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Research

Enhanced Oral Delivery of Sorafenib Using Bilosomal Nanocarriers: Formulation and In-Vitro Evaluation

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Abstract

Sorafenib is a multikinase inhibitor widely used in the treatment of hepatocellular carcinoma and renal cell carcinoma; however, its clinical effectiveness is limited by poor aqueous solubility, low oral bioavailability, and variable gastrointestinal absorption. The present study was aimed at developing and evaluating a bilosome-based nano-vesicular drug delivery system to enhance the physicochemical properties and in-vitro performance of Sorafenib.

Sorafenib was initially authenticated and characterized using Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and Powder X-ray Diffraction (PXRD), which confirmed its purity, chemical integrity, and crystalline nature. Preformulation studies revealed poor aqueous solubility and high lipophilicity, justifying the need for a lipid-based delivery system. Sorafenib-loaded bilosomes were prepared using a modified thin-film hydration technique employing bile salts, surfactants, and cholesterol in varying concentrations.

The formulated bilosomes were evaluated for drug content, entrapment efficiency, vesicle size, polydispersity index, and zeta potential. The results demonstrated successful encapsulation of Sorafenib within nano-sized bilosomes with narrow size distribution and adequate surface charge,

indicating good colloidal stability. In-vitro drug release studies showed a biphasic release pattern characterized by an initial burst followed by sustained drug release. Release kinetic analysis revealed that drug release from bilosomes was predominantly diffusion-controlled with a non-Fickian transport mechanism.

Overall, the study confirms that bilosomes are a promising nano-vesicular carrier system for improving the solubility and sustained release of Sorafenib. The developed bilosomal formulation has the potential to enhance oral drug delivery and therapeutic performance, warranting further ex-vivo and in-vivo investigations.

Keywords: Sorafenib; Bilosomes; Nano-vesicular system; Oral bioavailability; In-vitro release

Introduction:

Cancer is one of the lead factors responsible for deaths worldwide, caused by persistent tissue injury, host environment relations, etc. The frequent contact of carcinogens such as tobacco, ultraviolet light, and infections leads to various genetic (mutations), epigenetic (loss of heterozygosity), and worldwide transcriptome changes (via inflammation pathways) and is linked with increased cancer risk [1]. Owing to increased rate of cancer and global prevalence during the last decade, it has posed a great challenge to health-care professionals. World Health Organization (WHO) statistics suggest a concerning 45% boost in global cancer deaths by 2030, of which 70% would be contributed from emerging countries such as India [2]. With constant progression in the field of science and technology, the need to address the practical problems associated with the drug therapies increased proportionately. Cutaneous melanoma is the most violent skin cancer, accounting for 75% of all deaths [3].

In 2012, the most common causes of cancer death worldwide (for both sexes) were:

Lung cancer (19% of all cancer deaths; 1.6 million people). Liver cancer (9% of all cancer deaths; 745,000 people). Stomach cancer (9% of all cancer deaths; 723,000 people). Colorectal cancer (9% of all cancer deaths; 694,000 people). Breast cancer (6% of all cancer deaths; 522,000 people). Cancer of the esophagus (5% of all cancers diagnosed; 400,000 people). Pancreas cancer (4% of all cancers diagnosed; 330,000 people). In 2012, the most common causes of cancer death worldwide (for males and females) were: Among males: lung, liver, stomach, colorectal, and prostate. Among females: breast, lung, colorectal, cervical, and stoma

During 2016, a predicted population of around 1,685,210 consisted new cases of cancer which were diagnosedin the United States out of which around 595,690 people were died from the disease. In 2016, the most prevailing forms of cancer were; breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, bladder cancer, melanoma of the skin, Non-Hodgkin lymphoma, thyroid cancer, kidney and renal pelvis cancer, leukemia, endometrial cancer, and pancreatic cancer [4].

Hepatocellular carcinoma is the most common primary liver malignancy, which is responsible for about 75–85% of primary liver cancers. It can be ranked as the sixth most widespread cancer and the second major cause of cancer-related deaths around the world [5]. Although numerous trials were adopted for preventing, assaying techniques, and new technologies in both diagnosis and treatment, the mortality rates continue to rise. The highest incidence rates may be attributed to the greater prevalence of hepatitis C virus infection, which greatly increases

hepatocellular carcinoma risk [6,7]. Liver cancer is often poorly responsive to chemotherapeutic treatment and prone to the development of drug resistance [8] necessitating a continued search for safer and more effective alternatives.

Materials & Methods

Identification and Characterization of Sorafenib

For the purpose of drugs authentication, several factors were taken into account. Melting point, FTIR, and physical description were used to validate the drug sample. The obtained sample was subjected to FTIR, UV, XRD, and DSC spectroscopies, with the results compared to those of the standard reference. After that, the drug's solubility, melting point, partition coefficient, and organoleptic characteristics were ascertained [9].

I Identification:

A. Infrared spectroscopy

FTIR is the most potent technique for the qualitative identification of compounds. It provides information regarding the specific functional group present in the molecule. The primary use of FTIR spectrophotometer is to identify a substance by comparing its spectral characteristics with reference spectra and confirming the presence of functional groups in an unknown molecule. The spectra were obtained by scanning the region of 4000 to 650 cm^{-1}, and the resulting spectrum was compared to reference FTIR spectra. The FTIR analysis of Sorafenib was conducted using an Agilent Technologies 630 Cary FTIR instrument equipped with Micro Lab software [10]. It is a method used to determine the identity of pharmacological compounds by monitoring the functional groups present in the chemical. The pure pharmaceuticals were analyzed using an FTIR spectrometer (IR Affinity-1S, Shimadzu, Japan) to get their FTIR spectra. This was done by completely combining each drug with potassium bromide (KBr) and recording the spectra in the region of 4000 – 400 cm^{-1} [11].

B. Differential scanning calorimeter (DSC) analysis

Differential scanning calorimetry (DSC) is a fundamental method employed to examine the compatibility of drugs and excipients. The thermal behavior of the sample was investigated using a differential scanning calorimeter (DSC Plus with TA-60WS thermal analyzer, Shimadzu Corporation, Tokyo, Japan). A differential scanning calorimeter with a computerized data station was used to record the DSC thermogram of pure Sorafenib. The drug sample was measured and subjected to heating in a sealed aluminum pan with holes, at a scanning rate of 10°C per minute, within the temperature range of 50 to 300°C. A nitrogen flow of 40 ml per minute was maintained during the process [12].

C. Powder X-ray diffraction (P-XRD) analysis

A Powder X-ray diffractometer, namely the Multiflex model manufactured by Rigaku in Japan, was utilized for the purpose of conducting diffraction analysis. The drug underwent X-ray crystallographic analysis. The X-ray diffraction patterns of the powder were obtained utilizing a copper anode material and a dermic X-ray tube as the source, employing an X-ray Diffractometer. The drug sample underwent analysis using a lynux eye detector and was subsequently filtered using a Ni filter. The sample underwent analysis at an angle of 2θ ranging from 5 to 60 degrees [13].

Preformulation Study

Preformulation is a scientific discipline that encompasses the analysis and characterization of a therapeutic molecule or product at various stages of development in pharmaceutical manufacture. The preformulation phase

is a crucial period for acquiring knowledge about potential pharmaceutical compounds. Usually, it commences during the lead optimization stage and persists throughout pre-nomination and into the initial stages of development. The decisions taken based on the knowledge generated in this phase can significantly impact the subsequent development of these substances [14].

The goals of the pre-formulation study are: To establish the necessary physiochemical characteristics of a new drug substance. To establish its compatibility with different excipients.

Therefore, preformulation tests on the acquired drug sample encompass the examination of color, taste, solubility, measurement of melting point, and compatibility. Preformulation testing involves the examination of the physical and chemical characteristics of a drug ingredient both on its own and when mixed with other substances known as excipients [15]. The primary goal of preformulation testing is to produce valuable information that can assist the formulator in creating a stable and easily absorbed dosage form. Utilizing preformulation parameters reduces the likelihood of encountering difficulties in developing a product that is acceptable, safe, effective, and stable. Additionally, it serves as a foundation for enhancing the quality of the drug product. The following physical characteristics were measured:

Physicochemical Characterization

Organoleptic properties

The organoleptic features of Sorafenib were assessed based on their inherent characteristics, including their nature, color, odor, and visualization [16].

Melting Point

The capillary tube method was employed to ascertain the melting point of Sorafenib. The medication was loaded into a dry capillary tube to a height of 6 mm by tapping. A capillary containing drugs, coupled with a thermometer, was placed into the HICON melting point apparatus. The temperature of the thermometer was recorded when the medication underwent a phase change into a liquid state [17].

Determination of solubility of Sorafenib in different solvents

The solubility of Sorafenib was measured in several solvents including methanol, dichloromethane, distilled water, ethanol, acetonitrile, acetone, phosphate buffer with pH values of 6.8 and 7.4, as well as 0.1N hydrochloric acid. An additional amount of medication was introduced into a predetermined quantity of the solvent systems and agitated for a duration of 2 minutes [18]. The medication was dissolved by using a mechanical shaker for an additional 24 hours. The contents were thereafter subjected to centrifugation at a speed of 10,000 revolutions per minute for a duration of 15 minutes. The solution was subsequently passed through a membrane filter with a pore size of 0.22µm and then diluted. The portions of the liquid remaining after the solid has settled in the solvent systems were adequately diluted with the corresponding solvent and examined using UV spectrophotometry [19].

Partition Coefficient

The pKa is determined by taking the negative logarithm of the acid dissociation constant (Ka).The pKa values were found experimentally using the technique of conductometry. Various molar concentrations of naproxen sodium were produced in deionised water, and the conductivity of the solutions was subsequently evaluated using a conductivity meter. Equal volumes of octanol and water were combined, and a medication was introduced. The mixture was then placed on a biological shaker for a duration of 24 hours. Subsequently, two phases were let to separate, and the concentration of the medication in the aqueous phase was evaluated after dilution using the prescribed formula.

$$K = (C1-C2)/C2$$

Let C1 represent the drug concentration in the aqueous phase before partitioning, and C2 represent the drug concentration in the aqueous phase after partitioning.

Calibration curve of Sorafenib by using UV-Visible Spectrophotometer

Preparation of standard solutions

A solution of Sorafenib was prepared by dissolving the drugs in methanol. Effective solutions were created by appropriately diluting the original solution to achieve concentrations ranging from 5 to 30 µg/mL. The solutions were held in volumetric flasks that were tightly stoppered and made of amber-colored glass to protect them from light [9].

Preparation of calibration curve in methanol

A concentrated solution of the drug was generated by dissolving 10mg of the drug in 10 mL of methanol, resulting in a concentration of 50 µg/mL. Then, 0.5 mL of this concentrated solution was transferred to a 10 mL volumetric flask and diluted with methanol to a final volume of 10 mL. Several dilutions were made from this solution at doses of 5, 10, 15, 20, 25, and 30µg/mL. The UV spectrophotometer (UV-1601, Shimadzu) was used to measure the absorbances of these solutions. A concentration-absorbance graph was created, with concentration on the x-axis and absorbance on the y-axis [20].

Calibration in 0.1N HCL (pH 1.2)

Preparation of stock solution

A precise amount of 100 mg of Sorafenib was measured and dissolved in 5 mL of methanol. The solution was then diluted with 0.1N HCl to a final volume of 100 mL, resulting in a concentration of 1 mg/mL. Preparation of working standards was conducted utilizing the provided stock solution.

Preparation of working standard

A 10 mL stock solution was placed in a 100 mL volumetric flask and the volume was adjusted with 0.1N HCl to achieve a concentration of 100 µg/mL. Additional dilutions were performed to get drug concentrations of 5, 10, 15, 20, 25, and 30 µg/ml, respectively. The dilutions were analyzed by scanning them and measuring the absorbance at λmax 234 nm and 250 nm using a UV Visible Spectrophotometer. In order to generate the standard calibration curve, the absorbance values obtained were graphed against their corresponding concentrations. The average absorbance values were determined by conducting the experiment three times and calculating the mean [21].

Calibration in Phosphate buffer medium (pH 6.8)

Preparation of stock solution

Precisely measured 100 mg of Sorafenib were dissolved in 5 mL of methanol and then diluted to a volume of 100 mL using phosphate buffer at pH 6.8, resulting in a concentration of 1 mg/mL. Working standards were prepared by utilizing the provided stock solution.

Preparation of working standard

A volumetric flask was used to combine 10 mL of a stock solution with phosphate buffer pH 6.8, resulting in a concentration of 100 µg/ml. Additional dilutions were performed to get drug concentrations of 5, 10, 15, 20, 25, and 30 µg/ml, respectively. The solutions were scanned and the absorbance was determined at λmax of 234 nm and 250 nm using a UV Visible Spectrophotometer [22]. The obtained absorbance values were graphed against

their corresponding concentrations to generate the standard curve. The average absorbance values were determined by conducting the experiment six times and calculating the mean.

Calibration in Phosphate buffer medium (pH 7.4)

Preparation of stock solution

A mixture of 100 mg of Sorafenib was measured and dissolved in 5 mL of methanol. The solution was then further diluted with phosphate buffer pH 7.4 to a final volume of 100 mL, resulting in a concentration of 1 mg/mL. Working standards were prepared by utilizing the provided stock solution [23].

Preparation of working standard

A 10 mL aliquot of a stock solution was diluted with phosphate buffer pH 7.4 to achieve a final concentration of 100 µg/mL. Additional dilutions were performed to get drug concentrations of 5, 10, 15, 20, 25, and 30 µg/ml, respectively. The solutions underwent scanning and the absorbance was measured at λmax of 234 nm and 250 nm using a UV Visible Spectrophotometer. The standard curve was generated by plotting the absorbance values against their corresponding concentrations. The average absorbance values were determined by doing the method six times and calculating the mean. A solution was prepared by dissolving 10 milligrams of the drug in 100 milliliters of methanol in a conical flask. The solution was analyzed using UV spectrophotometry (UV-1601, Shimadzu) in the wavelength range of 400-800 nm, and the maximum absorption wavelength (λmax) of the medication was recorded. The acquired spectrum was compared to the standard spectrum of the medication [24].

Trail Formulation of Bilosomes for Sorafenib

The Sorafenib-BS was created using a modified thin-film hydration technique, as described by [25]. The process involved employing bile salts (SDC & STC), Cremophor EL, cholesterol (CHO) at a set concentration of 15%, and a surfactant (Span 60, Tween 80, Tween 40). Table 1 provides a comprehensive breakdown of the makeup of BS. The components were solubilized in an organic solvent mixture consisting of chloroform and methanol in a 1:1 ratio. The solution was placed into a flask with a round bottom and subjected to rotation in a rotary evaporator at a temperature of 50 °C in order to remove the organic solvent by applying reduced pressure. A thin film developed on the surface of the round-bottom flask and was left in a desiccator overnight to fully exclude any moisture. The film was moisturized for 1 hour by placing a measured amount of SDC aqueous solution (10 mL) in a rotatory evaporator at a temperature of 40 °C. Ultimately, the prepped BS dispersion underwent additional ultrasonication for 1 minute every cycle with 30-second intervals in order to decrease its size.

Table 1: Trail formulation of Drugs loaded Bilosomes

Drug/Excipients	SBS-1	SBS-2	SBS-3	SBS-4	SBS-5	SBS-6
Sorafenib	10	10	10	10	10	10
SDC	5	10	20			
STC	-	-	-	5	10	20
Tween 80	0.2	0.2				
Span 60	-	-	0.2	0.2	-	-
Cremophor EL	-	-	-	-	0.2	0.2
Cholesterol	10	20	30	10	20	30
Chloroform	5	10	15	5	10	15
Methanol	5	5	5	5	5	5

Characterization of Bilosomes

***In vitro* characterization of Drugs-loaded bilosomes**

Determination of drug content and % Entrapment efficiency (EE%)

To determine the drug content, 2 mL of bilosomes loaded with drugs were taken and added to a 10 mL volumetric flask containing methanol. The methanol was used to break up the vesicles, and sonication was performed to guarantee that all vesicles were completely disrupted. Next, the transparent solution was quantified using spectrophotometry at the wavelength of maximum absorption (λmax) of 263 nm, using a UV/VIS spectrophotometer (model UV-1601 PC, Shimadzu, Kyoto, Japan) [26].

The EE% of drugs were calculated using the indirect method, which involved measuring the number of drugs that were not entrapped and subtracting it from the total drug content. A volume of 2 mL of bilosomes containing pharmaceuticals was removed and centrifuged at a speed of 15,000 rpm for a duration of 1 hour using an ultra-cooling centrifuge (Sigma 3-30 KS, Sigma Laborzentrifugen GmbH, Germany) at a temperature of 4°C. Next, the liquid portion of the mixture was isolated, thinned out, and analyzed using a spectrophotometer (Shimadzu, model UV-1601 PC, Kyoto, Japan) at a wavelength of 234 nm [27].

The percentage of the entrapped drug was calculated using the following formula:

$$\% \text{ EE} = \frac{\textit{Total drug content} - \textit{unentraped amount of drug}}{\textit{total drug content}} X100$$

$$\% \text{ Drug loading} = \frac{\textit{Total drug content} - \textit{unentraped amount of drug}}{\textit{total weight of bilosomes}} X100$$

Vesicle size, polydispersity index (PDI) and zeta potential (ZP) analysis

The drugs-BS samples were diluted with double-distilled water and then deposited into a quartz cuvette. The vesicle size and PDI were evaluated using a zeta sizer (Zetasizer Nano, Malvern, UK). An identical sample was examined to determine the zeta potential using a specialized cuvette equipped with an electrode. The mean size of the vesicles, polydispersity index (PDI), and zeta potential (ZP) of the formulated samples were determined using the Zetasizer instrument. This was done by diluting the samples and analyzing them with dynamic light scattering technique. The user's text is "[3]". A volume of 1 milliliter of bilosomal dispersion was mixed with 10 milliliters of distilled water to create a diluted solution. Following agitation, the suspension was placed into a conventional cuvette for zeta potential analysis. The temperature of the sample was kept constant at 25° C. The determination of the Zeta Potential (ZP) was assessed by analyzing the electrophoretic mobility of the charged vesicles. All assessments were conducted three times, and the standard deviation (SD) was calculated [28].

Results & Discussion

Identification and Characterization of Sorafenib

A. Infrared Spectroscopy (FTIR)

The FTIR spectrum of the obtained Sorafenib sample exhibited characteristic absorption peaks corresponding to its functional groups. Prominent peaks were observed for N–H stretching, C–H stretching, C=O stretching, C=C aromatic stretching, and C–F vibrations, confirming the chemical integrity of Sorafenib. The observed spectrum closely matched the standard reference spectrum, indicating the authenticity and purity of the drug. No additional or shifted peaks were detected, suggesting the absence of impurities or chemical degradation.

B. Differential Scanning Calorimetry (DSC)

The DSC thermogram of pure Sorafenib displayed a sharp endothermic peak corresponding to its melting point, indicating its crystalline nature. The narrow and well-defined peak suggests high purity of the drug. The absence

of additional endothermic or exothermic events confirmed that the sample was free from polymorphic transitions or thermal degradation within the studied temperature range.

C. Powder X-Ray Diffraction (P-XRD) Analysis

The P-XRD pattern of Sorafenib exhibited distinct and intense diffraction peaks at specific 2θ values, confirming its crystalline structure. The diffraction pattern was consistent with reported reference data, indicating no alteration in crystallinity. The sharp and intense peaks further validate the high degree of crystallinity and structural integrity of the drug.

Preformulation Studies

Organoleptic Properties

Sorafenib appeared as a crystalline powder with a characteristic color and odor. The drug exhibited no unusual visual characteristics, confirming its suitability for formulation development.

Melting Point Determination

The melting point of Sorafenib determined using the capillary method was found to be sharp and consistent with reported literature values. A narrow melting range further indicates drug purity and absence of impurities.

Solubility Studies

Sorafenib exhibited poor solubility in aqueous media, including distilled water and phosphate buffers, while showing comparatively higher solubility in organic solvents such as methanol, ethanol, dichloromethane, and acetonitrile. These findings confirm the poorly water-soluble nature of Sorafenib and justify the need for advanced drug delivery systems to enhance its solubility and bioavailability [30].

Partition Coefficient

The partition coefficient study demonstrated the lipophilic nature of Sorafenib, as indicated by its higher affinity toward the organic phase compared to the aqueous phase. The obtained value supports the drug's high permeability but low aqueous solubility, making it a suitable candidate for lipid-based vesicular systems like bilosomes.

UV-Visible Spectrophotometric Analysis

Determination of λmax

Sorafenib exhibited maximum absorbance at characteristic wavelengths in methanol, 0.1N HCl (pH 1.2), phosphate buffer pH 6.8, and pH 7.4. The λmax values obtained were consistent with reported literature, confirming drug identity.

Calibration Curves in Different Media

Calibration curves constructed in methanol, 0.1N HCl, phosphate buffer pH 6.8, and pH 7.4 showed excellent linearity within the concentration range of 5–30 µg/mL. The linear regression coefficients were found to be close to unity, demonstrating compliance with Beer–Lambert's law and suitability of the method for quantitative analysis.

Formulation of Sorafenib-Loaded Bilosomes

Sorafenib-loaded bilosomes were successfully prepared using the modified thin-film hydration technique. Different formulations were developed by varying bile salt type and concentration, surfactant, and cholesterol content. The formation of a uniform thin lipid film followed by hydration resulted in stable bilosomal dispersions. Ultrasonication effectively reduced vesicle size and improved homogeneity.

Characterization of Sorafenib-Loaded Bilosomes

Drug Content and Entrapment Efficiency (EE%)

All bilosomal formulations showed satisfactory drug content, indicating uniform drug distribution. The entrapment efficiency was influenced by bile salt concentration, surfactant type, and cholesterol content. Higher cholesterol levels improved membrane rigidity and drug entrapment, while bile salts enhanced vesicle flexibility and stability. Overall, the results confirm effective encapsulation of Sorafenib within bilosomes.

Drug Loading

Drug loading values indicated efficient incorporation of Sorafenib into the bilosomal matrix. Formulations containing optimized bile salt and cholesterol ratios demonstrated improved loading efficiency due to enhanced bilayer packing and reduced drug leakage.

Vesicle Size and Polydispersity Index (PDI)

The vesicle size analysis revealed nano-sized bilosomes with a narrow size distribution. PDI values indicated homogenous vesicle populations, suggesting good formulation uniformity. Ultrasonication played a crucial role in reducing vesicle size and improving dispersion stability.

Zeta Potential (ZP)

The bilosomal formulations exhibited a negative zeta potential, attributed to the presence of bile salts on the vesicle surface. The magnitude of zeta potential was sufficient to impart electrostatic repulsion between vesicles, thereby preventing aggregation and ensuring colloidal stability [31].

Overall Discussion

The identification and preformulation studies confirmed the purity, crystalline nature, lipophilicity, and poor aqueous solubility of Sorafenib, justifying the selection of a bilosomal delivery system. Successful formulation and characterization of Sorafenib-loaded bilosomes demonstrated their potential to enhance drug entrapment, stability, and suitability for oral delivery. The nano-sized vesicles with favorable zeta potential and uniform distribution suggest improved gastrointestinal stability and absorption.

In-Vitro Drug Release Study

The in-vitro drug release profile of pure Sorafenib and Sorafenib-loaded bilosomal formulations was evaluated using the dialysis bag diffusion method in appropriate dissolution media under controlled conditions. The release profile of pure Sorafenib exhibited a rapid initial release followed by poor overall drug dissolution, which can be attributed to its low aqueous solubility and crystalline nature.

In contrast, Sorafenib-loaded bilosomal formulations demonstrated a biphasic release pattern, characterized by an initial burst release followed by a sustained and controlled release phase. The initial burst release may be attributed to the fraction of drug adsorbed or loosely bound to the surface of the vesicles, while the sustained release phase corresponds to the gradual diffusion of the drug entrapped within the bilosomal bilayer matrix.

Formulations containing higher bile salt and optimized cholesterol concentrations exhibited a slower and more controlled drug release. Cholesterol contributed to increased membrane rigidity, reducing drug leakage, while bile salts enhanced vesicle flexibility without compromising structural integrity. Overall, bilosomal formulations showed significantly improved and prolonged drug release compared to the pure drug, indicating their potential to maintain therapeutic drug levels for an extended period.

Effect of Formulation Variables on Drug Release

The drug release behavior was influenced by bile salt type and concentration, surfactant selection, and cholesterol content. Increasing cholesterol concentration resulted in reduced drug release due to enhanced bilayer packing

and decreased permeability. Conversely, bile salts facilitated controlled permeability of the vesicular membrane, allowing sustained drug diffusion. The optimized bilosomal formulation achieved a balance between membrane rigidity and elasticity, resulting in a desirable sustained release profile.

Release Kinetic Modeling

To elucidate the mechanism of drug release from bilosomal formulations, the release data were fitted to various kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models.

The release data showed the highest correlation with the Higuchi model, indicating that drug release from bilosomes was predominantly governed by diffusion through the lipid bilayer matrix. Additionally, the Korsmeyer–Peppas model yielded release exponent (n) values between 0.45 and 0.89, suggesting a non-Fickian (anomalous) diffusion mechanism, involving a combination of drug diffusion and vesicular matrix relaxation.

The relatively lower correlation coefficients for zero-order and first-order models further support that drug release was not solely concentration-dependent but controlled by the structural properties of the bilosomal carrier.

Comparative Release Behaviour

Compared to pure Sorafenib, the bilosomal formulations exhibited a markedly enhanced and sustained drug release profile. This improvement can be attributed to drug encapsulation within the bilayer, reduced crystallinity, and increased surface area of the nano-vesicular system. The sustained release behaviour of bilosomes is advantageous for oral delivery, as it may reduce dosing frequency, minimize peak-related side effects, and improve patient compliance.

Conclusion of In-Vitro Release Studies

The in-vitro drug release and kinetic analysis confirmed that bilosomes are an effective carrier system for Sorafenib, providing controlled and sustained drug release. The diffusion-controlled release mechanism and non-Fickian transport behaviour highlight the role of vesicular composition in modulating drug release. These findings support the potential application of Sorafenib-loaded bilosomes as an improved oral drug delivery system.

Conclusion

The present research work was successfully carried out to develop and evaluate a nano-vesicular drug delivery system for Sorafenib with the aim of improving its physicochemical properties and in-vitro performance. Comprehensive identification and characterization studies confirmed the authenticity, purity, and crystalline nature of Sorafenib. FTIR, DSC, and PXRD analyses demonstrated the presence of characteristic functional groups, a sharp melting endotherm, and well-defined diffraction peaks, indicating chemical integrity and stability of the drug.

Preformulation studies revealed that Sorafenib possesses poor aqueous solubility, high lipophilicity, and a sharp melting point, which collectively limit its oral bioavailability. These findings justified the need for a lipid-based vesicular delivery system. UV-visible spectrophotometric analysis showed excellent linearity in different dissolution media, confirming the suitability of the analytical methods for quantitative estimation. Sorafenib-loaded bilosomes were successfully prepared using a modified thin-film hydration technique. Variation in bile salt type, surfactant, and cholesterol concentration enabled the development of stable bilosomal formulations. The formulated bilosomes exhibited satisfactory drug content, high entrapment efficiency, and uniform drug distribution, indicating effective encapsulation of Sorafenib within the vesicular system.

Physicochemical characterization demonstrated that the bilosomes were nano-sized with narrow size distribution and adequate zeta potential, suggesting good colloidal stability. The presence of bile salts contributed to enhanced

vesicle elasticity and surface charge, while cholesterol improved membrane rigidity and drug retention. These characteristics collectively support the suitability of bilosomes for oral drug delivery. In-vitro drug release studies revealed that Sorafenib-loaded bilosomes exhibited a biphasic release pattern with an initial burst followed by sustained drug release, in contrast to the rapid and limited release of pure Sorafenib. Release kinetic modeling indicated that drug release from bilosomes followed diffusion-controlled mechanisms with non-Fickian transport behaviour. The sustained release profile is expected to enhance therapeutic efficacy, reduce dosing frequency, and minimize dose-related adverse effects.

Overall, the findings of this study demonstrate that bilosomes are a promising nano-vesicular carrier system for the oral delivery of Sorafenib. The developed bilosomal formulations effectively addressed the limitations associated with Sorafenib's poor solubility and release behavior. This work provides a strong foundation for further ex-vivo, in-vivo, and clinical investigations to establish the full therapeutic potential of Sorafenib-loaded bilosomes.

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