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DRUGS LOADED IN BILOSOMES FOR THE TREATMENT OF GASTROINTESTINAL CANCERS: A COMPREHENSIVE REVIEW

Shaima R. Banoon[1*], Athraa Haitham Sarhan[1], Fatima Jassim Ibrahim[1], Zahraa Abed Hussein[2], Abdolmajid Ghasemian[3]

[1]Department of Biology, College of Science, University of Misan, Maysan, Iraq
[2]Al-Manara College for Medical Sciences, Maysan, Iraq
[3]Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran

Abstract. Oral drug delivery remains the most widely studied and utilized method of drug administration due to the convenience, safety, compliance, and low cost associated with this route. Nevertheless, only a small fraction of orally administered investigational drug candidates successfully reach the market. One of the primary reasons for poor bioavailability and the high attrition rate of drugs undergoing Phase I clinical trials is the challenging permeability and solubility profile, which oral drugs must contend with. GI cancers have very unique challenges for drug carriers that are most noted for drug delivery to other tumors. GI cancers are of particular interest because conventional treatments fail to disseminate drugs to tumorous tissue adequately. This review aims to explore bilosomes as new, innovative, and unique surfactant-based nanocarriers that have been studied to effectively deliver biologically active drugs to the upper GI tract, especially for the treatment of organic and non-solid GI cancers like esophageal and pancreatic tumors. Bilosomes improved the stability and permeability of encoated drugs. As such, prevention of the degradation of the drug is essential to improve the local therapeutic effect and enhance oral absorption and bioavailability. Drug formulations that show a change in physicochemical properties, such as size, zeta potential, and pH, have also been shown to exhibit changeable release profiles from bilosomes. Therefore, more studies need to be done to fully understand how the drug-to-bilosome ratio can affect the physiological, morphological, and biopharmaceutical properties of the drug formulation. Further, exploring the insertion of new bile acids into bilosomes preparations can yield another avenue for increasing the viability of the end formulations for use with drug candidates with varied hygroscopicity and hydrophobicity. Moreover, modified bilosomes have been promising in this regard.

Keywords: *Bilosomes, gastrointestinal cancers, drug delivery, cancer therapy.*

Corresponding Author: *Shaima R. Banoon, Department of Biology, College of Science, University of Misan, Maysan, Iraq, e-mail: shimarb@uomisan.edu.iq*

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1. Introduction

Globally, cancer remains one of the most significant health issues. Patients with GI series cancer have a poor prognosis rating; not only do they suffer greatly from toxicity, but their life quality is also severely compromised (Ferlay *et al*., 2021). Among them, gastrointestinal (GI) cancers contribute to high mortality rates, necessitating effective treatment strategies. Conventional chemotherapy and radiotherapy are often accompanied

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by systemic toxicity, leading researchers to explore nanoparticle-based delivery systems (Bordry *et al*., 2021). In contrast, a new intelligent approach based on special natural biological properties and delivery is taking the spotlight: the use of natural lipids is becoming a burgeoning tool for biotherapy targeting tumors and cancer cells (Shukla *et al*., 2018; Calzoni *et al*., 2019). These abnormalities are repaired during metastasis, while normal cells stay intact. Bilosomes made of natural lipids have acquired interest in the field of oral controlled drug delivery technology (Zarenezhad *et al.,* 2023). The antimicrobial properties of nanoparticles in biofilms have been explored, indicating their potential in combating resistant cancer cells within the GI tract (Al-Saady *et al.,* 2022).

Bilosomes are closed bilayer structures of non-ionic amphiphiles correlated to non-ionic surfactant vesicles but incorporating bile salts (Rajput & Chauhan, 2017). This innovation could aid in the oral delivery of biological actives for sustained release. Several research groups have successfully utilized bilosomes for glucose monitoring, oral vaccine delivery, and other drug applications. The unique properties of bile salts support the complexation of actives with phospholipid vesicles to facilitate cellular interaction and internalization (Wilkhu *et al*., 2013; Salapa *et al*., 2020). Recent developments in the formulation and characterization of bilosomes and various applications in delivering drugs, proteins, peptides, and vaccines are summarized. An overview of the advantages and potential challenges in the development of bilosomes is also provided.

1.1. Overview of Gastrointestinal Cancers

GI cancers are one of the leading causes of cancer mortality worldwide, posing a severe economic and social burden on society and include cancers of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, and anus (Bordry *et al*., 2021; Xie *et al.,* 2021). Other diseases of the GI tract include jaundice due to liver obstruction, hepatic encephalopathy due to liver failure, and esophageal varices due to portal hypertension, all of which can also lead to death. According to the International Agency for Research on Cancer (IARC), GI cancer has the highest incidence accounting for 112.9 per 100,000 individuals, with the highest mortality of 58.8 per 100,000 individuals. According to published data, GI cancer accounts for 45% of cancer-related death in China, overtaking lung cancer (Dyba *et al.,* 2021; Gu *et al*., 2022). GI cancers are more common in males than females. Among GI cancers, liver cancer is one of the leading causes of cancer deaths in both males and females. In men, stomach cancer ranks fourth in mortality and esophageal cancer ranks sixth (Ilic & Ilic, 2022). In women, stomach cancer is ranked sixth and pancreas cancer is ranked seventh. In addition, gallbladder cancer is more prevalent in females than males, and rectum cancer is more common in males than females. According to recent studies, oxidative stress plays a crucial role in tumor progression, with oxidized low-density lipoproteins (oxLDL) contributing to inflammation and cancer metastasis (Falih *et al*., 2021). This connection highlights the importance of drug carriers capable of targeting oxidative stress pathways in GI cancer therapy.

GI cancer is one of the most common and aggressive malignancies (Salapa *et al.,* 2020). Fighting GI cancer is challenging due to the pathogenesis of the disease, which involves multiple pathways and numerous molecules. As GI cancer may have no or few obvious symptoms in the early to intermediate stage, it is usually detected at an advanced stage, which leads to a poor prognosis. Given the combination of surgery, endoscopy, radiotherapy, targeted therapy, chemotherapy, and immunotherapy, GI cancer remains one of the most deadly tumors, accounting for 26% of cancer mortality globally.

Therefore, the search for potential materials for improving the therapeutic effect on GI cancer is urgently needed.

1.2. Challenges in Current Treatment Approaches

GI cancers, encompassing malignancies like esophageal, gastric, liver, pancreatic, and colorectal cancers, are among the leading causes of cancer-related morbidity and mortality globally. Their unique anatomical and physiological characteristics, such as the rich vascular supply of the GI tract and the presence of specialized cell types, can be leveraged for the targeted delivery of drugs, especially anti-cancer agents like chemotherapeutics and biotherapeutics. However, despite recent advances in pharmacology and drug formulations that have improved treatment efficacy, GI malignancies remain difficult to treat, presenting a significant challenge to modern medicine (Balakrishnan *et al.*, 2017; Salapa *et al*., 2020). The limitations of traditional and metal nano drug delivery systems are notable, including a lack of chemical and structural versatility, toxicity, side effects, low efficiency, the induction of unwanted immune responses, and the retention of the majority of the administrated dose outside the desired target organs or tissues (Jamkhande *et al*., 2019), have led to the development of novel nano-based formulations. The role of nanoparticles in gene therapy has been extensively reviewed, underscoring their potential for precise and efficient delivery of therapeutic agents to cancer cells (Rabeea Banoon *et al*., 2024). Additionally, artificial intelligence-driven nanotechnology development has further optimized nanoparticle design for enhanced cancer treatment efficacy (Hassan *et al.*, 2023).

Against this backdrop, novel and effective therapeutic agents, specifically drug delivery vehicle structures or lipid nanocarriers have emerged, promising to address these challenges. Bio-inspired spherical vesicular carriers mimicking biological entities, such as viruses and cells, have shown great potential for drug delivery systems (Pensado *et al.*, 2014; Pimentel-Moral *et al.*, 2018). This biotechnology represents the frontier in drug delivery technology for the treatment of GI cancers. Lipid bilayer-cored bio-inspired vesicles (i.e., bilosomes) are exceptional nano-sized drug delivery systems characterized by unique amphoteric bilayer structures. They consist of a phospholipid core encapsulated within a giardial bile salt polymer shell with a natural bioactivity profile. Bilosomes have the capacity to encapsulate a vast range of lipophilic compounds and proteins with advantages over liposomes and polymeric micelles. These bio-inspired nanocarriers have the potential to overcome biological barriers while withstanding enzymatic degradation and substantially improving the oral bioavailability of drug compounds otherwise poorly absorbed by the intestine (Wang *et al.*, 2021). Current treatment strategies for GI malignancies are detailed with regard to their limitations and possible improvements. Past and current attempts in the development of bile salt-based drug delivery systems, specifically bilosomes, are reviewed. Finally, strategies for the development of bile salt-breakable bilosomal formulations for the transportation of anticancer compounds in the colorectal and pancreatic drug delivery routes, capabilities of addressing the unanswered challenges posed by current treatment approaches, are discussed.

2. Bilosomes as Drug Delivery Systems

Bilosomes are novel drug delivery systems that are gaining popularity across academia and industry (Zarenezhad *et al*., 2025). Bilosomes are bilayered vesicles that combine the biocompatibility of liposomes, the targeting potential of niosomes, and the

ability of bile salts to enhance drug penetration across the intestinal membrane. Bilosomes consist of a phospholipid bilayer shell coated with bile salts and can incorporate hydrophilic and hydrophobic drugs. Composition modifications in terms of the molar ratio of bile salts and phospholipids lead to variations in physicochemical and stability parameters. Several studies have demonstrated the potential of bilosomes to deliver drugs, proteins, peptides, and vaccines (Rajput and Chauhan, 2017). Furthermore, advancements in computational drug discovery have identified anti-cancer compounds targeting RNA-binding proteins like RBFOX1, which could be effectively delivered using bilosomes (ul Qamar *et al.,* 2025).

Bilosomes are vesicular carriers with a core-shell structure composed of a phospholipid bilayer shell. The core of bilosomes consists of bile salts (natural surfactants) (Zarenezhad *et al.,* 2023). Bilosomes are designed to deliver therapeutic moieties such as drugs, proteins, peptides, or a combination of drugs and other bioactives. The bile salt coating can improve the oral delivery of bioactives by enhancing penetration and absorption across the intestinal membrane and improving lymphatic transport. Amphipathic bile salts also stabilize the vesicles against bile salts and enzymatic degradation in the GI tract (El-Nabarawi *et al.,* 2020). This makes bilosomes an attractive alternative to liposomes and niosomes.

2.1. Definition and Characteristics of Bilosomes

Bilosomes are bile salt-based drug delivery systems with unique structural and functional characteristics that enable them to transport drugs successfully through different biological barriers. Bilosomes are vesicles with a multi lamellar lipid structure formed by self-assembly when bile salts are mixed with phospholipids. These vesicles are typically between tens and hundreds of nanometers in diameter or 0.05 to 5 μm. In addition to their membrane phospholipid layer, bilosomes have a lipid bilayer structure around the aqueous core stabilized by bile salts, making them more resistant to salts and bile acids compared to pure liposomes. Bilosomes have natural mucoadhesive properties due to their ability to interact with other polyanionic macromolecules (Rajput & Chauhan, 2017; El-Nabarawi *et al*., 2020). The glycoprotein mucins formed in the GI tract have a low isoelectric point, mostly around 5-6, and have a high negative charge, to which bile salt-coated vesicles can adhere. With the help of an enterocyte endocytosis or a transcytosis pathway, this mucoadhesion can improve the uptake of the vesicles, making them good candidates for the oral delivery of drugs. Bilosomes can also avoid the acidic environment of the stomach and have good permeation with a vesicle-induced permeability (VIP) value in the range for good permeability (>1.0). With these unique structural and functional properties. Their ability to encapsulate a wide range of therapeutic agents, from chemotherapeutics to genetic materials, makes them highly versatile. A recent study on graphene oxide nanoparticles and doxorubicin demonstrated significant cytotoxic effects against colorectal cancer cells, reinforcing the potential of nanocarrier-based delivery systems (Banoon & Ghasemian, 2022).

Phospholipids are biodegradable compounds available from natural sources, rendering bilosomes biocompatible. Generally recognized as safe (GRAS) by the FDA and Generally Accepted as Safe (GAS) by the European Commission, different types of phospholipids can be used in bilosomal formulations. For instance, the phospholipid filler used with bile salts may either be phosphatidylcholine (PC), which has a high drug encapsulation efficiency and good membrane fluidity but is more costly, or hydrogenated soy phosphatidylcholine (HSPC), a semisynthetic plant-derived egg phospholipid (Singh

et al., 2017). The protein-to-surfactant ratios of bilosomes with phospholipids affect bilosomal size and charge, and the use of nonionic surfactants may reduce irritate action and tolerance while lowering cost. Biocompatibility testing in a suitable in vitro and in vivo model with a standard positive and negative control group should be performed early in the formulation development to avoid late failures (Zarenezhad *et al.*, 2023).

2.2. Advantages of Bilosomes over Conventional Drug Delivery Systems

In general, lipid nanocarriers have advantages of low toxicity and high drug load to controlled delivery. However, some of them are susceptible to the gut lumen and are lysed by enzymes and bile acids. Hence, preparation of bilosomes containing bile salts have overcome this problem in GI tract (Liu *et al*., 2022). The prescription of modern medicines, which include biological macromolecules, is usually with water. These drug formulations are not always safe and effective, as they may be capable of causing systemic and off-target effects to normal organs. Moreover, there in vivo application often leads to undesired stimulation of biological fluids, resulting in their rapid clearance before reaching the target tissues (Rajput & Chauhan, 2017). Hence, there is a growing interest in devising efficient drug-carrier systems capable of transporting chemicals through physiological fluids without degrading their chemical nature.

Table 1. Advantages of bilosomes over other lipid nanocarriers

Nanocarrier	Advantages	Disadvantages
Bilosomes	Containing positively and negatively charged molecules, high loading of cargo, targeted delivery, low toxicity, bioavailability, resistance and stability in GI tract,	Low aquatic solubility, sensitivity to light, oxygen and light
Niosomes	Surfactants are available for their synthesis, containing positively and negatively charged molecules, targeted delivery, low toxicity, stability at osmotic conditions, bioavailability	High costs, aggregation possibility, time-consuming techniques, low physical stability, drug leakage, insufficient loading capacity, low shelf life, high protein and peptide required, Gastric irritation, low lymphatic transport
Liposomes	Containing positively and negatively charged molecules, high loading of cargo, targeted delivery, low toxicity, bioavailability, surface and pharmacokinetic modifiable	High cost, Leakage of cargo, oxidation and lysis, low solubility and stability in liquid state, short half-life, disruption and low permeability in GI tract, low lymphatic transport, needing special storage conditions
Exosomes	Natural ability to transfer cargo, mimics cell membrane, improve bio-distribution,	High cost, side effects, low load and insufficient therapy, sensitivity to GI tract, difficult in quality control, difficult to store and transportation, physiochemical instability, preferred for protein carriage
Ethosomes	Ease of preparation, biodegradable, low toxicity, softness, malleability,	Oxidative degradation, purity of natural phospholipids, high cost, lack of analytical method for drug delivery, variability of distribution kinetics

There are various drug delivery systems such as lipid nanocarriers, polysaccharides, polymeric nanoparticles, solid lipid nanoparticles, dendrimers, and nanoemulsions (Torchilin, 2007; Zhang *et al*., 2013; Wang *et al.*, 2019; Peer *et al.*, 2020). However, these traditional carriers often have shortcomings, including biodegradability, stability, and food intake. Liposomes, the first biocompatible nanocarrier prepared by Bangham et al., are biodegradable particles that could solubilize, transport, and deliver hydrophilic or hydrophobic molecules. However, they are susceptible to the GI tract and have poor intestinal absorption, similar to all phospholipid-based carriers (Table 1). Non-toxic, controlled, and targeted drug delivery, biocompatibility, and degradability are provided by lipid nanocarriers. Compared to other lipid-based carriers, bilosomes offer enhanced mucoadhesion, stability, and bioavailability. Research on the conjugation strategies of functionalized iron oxide nanoparticles as malaria vaccine carriers has highlighted the adaptability of similar nanocarriers in targeted drug delivery applications (Al-Abboodi *et al*., 2021).

3. Drugs Utilized in Bilosomes for Gastrointestinal Cancers

GI cancers are among the most prevalent malignancies worldwide, with high morbidity and mortality rates. Gemcitabine has been used as a chemotherapeutic drug to treat various cancers, including GI tumors. However, due to the rapid metabolism of gemcitabine by the enzyme deoxycytidine kinase and its poor hydrophobic nature, a high dose of gemcitabine needs to be administered, affecting normal cells and causing dose-dependent toxicity. The low permeability of drug permeation across the intestinal epithelia is due to its higher hydrophilicity (Log P=-0.92) (Nakamura *et al*., 2012).

Cisplatin is used for the treatment of various tumors, including those of the GI tract. However, it is associated with systemic toxicity and drug resistance (Lee *et al.*, 2022). Encapsulation of cisplatin in bilosomes has been used to deliver the drug across the intestinal barrier and contribute towards healing peptic ulcers (Limongi *et al*., 2021). Curcumin (diferuloylmethane) is a hydrophobic polyphenol compound with anti-cancer properties. It has low bioavailability due to its hydrophobic nature. Nano-formulation of curcumin in bilosomes has been proposed for effective targeting of colorectal cancer at low doses (Salapa *et al*., 2020; Abbas *et al*., 2022). 5-Fluorouracil (5-FU) is an anti-metabolite drug used as a chemotherapeutic agent against numerous types of cancers. Bilosomal nanocarriers of 5-FU (nBLA) have been synthesized to improve the bioavailability of the drug due to high lipid nature and long-term stability (Faustino *et al*., 2016). Sintilimab is a PD-1 inhibitor and a monoclonal antibody used for the treatment of cancers. Bilosomes containing sintilimab (BLSM) and pitavastatin have been formulated to deliver the drug via oral administration for enhanced therapeutic efficacy against GI tumors (Sen *et al.*, 2024). Recent findings suggest that niosomes containing isomeldenin plus lupeol induce apoptosis in several cancer cell lines and modulate apoptotic gene expression, indicating the potential for bilosomes in similar applications (Ali *et al.*, 2024). Furthermore, medicinal plant extracts have been identified as promising candidates for respiratory virus treatments, suggesting their potential incorporation into bilosomal formulations for multi-target therapy (Alrashedi *et al*., 2021).

3.1. Common Chemotherapeutic Agents

Focusing on traditional chemotherapeutic agents, several compounds are noted for their effectiveness against GI cancers and compatibility with bilosomal delivery. Based

on an extensive review of the literature, the anticancer drugs currently employed and explored for bilosomes are summarized as follows, along with their mechanisms of action.

5-FU inhibits the enzyme thymidylate synthase and starves the cell of dTMP required for DNA synthesis and repair. This mechanism of action makes 5-FU most effective against rapidly dividing cells, as found in solid tumors. While having broad-spectrum efficacy against various cancers, 5-FU is most commonly employed in the treatment of GI cancers (Entezar-Almahdi *et al.*, 2020; Salapa *et al*., 2020)

Doxorubicin, an anthracycline antibiotic, exerts its anticancer effects via multiple mechanisms. Doxorubicin intercalates into DNA and disrupts topoisomerase II activity, leading to inhibition of DNA replication and repair. It can also bind to cell membranes and generate reactive oxygen species, resulting in membrane lipid peroxidation and apoptosis (D'Angelo *et al*., 2022).

Paclitaxel is a natural compound derived from the Pacific yew tree. The cancer cell growth inhibition mechanism of paclitaxel has been related to its action on microtubules, where paclitaxel binds and stabilizes the polymerized form of tubulin, blocking the cells in the G2/M phase of the cell cycle of cancer cells (Sharifi-Rad *et al.,* 2021).

Gemcitabine is widely used in the treatment of pancreatic cancer. This nucleoside analogue is used as a prodrug that requires conversion into its active phosphorylated forms, gemcitabine monophosphate, and gemcitabine diphosphate. These metabolites interfere with the incorporation of deoxynucleotides into newly synthesized DNA strands, leading to DNA chain termination and inhibition of DNA synthesis and repair (Han *et al*., 2022).

3.2. Emerging Targeted Therapies

In contrast to conventional chemotherapy, emerging targeted therapies including drug antibodies, peptides, and a variety of natural products and conjugates are being employed in bilosomes to treat GI cancers, thereby improving drug targeting effectiveness. These drug carriers show the potential of improving oral bioavailability of poorly water-soluble agents by bringing them into a solution or stabilizing them in a specific state prior to absorption, and utilizing the lipophilicity of the drug to enhance permeability. Natural products isolated from plants, animals, fungi, or marine sources have shown mechanisms against GI tumors regardless of the phase of the life cycle (Salapa *et al*., 2020; Xie *et al*., 2020). Novel drug antibodies or antibody-drug conjugates (ADCs) have emerged as a differentiated, potent, and selective class of biologics to address unmet needs, promising a new wave of innovation and market opportunities. Peptides, alike antibodies, show the potential to interrupt the tumor cells interaction and cease cellular proliferation.

4. Mechanism of Action of Drugs in Bilosomes

Bilosomes may undergo sedimentation or aggregation, rendering the vesicle dispersion ineffective for drug delivery. Additionally, passage through the protective mucus layer of the GI tract may prevent the accumulation of the vesicles in the enterocyte due to the negatively charged phospholipid coating of the vesicles, causing a repulsive electrostatic interfacial barrier. Mechanisms underlying the action of drugs loaded in bilosomes in vivo before drug release are demonstrated due to anatomical and physiological features, locally administered bilosomes can take advantage of the EPR

effect, which is one of the crucial factors for drug delivery to solid malignancy (Shukla *et al*., 2018; Wang *et al.,* 2021; Zarenezhad *et al.,* 2023). Unlike normal tissues, tumor-associated blood vessels are composed of endothelium with high porosity contributed by fenestration and lack of tight junctions. In addition, the higher interstitial fluid pressure, dense extracellular matrix, and outward solvent flow due to a higher concentration of macromolecules in the interstitial space create a tendency for extravasated nanoparticles to remain in the tumor tissues instead of returning to blood circulation. Such enhanced permeability and retention effects have been utilized in drug targeting for both conventional and nano-based therapies. After drug release into the GI tract, bilosomes could still accumulate in the GI cancer tissues via mechanisms other than EPR (Figure 1). The effect of aerobic exercise on tumor size and gene expression in breast cancer models has demonstrated the importance of metabolic influences on drug efficacy, which could be leveraged in bilosome-based therapies (Banoon *et al.,* 2023).

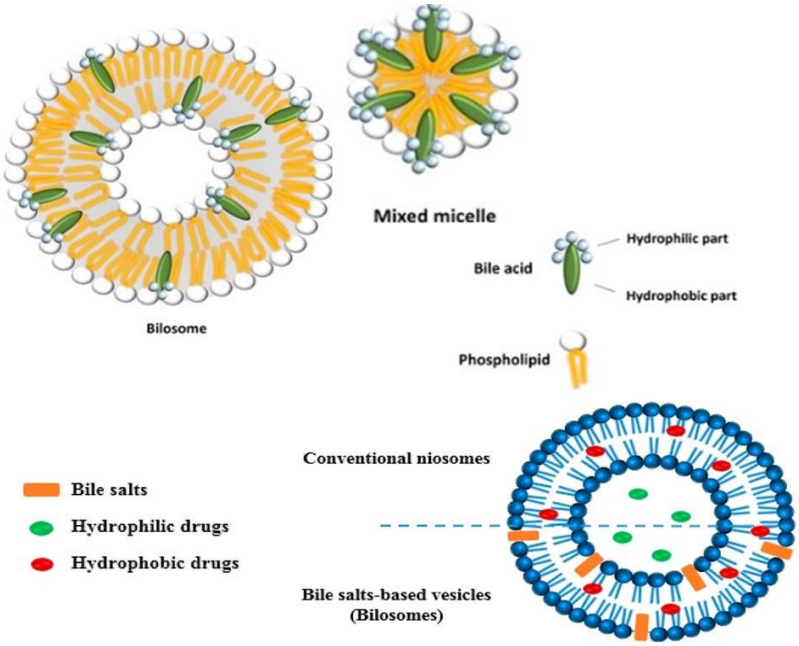

Figure 1. Bilosomes structure including phospholipids and bile salts which contain and carry both hydrophobic and hydrophilic drugs

Bilosome nanoparticles could adhere to the mucus layer covering the surface of the GI tract due to electrostatic interactions. The mucus penetrability would allow the bilosome nanoparticles to moot viscous resistance and ambulate nearer to the epithelial surface for transcellular transport. Bilosome nanoparticles may be internalized also by passive transport mechanisms including caveolae-mediated endocytosis and micropinocytosis, swelling invaginative pits that delves into the cytoplasm to form vesicles and allow for cellular uptake of bigger particles (Wang *et al.*, 2021; Zarenezhad *et al*., 2023). After biliary excretion from the liver, bile salts derived from tumor tissue could function as an intrinsic biotargeting moiety to bind thaimine salt and bile acid-conjugates drugs to the bile acid receptors of apical sodium-dependent bile salt transporter (ASBT) on the apical membrane of intestinal epithelial cells (Nallamothu *et al*., 2023). Thereafter, the drug release may be triggered by the low pH in the endosomal compartment. Following the saturation of ASBT, the internalized vesicles may further fuse with lysosomes where

endosomal drug release occurs before drug migration into the cytosol. Following the endosomal escape, drugs would be translocated into the cell nucleus to exert their therapeutic efficacy. Thus, montmorillonite clay nanodisks stabilized by polycation-chitosan and bile salts including soduim cholate and soduim deoxycholate have been fabricated and employed as the pH-responsive nanocarrier to co-deliver fluorouracil and doxorubicin in vitro (Deng, 2022).

4.1. Enhanced Permeability and Retention (EPR) Effect

A large number of anticancer agents such as lipophilic drugs have been found ineffective in treating various cancers such as hepatocellular carcinoma (HCC) and colon cancer due to experimental and clinical obstacles of drug delivery. Poor pharmacokinetics, unexpected delivery to non-target organs, incomplete or inadequate intracellular uptake, and consequent side effects are some of the major limitations of effective drug delivery for cancer therapy (Pavlović *et al*., 2018; Kharouba *et al*., 2022). One prominent problem in conventional anticancer therapies is the inability of the drug to penetrate the blood-tumor or lymph-hyperplasia barrier. These circumferential barriers prevent the transport of therapeutic agents, including bioactive macromolecules such as anticancer drugs, monoclonal antibodies (mAbs), small interfering RNA (siRNA), DNA/cDNA, proteins, and other biological macromolecules from blood flow to the tumor intracellular space and vice versa. Drugs that reached the tumor tissue are rapidly reabsorbed into the blood circulation and lymphatic system due to the high interstitial fluid (IF) pressure. Tumors such as those in the GI tract, brain, breast, liver, lung, pancreas, prostate, ovary, and stomach that block the movement of nanocarriers usually produce vascular endothelial growth factor (VEGF) continuously to promote abnormal angiogenesis. Due to genetic mutation-induced hyperpermeability of blood vessels, unwanted hole-like fenestrae are formed in their walls that are >500 nm in diameter compared to 10–30 nm in normal blood vessels. As a result, macromolecules and nanocarriers of >500 nm diameter have high blood retention in tumors vascularized by immature blood vessels. Undesired rapid uptake by the liver, spleen, lymph nodes, and lungs is prevented by shortening the individual circulation life of the macromolecules and nanoparticles compared to the longer plasma half-lives of such nanocarriers in the rest human organ tissues (Amin *et al*., 2022; Ejigah *et al*., 2022). Tumor penetration and interstitial diffusion of the macromolecules also become favorable due to the absence of uploaded ECM in the vascular wall and between tumor cells (Mohammadabadi *et al*., 2020). Nanocarrier accumulation in tumors is also enhanced by a leaky lymphatic system of immature and dilated lymphatic vessels. Interstitial uptake of components of blood solutes, lipoproteins, and inflammatory mediators from the tumor tissue is made constant due to an increased vascular to IF ratio (Tee *et al*., 2019). Consequently, the observed higher concentration of such molecules in the surrounding fluid environment of the tumor is >2-, 5-, 10-, 20-, and >100-fold higher than in the normal surrounding parenchyma of the heart, thymus, liver, spleen, kidney, and lungs, respectively. Collectively, these tumor-associated pathophysiological conditions lead to targeting the macromolecules and nanoparticles to the tumor tissue by a unique mechanism called enhanced permeability and retention (EPR) effect (Landsiedel *et al.*, 2012).

4.2. Intracellular Drug Delivery Mechanisms

Bilosomes, the latest generation of vesicles prepared with the natural surfactant bile salts and phospholipids, are considered promising for oral delivery, treatment of tumors, and local immunization against gut pathogens. The main driving concept for this vesicle technology is related to the high toxicity of several anti-cancer molecules when administered systemically. The aim is to use bile salts, the main components of the GI tract, as carriers to deliver therapeutics to intestinal malignant tumors. This specific drug targeting mechanism is based on the ability of bile salts to bind to and solubilize cholesterol-rich microdomains in the cell membranes, enhancing the endocytic engulfment of the vesicles by target cells. Furthermore, the vesicles can be delivered to the lymphatic system via M cells, immunizing gut-associated lymphoid tissues (Abdel-Moneum *et al*., 2023).

A better understanding of the mechanisms involving increased penetration of bile salts for drug delivery at the cellular level, including digestion, uptake, and intracellular metabolism, is currently needed. In addition, the design of liposome constructs triggered by specific stimuli (pH, temperature, or enzyme-triggered) may be an important enhancement in oral drug delivery. Fully understanding the presented mechanisms, reacting accordingly in modifying the biles, or engineering liposome structures may lead to smarter vesicular carriers with greater ability to traverse the GI tract and reach systemic circulation. Beyond drug delivery applications, biles have also been indicated as decisive factors for the onset of various diseases (Abdel-Moneum *et al*., 2023; Zarenezhad *et al*., 2023).

5. Preclinical Studies on Bilosomes in Gastrointestinal Cancers

Bilosomes, a new generation of non-viral drug delivery nanoparticles composed of a phospholipid, bile salt, and bile salt-co-PEG-silica, have emerged as an effective carrier to overcome the GI barrier. Bilosomes can enhance the oral bioavailability of drugs by incorporating excipients that facilitate transport across the gut wall via absorption mechanisms such as transcellular passage; paracellular transport, cell-endocytosis; or via the lymphatic system. Various classes of drugs such as anti-cancer drugs, antibiotics, antiviral agents, and others have been implemented in bilosomal formulations and proved highly effective. Investigating the genetic mutations associated with major human disorders has provided insights into how genetic factors influence drug responses, reinforcing the need for precision drug delivery (Banoon *et al*., 2022).

Numerous in vitro studies have evaluated the effect of bile-salt-loaded bilosomes on cancer targeting using various cell lines, including COLO205, SNU-16, Caco-2, McCoy, NCM-287, HT-29, and AGS (Alhakamy *et al*., 2021; Alamoudi *et al*., 2023). In addition, the in vitro biodegradation of bilosomes has been studied by adding them to rat gastric juice, rat intestinal juice, rat gut homogenates, and pepsin, leading to the complete degradation of liposomes over 12 h. Moreover, bile-salt-loaded bilosomes have been used to evaluate their effect on antagonizing NCI-N87 gastric cancer cell-induced jejunal injury and inflammation in a co-culture model of NCI-N87 gastric cancer cells and Caco-2 small intestinal cells. As a result, bile-salt-loaded bilosomes inhibited the migration of NCI-N87 gastric cancer cells to Caco-2 cells 1.5 times greater than bilosomes without bile salt (Salapa *et al*., 2020). Other than this, a mucoadhesive taxane-based multi-drug combination was also designed to treat advanced GI cancer using in vitro and in vivo BA-odontosphere models. These results warrant further in vivo studies

to assess the safety of bilosome formulations. Furthermore, the loading capacity of drugs into bilosomes could be improved by optimizing the ratio of bile salts, silica, and lipids or using ceramide biocompatible lipids.

5.1. In vitro Studies

The popularity of the bilateral healthcare system has significantly increased the progression of research targeting various diseases, especially cancer (Rajput & Chauhan, 2017). Esteemed institutes and pharmaceutical companies have devoted themselves and their resources to enhancing drug delivery systems for various cancers. In vitro studies are initial laboratory studies done to verify the action of the pharmaceutical preparations on the cultured cells. Progressing toward formulation development, in vitro studies are the first step often undertaken before animal studies of the medicinal formulation.

Taking a closer look at in vitro studies, A 2^3 full factorial design was employed to optimize the formulation of bilosomal gel. In this context, the type of bile salt (SDC/GC) and the concentration of cholesterol (1.5 and 2.5% w/w) and phospholipid (1.5 and 2.5% w/w) were taken as independent variables. The type of bile salt (SDC or GC) and the concentration of phospholipid and cholesterol influenced the particle size and % entrapment efficiency significantly. The optimized formulations developed with SDC had smaller vesicles than those developed with GC, which is attributed to the stronger membrane fluidity imparted by SDC compared to GC (Abdallah *et al*., 2024). Higher levels of phospholipid and cholesterol, umilast loaded bilosomes with 0.5% semaglutide and 500 nm size showed an efficient entrapment of umilast up to 99%, compared to only 25% with plain liposomes. Mean vesicle sizes, polydispersity index, zeta potentials were analyzed by photon correlation spectroscopy and were determined to be 422 ± 16 nm, 0.57, ∼−35 mV respectively (Elkomy *et al*., 2022; Abdel-Moneum *et al*., 2023).

5.2. In vivo Studies

In vivo studies were conducted to evaluate the efficacy of bilosomes in GI cancers using animal models, detailing the significant findings related to drug efficacy and delivery. The in vitro and in vivo data are presented, providing insights into the actual impact of bilosomes in various scenarios. The first study looked at the efficacy of P-glycoprotein inhibitors in drug-resistant gastric cancer. During the course of treatment with drugs P-glycoprotein (P-gp) and its inhibitors were administered to gastric cancer mice. P-gp-MDR1 levels were evaluated by qRT-PCR. The mean tumor volume of each group was calculated and compared. Toxicity and side effects were measured by considering Normal blood cell count of WBCs, RBCs and PLTs as well as Normal serum levels of BUN, ALT and AST. The results indicated that the tumor sizes were nearly equal before treatment, but after treatment with drugs PBS, 5-FU +P-gp, 5-FU + chloroquine, 5-FU + shRNA-PP2A and 5-FU + chloroquine + shRNA-PP2A) the tumor volume was decreased. The tumor volume of drug combinations in DLBCL was notably decreased. On the other hand, the tumor volumes in groups PBS, 5-FU +P-gp were gradually increasing and became the largest compared to other groups.

The physical signs and symptoms of drug toxicity in mice were weighted along with blood analysis and were compared with the normal. In the observation group, only ALT showed slight elevation while in the chemotherapy and control group, the WBCs and PLT counts significantly and dramatically decreased whereas the level of BUN, ALT and AST was increased (Salapa *et al.,* 2020). The finding showed that using drug combinations led

to positive synergistic effects in drug-resistant GC, meanwhile, prevented significant toxicity. In the second study, bilosomes loaded with an anticancer drug were prepared, optimized and characterized for oral delivery. The characterization was done to assess morphology and size through different microscopy techniques including AFM, SEM, MFM etc of the bilosome. It was observed that the drug was released in a controlled manner over 72 hours and no major release of drug occurred at 48 hours. Later the bilosome was tested for toxicity in Caco-2 cells and the results showed that bilosome is non-toxic even at a high concentration of 1000 μg/mL (Parayath, 2016). After the confirmation cytotoxicity, study was performed using the bilosome and free drug Paclitaxel with varying concentrations from 1.56 μg/mL to 50 μg/mL. It was found that at a concentration of 1000μg/mL of the paclitaxel loaded bilosome, the cells were 50% less viable compared to the control untreated cells. In conclusion prospective novel formulation such as bilosomes should be further explored in the oral delivery of anticancer drugs.

6. Clinical Trials and Applications

To investigate the therapeutic efficacy of a bilosomal formulation containing PHA-739358, a potent anti-cancer drug, a clinical trial is currently underway in South Korea. Patients with advanced or unresectable digestive tract cancers are being recruited for the study which applies to those aged 19 or older with histological evidence of stomach, small intestine, esophagus, colon, or rectal cancer. Exclusion criteria include concomitant malignancy, pregnancy, peripheral nerve involvement, respiratory illness, psychiatric disorders, and other conditions deemed unsuitable by investigators. After signing informed consent forms, patients undergo tests that include blood and urine tests, physical examinations, tumor tissue tests, vital sign measurements, and imaging scans (Computed Tomography or Magnetic Resonance Imaging). One week before treatment, patients receive a 21-day “Observation Phase” during which daily oral intake of PHA-bilosomes is initiated (NCT05920000) (Salapa *et al*., 2020).

Apart from these proposed applications, many opportunities exist for the clinical use of bilosomes in both therapeutic and preventive drug delivery. This includes the delivery of therapeutic peptides, proteins and anti-cancer chemotherapeutics to breast, colon, lung, and pancreatic cancers. Likewise, vaccines against pathogens such as Schistosoma japonicum, Mycobacterium bovis, and indelible print virus may also be delivered via bilosomes. To address pharmacokinetic and bioavailability issues, various formulations of bilosomes have been developed, including biorhythmic/ chronopharmacological liposomes that time-release drugs based on biological rhythms (e.g. the pharmacokinetics of acyclovir on circadian rhythms). Other formulations include stimuli-sensitive liposomes that release drugs only in response to external stimuli/hyperthermia. Additionally, antimicrobial cationic liposomes have been designed to combat antibiotic resistance in infections by E. coli and S. aureus (Rajput & Chauhan, 2017). Several clinical trials are currently evaluating bilosomal formulations for cancer therapy. Research on protein-conjugated superparamagnetic iron oxide nanoparticles has indicated their efficiency as vaccine delivery systems, suggesting potential applications in immunotherapy for GI cancers (Al-Abboodi *et al.,* 2024).

6.1. Ongoing Clinical Trials

A wide range of bilosomes and pertinent health claims of bilosome formulations that have been evaluated in ongoing clinical studies are summarized in Table. The current bilosomal formulations being assessed in clinical investigations for the treatment of GI tumors include curcumin-laden bilosomes, gefitinib-loaded bilosome, and doxorubicin-hyaluronan-connected bilosomes (HNs). A number of orthogonal pathways involved in the transport of bilosomes loaded with curcumin are being examined in a phase 0 clinical trial. In a phase II research, the tolerability and pharmacokinetics of curcumin-bilosomes in cancer patients with GI cancer is assessed. In the ongoing trial, the safety and efficacy of curcumin-bilosomes in patients with solid tumors are evaluated (Salapa *et al*., 2020). An ongoing phase I clinical investigation is evaluating the bioavailability and adherence of gefitinib in subjects with gastric cancer receiving oral treatment with gefitinib-bilosomes. Doxorubicin-HN bilosomes are the subject of ongoing phase I trials assessing the toxicology and pharmacokinetics of intravenously injected doxorubicin-HN bilosomes in patients with advanced solid tumors (Hamad *et al*., 2024).

In phase 0 clinical research, the transport of curcumin-laden bilosomes through various orthogonal pathways, including the modulation of permeation and efflux transporters, is being evaluated in subjects with Barrett's esophagus. In healthy human volunteers, the safety and pharmacokinetics of curcumin-bilosomes are assessed in an ongoing phase II study. An ongoing phase II trial investigates the safety and efficacy of curcumin-bilosomes in cancer patients with GI cancer (oesophageal, gastric, pancreatic, biliary tract) (Storka *et al.*, 2015; Gota *et al.*, 2010).

6.2. Potential Future Directions

As novel drug delivery systems, bilosomes studies can focus on formulation optimization, mechanisms of action, targeted delivery, in vivo studies, applications in biologics, regulatory considerations, patient-centric studies and stability and storage (Rajput & Chauhan, 2017; Salapa *et al*., 2020; Zarenezhad *et al*., 2023).

7. Formulation and Characterization of Bilosomes

Bilosomes can be formulated using the novel technique of thin film hydration, which is a cost-effective and simple process. Initially, the lipids and bile salts are mixed in different ratios and dissolved in an organic solvent, which could be chloroform, methanol, or ethanol. A stream of nitrogen gas is passed into the solution under a water bath at 40-60 °C until a thin film is formed. The formulations are hydrated using pH-7.4 phosphate buffer saline solution. To enhance the bilosome formation, the formulated batches can be sonicated for 15 minutes (with 5-second pulses) and soaked for 12 hours in an incubator at room temperature. The formulations were characterized for particle size, zeta potential, polydispersity index, entrapment efficiency, stability, and in-vitro drug release (Zafar *et al.,* 2021). The entrapment efficiency can be calculated by centrifuging the formulations at 15000 rpm for 45 minutes at a temperature of 4 °C and using an ultra-filtration membrane 10 KDa. The encapsulated amount of drug can be determined using UV spectrophotometry at 220 nm, and the unentrapped drug can be used to evaluate the entrapment efficiency. The drug leakage from the formulation to the supernatant can be used to evaluate the percentage of drug leakage.

7.1. Bilosome Preparation Excipients Determine the Lipid Composition

Bilosomes can be formulated using ingredients such as phosphatidylcholine, cholesterol, deoxycholic acid, sodium taurodeoxycholate, glyceryl-monostearate, glyceryl-distearate, olivamine, labrafac lipid, deionized water, and PEG-4000 (Rajput & Chauhan, 2017). Natural lipids like gelucire have been used in the preparation of bilosomes, while sunspray, hydroxypropyl methylcellulose, polysorbate 80, and propylene glycol are used as excipients in various nanocarriers. The compatibility of excipients can be determined by differential scanning calorimetry (DSC) or Fourier transform infrared spectroscopy (FT-IR).

7.2. Physical Characterization of Bilosomes

A critical first step in the characterization of drug delivery systems is the evaluation of their physicochemical properties. Physicochemical characterization will provide information about the bulk physical properties of bilosomal formulations, which includes lipid composition, excipients, drug loading, zeta potential, particle size, and polydispersity index. The naked bilosomes can be characterized using a laser diffraction method, optical microscopy, cryogenic transmission microscopy (cryo-TEM), and matrix-assisted ultraviolet laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS).

7.3. Lipid Composition and Excipients

At present, researchers are consistently examining numerous excipients to formulate stable bilosomes. Bilosomes are composite structures composed of phospholipids and bile salts. Bile salts are pharmaceutically approved amphiphiles that are actively secreted in the GI tract. Because of this, they can be employed as bile acid phospholipid mixtures for the preparation of bilosomes. Several bile salts (sodium deoxycholate, sodium taurocholate, sodium chenodeoxycholate, sodium cholate and sodium deoxycholate) and phospholipids such as egg phosphatidylcholine (EPC), soy phosphatidylcholine (SPC) and hydrogenated soy phosphatidylcholine (HSPC) have been evaluated in the past for the formulation of bilosomes (Rajput & Chauhan, 2017).

In a similar approach, a study prepared and characterized bilosomes composed of soy phosphatidylcholine and sodium cholate. Formulation stability was studied under different pH and temperature conditions. A summary of possible excipients employed in the preparation of bilosomes is given in Table. Several studies have been published that investigate lipid composition in the formulation of bilosomes. In a study, the influence of co-formulated Corn Oil, Glyceryl Monostearate, and Sucrose on the formation and properties of bilosomes was examined. Results from the study concluded that microemulsion production and properties were influenced by Gulf Optical Composition and that Homogenization was a critical step for consistent microemulsion formation in the lab. Additionally, Employing a w/o emulsion to optimize the preparation with corn Oil was necessary to achieve high oil-phase encapsulation efficiency was determined (Bhairy *et al*., 2020; Abdallah *et al.*, 2024).

7.4. Physicochemical Characterization Techniques

The physicochemical characterization of bilosomes is performed to assess the characteristics of the formulations that affect the drug delivery properties of bilosomes. In bilosomes, bile salts and phospholipids play a major role in providing structural integrity to the vesicles and modulating drug release. The physicochemical

characterization techniques for bilosomes include matrix particle size, shape, and surface charge; Particle Size Analyzer; Transmission Electron Microscopy; Scanning Electron Microscopy; Zeta potential Analyzer; In-vitro drug release study; and Stability studies. In the in-vitro drug release study, the release of the drug from the lipid formulations is assessed by using a dialysis membrane apparatus or Franz diffusion cell (Zafar *et al*., 2021).

The drug release is assessed using the appropriate diffusion equation models or kinetic models such as Zero order kinetics, First order kinetics, Higuchi model, and Korsmeyer-Peppas equation model which will provide information regarding the principles governing the pattern of drug release. The stability of the formulations is assessed under the usual conditions (temperature and humidity) of storage. It is performed for a determined period daily and then at every week interval till three months by evaluating the particle size and percentage entrapment which ensures stability (Abdelbary *et al*., 2016; Rajput & Chauhan, 2017).

A study was performed to assess the anti-cancer activity of oleoylethanolamide. Bilosomes were developed with soya phosphatidylcholine, cholesterol, and sodium taurocholate for drug encapsulation and its subsequent in vitro cytotoxicity and biodistribution evaluation (Yamagata *et al.,* 2021). Bilosomes showed an average size range of ~186.33±22.225 nm with positive zeta potential (+7.13±2.423 mV). Transmittance electron microscopy showed nano-sized bilosomes with a smooth structure. The maximum drug encapsulation efficiency was ~85.222% with ~36% skin permeation enhancement (Lacatusu *et al*., 2019). The cellular uptake studies confirmed a significant enhancement of cellular uptake (more than 70%) at various time intervals with bilosome formulation compared to the free drug. Biodistribution studies showed high drug deposition in the intestine, liver, and brain. The in vitro cytotoxicity studies showed that oleoylethanolamide-loaded bilosome exhibited significantly ($p < 0.001$) high cytotoxicity against colorectal cancer cells (HT-29) and liver cancer cells (HepG2) compared to the drug suspension. The prepared bilosomes and oleoylethanolamide showed no signs of edema or skin irritation, indicating the safety profile of the formulation.

The focus on preparing the bilosome complex with the antitumor drug doxorubicin, confirmed the doses, the dynamics of the cytotoxicity effect of doxorubicin in a mono dose, and studied the antitumor activity of the drug (Hegazy *et al*., 2022; Sultan *et al.,* 2023). The studies determined the toxic effects of the gel on the cellular metabolism and growth of tumors. For the first time, new composite structures were created based on doxorubicin and modified liposomes and studied their cytotoxic action against the HeLa line. There was a direct dependence of the cytotoxic effect on the dose of the polymer, with the maximum suppression of the growth of tumor cells at a concentration of 50 μg/ml. The minimum inhibition of tumors was recorded at a dose of 0, 1, and 10 μg/ml. The developed bilosomes allow for a significant reduction in the damaging effects of the preparations of doxorubicin and its dosage forms while maintaining high efficiency. Formulations of doxorubicin in modified liposomes and their gel forms of the drug have been created, investigated, and proposed for the treatment of cervical cancer.

Bilosomes have been applied for the efficient delivery of anticancer agent Doxorubicin (Dox). Dox-loaded bilosomes were characterized by average size ~ 170 ± 7 nm, entrapment efficiency ~77% as observed by transmission electron microscopy. The release of Dox from bilosomes was 90% at pH 5.5-treated media which followed the

Higuchi model and Fickian diffusion mechanism. Intracellular delivery of Dox and its anticancer effectiveness were examined for bilosomal formulation on A549, MCF7 and HeLa cancer cell lines. The Dox-loaded bilosomes showed 10-28-fold higher cytotoxicity than free Dox. These results suggested that bilosome formulation enriched with G/lipodextrin conjugates can be used as a nanocarrier for enhanced intracellular delivery of Dox (Wang *et al.*, 2014; Orabi *et al*., 2022).

7.5. In vitro Efficacy Studies

Focusing now on the in vitro efficacy studies, it can be seen that multiple experimental evaluations of the efficacy of bilosomal drug delivery have been experimentally evaluated on controlled laboratory settings, making it applicable to evaluate the efficacy of bilosome drug delivery using in vitro studies. As gathered information suggests, PVP-Fe3O4@bilo-MoS2-APC, PVP-Fe3O4@bilo-MoS2-APC/F126 MA, and PVP-Fe3O4@bilo-MoS2-APC/F127-MA nanocomplexes had a significant decrease in motility for all point mutations relative to the untreated or separately treated groups (saline, PVP-Fe3O4, PVP-Fe3O4@bilo-Control). Transwell chamber analysis suggested that changes in cell motility corresponded to changes in cell invasion capability (Salapa et al., 2020). Results suggest that bilosome drug delivery is effective at reducing invasiveness and motility in cell lines that express multiple gain-of-function K-ras mutations, further establishing their effectiveness across multiple cancer genomic backgrounds. zA delves more into the mechanisms through which bilosomes act on cell lines expressing similar genomic changes. Western blotting suggests that there is a difference in the effect of agents on potential signaling pathways with regards to the type of K-ras mutation (Parayath, 2016). It also suggests that combined treatment with bilosomes and the dominant negative expression of the signaling approaches may be most effective in reducing motility and potentially aggressiveness across cancers with similar background concerns.

7.6. In vivo Safety Profiles

The biodistribution of the drug is told by in vivo safety profiles. The biliary toxicity of the bilosomes is examined from the biodistribution in various organs and by histopathological studies in the liver. Biodistribution drug determination in organs and tissues like major organs, the stomach, intestine, liver, spleen, lungs, kidneys, heart, and brain is performed using an in vivo fluorescence imaging system. Fluorescence images of organs are taken at various time points to evaluate and explore the biodistribution of bilosomes on oral administration. To study the localization and accumulation in organs and tissues post-oral administration, fluorescence microscopy is used. The localization of the bilosomes is studied at 1, 2, 6, and 24 hours of oral administration. The fluorescence intensity of the livers, spleens, stomachs, and other tissues from fluorescence micrographs shows accumulation and localization of fluorescence at all the time points taken, indicating the good stability of bilosomes and the retention action of bile salt and surfactant. At 24 h the intensity decreases, but for 15 nm and 50 nm, higher accumulation is in the liver, which means they can also be a carrier for drugs targeting the liver and gallbladder (Shukla *et al.*, 2008; Rajput & Chauhan, 2017; Mondal *et al.*, 2022).

For further confirmation of hepatotoxicity or other organ toxicity of the bilosomes, the biochemistry profile parameters of ALT, AST, ALP, and TP in serum and plasma are evaluated. Any drug-induced alteration in any of these values indicates organ injury or

damage. The levels of ALT, AST, and ALP are mostly normal, which indicates no damage to liver efficacy. The levels of TP are slightly elevated which can be due to the change in biliary function, which stems from NTPC, internanocrystallization or formation of micelles. The hematological profiles of RBC, WBC, HB, HCT, and PLT are almost normal and don't have significant differences from the control, indicating bilosomes do not have potential problems. The histopathological studies of the liver indicate the safety profile of the bilosomes (Abdallah et al., 2024).

8. Regulatory Considerations and Market Approval

Bilosomes promise to be a remarkable potential strategy for treating GI cancers via oral route that needs further research explorations. Topics thoroughly discussed include GI cancer biology and the treatment landscape, oral drug delivery systems in clinical and pre-clinical research, bilosomes in the treatment of gastric, intestinal and CRC drug delivery, challenges and usefulness of bilosomes for GI drug delivery, biocompatibility, safety and regulatory considerations of bilosomes in drug delivery applications.

Market Approval Considerations: It is highly important to consider market approval regarding product development. The origin of the product and the nature of the biocomponents (e.g. plant, microorganism or animal) are the determinants to classify and decide on the appropriate regulatory pathways. Furthermore, the toxicology assessments and characterisation tests depend on the nature of the biocomponents. There are two pathways for market approval as a drug in the USA; one is the New Drug Application (NDA) for new active ingredients and the Abbreviated New Drug Application (ANDA) for generic active ingredients (Salapa *et al.*, 2020). A preclinical assessment on biocompatibility and toxicology is required prior to Phase 1, the clinical phase. A biocompatible product is defined as a product that does not produce unwanted effects in the biological environment.

Regulatory Pathways for Drug Delivery Systems

Regulatory agencies such as the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA), Health Canada and others have recognized the urgency of establishing a streamlined regulatory pathway for drug delivery systems (DDSs). Investigators developing CCS platforms to demonstrate the efficacy of their technologies must obtain regulatory approval. Regulatory processes are a major obstacle for commercializing CCS technologies. Moreover, it is a common misconception among investigators that only the DDS and drug must be characterized for regulatory submissions. Many other important considerations exist, such as the manufacturing process, stability studies, safety, environmental impact studies, transportation and misuse studies. The target audience for this white paper is thus those investigators who are still in the bench-top phase of CCS research. They must consider the regulations relevant to their DDS and attempt to eliminate experiments and complex formulations that are not compatible with the conditions of those regulations, thus maximizing the chances for FDA approval. Drug delivery and dosage administration systems influence the course of treatment and the therapeutic effect of the drug itself. These systems cover a wide variety of treatment types and routes for administration, including solid drug forms, parenteral injections, inhalation, nasal drugs and ocular and transdermal preparations and have different blood circulation and excretion properties (Rajput & Chauhan, 2017). On the market and in development, there are both complex technologic systems and very simple

preparations. Based on the drug administration route, those systems fall into three groups: invasive systems, non-invasive systems and drug-polymer interactions (Kim *et al*., 2018).

9. Future Perspectives

With the increasing prevalence of GI cancers worldwide, the demand for drug delivery systems capable of specifically targeting the GI tract has grown. Bilosomes could be designed as oral drug delivery systems loaded with various therapeutic agents to treat GI cancers. The efficacy of bilosomal drug delivery systems for GI cancer treatment has been demonstrated in vitro and in vivo (Salapa *et al*., 2020; Garbati *et al*., 2024). Drug-loaded bilosomes can specifically target the GI tract after oral administration, with 1-98% accumulation in the small intestine, 0.08-68% accumulation in the colon and 38% accumulation in the liver for bilosomes with an appropriate surfactant-to-phospholipid ratio (2:1).

Bilosomes can be loaded with several therapeutic agents, including anticancer drugs (such as 5-FU and cisplatin), siRNA, mRNA and vaccines, to treat GI cancers. The anticancer efficacy of drug-loaded bilosomes has been demonstrated in vitro and in vivo. Bilosomes can also be used to encapsulate multiple therapeutic agents, either in single or separate nanocarriers, to improve the anticancer efficacy of combination therapy. Bilosomes encapsulating siSOX9 and 5-FU effectively inhibited the growth of colon cancer and did not induce toxicity in the other GI tissues.

The current progress of bilosomes as drug carriers for treating GI cancers has been reviewed and future research directions have been proposed. Further studies are required to confirm the safety and efficacy of bilosome formulations in humans. Bilosomes can be functionalized with targeting ligands to enhance the specificity of drug-loaded bilosomes to GI cancer cells. Additionally, trending algorithmic approaches on platforms like Twitter have facilitated the dissemination of emerging research findings, enabling real-time discussions and collaborations in the scientific community (Hassan *et al*., 2024).

10. Future Research Directions

The field of personalized cancer therapy is growing rapidly. Newer treatment options directed toward more individual approaches are desired to maximize individual response to anticancer treatment while minimizing negative side effects. As drug treatment protocols become more individualized, strategies to drug delivery are being developed to enhance the bioavailability and pharmacological activity. In particular, drug delivery systems based on nanoparticles (NPs) are becoming of growing interest in order to meet various physicochemical properties such as size, shape, porosity and surface characteristics of the engineered structure (Salapa *et al*., 2020). A wide variety of disease-targeted NPs are currently being researched with applications in GI cancer therapy. These NPs have desirable drug delivery characteristics that include controllable sizes and multimodal surface functionalization capable of site-specific targeting. Engineered NPs have shown superior tumor-targeting efficacy due to their unique properties that allow for the passive targeting of diseased tissues via excess EPR effect. Additionally, the application of stimuli-responsive NP-based drug delivery systems may enable contingent drug release according to environmental or internal triggers and in this way reduce systemic release and toxicity (Rajput & Chauhan, 2017). Strategies to overcome drug resistance such as co-delivery of anticancer agents and modulating membrane lipid

compositions are being examined to enhance treatment efficacy. According to these and other aspects, formulation optimization, mechanisms of action, targeted delivery, in vivo studies, applications in biologics, regulatory considerations, patient-centric studies and stability and storage can be considered in future studies. Modified bilosomes have been also promising for efficient carriage and delivery of medicines.

11. Conclusion

Efficient treatment of gastrointestinal (GI) cancers necessitates considering of lowering side effects and increasing the delivery without effect of off-targets. Additionally, long-term toxicity of nanocarriers should be considered as it is low for lipid nanocarriers. Among them, bilosomes have developed for GI diseases drug and vaccines delivery. The advantages of bilosomes include high drug loading and resistance to acids as they contain bile salts within membrane of vesicle bilayer. Bilosomes have utilized for various drugs delivery into the GI cancers. Moreover, modified bilosomes have been promising in this regard. The formulation optimization, mechanisms of action, targeted delivery, in vivo studies, applications in biologics, regulatory considerations, patient-centric studies and stability and storage can be considered in future studies. Modified bilosomes have been also promising for efficient carriage and delivery of medicines.

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