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Development and Evaluation of Sea Buckthorn Oil Loaded Niosomes

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ABSTRACT

The solubility of SBT oil was determined in various organic solvents by visual examination. Niosomes were formulated employing thin-film hydration method, wherein SBT, Span 40, and cholesterol, were dissolved in chloroform, to form film. The film was then hydrated using phosphate buffer pH 7.4. The SBT loaded niosomes were characterized to vesicle size, polydispersity index, and zeta potential (Dynamic light scattering), morphology (High-resolution transmission electron microscopy) and %entrapment efficiency (ultracentrifugation method). The % cumulative drug release of plain SBT oil and SBT loaded niosomes were determined employing dialysis membrane technique. Higuchi, Korsmeyer-Peppas, first-order, and zero-order models were applied to analyze the release kinetics of the selected formulation. The best SBT niosomal formulation was incorporated into hydrogel, and characterized for rheology and texture profile analysis. The solubility of SBT oil was determined in various organic solvents by visual examination. Niosomes were formulated employing thin-film hydration method, wherein SBT, Span 40, and cholesterol, were dissolved in chloroform, to form film. The film was then hydrated using phosphate buffer pH 7.4. The SBT loaded niosomes were

characterized to vesicle size, polydispersity index, and zeta potential (Dynamic light scattering), morphology (High-resolution transmission electron microscopy) and %entrapment efficiency (ultracentrifugation method). The % cumulative drug release of plain SBT oil and SBT loaded niosomes were determined employing dialysis membrane technique. Higuchi, Korsmeyer-Peppas, first-order, and zero-order models were applied to analyze the release kinetics of the selected formulation. The best SBT niosomal formulation was incorporated into hydrogel, and characterized for rheology and texture profile analysis. It followed the zero-order kinetics model with correlation coefficient value of 0.9822. Stability studies revealed SBT loaded niosomal formulation was quite stable. Hydrogel formulation revealed optimum rheological and textural attributes with flow index of 0.18, which indicated shear thinning and pseudoplastic behavior (n<1).

Introduction

Sea buckthorn (*Hippophae rhamnoides Linnaeus*) is a flowering plant (Angiosperm) of the order Rosales and Elaeagnaceae family. Sea buckthorn is morphologically described from a bush to small tree, with different growing thorns all around the plant, and it naturally grows in locations near to the sea, specific traits which build up its name. It is stated that its latin name *Hippophae rhamnoides* comes from ancient Greece, from the words “hippo”-horse- and “phaos”-shine (Franquesa et al., 2020). It is a rich source of bioactive components (including fatty acids, phytosterols, tocopherols, carotenoids, or flavonoids), has found wide application in the cosmetic, food and medical markets (Waglewska et al., 2022).

Sea buckthorn has been identified as possessing a wide range of health advantages, such as lower cholesterol, platelet aggregation, blood pressure, blood sugar and in treating tumours, stomach ulcers, and skin diseases (Yan-Jun et al., 2021). Despite its many advantages, so colloidal vesicle systems are emerging as an essential strategy for improving oil health properties (Misiaszek et al., 2022).

Nanocarriers are nanoscale materials that have the potential to enhance the bioavailability and efficacy of poorly soluble drugs. Nanoparticles can be designed to fulfill particular specifications and can be

made from a variety of materials, including dendrimers, carbon nanotubes, nanogels, carbon dots, iron oxide nanoparticles, polymeric micelles, and liposomes (Su et al., 2020). The small size nano-carriers have been shown to facilitate the movement of select biological barriers, such as the blood-brain barrier, and can enhance the delivery of the drug to particular tissues or cells (Ulldemolins et al., 2021). Nanocarriers have the potential to protect drugs from degradation and enhance their stability, thereby enhancing their therapeutic efficacy (Wahab et al., 2022).

Niosomes are an upcoming approach of nanocarriers in enhancing the solubility and bioavailability of poorly soluble drugs. They offer several advantages over conventional drug delivery systems and have the potential to significantly improve the effectiveness of a wide range of drugs. Niosomes have gained significant attention and research efforts due to their numerous advantages over alternative nanocarriers.

Hydrogel is a water-based substance with a soft consistency and moisturizing properties that can be used in various industries, including medical technology, wound care, and drug delivery systems. Hydrogel is a 3D network of hydrophilic polymer material that quickly absorbs and retain water (or other fluid). It can be formulated to degrade, dissolve, or maintain chemical stability, depending on its use. Hydrogel comes in two forms: solid and liquid. Its solid form is often rolled or sliced into sheets for wound dressings, while liquid form can be injected into the tissue or other parts of the body. Hydrogel helps in sustained release, control release, improved shelf-life, oral administration and penetration of drugs into the body.

The primary goal of this study was develop SBT-loaded niosomal formulation and gel formulation and further characterization, like (1) Entrapment efficiency (2) Solubility studies (3) *In vitro* release studies (4) Rheology and texture analysis.

Materials and Methods

The experiment was carried out during 2023-24 at Department of Pharmaceutical Sciences, Shoolini University of Biotechnology and Management Sciences, Solan. The pure Sea Buckthorn seed oil of *Hippophae rhamnoides* (Sea Buckthorn) was obtained from Deveherbes, A2-92, Janak Puri, New Delhi. Solvents such as ethanol, methanol, dimethyl sulfoxide (DMSO), span40, cholesterol, chloroform, hexane, butanol, diethyl ether, dicetyl phosphate (DCP), Carbopol 934, triethanolamine (TEOA), were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Salts for the preparation of buffer like disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride were supplied by Loba Chemie

Pvt. Ltd., Mumbai, India. Highly calibrated and sophisticated instruments were used in the study to conclude the precise results. Throughout the study, samples were accurately weighed using weighing balance from Shimadzu Corporation, Philippines; glass wares were dried in hot air oven, Setwin from Shree Sati Scientific Industries, Ambala, India. For solubility studies thermostatically controlled water bath shaker, from M/s rei motors, Mumbai, India was used. For analytical method development UV-Spectrophotometer, Evolution^{TM} 201 from Thermo Scientific, India was employed. Major instruments employed for the formulation like magnetic stirrer was from M/s Remi motors, Mumbai, India. For characterization of the formulations, refrigerated centrifuge (Model no. CPR-24 plus) was from Remi Electronik Ltd., Mumbai, India and Particle Size Analyzer was from Litesizer 500 installed at (Shoolini University, Solan, India). For morphological analysis, High- Resolution Transmission Electron Microscopy (HR-TEM) Hitachi, Japan (Model: H-7500) was used which is installed at Punjab University, Chandigarh. For hydrogel characterization, Texture analyzer (Model: TA- CTX), Rheometer (Model:RSX-CPS) installed at Ametek Brookfield, Mumbai, India.

Results and Discussion

Preparation of calibration curve by UV-spectrophotometer

The stock solution prepared in chloroform was scanned in UV, and the maximum absorbance was observed at λ_{max} 320nm. From the stock solution, dilutions were prepared in the range of 5-20 µg/ml in chloroform and absorbance was measured at 320 nm. The standard calibration curve of sea buckthorn in chloroform was plotted by taking concentration on X-axis and absorbance on Y-axis (Figure 1). The calibration curve was linear with r^2 as 0.997 and followed the Beer's Lambert law.

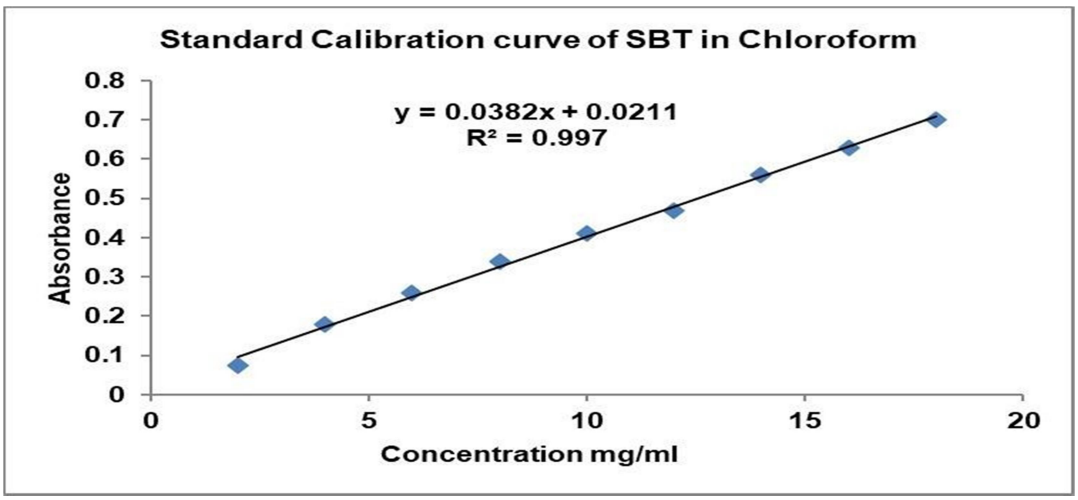


Figure 1. Standard calibration curve of SBT in choloroform

Solubility studies

To develop niosomes of SBT, firstly it should be soluble in the organic solvents. The solubility of SBT oil in various solvents was investigated visually and is depicted in the table 1. The sea buckthorn oil was maximum soluble in chloroform vis-à-vis other solvents.

Table 1. Solubility of Sea buckthorn seed oil in various solvents

S.No.	Solvent	Solubility
1	Ethanol	Not soluble
2	Methanol	Not soluble
3	Chloroform	More soluble
4	Hexane	Less soluble
5	Butanol	Less soluble
6	Diethyl ether	Less soluble

Sea buckthorn-loaded niosomes

Table 2. shows the composition of various SBT-loaded niosomes. As the main aim during the formulation of SBT loaded niosomes was to have the maximum drug loading, hence the concentration of cholesterol, span 40 were kept constant, while increasing the SBT oil concentration.

Table 2. The composition of different SBT loaded niosomal formulations

Formulations	Span 40 (w/v%)	Cholesterol (w/v%)	Chloroform (ml)	Sea buckthorn oil (w/v%)
F1	0.7051	0.733	10	5
F2	0.7051	0.733	10	10
F3	0.7051	0.733	10	15
F4	0.7051	0.733	10	20

Physiochemical characterization of SBT-loaded niosomes

The entrapment efficiency, particle size, PDI and drug loading of SBT-loaded niosomes are shown in the table 3. The mean particle diameter of all the niosomes prepared showed nanoscale dimensions and a uniform distribution of particles sizes. It was observed that with increase in the SBT oil concentration, the particle size increased. This is because higher oil concentration can lead to formation of larger vesicles due to the increased hydrophobic interaction between oil molecules and surfactants tails (Soni et al.,2024).

The observed zeta potential values exhibited negatively across all samples. The selection of optimized formulation was done on the basis of its size and the optimized formulation is chosen as F1 with a particle size of 212.9nm and PDI 0.248, entrapment efficiency (68.6%) respectively. High entrapment efficiency in F1 because smaller niosomes have a high surface area-to-volume ratio, which allows for more efficient encapsulation of the drug (SBT oil) within the vesicles (Nowroozi et al., 2018).

Formulations	Particle Size (nm)	PDI	Zeta Potential (mV)	(%) Entrapment Efficiency
F1	212.9nm	0.248	- 39.6mV	68.6%

F2	235.3nm	0.338	- 49.3mV	47.8%
F3	358.1nm	0.319	- 66.1mV	60.9%
F4	417.9nm	0.39	- 66.4mV	39%

Table 3. Physiochemical characterization of SBT-loaded niosome

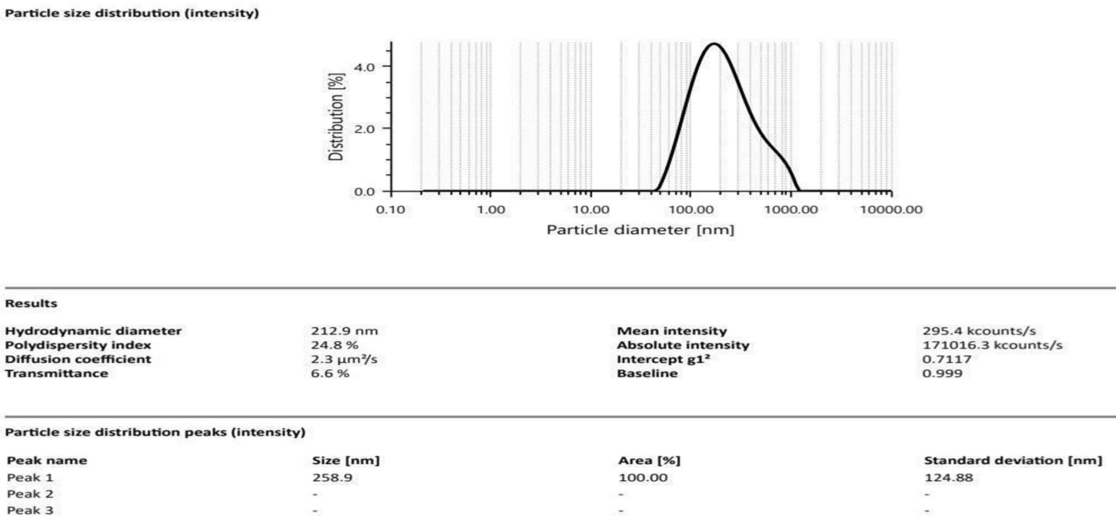


Figure 2. Particle size results of optimize Formulation

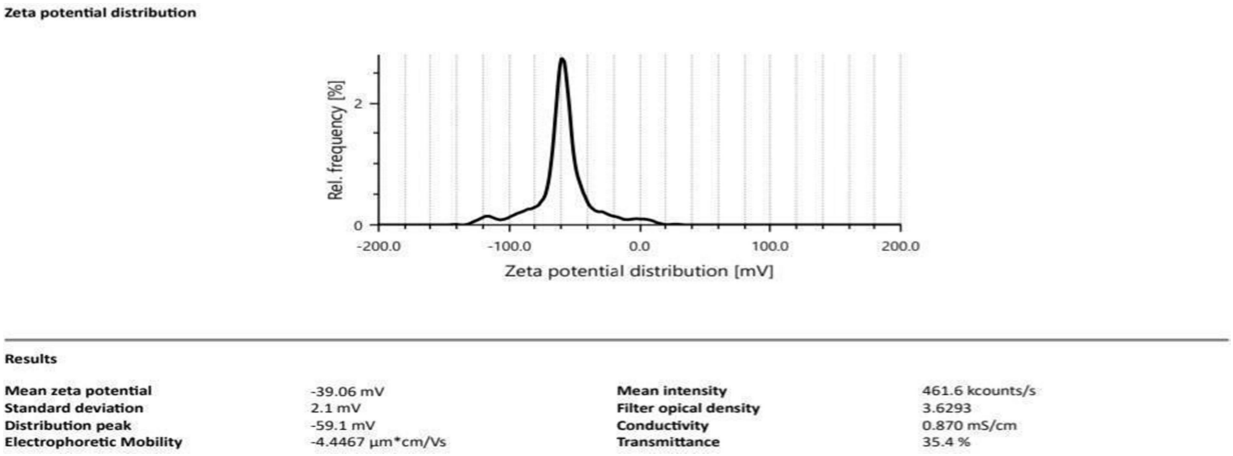


Figure 3. Zeta potential result of optimize formulation

Morphology of SBT-loaded niosomes

On the basis of particle size, zeta potential and PDI, F1 formulation was selected for HR- TEM. The high-resolution transmission electron microscopy (HR-TEM) images are presented in Figure 5.4. It is evident from that the SBT-loaded niosomes exhibits a predominantly spherical morphology, although some particles may possess a slightly elliptical shape. The noisome particles exhibit a high degree of dispersion and absence of any form of aggregation (Silva et al., 2019).

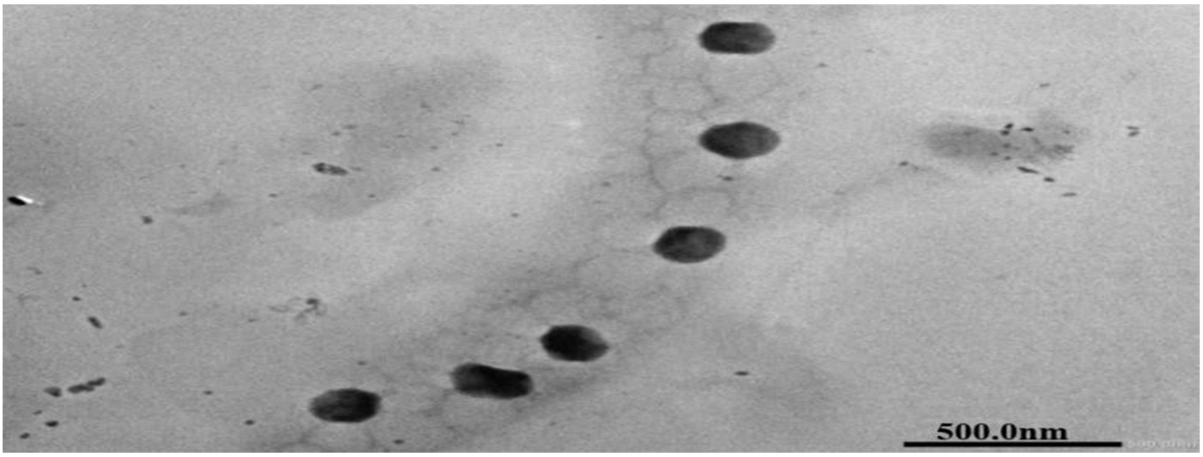
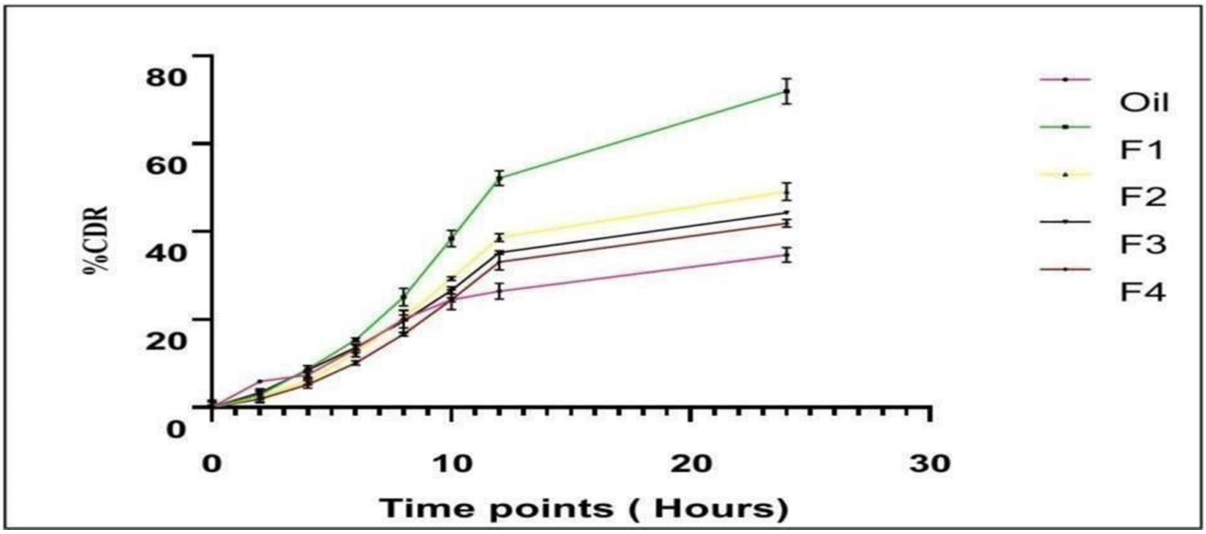


Figure 4. HR-TEM photomicrograph of optimized F1 formulation

***In vitro* evaluation of SBT-loaded Niosomes**

In vitro drug release of all the prepared niosomal formulation is shown in the Figure

5. It is vivid from the release profile that SBT from all the niosomal formulations was linear during first 6 hours, and then followed a sustained release. A diffusion- mediated process controlled the release of the SBT from all formulation during initial phases, as it followed the linear trend. The release of sea buckthorn from formulation F1 and F2 was slightly faster than from formulation F3 and F4. This may due to a difference in the composition of the niosomes in the formulations, as F1 and F2 have high

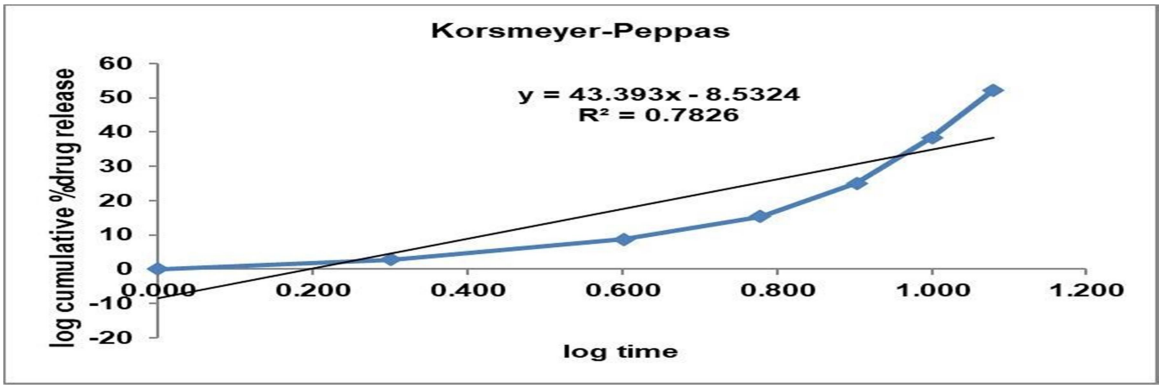


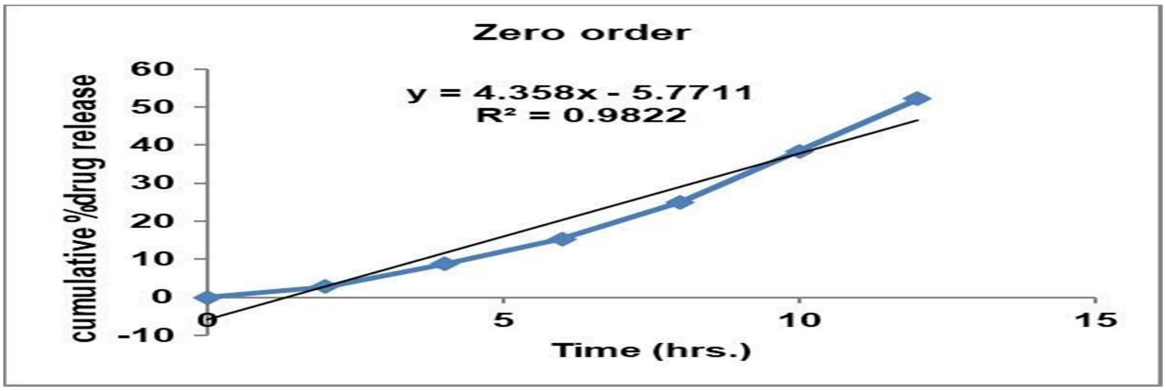
concentration of SBT encapsulated in the Niosomal vesicles. During the later time intervals, the release of SBT oil from all formulations was relatively slow, indicating that the niosomes were effectively encapsulating the sea buckthorn and preventing its premature release. Overall, the *in vitro* release data suggests that the sea buckthorn- loaded niosomes have the potential to be used for sustained delivery of sea buckthorn.

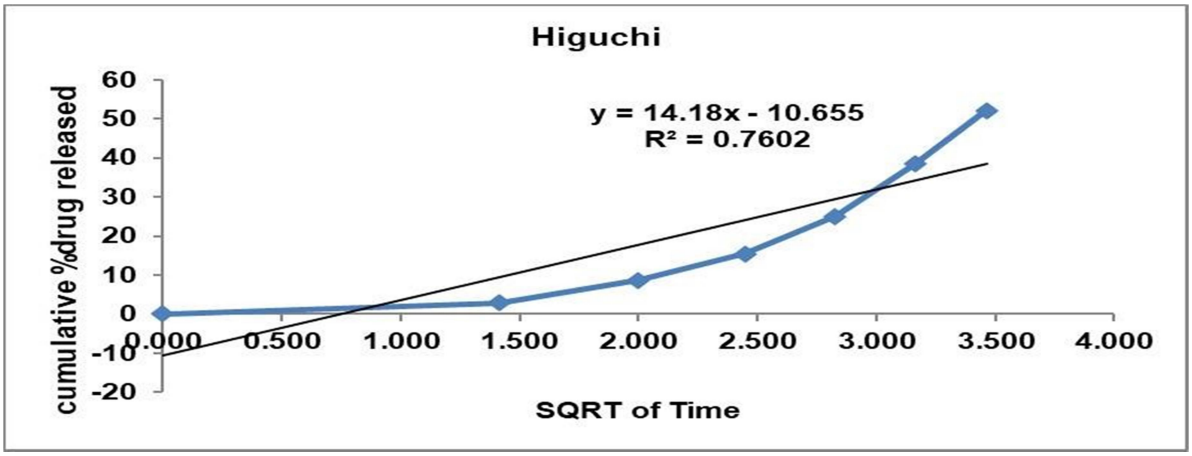
Figure 5. *In vitro* release comparison of SBT-Oil & formulations

***In Vitro* release Kinetics**

The drug release models showed that a zero-order kinetic model best fit the data, indicating a constant drug release rate regardless of remaining concentration, with an high R-squared value of 0.9822 in F1. Formulations F1, F2 and F4 followed zero-order kinetics, suggesting a similar release pattern. However, F3 followed a first-order kinetic model indicating a release rate dependent on the remaining drug concentration.


Korsmeyer-Peppas
y = 43.393x - 8.5324
R² = 0.7826
log cumulative %drug release
log time
60
50
40
30
20
10
0
-10
-20
0.000
0.200
0.400
0.600
0.800
1.000
1.200


Zero order
y = 4.358x - 5.7711
R² = 0.9822
cumulative %drug release
Time (hrs.)
60
50
40
30
20
10
0
-10
0
5
10
15


Higuchi
y = 14.18x - 10.655
R² = 0.7602
cumulative %drug released
SQRT of Time
60
50
40
30
20
10
0
-10
-20
0.000
0.500
1.000
1.500
2.000
2.500
3.000
3.500
4.000

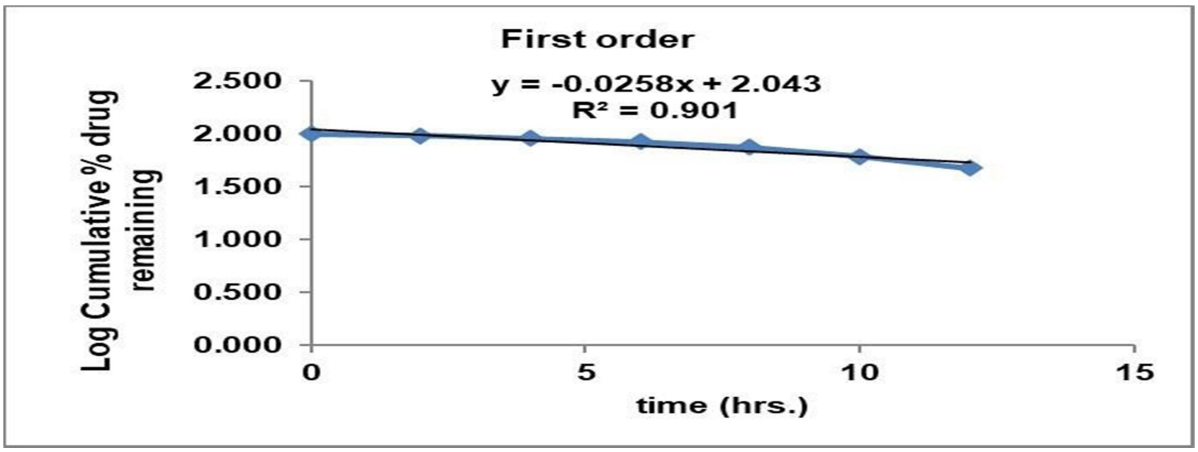
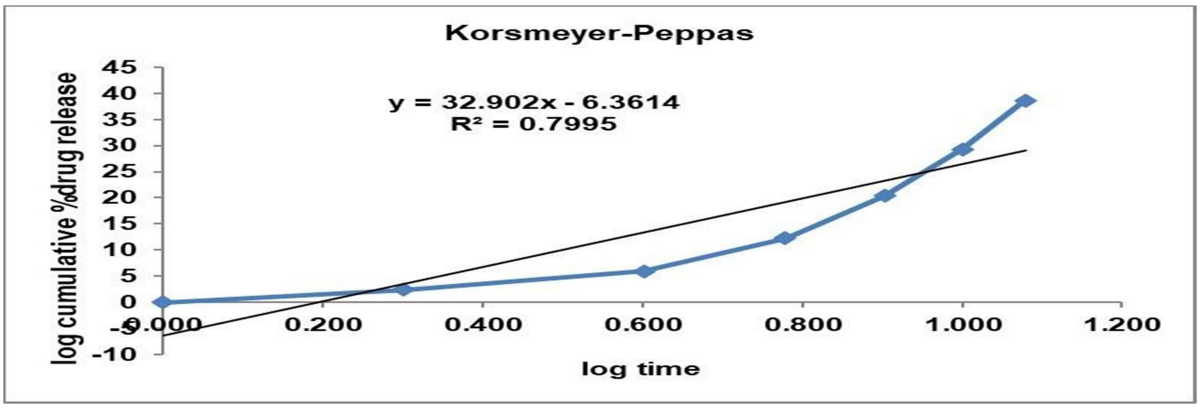
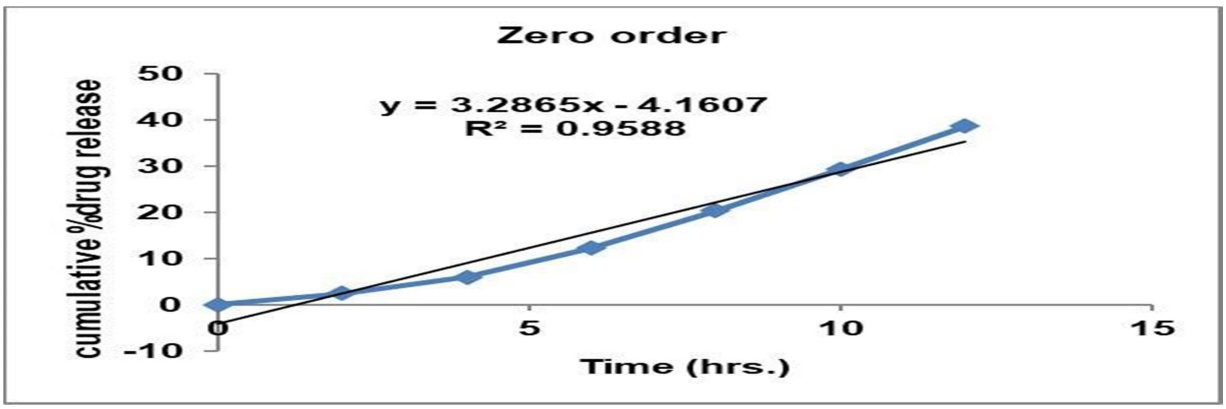
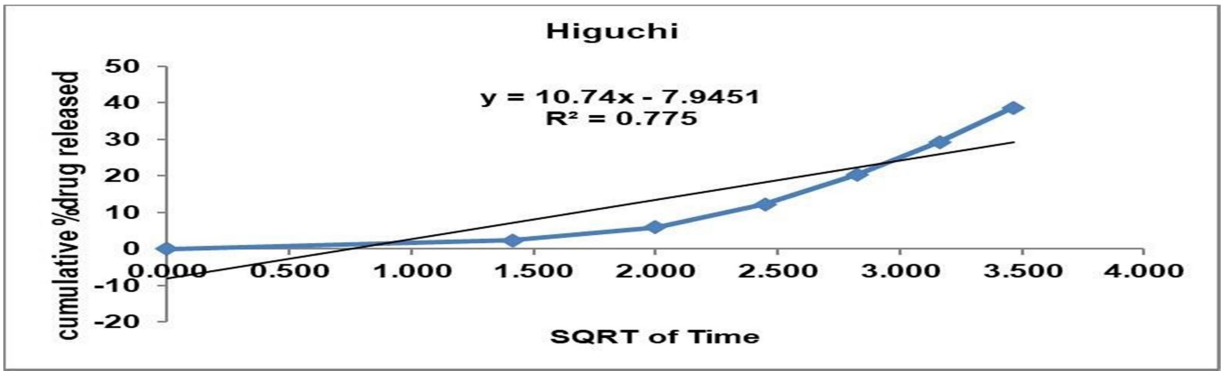

First order
y = -0.0258x + 2.043
R² = 0.901
Log Cumulative % drug remaining
time (hrs.)
2.500
2.000
1.500
1.000
0.500
0.000
0
5
10
15

Figure 6. F1 release kinetics







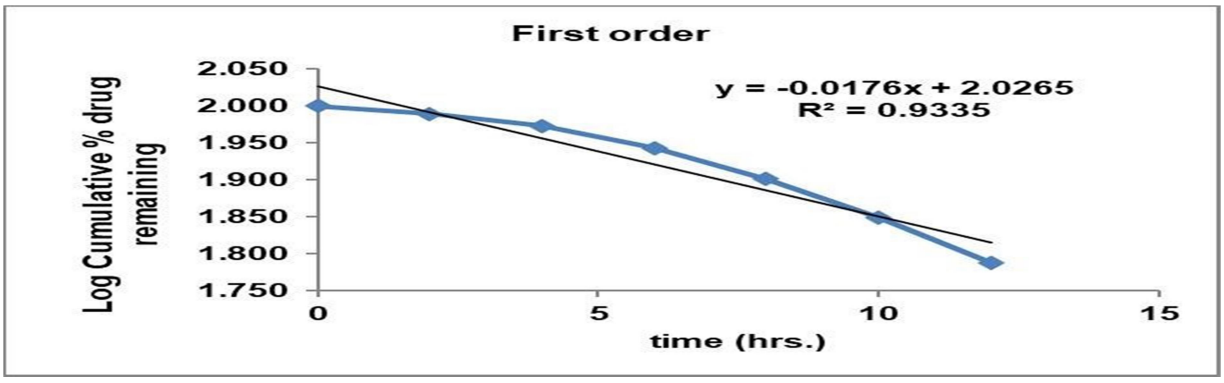
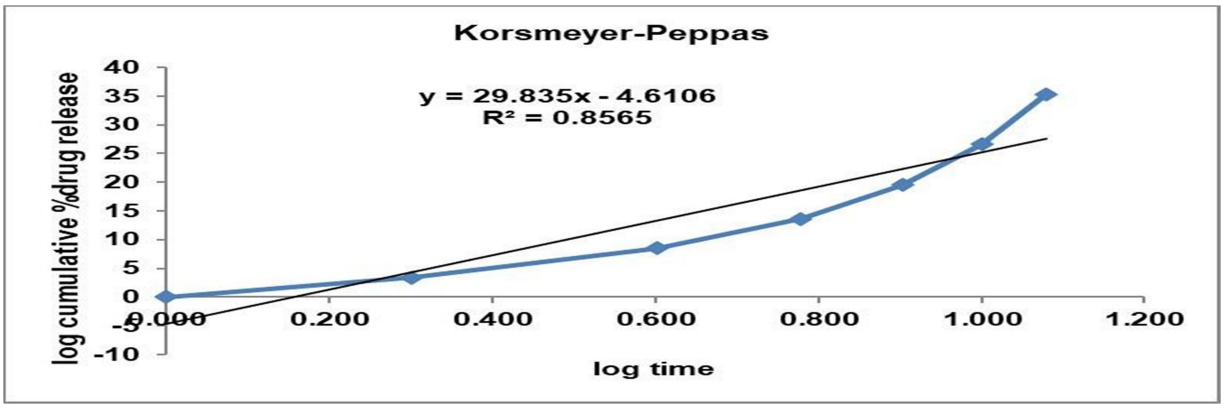
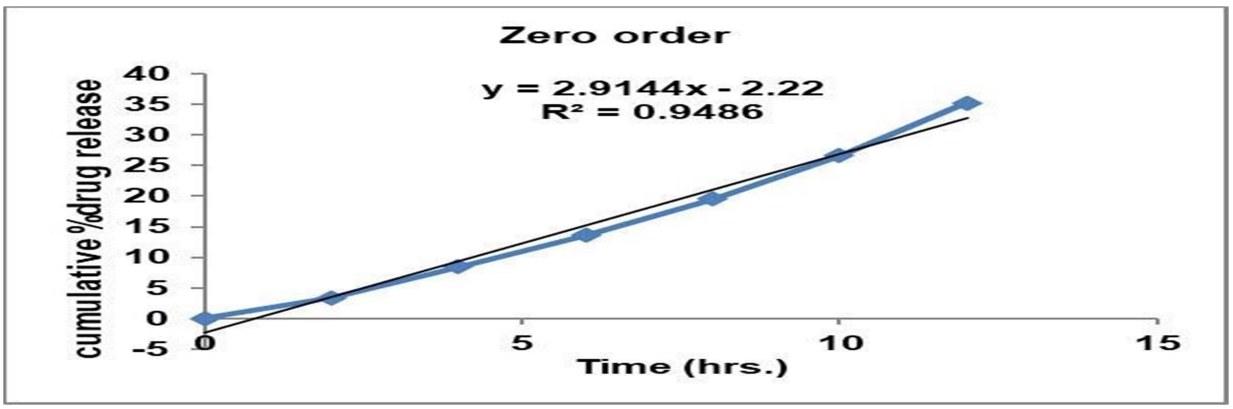
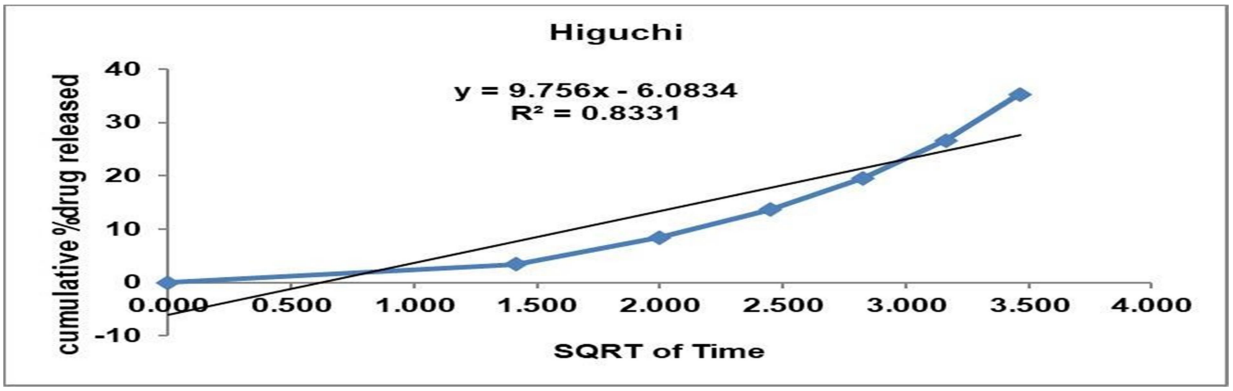


Figure 7. F2 Release kinetics







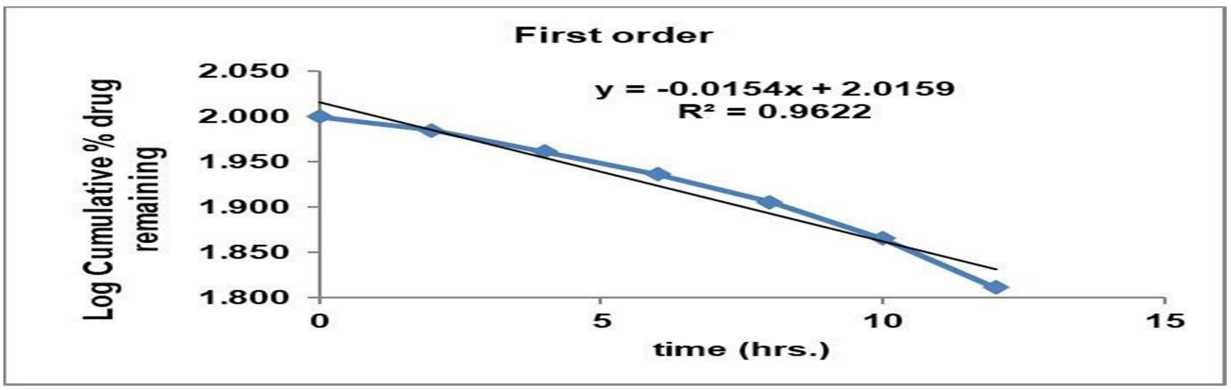
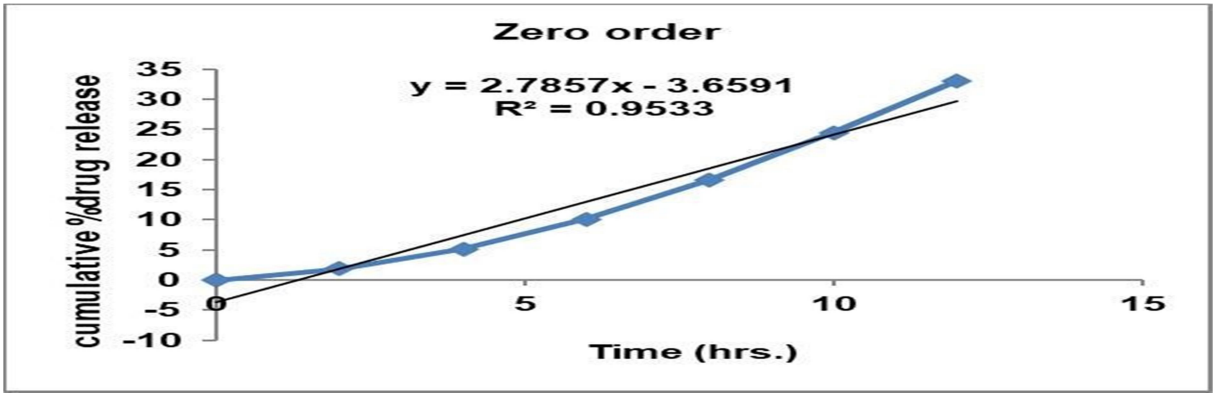
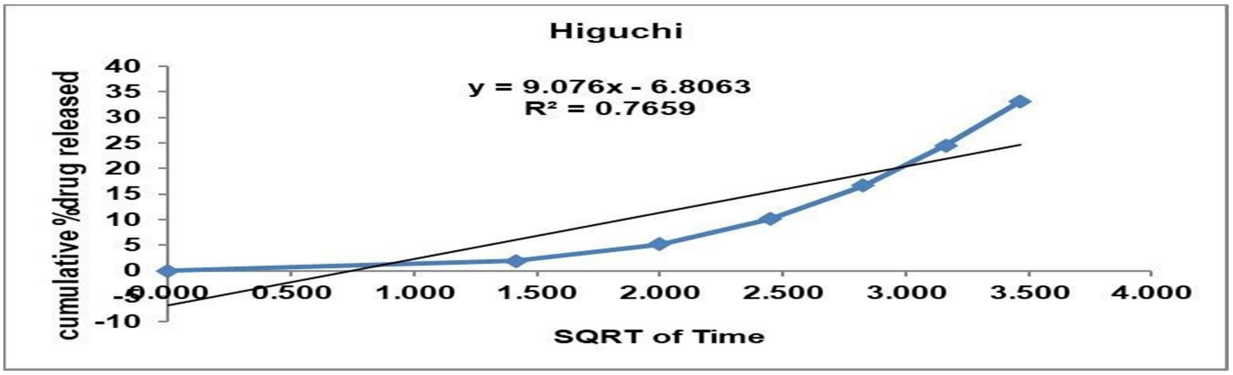
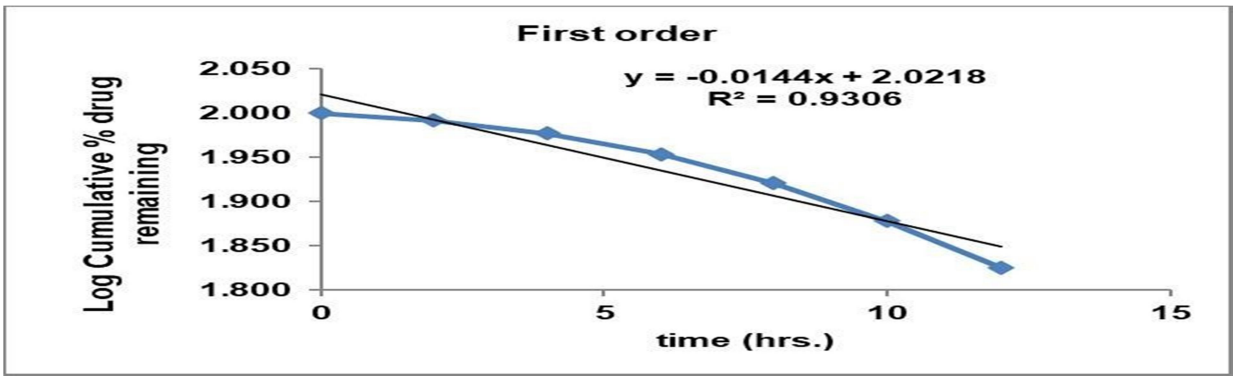


Figure 8. F3 Release Kinetics







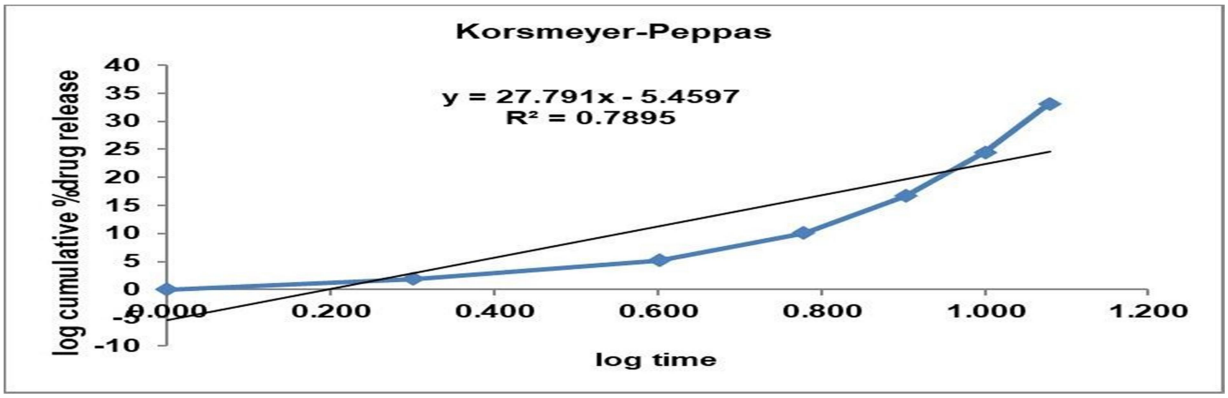


Figure 9. F4 Release kinetics

Table 4. Release kinetics table

Formulations	Zero order (r2)	Higuchi (r2)	Korsmeyer-Peppas (r2)	First order (r2)
F1	0.9822	0.7602	0.7826	0.901

F2	0.9588	0.775	0.7995	0.9335
F3	0.9486	0.8331	0.8565	0.9622
F4	0.9533	0.7659	0.7895	0.9306

Stability studies

The stability assessment of the optimal niosomes formulation was conducted under controlled conditions, i.e., at a relative humidity (RH) of 55% and a temperature of 25°C. The drug content at day 0 was compared with the drug content after two months, resulting in values of 68.6 and 54.88 respectively. Based on the available evidence, it can be conclusively stated that the niosomes exhibited stability for a minimum duration of two months.

S. No	Days	%Drug content
1.	0	68.6
2.	60	52.88

Table 5. Drug content in niosomes

Hydrogel Rheology and Texture analysis

Rheology Analysis

Because it is crucial to the flow properties of the materials while blending, loading into containers, storage, and applying to the skin, the rheological characteristics of the created gels was investigated (Dragiceivic et al., 2018 Abdulbaqi et al., 2018). The rheological profile of optimized gel formulation (i.e SBT-loaded niosomal gel) pertaining to viscosity and shear rate illustrated in Figure 10. And their Herschel- Bulkey model graphs between log e shear stress and log e shear rate have been shown in Figure 11. Values of rheology-specific parameters of the developed systems have been displayed in Table 6.

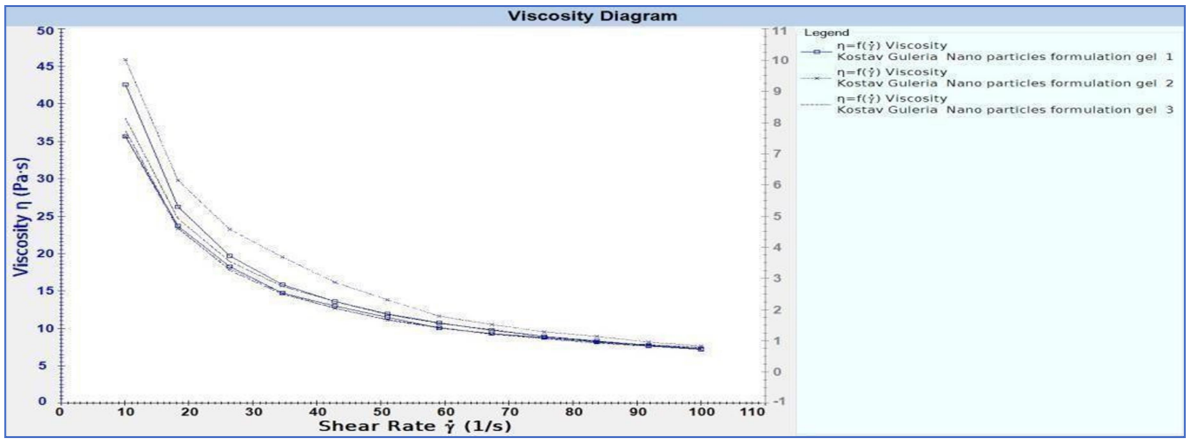


Figure 10. Plot of viscosity (η) vs. shear rate (γ) of niosomal gel formulation

Shear-thinning flow response was observed for gel based on the Herschel-Bulkey model and power law equation n (0.18) <1. Literature reports that shear-thinning is typified by a continuous decline in viscosity, which indicates a gradual decline of polymer entanglement with rising shear stress. Semisolid dosage forms should be “thick” outside of application and “thin” during application, hence shear-thinning is a desired characteristic.

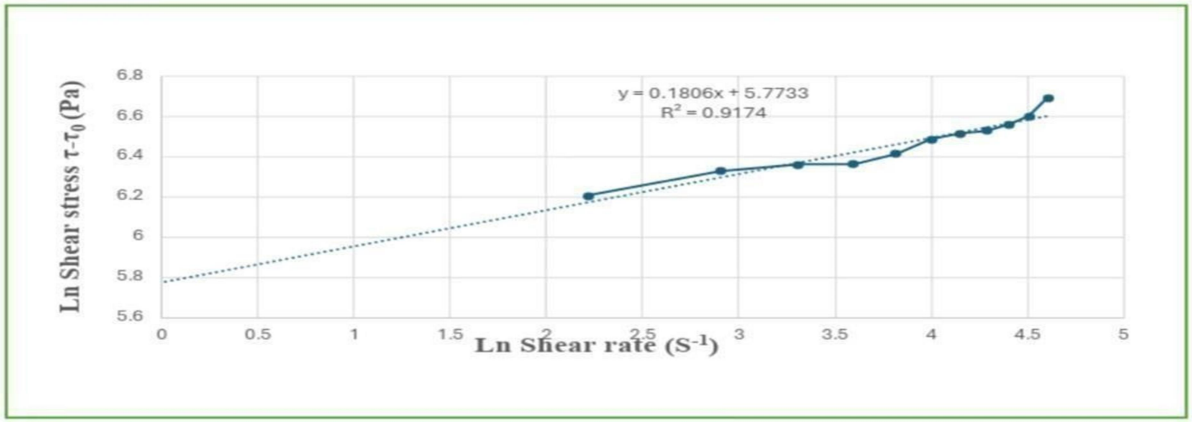


Figure 11. Linear plot between loge shear stress and loge shear rate of optimized formulation

The yield value of SBT-loaded niosomal gel was 31.8 Pa respectively, which fall within the desirable yield range (<500Pa) for the topical drug administration (Shahin et al., 2011). Consistency index (k) is the viscosity of gel in total absence of shearing and is related to the visual appearance of the product. K value for SBT-loaded gel was found to be 32.59 Pa.

Table 6. Rheological analysis results of optimized niosomal gel formulation

Formulation	N	K(Pa)	r2	Yield value (Pa)
SBT-Loaded Niosomal Gel	0.180	32.59	0.9174	31.8

Texture analysis

The Hardness/firmness, cohesiveness and adhesiveness were determined as texture parameters of the sample3 and sample2. The texture parameter of the niosomal gel sample3 and sample are shown in table no 7, figure no. 12. Sample 3 and Sample2 formulation exhibited hardnes/firmness (156.5 g) and (143.1 g), and adhesiveness (2.46 mj), and (5.41), cohesiveness (1.14 g) and (1.39). Hence, hydrogel exhibit good hardness/firmness, cohesiveness and adhesiveness.

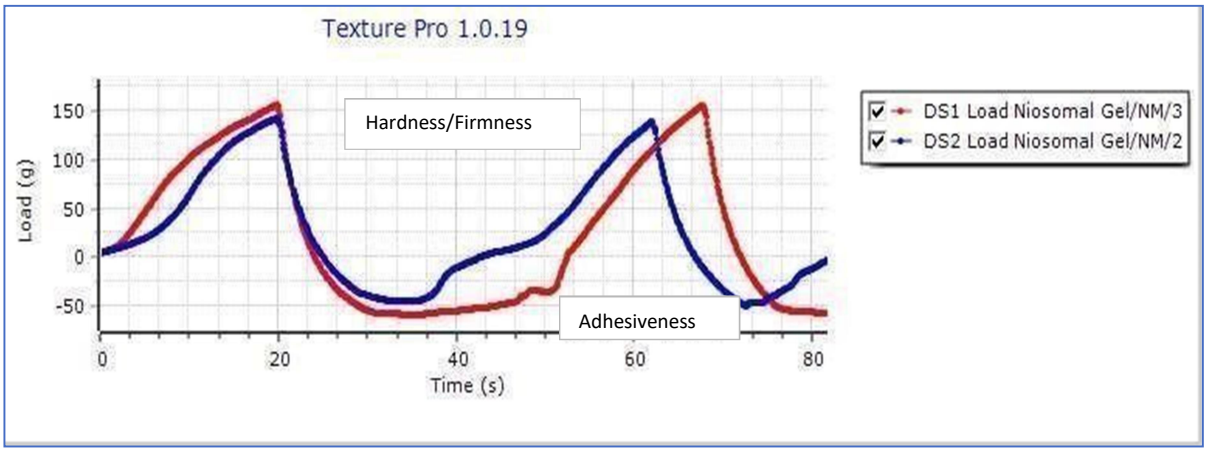


Figure 12. Graph representing texture analysis of niosomal gel formulation

Table 7. Texture analysis results of niosomal gel formulation

S.no	Niosomal Gel 2	Hardness (g)	Adhesiveness (mj)	Cohesiveness
1.	Sample 3	156.5	2.46	1.14
2.	Sample 2	143.1	5.41	1.39

Conclusion

The study aimed to develop and characterize SBT-loaded niosomes and their gel formulation, achieving significant milestones in formulation optimization and characterization. Sea buckthorn (SBT) solubility was notably favorable in chloroform compared to other solvents, facilitating efficient encapsulation within niosomes. Among the formulations tested, F1 stood out with an optimized particle size of 212 nm and an impressive entrapment efficiency of 68.6%. To quantify the release kinetics, various release kinetics models were used, including Higuchi, Korsmeyer-Pappas, Zero-order, and First order. Among these models, the zero-order model exhibit the highest correlation coefficient i.e 0.9822, indicating controlled and sustained release characteristics. These promising results prompted the selection of F1 for further development into a gel formulation, leveraging its optimal particle size (212.9nm) and high drug entrapment (68.6%). Rheological analysis of the gel formulation revealed a shear-thinning behavior with a viscosity of 7.5 Pa.s, indicative of pseudoplasticity, and a flow index ($n < 1$) suggesting non- Newtonian fluid behavior. Texture analysis demonstrated that the niosomal gel exhibited desirable attributes such as significant hardness (156.5g), moderate adhesiveness (2.46g), and good cohesiveness (1.14). Overall, this study successfully established a robust SBT-loaded niosome delivery system characterized by stability, good particle size, good entrapment efficiency, good release and favorable rheological properties. Further, the SBT loaded niosomal hydrogel can be evaluated in suitable animal model for preclinical efficacy.

References

1. De Silva L, Fu JY, Htar TT, Muniyandy S, Kasbollah S, Wan kamal WH, Chuah LH. Characterization, optimization, and in vitro evaluation of Technetium-99m- labeled niosomes. International journal of nanomedicine. 2019 Feb 12:1101-17.

2. Jasniewska A, Diowksz A. Wide spectrum of active compounds in sea buckthorn (Hippophae rhamnoides) for disease prevention and food production. Antioxidants.2021 Aug 12;10(8):1279.

3. Sulthana S, Chary PS, Bhavana V, Pardhi E, Singh SB, Mehra NK. Development and evaluation emulgel for effective management of the imiquimod-induced psoriasis. Inflammopharmacology. 2023 Feb;31(1):301-20.

4. Somagoni J, Boakye CH. Godugu C, Patel AR< Mendonca Faria HA< Zucolotto V, Singh M. Nanomiemgel-a novel drug delivery system for topical application-in vitroand in-vivo evaluation. PloS One. 2014 Dec 29; 9 (12):115952.

5. Sola Marsinach M, Cuenca AP. The Impact of sea buckthorn oil fatty acids on human health. Lipids in health and Disease. 2019 Dec;18(1):1-1.

6.Shahin M, Abdel Hady S, Hammad M, Mortada N. Novel jojoba oil-based emulsiongel formulations for clotrimazole delivery. Aaps Pharmscitech. 2011 Mar; 12:239-47.

7.Waglewska E, Misiaszek T, Bazylinska U. Nanoencapsulation of porly soluble sea- buckthorn pup oil in bile salt-origin vesicles: Physicochemical characterization and colloidal stability. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2022 Aug 20; 647:129113.