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Design and In-Vitro Evaluation of Ether-Injection–Derived Span-20 Niosomes as Nanocarriers for Atorvastatin

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ABSTRACT

Niosomes or nonionic surfactant vesicles attracted great interest in the field of modern drug delivery systems due to its distinctive properties including low toxicity, ease of handling and storage, chemical stability, biodegradability, and biocompatibility. HMG-CoA reductase inhibitors like atorvastatin are widely prescribed in the treatment of hyperlipidemia. This study focused on evaluating the effect of varying surfactant concentrations on the dissolution behavior and potency of Atorvastatin formulations prepared by the ether injection technique using Span 20 surfactant. Six laboratory-prepared formulations (F1–F6) were developed and compared with three marketed products (S1–S3). A standard calibration curve was obtained with an R^2 value of 0.9946, enabling the calculation of dissolution data for both the six niosomal formulations of the drug and the marketed drug. Dissolution testing revealed that formulations F1–F5 exhibited slow and limited drug release, with cumulative release values plateauing below 48% even after 90 minutes. In contrast, formulation F6 showed a gradual and consistent increase in drug release, reaching 95.40% at 90 minutes, closely resembling the near-complete release observed in the marketed products. Potency analysis demonstrated that F6 achieved 99.24%, meeting USP specifications, while formulations F1–F5 had lower potencies ranging from 42.90% to 77.16%. The marketed products consistently complied with both dissolution and potency standards. Overall, the results indicate that F6 with a ratio of 1:3.5:1 of drug, surfactant, and cholesterol respectively, is the only prepared formulation meeting both criteria, suggesting that its optimized surfactant concentration enhances Atorvastatin release and ensures acceptable drug content, making it a promising candidate for improved therapeutic efficacy.

Keywords: Atorvastatin; Cholesterol; Dissolution; Ether Injection Method; Formulation; Hyperlipidemia; Niosomes; Potency; Span 20; Surfactant.

1. Introduction

Atorvastatin is a popular antihyperlipidemic medication which belongs to the statin class and exerts its pharmacological action through the inhibition of the HMG-CoA reductase enzyme [1,2]. It is widely prescribed to reduce high blood cholesterol levels and prevent heart disease. Though therapeutically effective, poor aqueous solubility and low oral bioavailability, usually reported at 12-14%, are significant drawbacks of atorvastatin [3,4]. Low solubility and extensive first-pass metabolism at both the liver and intestinal mucosa are the major contributing factors [5].

Therefore, some new techniques in drug delivery are needed in order to overcome such limitations. Amongst them, the niosomes have emerged as a promising carrier. Niosomes, the microscopic vesicles made up of non-ionic surfactants, house both lipophilic and hydrophilic drugs. They improve bioavailability and prolong the release with enhanced stability of the drug [6]. Intercalation of atorvastatin in niosomes shields the drug from rapid degradation, thus allowing its controlled release [7,8]. The ether injection technique was adopted for the formulation of atorvastatin-loaded niosome in the present study.

This study has aimed to check the drug potency and in vitro drug release of the formulations and compare these findings to the marketed products. If fruitful, this formulation may enhance therapeutic outcomes, reduce the dosing frequency, and thus provide a more potent lipid-lowering therapy to the patients.

1.1. Study Objectives

i. Prepare niosomal vesicles with atorvastatin by injecting them with ether.

ii. The efficiency of drug encapsulation can be improved by optimizing the surfactant and cholesterol makeup.

iii. The determination of potency of the prepared niosomal compositions.

iv. Comparison among the potency of formulations with the marketed products of atorvastatin.

v. In vitro studies of drug release will be undertaken in order to investigate the pattern of atorvastatin release from the niosomal system.

vi. Evaluation of the medication release characteristics of commercial formulation and niosomal atorvastatin.

2. Materials and Methods

2.1. Materials

Atorvastatin calcium was gifted as a gift sample by a renowned pharmaceutical company for research purposes. Span 20, cholesterol, diethyl ether, chloroform, and 0.1N hydrochloric acid (HCl) used in the study were of analytical reagent grade and collected from the Pharmacy department laboratory, Bangladesh University. The solvent was distilled water, and all of the compounds were of analytical purity. A Shimadzu UV-1800 double-beam spectrophotometer (from Japan), a dissolution apparatus and an electronic balance (AS 220.R2 PLUS) were used and statistical data were processed with Microsoft excel.

2.2. Preparation of Atorvastatin-loaded niosomes by ether injection method

Atorvastatin-loaded niosomes were produced with the ether injection technique, a commonly used method for preparing nano and microscale vesicles [9]. Initially, a mixture was prepared for each formulation containing span 20 and cholesterol in different ratios as shown in the formulation chart F1–F6. After that, the mixture of weighed amounts of cholesterol and surfactants (Span 20) placed in a bath of water at 55-60°C. Apart from that, 100 mg of Atorvastatin was dissolved in 10 mL of chloroform in another test tube.

Table 1. Formulations of Atorvastatin Niosomal preparation (F1-F6) varying different concentrations of Span-20 surfactant

Formulation code	Atorvastatin (mg)	Span-20 (mg)	Cholesterol (mg)	Ratio	Chloroform (mL)	Final volume (mL)
F-1	100	100	100	1:1:1	10	300
F-2	100	150	100	1:1.5:1	10	350
F-3	100	200	100	1:2:1	10	400
F-4	100	250	100	1:2.5:1	10	450
F-5	100	300	100	1:3:1	10	500
F-6	100	350	100	1:3.5:1	10	550

Following that, this organic mixture was drawn into a syringe and injected dropwise using a microsyringe at a rate of 1 mL/min while being continuously stirred with the aid of a magnetic stirrer. Upon injection, the chloroform rapidly vaporized due to the high temperature, leading to the formation of niosomal vesicles as the surfactant and cholesterol self-assembled in the aqueous phase [10]. After complete addition, the suspension was stirred for 30 minutes to ensure full evaporation of the solvent. To further reduce vesicle size and obtain uniformity, a probe sonicator was used to sonicate the dispersion for five minutes. After then, the niosomal solution was kept at 4°C for additional analysis. Each formulation was coded as indication in Table 1.

2.3. Calibration curve preparation

Using a volumetric flask, 10 mg of atorvastatin calcium was taken in 100 mL of 0.1N HCl to create a stock solution. This yielded a 10 µg/mL stock solution after 10 mL was filtered and diluted to a final level of 100 mL using distilled water. Serial dilution was used to create particular concentrations (1, 3, 5, 7, and 9 µg/mL) from this stock solution. The absorbance of these standard solutions at 246 nm was measured using a UV-Visible spectrophotometer equipped with a cuvette with a 1 cm path length. To ascertain linearity and compute unknown sample concentrations, a calibration curve was plotted with concentration vs. absorbance [11]. The curve, shown in Figure 1, was utilized for additional drug assessment because it demonstrated strong linearity.

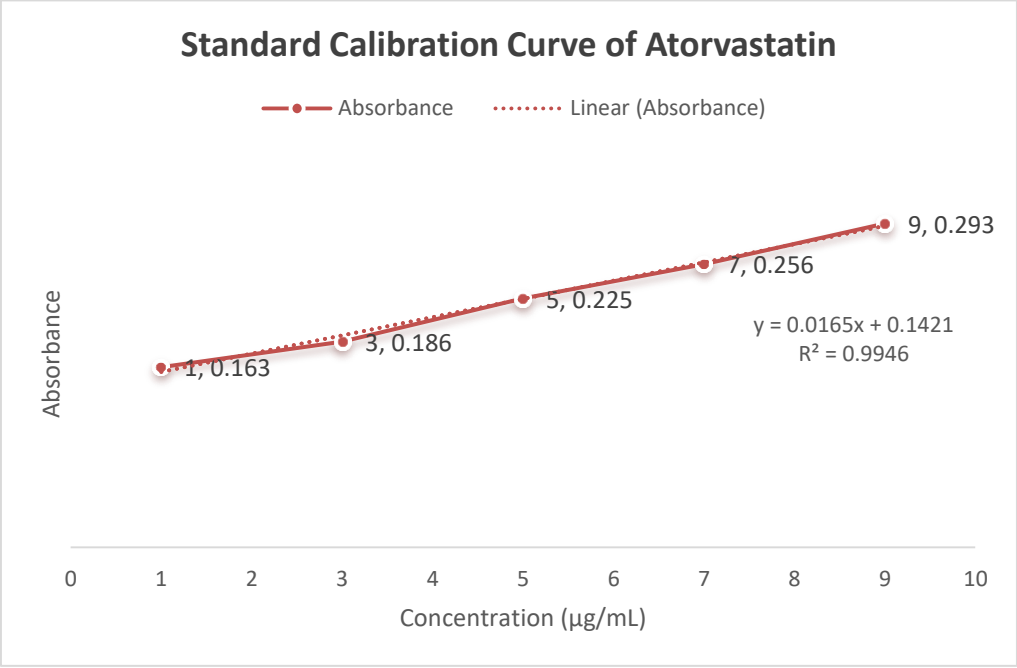

Figure 1. Standard calibration curve of Atorvastatin

2.4. Dissolution studies

To ascertain the percentage of medication released over time, the niosomal formulation of atorvastatin's in-vitro drug release profile was examined. Using 900 mL of 0.1N HCl as the dissolving media, the USP dissolving Apparatus II (Paddle type) was used to dissolve the material at 37 ± 0.2°C. The paddle was rotated at 100 rpm for 90 minutes. Samples of 10 mL were removed at regular intervals (15, 30, 45, 60, 75, and 90 minutes) and swapped out for new medium to preserve sink conditions. After the samples were filtered, absorbance at 246 nm was measured

using a UV-Vis spectrophotometer to determine the percentage of medicine release [12,13]. The traditional calibration curve was used to determine the medication concentration. The percentage of drug release was calculated using Microsoft excels and the results were shown in Table 2.

2.5. Drug content determination

To estimate the drug content, three commercially available brands of Atorvastatin tablets (10 mg) were procured. From each brand, ten tablets were selected, crushed into fine powder, also blended thoroughly. A 100 mL volumetric flask was filled with a precisely weighed powder sample that contained 10 mg of atorvastatin. To this, a sufficient volume of 0.1N HCl was added.

The solution was then sonicated to ensure complete dispersion and filtered to eliminate undissolved particles. Ten milliliters of the clear filtrate were extracted and diluted with distilled water to make 100 mL. The absorbance of this diluted solution was measured at 246 nm using a UV-Visible spectrophotometer [14,15,16].

By comparing the absorbance results to the calibration curve, the drug concentration was ascertained and the potency was calculated using Equation 1.

$$\text{Potency} = \frac{1o \times \text{Concentration of sample} \times \text{Absorbance of formulation}}{\text{Absorbance of standard}} \quad (1)$$

3. Result and Discussion

This work aimed to evaluate the influence of varying surfactant concentrations on the dissolution behavior of Atorvastatin tablets. Six formulations (F1–F6) containing different surfactant levels were developed, and their cumulative drug release profiles were compared with three marketed reference products (S1–S3). The comparative dissolution results are summarized in table 2 and presented in Figure 2.

Among the test formulations, F6 exhibited the highest drug release, reaching 95.40% at 90 minutes, and showed a dissolution profile most comparable to the marketed preparations, which displayed near-complete release across all time intervals. In contrast, formulations F1–F4 displayed slow and limited drug release, with values generally plateauing below 40–48% even after 90 minutes. Formulation F5 demonstrated the poorest performance, releasing only 25.58% of the drug at 90 minutes, far below the reference products.

Formulation F6 showed a gradual and consistent increase in drug release, beginning with 24.49% at 15 minutes and rising progressively to 42.49%, 55.41%, 74.94%, 80.67%, and 95.40% at 30, 45, 60, 75, and 90 minutes, respectively. Although F6 eventually reached more than 80% release at 75 minutes, it demonstrated the best dissolution characteristics among the test batches and shows potential for improved Atorvastatin release and enhanced bioavailability.

All marketed samples (S1–S3), however, exhibited rapid and complete dissolution from the earliest time point, confirming compliance with standard quality requirements. Dissolution values exceeding 100% may be attributed to assay values slightly above the labeled claim and minor analytical variations, which are within acceptable USP limits.

Table 2. Comparative study of dissolution profile among six F1 to F6 and S1 to S3

Formulation	Time for percentage of drug release					
	15 min	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
F1	20.78	29.23	33.69	37.1	37.5	40.32
F2	22.41	24.78	29.69	38.6	39.5	39.69
F3	21.32	29.14	34.78	34.96	36.69	38.78
F4	24.05	27.96	28.32	30.78	39.69	48.05
F5	9.76	13.58	16.3	20.12	22.3	25.58
F6	24.49	42.49	55.41	74.94	80.67	95.4
S1	100.49	101.4	102.76	104.67	104.7	105.3
S2	102.30	103.30	103.94	104.12	104.37	104.5
S3	100.67	101.67	103.76	104.76	105.1	105.5

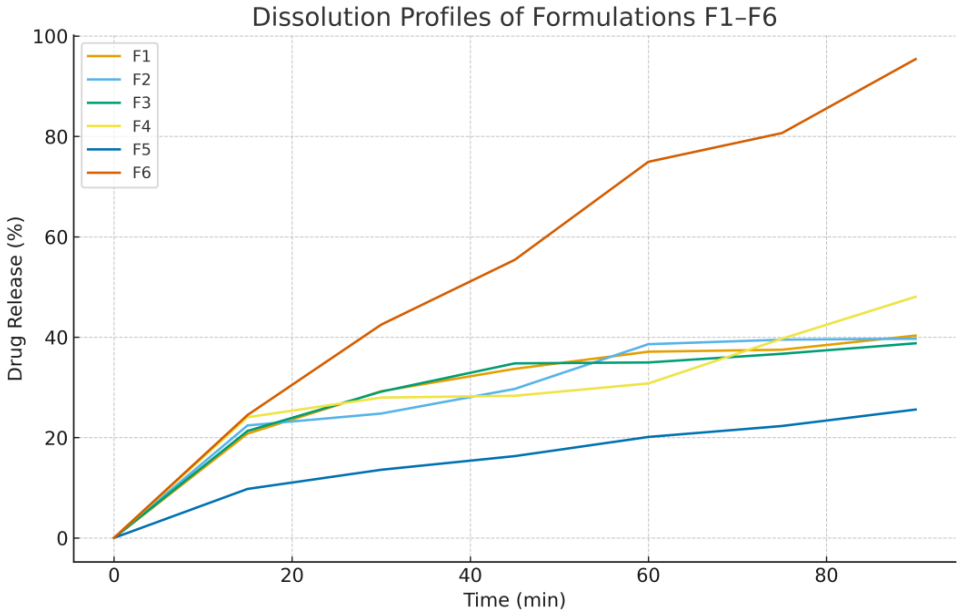

Figure 2. Comparison of dissolution profile among six formulations F1-F6

Additionally, potency comparisons were conducted between the prepared formulations (F1–F6) and the three marketed products (S1, S2, and S3). Table 3 and Figure 3 presented the potency values of both the formulated niosomes and the commercial samples. Among the marketed products, S2 exhibited the highest potency at 101.38%, consistent with USP requirements (95%-105%). For the prepared formulations, F6 showed the highest potency at 99.24%, closely aligning with the marketed drugs and falling within acceptable USP limits. In contrast, formulations F1–F5 demonstrated significantly lower potencies, ranging from 42.90% to 77.16%, and therefore did not meet the required potency specifications. Overall, while all marketed products complied with potency standards, only formulation F6 met the USP criterion among the prepared niosomes.

Table 3. Potency of formulation and potency of sample tablet are given below:

Sample	Absorbance	Potency (%)
F1	0.223	77.16
F2	0.183	63.32
F3	0.124	42.90
F4	0.125	43.25
F5	0.127	43.94
F6	0.287	99.24
S1	0.278	96.19
S2	0.293	101.38
S3	0.284	97.89

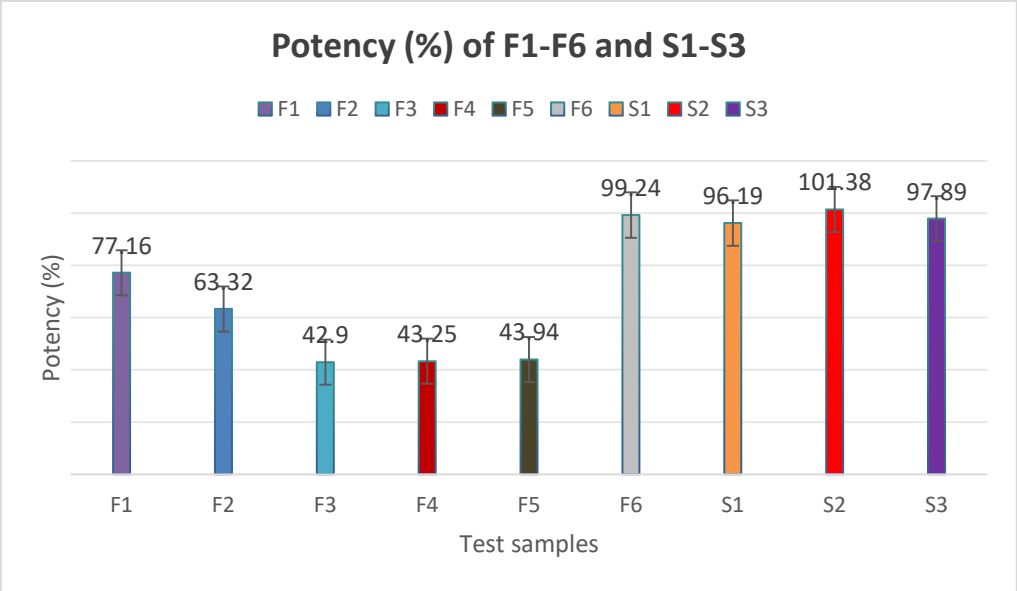

Figure 3. Potency of formulations F1-F6 and samples S1-S3

The observed differences in dissolution and potency among the six prepared Atorvastatin formulations (F1–F6) can largely be attributed to variations in surfactant concentration and composition. Among the six formulations, F6 consistently showed superior performance, exhibiting the highest drug release (95.40% at 90 minutes) and achieving potency within USP specifications. Its dissolution profile was the closest to the marketed products (S1–S3), which displayed rapid and complete drug release along with high potency values (96.19%–101.38%). This finding aligns with prior studies reporting that increased surfactant levels in niosomal systems enhance drug solubilization, vesicle stability, and drug release by reducing interfacial tension and improving wetting and dispersion of the drug within the vesicles [17]. In contrast, formulations F1–F5 showed inadequate dissolution and substandard potency, indicating insufficient drug content and poor release characteristics. The findings confirm that F6 is the only formulation that meets both potency and dissolution criteria, suggesting that its optimized surfactant ratio enhances drug release and ensures acceptable drug content.

4. Conclusion and Further study

The niosomal drug delivery method is one of the remarkable advancements in nanotechnology and medication delivery. As niosomes are often stable in nature, they clearly have a preferred drug delivery mechanism over alternative dosage configurations and strategy to improve the oral bioavailability, stability, and high penetration of the medicines encapsulated in niosomes through biological membranes. The objective of the present study is to create atorvastatin formulations using various surfactant and cholesterol in distinct ratios and assess its in vitro efficacy. A niosome formulation that provides high potency and solubility percentage of drug release is considered optimum or best. These facts suggest that niosomes could be employed to increase atorvastatin's potency and dissolution percentage of drug release. To investigate regarding these formulations, the following studies should be carried out to provide a concrete conclusion-

i. More variations are possible with the niosomal formulations; hence, further research is required for better results.

ii. The in vitro studies need to be continued for the testing of potency and percentage of dissolution before entering into applications at a clinical or market level.

iii. In this study, only in vitro potency and dissolution percentage of Atorvastatin were determined, further studies are needed to establish its reliability and effectiveness.

iv. Further in vitro and in vivo studies will be required to fully validate the formulation.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

Md. Mahbubol Alam took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing and editing. Ahsan Ullah, Md. Wasim Kazi and Fahamida Binta Anower analyzed data. Most. Ashrafia Sultana and Md. Jewel Islam were involved in the design of the manuscript. Abul Kalam Azad and Md. Sakib Mia reviewed the manuscript. All authors have read and approved the final manuscript.

Availability of data and materials

Supplementary information is available from the authors upon reasonable request.

Ethical Approval

Not applicable for this study.

Informed consent

Not applicable for this study.

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