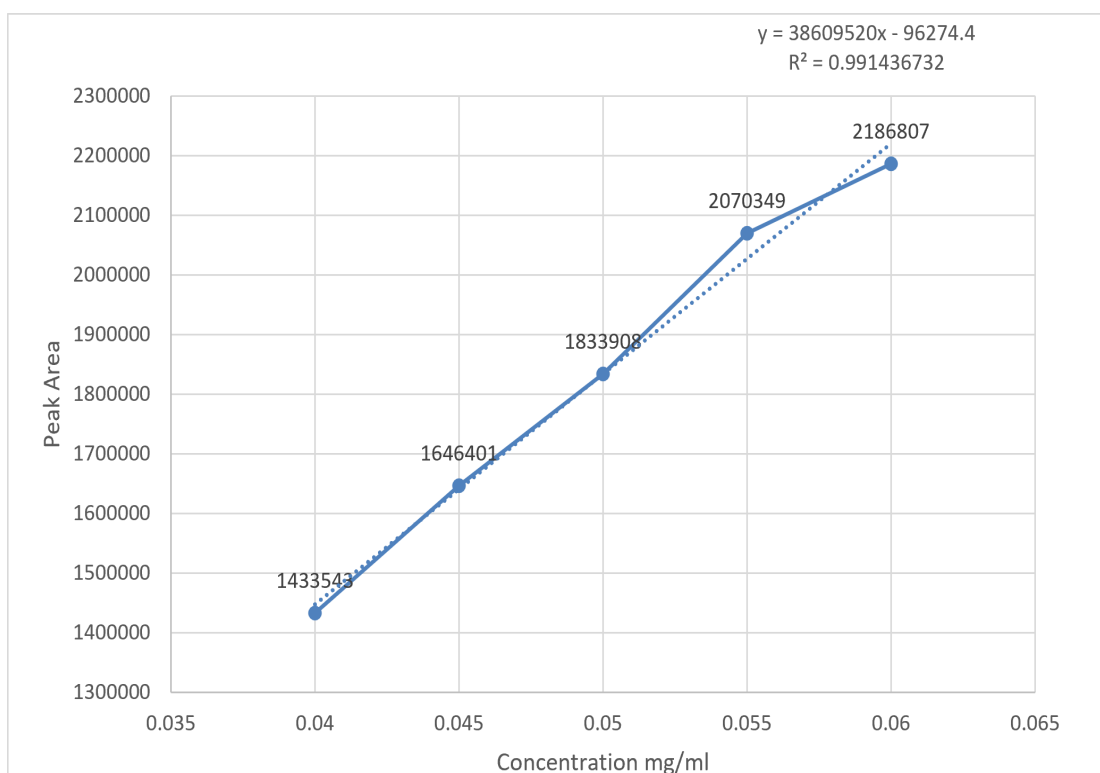


FIGURE 4.1: Concentration vs Peak Area relationship for Calibration Curve of Drug.

4.3 Formulation Optimization by Statistical Experimental Design

Formulation was optimized using three factorial (3^3) Box Behnken Design (BBD) implemented in Design Expert® software.

The design produced 15 experimental runs, including 3 center points, polynomial quadratic models were fitted to describe how the variables of the formulations relate to their relative responses.

TABLE 4.2: BBD originated Eu-NTF-SLNs responses for experimental and predicted values

Run	Independent Variables			Observed Values			Predicted Values		
	Compritol® 888 ATO Conc. (mg) (A)	Surfactant Conc. (Total %) (B)	Eudragit® L-100 Conc. (mg) (C)	Particle Size (nm) (Y ₁)	Zeta Potential (mV) (Y ₂)	PDI (Y ₃)	Particle Size (nm) (Y ₁)	Zeta Potential (mV) (Y ₂)	PDI (Y ₃)
F1	100	2.0	1.0	245	−24.5	0.221	241.5	−25.19	0.2014
F2	200	2.0	1.5	256	−33.4	0.248	254.5	−32.84	0.2595
F3	300	1.5	1.5	395	−34.1	0.361	386.5	−35.1	0.3136
F4	300	2.0	1.0	342	−27.8	0.287	352	−27.36	0.3229
F5	200	2.0	0.5	248	−22.3	0.233	243	−22.61	0.2052
F6	100	1.0	1.0	318	−25.2	0.421	308	−25.64	0.3851
F7	200	1.5	1.0	228	−31.8	0.189	227.67	−31.83	0.2123
F8	200	1.0	1.5	336	−34.6	0.392	341	−34.29	0.4197
F9	300	1.0	1.0	448	−28.5	0.472	451.5	−27.81	0.4916
F10	200	1.5	1.0	224	−32.5	0.189	227.67	−31.83	0.2123
F11	100	1.5	1.5	262	−33.2	0.242	267	−33.08	0.2501
F12	200	1.0	0.5	321	−21.5	0.409	322.5	−22.06	0.3975
F13	200	1.5	1.0	231	−31.2	0.189	227.67	−31.83	0.2123
F14	100	1.5	0.5	236	−22.7	0.114	244.5	−21.7	0.1614
F15	300	1.5	0.5	384	−23.9	0.334	379	−24.03	0.3259

TABLE 4.3: Model statistics for the responses generated by the Box–Behnken Design.

Response	Model F-value	p-value	R^2	Adjusted R^2	Predicted R^2	Adeq Preci- sion	Lack of Fit p-value
Particle Size (Y_1)	78.28	< 0.0001	0.9930	0.9803	0.8920	27.4643	0.0733
Zeta Potential (Y_2)	34.59	0.0006	0.9842	0.9557	0.7836	16.3558	0.2414
PDI (Y_3)	8.28	0.0157	0.9371	0.996	0.8239	7.9213	0.3512

All three response models were reported as statistically significant at $p < 0.05$, demonstrating that the selected formulation variables influenced the measured SLN characteristics [113].

The lack-of-fit tests were non-significant ($p > 0.05$), indicating an acceptable fit of the proposed models to the experimental observations.

R^2 values of 0.9930 for particle size, 0.9842 for zeta potential, and 0.9371 for PDI further indicated close agreement between observed and model-predicted responses.

4.3.1 Influence of Formulation Variables on Particle Size (Y1)

Particle size is an important quality attribute because it can influence colloidal stability, release behavior, and intestinal uptake. The quadratic particle-size model was of highly significance, with $F=78.28$ and $p<0.0001$, and produced an $R^2=0.9930$. Thus, the observed variation of 99.30% in particle size was represented by the fitted model.

4.3.2 Polynomial Model Describing Particle Size

$$\begin{aligned}
 Y_1 = & 227.67 + 63.50A - 41.50B + 7.50C \\
 & - 8.25AB - 3.75AC - 1.75BC \\
 & + 69.79A^2 + 40.79B^2 + 21.79C^2
 \end{aligned}
 \tag{4.1}$$

The t-test (ANOVA) showed that lipid concentration (A) exerted most pronounced enlarging effect on particle diameter ($p < 0.0001$), meaning that a higher Solid Lipid content produced considerably larger particles.

A likely explanation is that the lipid phase becomes more viscous, which lowers the efficiency of homogenization and favours the formation of bigger particles [114].

In contrast, total surfactant conc. (B) had a predominantly negative effect ($p < 0.0001$), so more surfactant levels led to smaller particles.

Greater amount of surfactant reduces interfacial tension and stabilizes the droplets formed during emulsification, thereby yielding finer nanoparticles [115].

Eudragit[®] L100 concentration (C) had a relatively weaker influence that did not reach statistical significance ($p = 0.0869$). The significant quadratic terms A^2 , B^2 , and C^2 indicated that the relationships between the formulation variables and particle size were nonlinear [115].

TABLE 4.4: Summary of Characteristics for Particle Size

Factor	F-value	p-value	Remarks
A	323.77	< 0.0001	Statistically Significant
B	138.29	< 0.0001	Statistically Significant
C	4.52	0.0869	Not Statistically Significant
A^2	180.51	< 0.0001	Statistically Significant
B^2	61.66	0.0005	Statistically Significant
C^2	17.60	0.0085	Statistically Significant

4.3.3 Effect of Formulation Variables on ZP (Y2)

ZP reflects the electrical charge carried at the surface of nanoparticles and is commonly used to judge their stability of the suspended particles. The second-order polynomial model describing zeta potential proved statistically significant ($F=34.59$, $p=0.0006$), and its R^2 of 0.9842 indicates a close fit to the experimental data.

4.3.4 Polynomial Model Describing Zeta Potential

$$\begin{aligned}
 Y_2 = & -31.83 - 1.09A + 0.225B - 5.61C \\
 & + 0AB + 0.075AC + 0.500BC \\
 & + 2.40A^2 + 2.93B^2 + 0.954C^2
 \end{aligned} \tag{4.2}$$

Of the parameters examined, Eudragit[®] L-100 concentration (C) had the greatest influence on ZP ($F = 250.29$, $p < 0.0001$). A higher polymer content shifted the values toward greater negativity, which is explained by the ionization of the carboxylic groups in polymer [116]. Compritol[®] ATO 888 concentration (A) also had a significant effect on zeta potential ($p = 0.0279$), while surfactant concentration (B) did not ($p = 0.5538$). The significant quadratic terms A^2 and B^2 pointed to nonlinear relationships between these two variables and the surface charge of the nanoparticles. The negative zeta potential values obtained suggest that electrostatic repulsion between the particles was adequate, which limits aggregation and enhances the stability of the formulation [117].

TABLE 4.5: Model Statistically Significant terms for zeta potential

Factor	F-value	p-value	Remarks
A	9.40	0.0279	Statistically significant
B	0.4023	0.5538	Not Statistically significant
C	250.29	< 0.0001	Statistically significant
A^2	21.20	0.0058	Statistically significant
B^2	31.47	0.0025	Statistically significant

4.3.5 Factor Effects on Particle Size Distribution Uniformity (Y3)

PDI provides information about the breadth of particle-size distribution, with lower values corresponding to a narrower and more uniform nanoparticle population. The quadratic PDI model was reported with $F = 8.2$, $p = 0.0517$, and an R^2 value of 0.9347.

4.3.6 Second-Order Equation for PDI

$$\begin{aligned}
 Y_3 = & 0.2123 + 0.0570A - 0.0881B + 0.0191C \\
 & + 0.0037AB - 0.0252AC + 0.0080BC \\
 & + 0.0401A^2 + 0.0978B^2 + 0.0103C^2
 \end{aligned} \tag{4.3}$$

Raising the amount of Compritol[®] ATO 888 (A) produced a significant increase in PDI ($p = 0.0148$), which means that a higher lipid content widened the particle size distribution. Surfactant concentration (B), on the other hand, lowered PDI significantly ($p = 0.0024$), highlighting its part in yielding more uniform nanoparticles [113]. Eudragit[®] L100 concentration did not have a statistically significant influence ($p = 0.2756$). Of the quadratic terms, only B^2 had a significant effect on PDI ($p = 0.0051$). The model was reported with a predicted R^2 value of 0.8239 and adequate precision above 4, while remaining statistically significant according to the thesis analysis. These statistics were interpreted as showing that the model described the measured data within the experimental design space, although its predictive performance beyond the investigated conditions was comparatively more limited [118].

TABLE 4.6: Model Statistically Significant terms for PDI

Factor	F-value	p-value	Remarks
A	13.30	0.0148	Statistically Significant
B	31.80	0.0024	Statistically Significant
C	1.50	0.2756	Statistically Significant
B^2	22.66	0.0051	Statistically Significant

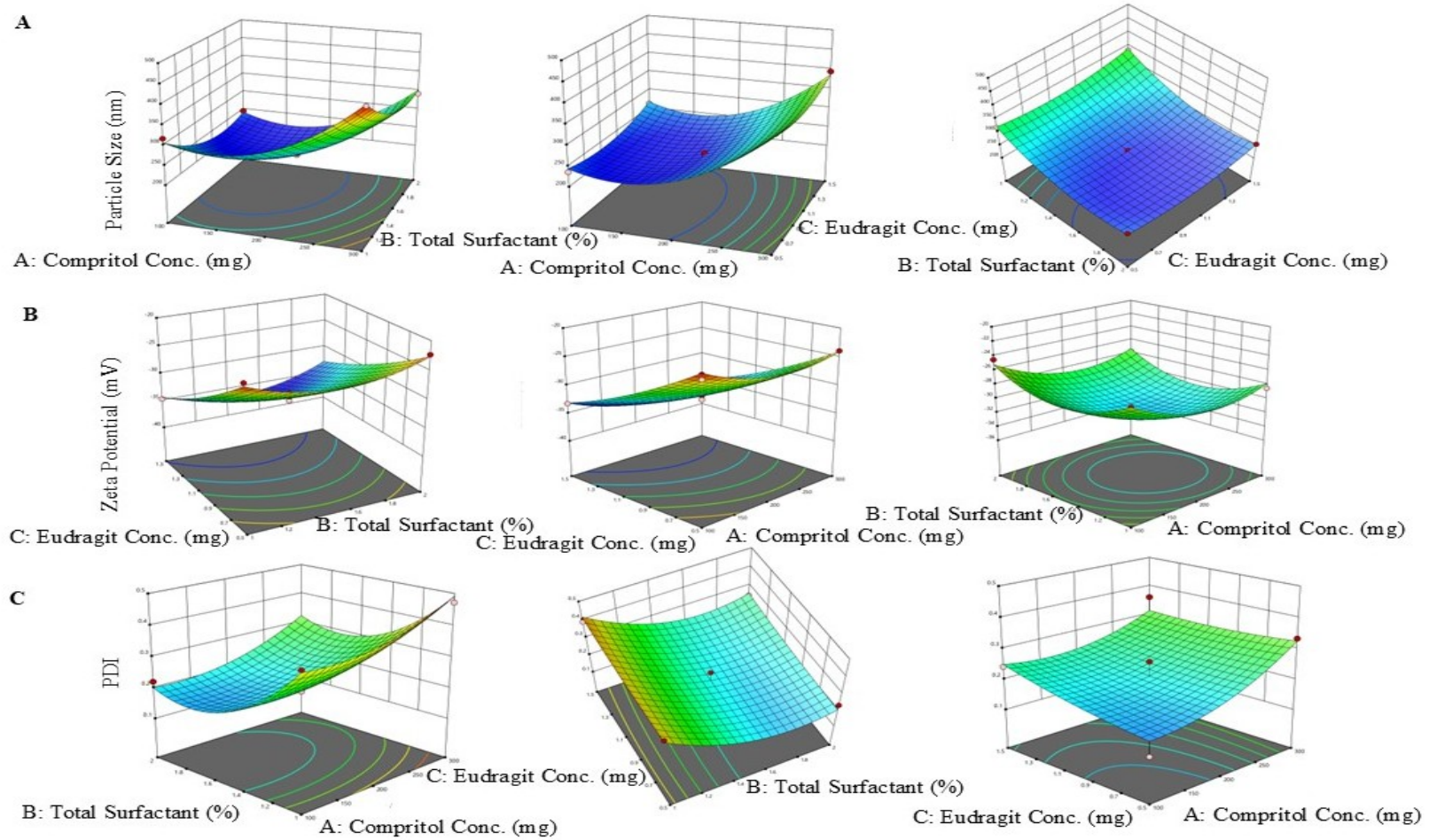


FIGURE 4.2: Summary of 3D plotting of all variables influence over (a) Particle Size (b) Zeta Potential (c) PDI using Box Behnken Design in Design Expert®

4.4 Analytical Characterization of SLNs

4.4.1 Measurement of Particle Size, Polydispersity and Zeta Potential

The Eu-NTF-SLNs were analyzed by DLS for size, PDI, and ZP, parameters that reflect the physical quality and storage stability of the finished product. The average diameter came to 228 ± 10 nm, placing the particles inside the nanometer window sought for oral administration.

Particles of this dimension expose a relatively wide area to the dissolution medium and may be taken up more readily across the intestine, which could translate into higher NFT bioavailability. This size most likely reflects efficient emulsification coupled with the stabilizing action of the surfactant combination chosen.[\[13\]](#).

A PDI of 0.189 ± 0.051 was recorded for the optimized batch, pointing to a fairly tight size distribution and a uniform particle population. Values under 0.3 are usually read as a sign of a near-monodisperse system.

The low figure here therefore hints at steady particle formation with little clumping, qualities that matter for repeatable drug release and for keeping the formulation stable.[\[119\]](#).

The Eu-NTF-SLNs showed a ZP of -31.80 ± 1.00 mV a sign of good colloidal stability. This negative charge arises mostly from Eudragit[®] L100, whose carboxylic groups ionize at the particle surface.

Magnitudes beyond roughly 30 mV are widely linked to enough electrostatic repulsion between particles to curb aggregation during storage.[\[102\]](#) The measured ZP thus backs the physical stability of the system. Taken as a set, the size, PDI, and ZP results show that the pH-sensitive SLN formulation for oral NFT delivery is stable, fairly uniform, and well dispersed.[\[100\]](#).

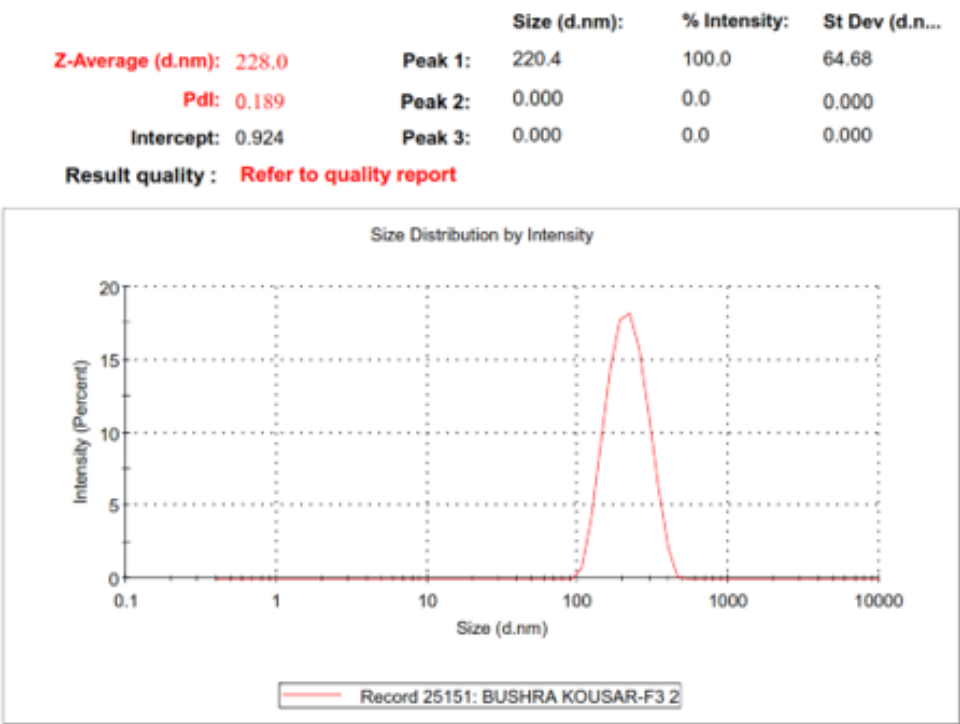


FIGURE 4.3: Particle Size and PDI of Eu-NFT-SLNs

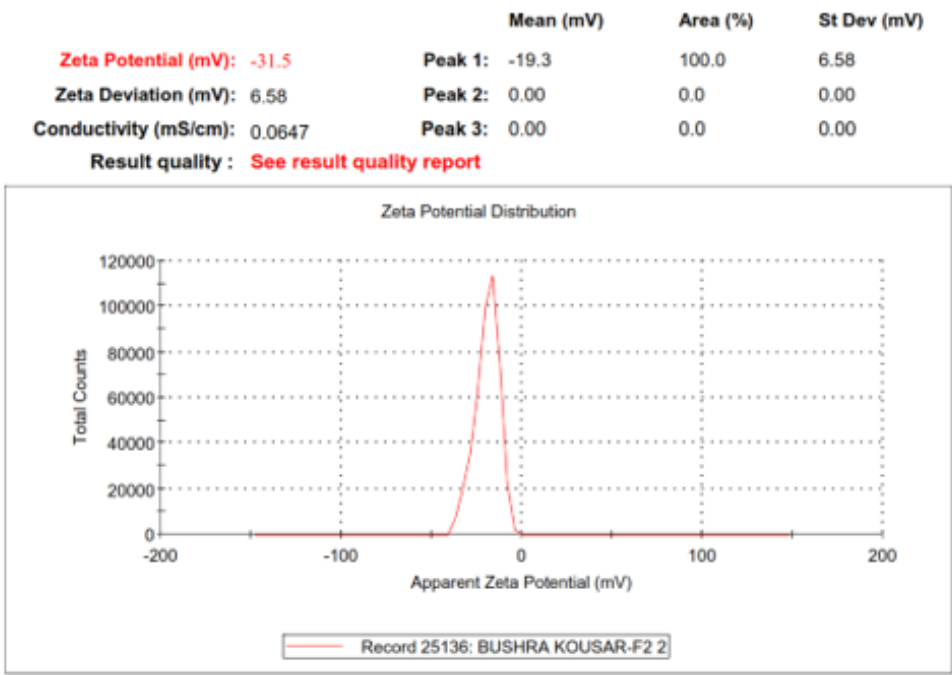


FIGURE 4.4: Zeta Potential of Eu-NFT-SLNs

4.4.2 Determination of Drug Entrapment and Drug Loading

Entrapment efficiency was measured for the NFT-SLNs with and without coating with Eudragit[®] L-100. The indirect approach was used, in which free NFT in the supernatant remaining, is measured by the validated HPLC method and the entrapped fraction was quantified from this value.

The NTF-SLNs achieved an EE of $85.960\% \pm 0.110$, while the Eu-NTF-SLNs produced an EE of $79.200\% \pm 0.260$ (Figure 4.2). Both formulations therefore retained a high proportion of NFT within the lipid system, although slightly greater drug entrapment was observed before application of the polymer coating.[\[120\]](#)

The relatively high EE of the NTF-SLNs may be related to the lipophilic character of lipid, which can provide a favorable lipid environment for incorporation of NFT molecules.[\[121\]](#) Solid lipid systems may also reduce movement of the drug into the surrounding hydrophilic environment during particle formation. Comparable results have been described for formulations based on glyceryl behenate, where strong drug-lipid affinity supported high encapsulation efficiency [\[122\]](#)

Coating with Eudragit[®] L100 produced a modest reduction in EE. This difference may result from partial migration of surface-associated NFT during polymer deposition as well as from the repeated centrifugation and redispersion steps required during coating [\[13\]](#) Formation of the polymeric layer may additionally change the distribution of drug near the particle interface and permit a small amount of NFT loss. Nevertheless, the coated system retained an EE above 75%, confirming that most of the drug remained successfully incorporated in the nanoparticles [\[124\]](#)

The measured EE values are within the range commonly documented for other drug incorporated nano-carrier systems, where EE of approximately 70%-90% are frequently observed. The maximize retention achieved in this study supports the suitability of lipid as a lipophilic carrier for drug and justifies Subsequent analysis of the formulation for sustained and pH-sensitive delivery.[\[124\]](#)

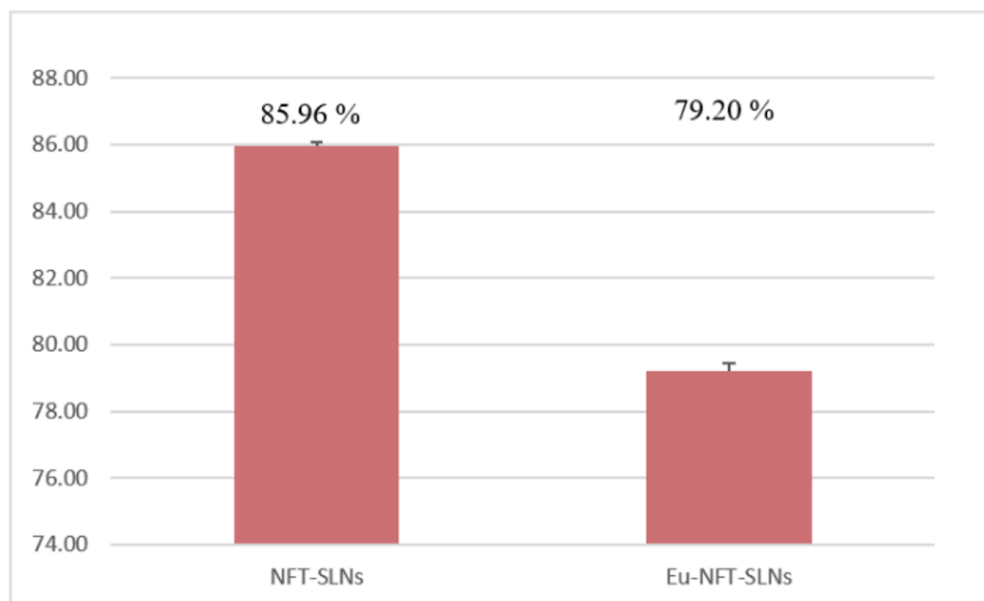


FIGURE 4.5: (%)EE of NFT-SLNs and Eu-NFT-SLNs

Drug loading (DL) was determined for the optimized NFT-SLNs and Eu-NFT-SLNs to establish how much NFT each formulation carries relative to its total weight which is calculated as 90%. The values indicate that the Compritol® ATO 888 matrix can hold a substantial amount of NFT within the particles. The Eu-NFT-SLNs formulation had a lower DL than the NFT-SLNs. This is expected, because the Eudragit® L100 layer adds to the total weight of the particles, and a small amount of drug may also be lost during coating and the repeated centrifugation steps, as discussed for EE. Even so, the DL of the Eu-NFT-SLNs remained adequate, which shows that the formulation can carry a useful drug payload for oral delivery [135].

4.4.3 Surface Morphology by Scanning Electron Microscopy

SEM was used to examine particle shape, surface appearance, and structural morphology of the optimized Eudragit® L100-coated NFT-SLNs. Representative micrographs of the optimized formulation are presented in Figure 4.3 at different magnifications to permit evaluation of both general particle form and finer surface characteristics.

The SEM micrographs showed that Eu-NFT-SLNs were predominantly spherical or near-spherical and possessed clearly defined particle boundaries. Particles appeared largely discrete and relatively uniformly distributed across the imaged field, supporting successful formation of the nanosystem. Most nanoparticles displayed smooth, continuous surfaces with few obvious irregularities, a feature compatible with effective incorporation of NFT within the lipid matrix. Such rounded morphology is commonly observed in lipid nanoparticles produced using high-shear homogenization together with ultrasonication [14].

At greater magnification, the nanoparticles retained a compact structure without obvious cracks or surface fractures. Their comparatively smooth exterior may be associated with deposition of Eudragit[®] L100 around the lipid particles, forming a thin polymeric outer layer.[76] Such a coating could contribute both to a more uniform surface and to improved physical stability. Similar attributes have been described for other polymers used to coat lipid nano-particles, where the external polymeric layer can reduce aggregation and limit premature drug release [103].

Only limited particle aggregation was evident in the SEM images, indicating successful stabilization effect by the combination of the two surfactants (Poloxamer 188 and Tween[®] 80). When Substantial aggregation is not present, it suggests that sufficient steric stabilization was maintained through formulation and subsequent lyophilization. Although occasional clusters were visible, the overall particle morphology remained fully retained, supporting the compatibility of the chosen materials and parameters [125].

SEM imaging revealed particles whose sizes lay in the nanometer range, and these values largely agreed with those obtained by DLS. Some discrepancy between the two techniques is to be expected. DLS measures the hydrodynamic diameter of particles suspended in a liquid, while SEM examines particles that have been dried and are held under vacuum [91].

Overall, SEM confirmed fabrication of Eu-NTF-SLNs with mainly spherical geometry, even surfaces, and limited agglomeration. These Surface morphology attributes are desired for an oral nano size particle system because they support

stability in physical state, more even distribution of incorporated drug, and consistent gastrointestinal emptying time, strengthening the potential of the developed system for improved NFT delivery.[126].

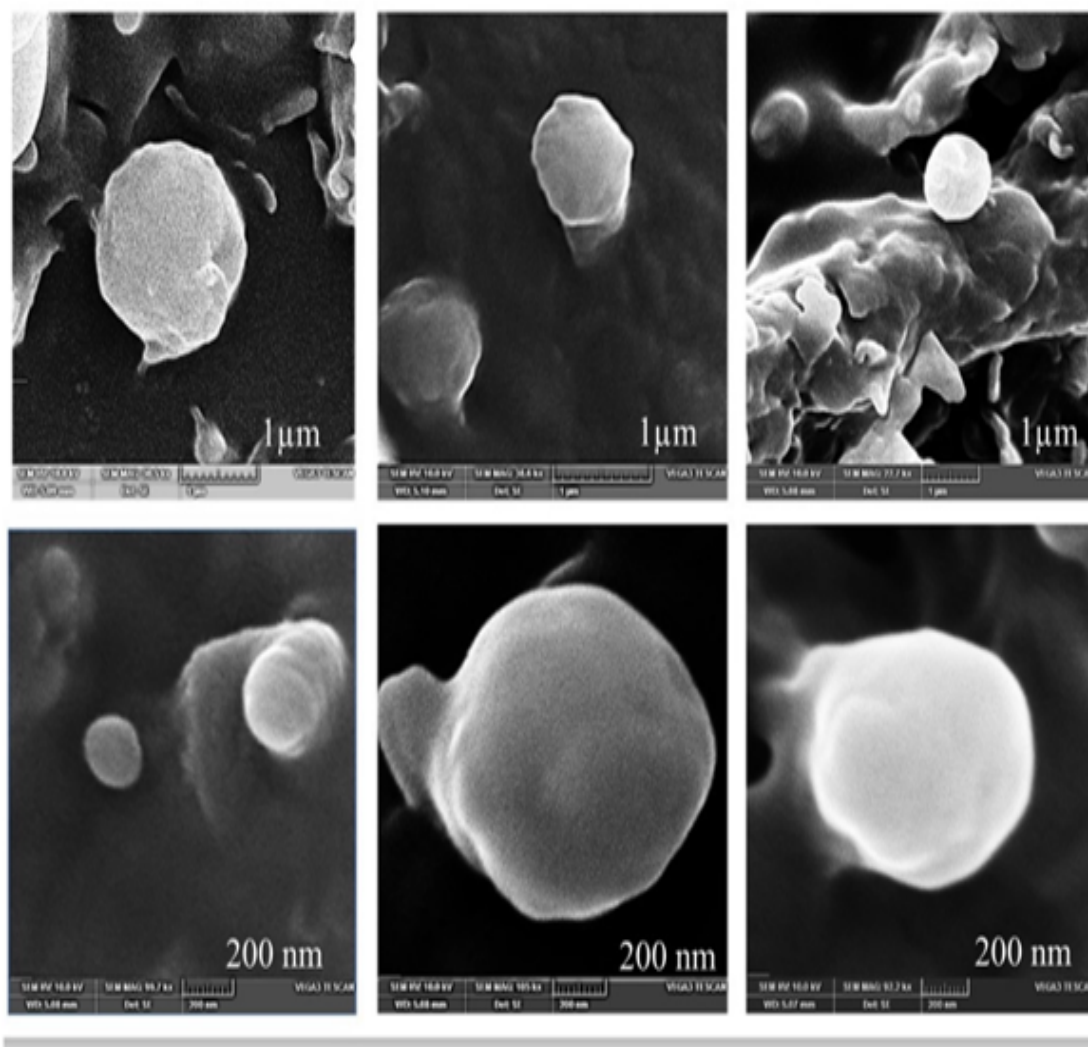


FIGURE 4.6: Surface morphology of Eu-NTF-SLNs at magnifications (a) $1\mu\text{m}$ (b) 200nm

4.4.4 Crystallinity Analysis by X-Ray Powder Diffraction

XRPD was used to assess the crystalline nature of pure NFT, Compritol® ATO 888, and the optimized Eu-NFT-SLNs (Figure 4.3). The purpose of this analysis was to determine the physical state of the drug inside the lipid matrix and to detect any change in crystallinity that occurred once the nanoparticles were formed [9, 10, 99].

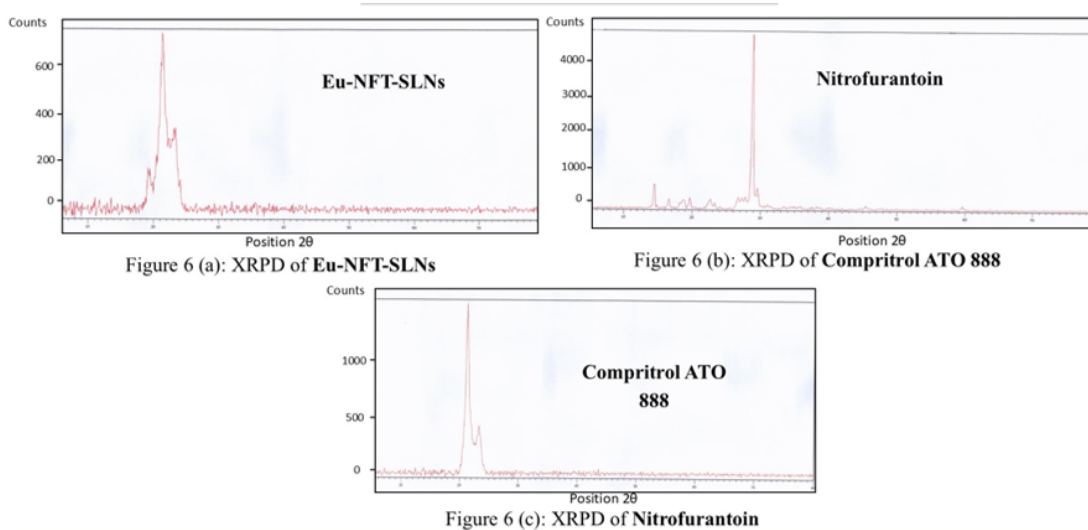


FIGURE 4.7: XRPD Diffractograms of (a) Eu-NFT-SLNs (b) Nitrofurantoin (c) Compritol[®] ATO 888

The XRPD pattern of pure NFT showed sharp, intense diffraction peaks, including a prominent reflection at about $2\phi = 29^\circ$, confirming its highly crystalline nature, consistent with previously reported patterns for the drug. This crystallinity is linked to NFT's limited aqueous solubility and slow dissolution, which can contribute to its poor oral bioavailability [9, 10].

Compritol[®] ATO 888 showed characteristic peaks at about $21\text{--}24^\circ$ (2ϕ), reflecting the ordered packing of glyceryl behenate and consistent with its known semi-crystalline polymorphic structure. This structure gives Compritol[®] ATO 888 a compatible lipid matrix for the encapsulation of drug and sustained release [11].

In the optimized Eu-NFT-SLNs, the characteristic NFT peaks were markedly reduced in intensity or absent, and the overall pattern was broader and weaker than that of pure NFT. This indicates a substantial loss of drug crystallinity, consistent with NFT being dispersed within the Compritol[®] ATO 888 matrix in a partially disordered or amorphous state. The homogenization, ultrasonication and solidification steps likely disrupted the native NFT crystal lattice, and the Eudragit[®] L100 coating may have further masked residual crystalline domains, supporting molecular-level dispersion of the drug within the lipid-polymer matrix [12].

Overall, the reduced crystallinity of NFT after incorporation into the Eudragit[®] L100-Eu-NFT-SLNs is favourable, since it is typically associated with improved wettability, faster dissolution, and potentially higher oral bioavailability.

These findings support successful encapsulation of NFT within the lipid–polymer system and its suitability as an oral delivery platform [13, 14].

4.4.5 Spectroscopic Compatibility Study by Infrared Analysis

The spectra of ATR-FTIR of NFT and the Eu-NFT-SLNs are shown in Figure X. Pure NFT gave bands at about 3300 cm^{-1} (N–H stretching), 3100 cm^{-1} (aromatic C–H) and 2920 cm^{-1} (aliphatic C–H).

A strong carbonyl band of the hydantoin ring appeared at about 1785 cm^{-1} , and a prominent band at about 1347 cm^{-1} was assigned to the nitro group. The many sharp bands in the fingerprint region confirmed the identity and purity of the drug [127, 129].

The Eu-NFT-SLN spectrum retained the N–H band near 3300 cm^{-1} and the aromatic C–H band near 3100 cm^{-1} , both with reduced intensity. It also showed excipient bands: an ester C=O band at about 1740 cm^{-1} from the lipid and Eudragit, and CH_2 bending at about 1470 cm^{-1} .

The strong ester carbonyl absorption of the excipients partly overlaps the drug's carbonyl region, so the NFT carbonyl band at 1785 cm^{-1} was not separately resolved in the formulation [127, 129].

The lower peak intensity and broadening of the NFT signals are consistent with the drug being entrapped within the lipid matrix and with overlap from the excipient bands. The characteristic N–H and aromatic C–H bands of NFT were preserved and no new bands appeared, so no chemical reaction between NFT and the excipients is indicated. The drug therefore remained chemically intact during preparation and is compatible with the formulation components [30].

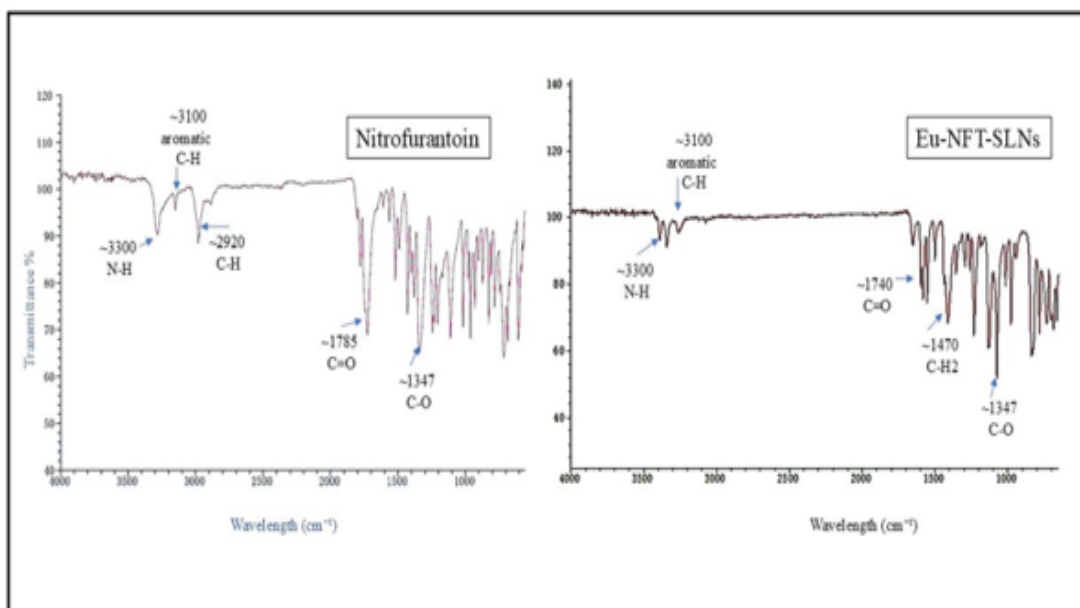


FIGURE 4.8: Graphical Representation of FTIR (A) Pure Drug NFT (B) Eu-NFT-SLNs

4.5 *In vitro* Assessment of Drug Release

Drug release from Eu-NFT-SLNs and NFT-SLNs was compared in two media. Artificial stomach medium (1.2 pH) was used to verify the Eudragit[®] L100 coating, because this polymer stays intact under gastric conditions. Phosphate-based buffer medium at 6.8 pH was used to study how the coating behaves when it dissolves at that pH.

In the pH 1.2 medium, Eu-NFT-SLNs released drug more slowly and in a more controlled way. Cumulative release rose slowly from roughly 9 to 10% at 2h to roughly 85 to 92% at 12h, and it approached complete release between 24h to 48h.

NFT-SLNs, in comparison, released drug faster in the same medium, with around 21-27% liberated in the first 2 h and near-complete release reached sooner.

The slower release of Eu-NFT-SLNs under acidic conditions is attributed to Eudragit[®] L100, which does not dissolve at low pH and so serves as an extra barrier to drug diffusion [131].

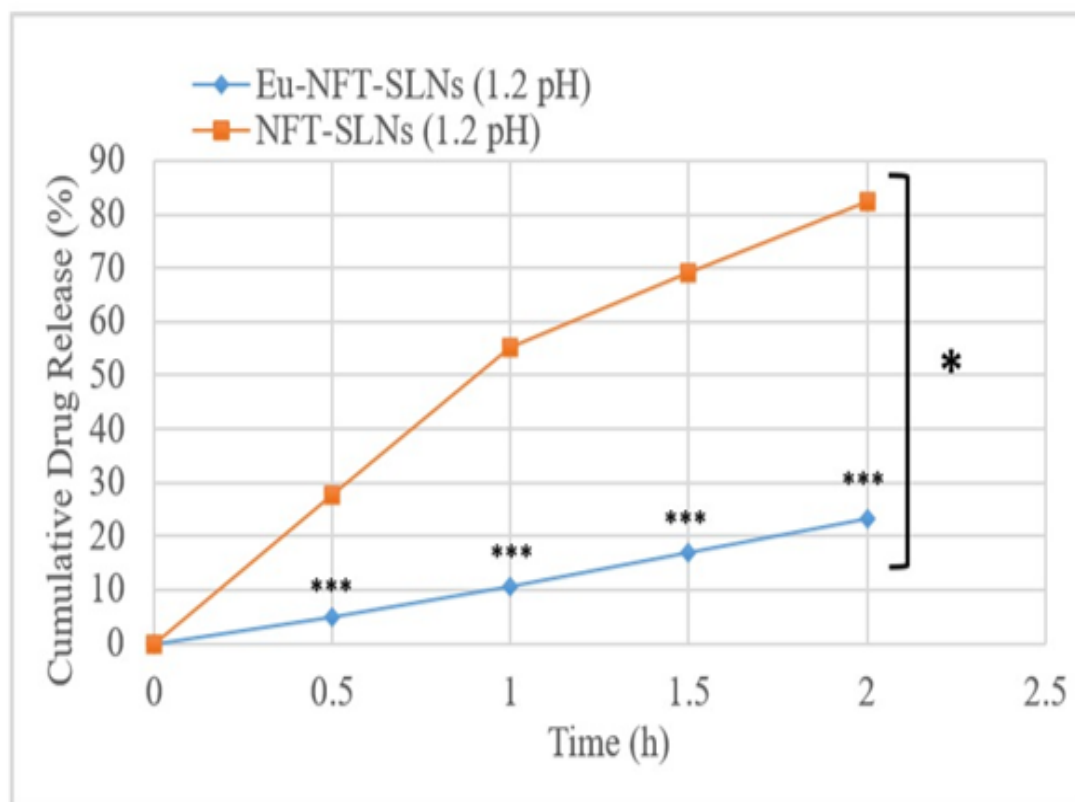


FIGURE 4.9: % CDR of NTF-SLNs and Eu-NTF-SLNs in gastric 1.2 pH

Both formulations released more drug at pH 6.8, where the dissolution conditions are more favourable. For Eu-NFT-SLNs, cumulative release climbed steadily from about 10% at 2 h to almost complete release within 24 h. NFT-SLNs, by contrast, released drug somewhat faster during the early stage of dissolution.

The gap between the coated and uncoated systems was widest in this early phase, and by the end both had released nearly all of their drug.

These results suggest that the lipid matrix alone gave sustained-release behaviour, and that the Eudragit[®] L100 coating added further control by reducing the initial burst and limiting drug diffusion [53].

The comparative profiles consequently support successful incorporation of pH-responsive behavior without eliminating the sustained-release effect provided by the solid lipid carrier [132].

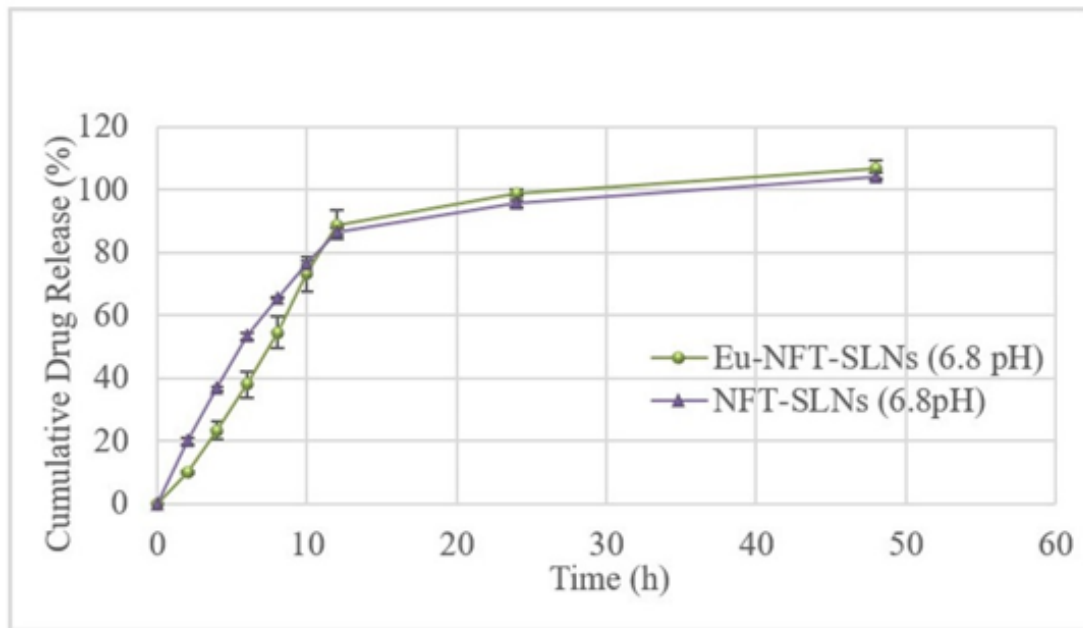


FIGURE 4.10: % CDR of NTF-SLNs and Eu-NTF-SLNs in 6.8 pH

After the profiles for dissolution had been generated, the data for the release of coated and uncoated SLNs were analyzed in DDSolver using mathematical kinetic models to investigate the mechanisms responsible for NFT release under both pH environments (acidic and intestinal).

TABLE 4.7: Comparison of Release Kinetic Model Parameters for Coated and Uncoated Nanoparticles at pH 1.2

Model	EU-NFT-SLNs (R^2)	EU-NFT-SLNs (AIC)	EU-NFT-SLNs (MSC)	NFT-SLNs (R^2)	NFT-SLNs (AIC)	NFT-SLNs (MSC)
Zero-order	0.9960	3.56	4.67	0.9556	28.37	2.02
First-order	0.9888	8.71	3.64	0.9925	19.45	3.80
Higuchi	0.8636	21.21	1.14	0.9963	26.99	2.29
Korsmeyer–Peppas	0.9999 $n = 0.68$	–18.25	9.03	0.9927 $n = 0.332$	21.32	3.43

Release of NFT from Eu-NTF-SLNs and NTF-SLNs in acidic medium (1.2 pH) was evaluated using all kinetic models. The two formulations showed multiple

best-fitting kinetic behavior. Eu-NFT-SLNs produced the strongest agreement with the Korsmeyer-Peppas model, which showed the highest R^2 value (0.9999), the lowest AIC (-18.25), and the highest MSC (9.03).

For the uncoated NFT-SLNs, the First-order model provided the best overall description, with the lowest AIC value of 19.45 and the highest MSC value of 3.80.

The strong Korsmeyer-Peppas fit for the coated nanoparticles indicates that acidic-medium release involved more than a single mechanism and likely reflected a mixture of molecular diffusion together with structural loosening of the matrix [127].

A release exponent of $n = 1.1217$ indicates that the drug was transported by a Super Case-II mechanism Eudragit[®] L100 layer around the SLNs probably acted as an extra diffusion barrier, holding back immediate NFT release and giving a more sustained profile [133]. The good fit of the Zero-order model also supports the the sustained influence of the lipid matrix and polymer coating together.

For the NFT-SLNs, on the other hand, First-order kinetics applied, meaning that the release rate depended on the amount of drug still present in the lipid matrix [123].

Without a polymeric barrier, the dissolution medium could reach the particle surface more easily, which led to faster drug diffusion and a higher initial release rate.

The uncoated particles released drug more quickly, yet the sustained-release effect of the solid lipid matrix was still clear, showing that SLNs can prolong release even without a pH-sensitive coating [134].

Neither formulation fitted the Higuchi model well, which implies that simple Fickian diffusion could not fully account for the release behaviour seen.

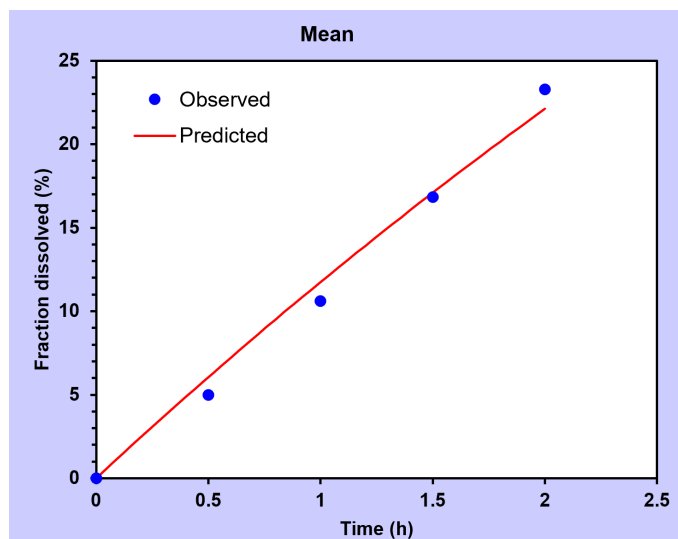


FIGURE 4.11: First-Order Release Kinetics Plot for Eu-NFT-SLNs at pH 1.2

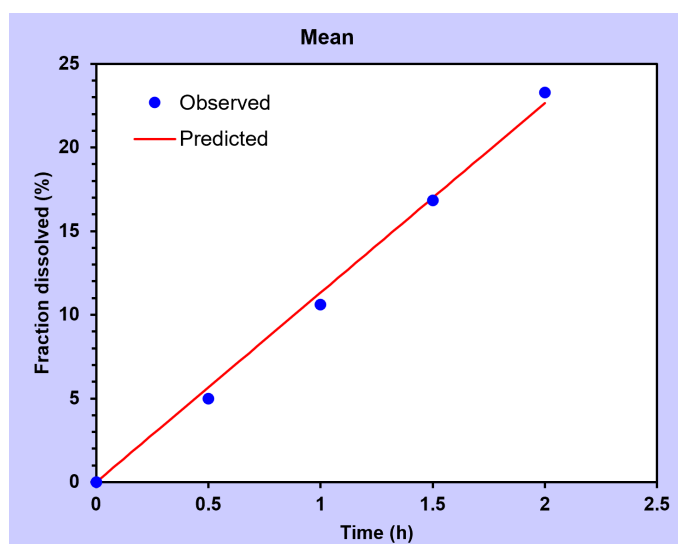


FIGURE 4.12: Zero-Order Release Kinetics Plot for Eu-NFT-SLNs at pH 1.2

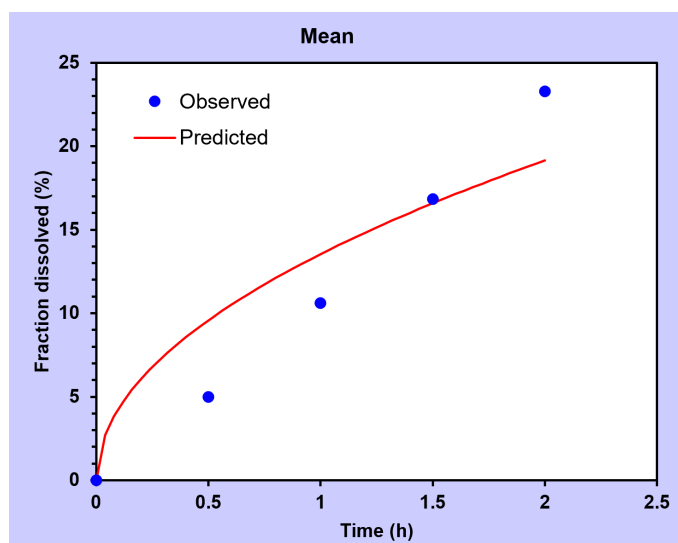


FIGURE 4.13: Higuchi Drug Release Kinetics Plot for Eu-NFT-SLNs at pH 1.2

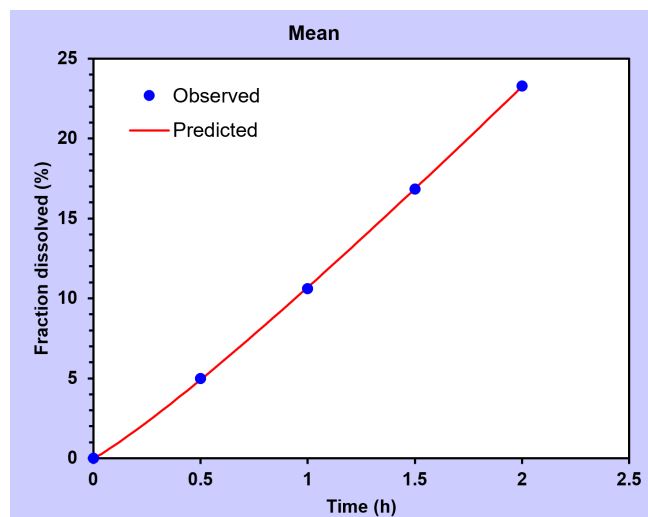


FIGURE 4.14: Korsmeyer-Peppas Release Kinetics Plot for Eu-NFT-SLNs at pH 1.2

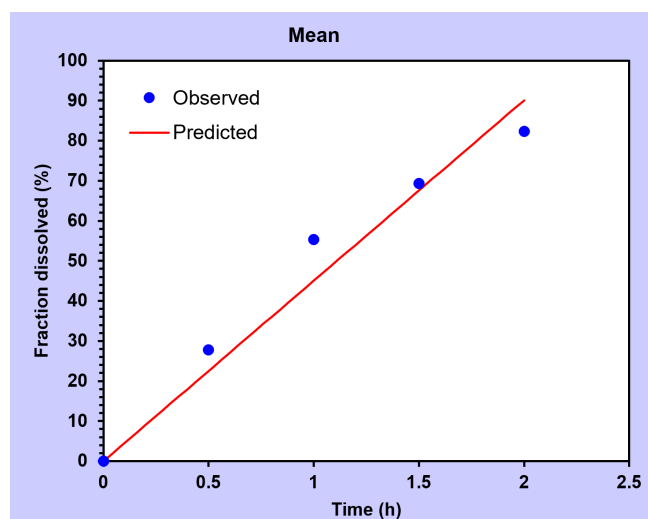


FIGURE 4.15: Zero-Order Release Kinetics Plot for NFT-SLNs at pH 1.2

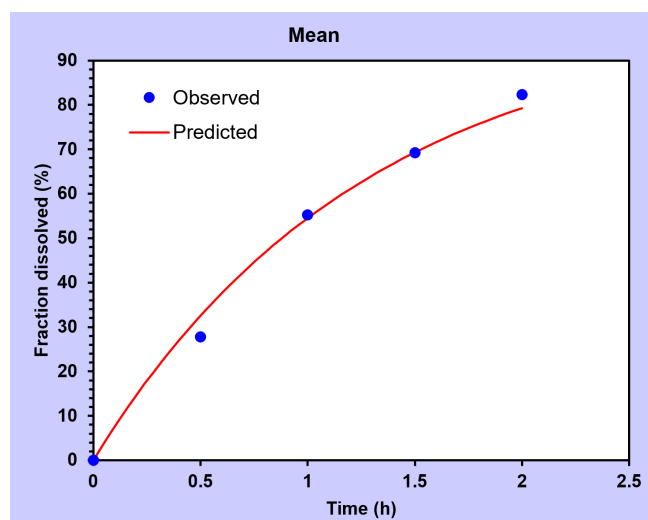


FIGURE 4.16: First-Order Release Kinetics Plot for NFT-SLNs at pH 1.2

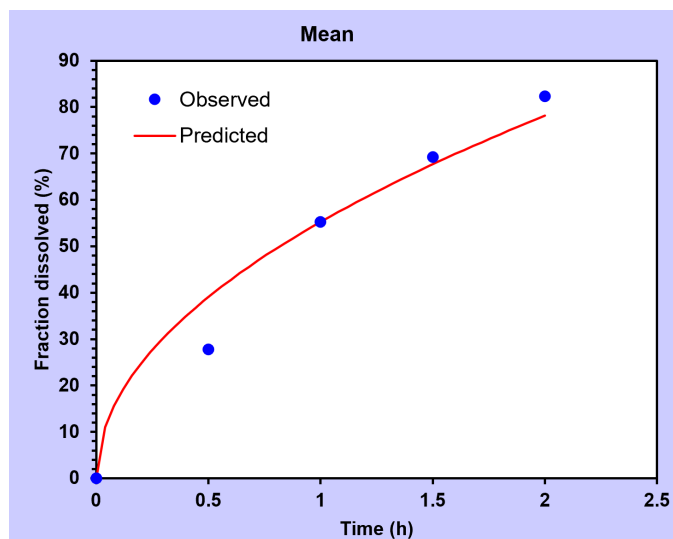


FIGURE 4.17: Higuchi Drug Release Kinetics Plot for NFT-SLNs at pH 1.2

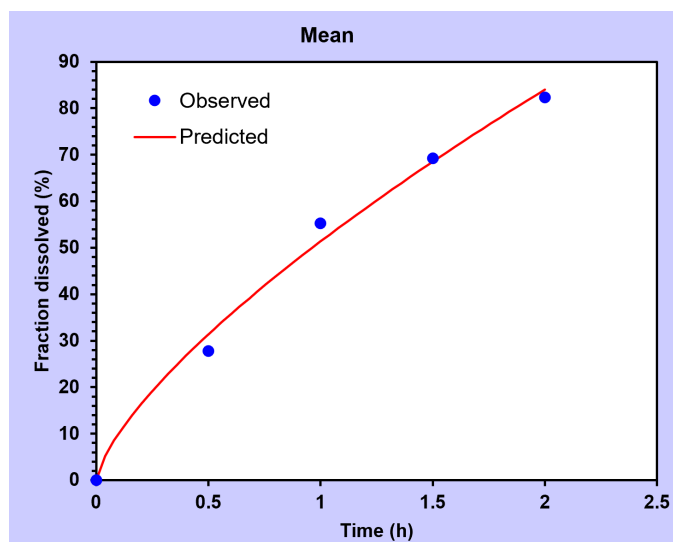


FIGURE 4.18: Korsmeyer-Peppas Release Kinetics Plot for NFT-SLNs at pH 1.2

TABLE 4.8: Release Kinetic Model Parameters Compared Between Coated and Uncoated Nanoparticles at pH 6.8

Model	R^2	Adjusted R^2	MSE	RMSE	AIC	MSC
For Eu-NFT-SLNs						
First-order	0.9414	0.9414	85.886	9.267	60.792	2.328
Zero-order	0.2442	0.2442	1107.669	33.282	83.805	-0.229
Hixson-Crowell	0.9549	0.9549	66.105	8.131	58.436	2.59

Table 4.8 continued from previous page

Model	R^2	Adjusted R^2	MSE	RMSE	AIC	MSC
Korsmeyer– Peppas	0.829 n = 0.4302	0.8045	286.497	16.926	72.433	1.034
Higuchi	0.8155	0.8155	270.383	16.443	71.114	1.181
For NFT-SLNs						
First-order	0.9887	0.9887	13.184	3.631	43.926	3.796
Zero-order	−0.2212	−0.2212	1425.21	37.752	86.074	−0.887
Hixson–Crowell	0.9339	0.9339	77.115	8.782	59.823	2.03
Korsmeyer– Peppas	0.8734 n = 0.3321	0.8553	168.881	12.995	67.676	1.157
Higuchi	0.7663	0.7663	272.747	16.515	71.192	0.766

To describe how NFT is liberated from Eu-NFT-SLNs and NFT-SLNs, the dissolution data were fitted to five mathematical models: zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas.

Among the models tested on Eu-NFT-SLNs, Hixson-Crowell achieved the top coefficient of determination ($R^2 = 0.9549$), so the fitted curve tracked the experimental release profile very closely (table 4.8). The same model also showed the lowest MSE (66.105) and RMSE (8.131), meaning its predictions strayed least from the observed values. Its AIC (58.436) was the smallest and its MSC (2.590) the largest of the group, which adds further weight to its selection as the best fit. The findings imply that NFT release from Eu-NFT-SLNs was governed chiefly by shifts in particle size and surface area as dissolution progressed [135]. While the enteric-coated lipid particles gradually eroded and dissolved in the medium, the area exposed for release kept changing, producing a profile that matches Hixson-Crowell kinetics.

The first-order model also correlated reasonably well for Eu-NFT-SLNs ($R^2 = 0.9414$), which hints that release partly depended on how much drug remained inside the formulation [133]. Still, its lower R^2 and poorer statistical indicators than those of Hixson-Crowell show that it was not the dominant mechanism. Fits

from the Higuchi ($R^2 = 0.8155$) and Korsmeyer-Peppas ($R^2 = 0.8290$) models were only moderate, indicating that diffusion contributed to release without being the controlling factor. Zero-order gave the poorest fit ($R^2 = 0.2442$), so the coated formulation did not deliver NFT at a steady, constant rate across the dissolution period [133].

The uncoated NFT-SLNs followed a different pattern. For them, the first-order model correlated best ($R^2 = 0.9887$) and also recorded the lowest MSE (13.184) and RMSE (3.631), the smallest AIC (43.926), and the largest MSC (3.796), so it described the data better than every competing model. A fit this close suggests that NFT release from NFT-SLNs was tied directly to the concentration of drug still held in the lipid matrix [79]. As NFT passed into the dissolution medium, the release rate declined gradually, since less entrapped drug remained to diffuse out.

Hixson-Crowell was also a good fit for NFT-SLNs ($R^2 = 0.9339$), implying that particle erosion and a changing surface area had some influence on release. Even so, the statistical parameters plainly favored first-order kinetics as the principal mechanism. The Higuchi ($R^2 = 0.7663$) and Korsmeyer-Peppas ($R^2 = 0.8734$) models indicated a moderate contribution from diffusion-controlled release, yet both explained the dissolution profile less well than first-order did. In addition, the negative R^2 for zero-order (-0.2212) confirmed that NFT-SLNs did not release their drug at a constant rate.

A side-by-side look at the two formulations shows that the Eudragit® L100 coating clearly shifted the release mechanism. NFT-SLNs mainly obeyed first-order kinetics, meaning release that depends on concentration, while Eu-NFT-SLNs were better represented by the Hixson-Crowell model. This implies that the polymer coating altered release by placing extra barriers in the path of drug diffusion and by encouraging release through slow matrix erosion and a shrinking surface area [75, 96, 131]. The change in kinetics after coating indicates that the nanoparticles' release properties were successfully tailored. The enteric coating shielded the formulation in acidic conditions and also helped produce a more sustained release at intestinal pH (6.8).[53]

To sum up, the kinetic analysis revealed that Eu-NFT-SLNs released drug through a modified mechanism characterized mainly by particle erosion and surface area change, whereas NFT-SLNs released NFT predominantly by concentration - dependent diffusion [136]. These outcomes confirm that the Eudragit[®] L100 coating is effective at regulating drug release and at improving the sustained-release performance of the developed SLN system.[137]

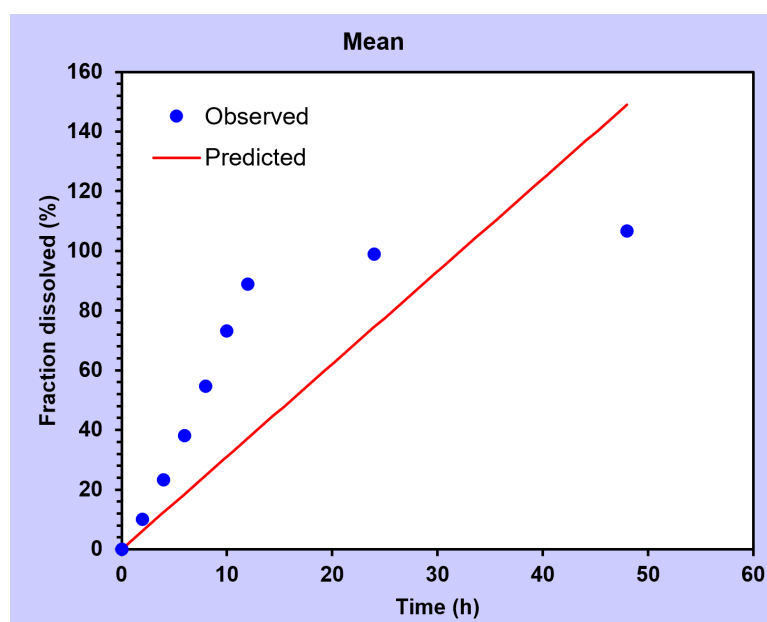


FIGURE 4.19: Zero Order Release Kinetics Plot for Eu-NFT-SLNs at pH 6.8

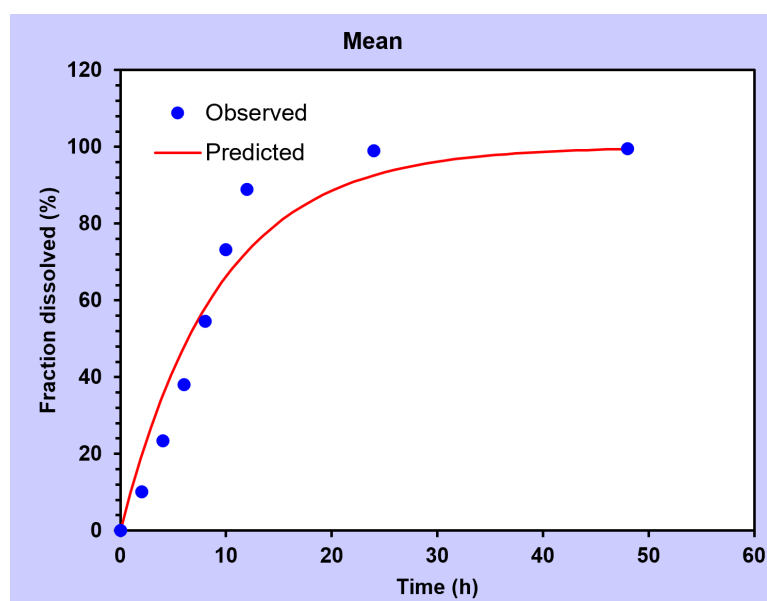


FIGURE 4.20: First-Order Release Kinetics Plot for Eu-NFT-SLNs at pH 6.8

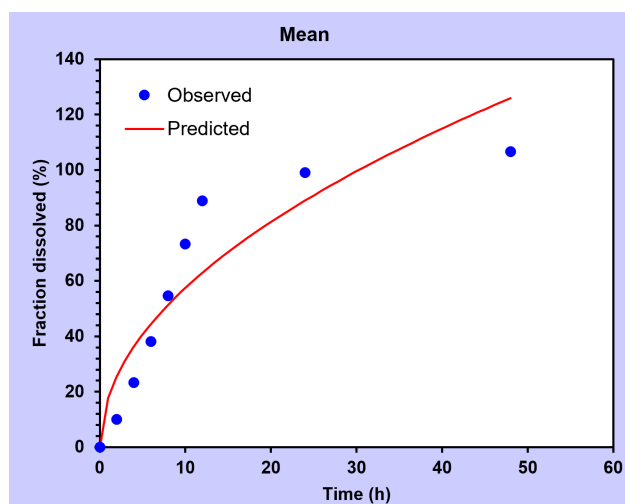


FIGURE 4.21: Korsmeyer-Peppas Release Kinetics Plot for Eu-NFT-SLNs at pH 6.8

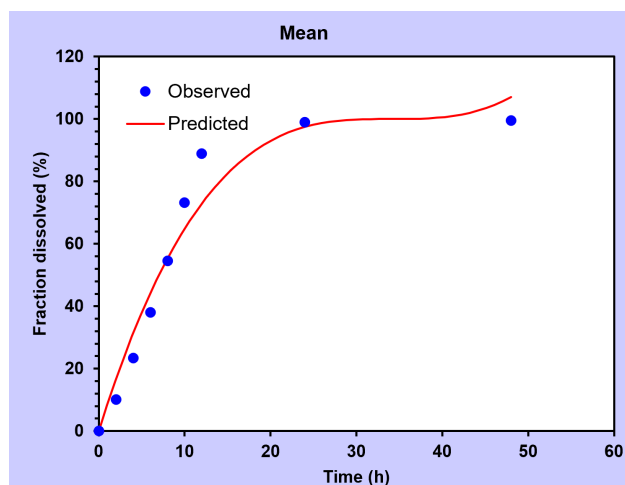


FIGURE 4.22: Hixson-Crowell Release Kinetics Plot for Eu-NFT-SLNs at pH 6.8

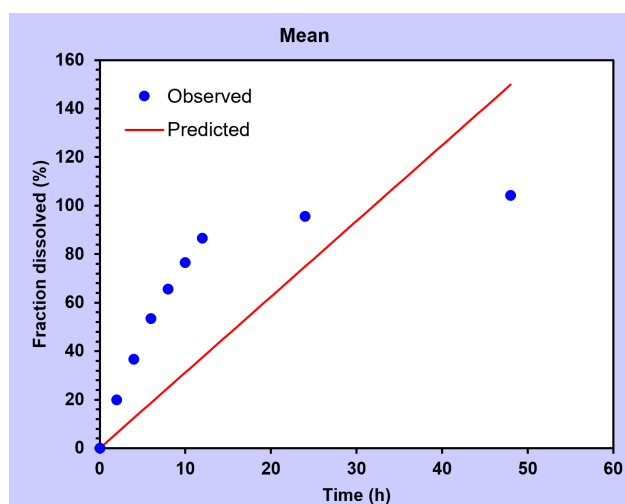


FIGURE 4.23: Zero Order Release Kinetics Plot for NFT-SLNs at pH 6.8

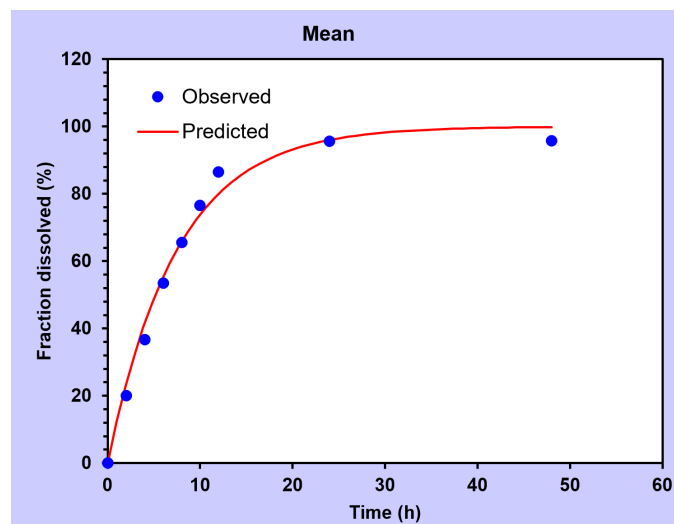


FIGURE 4.24: First Order Release Kinetics Plot for NFT-SLNs at pH 6.8

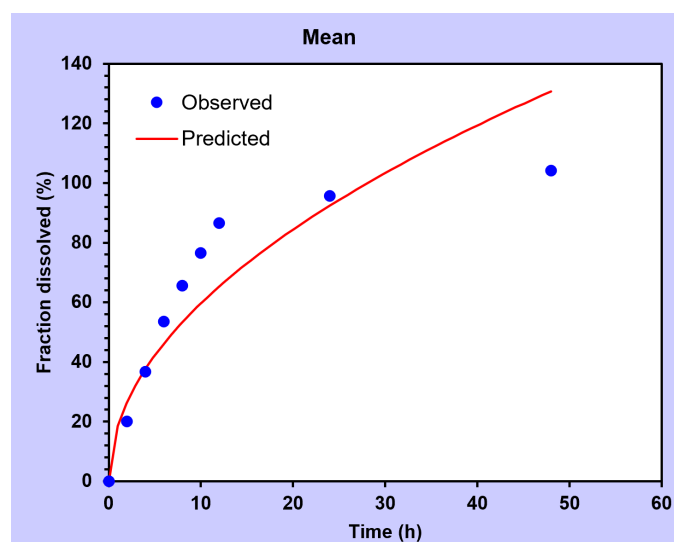


FIGURE 4.25: Higuchi Drug Release Kinetics Plot for NFT-SLNs at pH 6.8

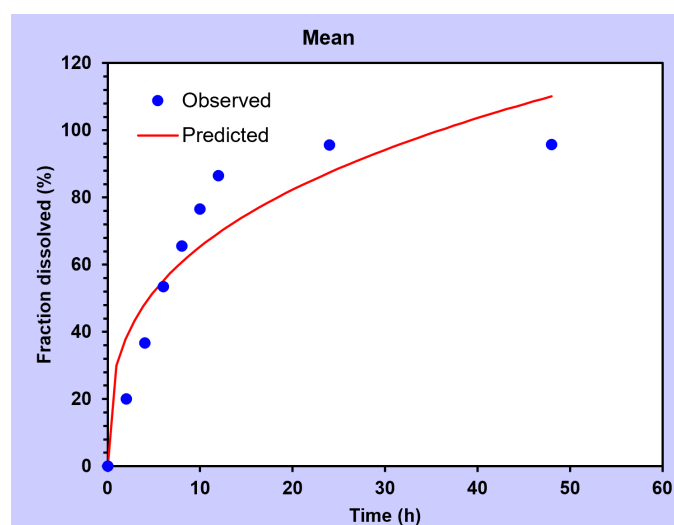


FIGURE 4.26: Korsmeyer-Peppas Release Kinetics Plot for NFT-SLNs at pH 6.8

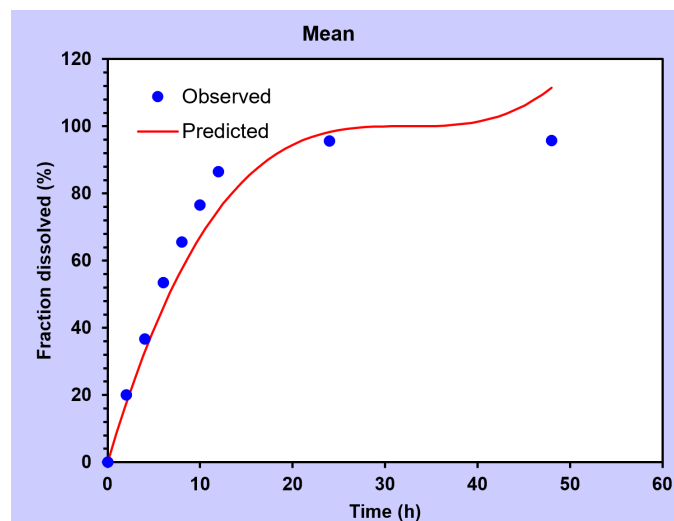


FIGURE 4.27: Hixson-Crowell Release Kinetics Plot for NFT-SLNs at pH 6.8

4.6 *In Vivo* Bioavailability Assessment in Animals

The oral pharmacokinetic behaviour of pure NFT, NFT-SLNs, and Eu-NFT-SLNs was evaluated after a single oral dose of 10 mg/kg in rats, with plasma drug concentrations analysed by non-compartmental analysis (linear trapezoidal method) using PKSolver. The resulting pharmacokinetic parameters are summarized below.

TABLE 4.9: Pharmacokinetic Parameters Compared Between Drug, NFT-SLNs and Eu-NFT-SLNs

Parameter	Pure NFT	NFT-SLNs	Eu-NFT-SLNs
$C_{\max}$ ($\mu\text{g/mL}$)	1.28	1.87	2.01
$T_{\max}$ (min)	20	120	240
$t_{1/2}$ (min)	16.6	601.8	643.8
AUC_{0-t} ($\mu\text{g}\cdot\text{min/mL}$)	37.13	394.91	1870.79
$\text{AUC}_{0-\infty}$ ($\mu\text{g}\cdot\text{min/mL}$)	37.39	416.52	2079.61
$\text{MRT}_{0-\infty}$ (min)	30.44	505.53	900.66
Cl/F	0.267	0.024	0.0048
($\text{mg}\cdot\text{kg}^{-1}/(\mu\text{g/mL})/\text{min}$)			
V_z/F ($\text{mg}\cdot\text{kg}^{-1}/(\mu\text{g/mL})$)	6.40	20.84	4.47
Relative Bioavailability	—	11.14-fold	55.61-fold

Pure NFT produced a steep, short-lived plasma concentration profile, reaching a $C_{\max}$ of $1.28 \mu\text{g/mL}$ within just 20 minutes of administration and declining rapidly thereafter, with negligible drug detectable beyond about 2 hours.

This behaviour is typical of a small, poorly water-soluble molecule administered as a simple oral solution: absorption occurs quickly wherever dissolved drug is available in the gastrointestinal fluid, but with no mechanism to sustain release, plasma levels fall as rapidly as they rise, and the elimination half-life was correspondingly short at only 16.6 minutes [127].

By contrast, both nanoparticle formulations produced far flatter, more extended plasma profiles. For the NFT-SLNs, $C_{\max}$ increased modestly to $1.87 \mu\text{g/mL}$, but this peak was reached much later, at 120 minutes, and measurable plasma concentrations persisted out to 2400 minutes (40 hours) post-dose.

The half-life extended to 601.8 minutes and the mean residence time (MRT) rose to 505.5 minutes, roughly a 36-fold and 17-fold increase, respectively, compared with the free drug. The area under the plasma concentration–time curve ($\text{AUC}_{0-\infty}$) rose from $37.39 \mu\text{g}\cdot\text{min/mL}$ for pure NFT to $416.52 \mu\text{g}\cdot\text{min/mL}$ for the NFT-SLNs, corresponding to an approximately 11-fold improvement in relative oral bioavailability.

This magnitude of improvement is consistent with what has been reported for other poorly soluble drugs once incorporated into solid lipid nanoparticles, where the lipid matrix slows gastrointestinal transit-dependent release, protects the drug from rapid metabolic or chemical loss, and thereby allows a much larger fraction of the administered dose to reach systemic circulation over time rather than being lost shortly after dosing, a pattern described broadly for oral SLN-based delivery systems [138] and for other optimized lipid nanoparticle formulations that produced markedly higher AUC and several-fold bioavailability gains relative to marketed or plain-drug references in rodent pharmacokinetic studies [139].

The Eu-NFT-SLNs formulation carried this improvement substantially further. $C_{\max}$ rose again, though only slightly, to $2.01 \mu\text{g/mL}$, while $T_{\max}$ was delayed even more, to 240 minutes — twice as long as the NFT-SLNs and twelve times longer

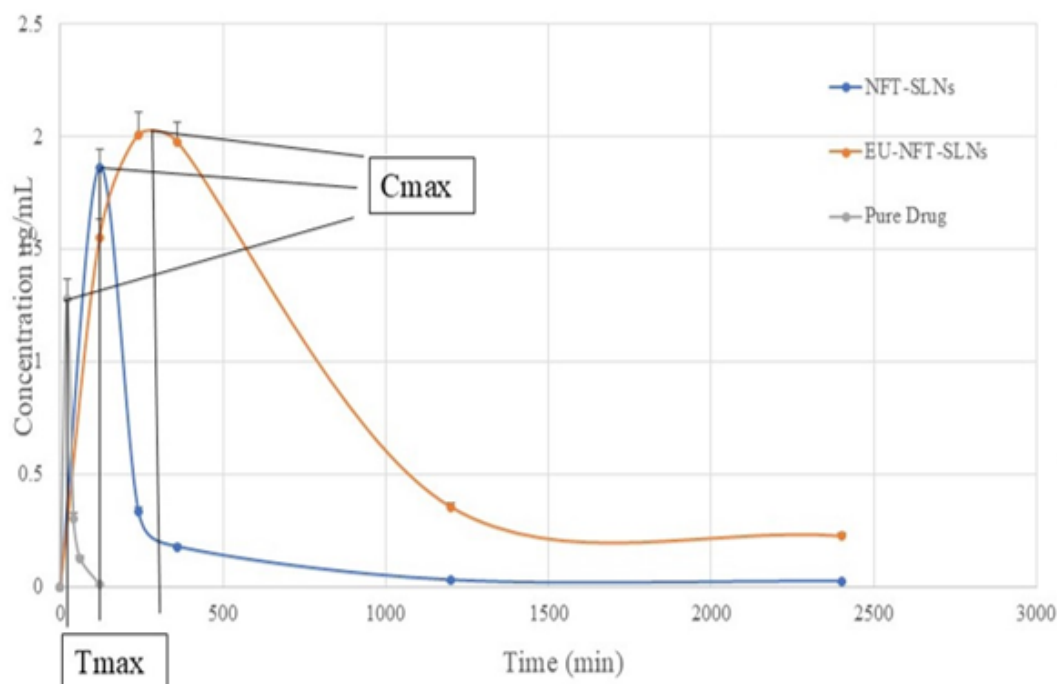


FIGURE 4.28: Concentration vs Time Graph plot for Pure NFT, NFT-SLNs and Eu-NFT-SLNs

than the free drug. The half-life (643.8 minutes) and MRT (900.7 minutes) were the longest of the three groups, and apparent clearance fell to $0.0048 \text{ (mg/kg) / } (\mu\text{g/mL})/\text{min}$, more than 55-fold lower than that of the pure drug and roughly 5-fold lower than the NFT-SLNs. $\text{AUC}_{0-\infty}$ reached $2079.61 \mu\text{g}\cdot\text{min/mL}$, representing an approximately 56-fold increase in bioavailability relative to pure NFT and a further 5-fold increase over the NFT-SLNs. The fact that $C_{\max}$ increased only modestly across the three formulations while AUC increased by one and then almost two orders of magnitude is an important observation that it indicates that the improvement in bioavailability was driven primarily by sustained, prolonged absorption rather than by a simple increase in the rate or amount of drug dissolving at any one time.

This pattern, a flatter, delayed peak accompanied by a large gain in total exposure, has been reported for other lipid-nanoparticle oral formulations, where SLN-based systems produced higher $C_{\max}$ and AUC together with increased relative bioavailability compared with marketed or free-drug formulations in rat pharmacokinetic studies [140].

The additional delay in $T_{\max}$ and the further reduction in clearance seen with the Eudragit-coated particles, compared with the NFT-SLNs, are best explained by the pH-dependent behaviour of Eudragit[®]L100 itself. This polymer is an anionic copolymer that remains un-ionized and insoluble in the acidic environment of the stomach but ionizes and dissolves once the pH rises above approximately 6, a property that is widely exploited to create enteric coatings that exclude gastric acid and release their payload only once the particle reaches the higher pH of the small intestine [141].

In the present formulation, this means the Eu-NFT-SLNs likely remained largely intact as they transited the stomach, limiting premature drug loss or degradation in the acidic gastric environment, and only began to release their payload once they reached the intestine, where absorption could then proceed over an extended period as the coating dissolved and the underlying lipid matrix gradually released the entrapped drug. This mechanism is consistent with reports that Eudragit[®]L100/L100-55-coated nanocarriers remain substantially protected in simulated gastric fluid and release their contents preferentially once exposed to intestinal-range pH, with coated carriers showing significantly less premature drug loss under acidic conditions compared with uncoated counterparts [140] and with in vivo evaluations confirming that Eudragit-based enteric nanoparticle coatings can meaningfully protect an orally administered drug from the gastric environment while permitting pH-triggered release further along the gastrointestinal tract [142]. A similarly designed core shell nanocarrier, with a pH-sensitive Eudragit[®]L100 outer layer controlling the release mechanism and protecting the encapsulated drug under simulated gastric conditions, has likewise been reported to support this kind of staged, protected release as a viable oral delivery strategy [143].

Taken together, these pharmacokinetic findings show a clear, stepwise improvement in the oral performance of nitrofurantoin as it progressed from free drug, to lipid nanoparticle encapsulation, to lipid nanoparticle encapsulation combined with pH-sensitive enteric coating. The free drug behaved as expected for a rapidly absorbed, rapidly cleared compound with minimal sustained exposure. Encapsulation within the Compritol[®] ATO 888 lipid matrix alone was sufficient to

produce an order-of-magnitude increase in systemic exposure and a substantial prolongation of residence time, most plausibly by protecting the drug from rapid loss and by slowing its release into the gastrointestinal fluid. The addition of the Eudragit®L100 coating built on this effect by preventing premature release in the stomach and concentrating drug release and absorption in the intestinal environment, which together nearly quintupled AUC relative to the uncoated particles and brought total bioavailability to roughly fifty-six times that of the free drug. These results support the rationale for combining a solid lipid matrix with a pH-responsive polymeric coating as a strategy for improving the oral bioavailability of nitrofurantoin, and are consistent with the broader body of literature describing similar two-tier lipid–polymer nanocarrier designs for other poorly soluble or acid-labile drugs [138, 139].

$$\text{Relative Bioavailability } F (\%) = \frac{\text{AUC}_{\text{NFT-SLNs}}}{\text{AUC}_{\text{Pure Drug}}} \times 100$$

$$\text{Relative Bioavailability } F (\%) = \frac{416.52}{37.39} \times 100$$

$$\text{Relative Bioavailability } F (\%) = 1114.1\%$$

$$\text{Relative Bioavailability } F (\%) = \frac{\text{AUC}_{\text{Eu-NFT-SLNs}}}{\text{AUC}_{\text{NFT-SLNs}}} \times 100$$

$$\text{Relative Bioavailability } F (\%) = \frac{2079.61}{416.52} \times 100$$

$$\text{Relative Bioavailability } F (\%) = 499.3\%$$

The relative bioavailability was calculated as **1114.1%** for the NFT-SLNs and approximately **499.3%** for the Eu-NFT-SLNs when compared with NFT-SLNs, which corresponds to an **11.1-fold** and a **5-fold** rise in drug exposure, respectively.

Several properties of the SLN system account for the improved pharmacokinetic performance of Eu-NFT-SLNs. Because the lipid matrix is at the nanoscale, it offers a large surface area that increases contact with the gastrointestinal mucosa and thereby promotes absorption[124]. NFT dissolves poorly in water, and the lipid core can enhance its solubility, whereas the surfactant layer keeps the particles well dispersed and stable inside the gastrointestinal environment. In addition, the Eudragit® L100 coating prevents the formulation from releasing its drug

prematurely in the acidic stomach and instead let's release happen in the intestine, so a greater share of the dose is available for absorption [125].

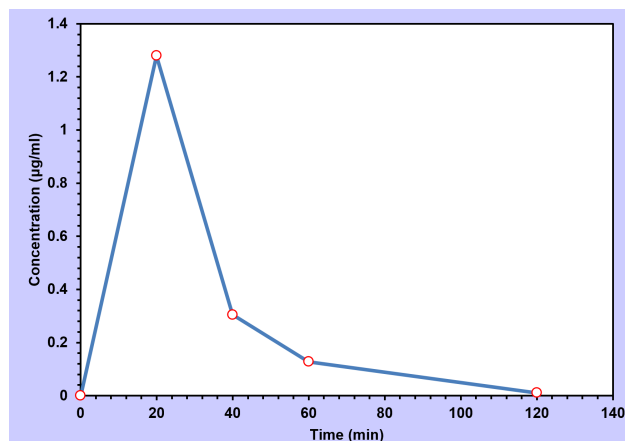


FIGURE 4.29: Concentration vs Time Graph plot for Pure NFT

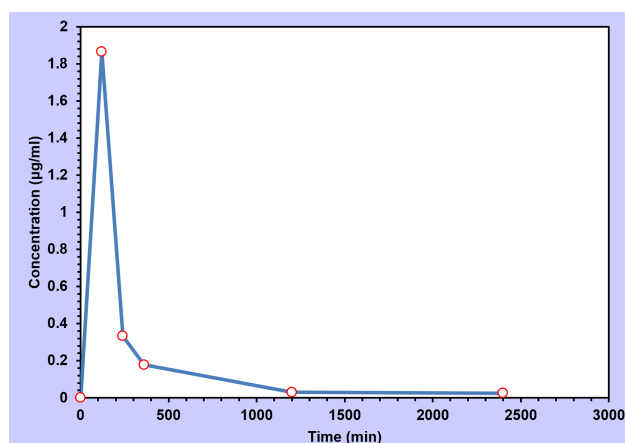


FIGURE 4.30: Concentration vs Time Graph plot for NFT-SLNs

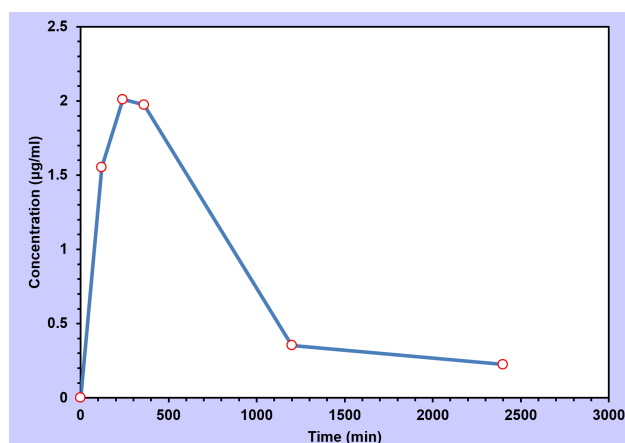


FIGURE 4.31: Concentration vs Time Graph plot for Eu-NTF-SLNs

Overall, the pharmacokinetic data show that incorporating NFT into pH - responsive SLNs made oral delivery more effective. Compared with NFT-SLNs, Eu-NFT-SLNs reached a greater peak concentration (C_{max}) at a later time point (T_{max}) and produced a bigger AUC, which suggests the drug was absorbed more efficiently and stayed available for longer [115]. Based on these results, the pH-sensitive SLN platform looks like a capable strategy for increasing the oral bioavailability of NFT.

4.7 Shelf-Life Assessment

Throughout the storage period, the optimized Eu-NTF-SLNs retained their physicochemical profile. No visible alteration occurred in color, texture, or overall appearance, indicating sound physical stability. Only minor deviations from the initial particle size, PDI, and zeta potential were recorded, and these were too small to affect the integrity or functioning of the nanoparticle system. Two factors likely explain this robustness: NFT was efficiently entrapped within the lipid matrix, and the polymer coating helped preserve particle structure while in storage. Overall, the formulation was most stable when kept under freezer conditions ($5^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and stayed fit for oral administration, while the other tested conditions were also acceptable to a certain degree.

TABLE 4.10: Summary of Eu-NFT-SLNs formulation for Stability at Multiple Storage Conditions

Storage Conditions	Condi-	Parameters	Initial	3 rd	6 th	12 th	18 th	24 th
Temp: $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$; RH: $60\% \pm 5\%$		Size (nm)	228	226				
		Size Distribution	0.189	0.36				
		PDI						
		Surface Charge (mV)	-31.8	-29.1				
		Drug Encapsulation (%)	79.20%	76.50%				

Table 4.10 continued from previous page

Storage tions	Condi- tions	Parameters	Initial	3 rd	6 th	12 th	18 th	24 th
Temp: 5°C ± 2°C; RH: 60% ± 5%		Size (nm)	228	231				
		Size Distri- bution Index (PDI)	0.189	0.195				
		Surface Charge (mV)	−31.8	−28.2				
		Drug Encapsu- lation (%)	79.20%	78%				
Temp: 40°C ± 2°C; RH: 75% ± 5%		Size (nm)	228	256.6				
		Size Distri- bution Index (PDI)	0.189	0.37				
		Surface Charge (mV)	−31.8	−13.1				
		Drug Encapsu- lation (%)	79.20%	75.61%				

The stability for entire shelf life of the NTF-SLNs and Eu-NTF-SLNs is planned to be monitored for 24 months under the recommended storage conditions. At set time points, each formulation will be tested for all the parameters i.e; PS, PDI or ZP so that any shift in their physical or chemical properties can be tracked. The data collected will then be used to judge the stability of the optimized formulation and its suitability for storage across the study period [108, 109].

Chapter 5

Conclusion and Future Recommendations

This study centered on the formulation and optimization of pH-sensitive SLNs loaded with NFT and with Eudragit® L100 as coating, as an approach for improving bioavailability through oral delivery.

An RP-HPLC method for the quantitative determination of NFT was developed and validated. It performed acceptably and was successfully used to measure drug content and EE, and to support the dissolution and pharmacokinetic studies.

A BBD was applied to examine how solid lipid concentration, Total surfactant quantities, and Eudragit® L100 concentration influence the major quality characters of the particles. The finalized formulation was prepared by combining hot homogenization with ultrasonication, produced a drug carrier at the nanoscale showing favorable physical and chemical characteristics.

The optimized Eu-NFT-SLNs had a particle size of 228 nm, a PDI of 0.189, and a zeta potential of -31.8 mV. EE was 85.96% for the NFT-SLNs and 79.20% for the Eu-NFT-SLNs. NFT was therefore incorporated efficiently into the lipid matrix, and the coating step lowered drug loading only slightly.

SEM confirmed that nanoparticles had formed, showing mostly spherical particles with fairly smooth surfaces and little aggregation, which reflects the stabilizing action of the chosen formulation components.

Once NFT was encapsulated, the XRPD patterns revealed a marked drop in the intensity of its typical crystalline reflections. This points to a lower degree of crystallinity, with the drug being molecularly or amorphously dispersed throughout the lipid carrier rather than existing as ordered crystals.

FTIR analysis detected the characteristic functional groups of both NFT and Eudragit[®] L100 in the optimized formulation, without any major peak shifts or losses. This indicates good compatibility between the drug and excipients and confirms that NFT was successfully incorporated into the SLNs.

The dissolution studies revealed pH-dependent release from the NTF-SLNs. In acidic medium (1.2 pH), the Eu-NTF-SLNs released only a small portion of the drug, showing that NFT was well protected from premature release under gastric conditions. In phosphate buffer (6.8 pH), release was considerably greater, which confirms that the enteric coating dissolved and let the encapsulated drug out. The NFT-SLNs, by comparison, released more drug in the acidic medium, highlighting the contribution of the polymer coating to site-specific release.

Rabbits dosed with Eu-NFT-SLNs absorbed considerably more NFT into the bloodstream than those given NFT-SLNs or the unformulated drug, according to the pharmacokinetic data. Several features of the formulation probably account for this. The particles are extremely small, the drug is well dispersed inside the lipid core, the Eudragit[®] L100 layer keeps the drug away from the acidic stomach, and release from the particles is gradual. Acting together, these properties improved uptake from the gut and kept NFT in circulation for longer.

Overall, coating NFT-loaded SLNs with Eudragit[®] L100 gave particles with desirable physical and chemical characteristics, release that responded appropriately to pH, and better performance in living animals. These findings suggest that this lipid nanoparticle system is a strong candidate for oral NFT delivery, with realistic potential to raise the drug's bioavailability and clinical effectiveness.

5.1 Future Recommendations

- i. Conduct stability studies according to ICH guidelines to evaluate the shelf life and storage stability of the optimized formulation.
- ii. Carry out pharmacokinetic studies in a larger group of animals to strengthen the statistical reliability and reproducibility of the results.
- iii. Use additional techniques such as Differential Scanning Calorimetry (DSC) to further evaluate thermal behavior, internal structures, and drug distribution.
- iv. Conduct studies in suitable infection models to establish the relationship between enhanced bioavailability and therapeutic efficacy.
- v. Investigate scale-up feasibility and process optimization for potential industrial manufacturing and commercialization.

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