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Development and Characterisation of Deep Eutectic Mixture of the Atorvastatin Calcium for Oral Drug Delivery

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Abstract:
The poor aqueous solubility and low oral bioavailability of atorvastatin calcium pose significant challenges in optimising its therapeutic efficacy. This study explores the formulation of deep eutectic solvents (DES) to enhance the solubility and bioavailability of atorvastatin calcium for oral delivery. Various DES formulations were prepared using choline chloride, choline bitartrate, and selected carboxylic acids. The formulations were characterised through physicochemical analysis, including pH, drug content, particle size, zeta potential, and Fourier-transform infrared spectroscopy. The most promising formulation, F12, exhibited a yellowish transparent appearance, a mean particle size of 74.21 nm, and a zeta potential of -22.3 mV, indicating good colloidal stability. In vitro drug release studies revealed a delayed release profile for F12 compared to the pure drug, potentially offering advantages in prolonged therapeutic effect and reduced dosing frequency. Kinetic modelling analysis suggested a diffusion-controlled release mechanism influenced by drug diffusion and polymer relaxation. Overall, this study demonstrates the potential of DES formulations in enhancing the solubility and bioavailability of atorvastatin calcium, paving the way for improved therapeutic outcomes and patient compliance.

Keywords: Atorvastatin calcium, deep eutectic solvents, solubility enhancement, bioavailability, oral delivery, controlled release, kinetic modelling.

1. Introduction

Atorvastatin Calcium (structure shown in Figure 1), a statin commonly prescribed for hypercholesterolemia management [1] and cardiovascular disease prevention [2], remains a cornerstone in clinical therapy due to its proven efficacy. However, despite its clinical benefits, Atorvastatin Calcium faces significant limitations arising from its poor aqueous solubility [3] and low oral bioavailability [4]. These issues substantially curtail its therapeutic potential. The drug exhibits an aqueous solubility of approximately 0.1 mg/mL [5], a characteristic that severely restricts its dissolution within the gastrointestinal tract [6]. This poor solubility is a critical factor contributing to its limited absorption when administered orally. Consequently, the oral bioavailability of Atorvastatin Calcium is around 12%, indicating that only a small fraction of the ingested dose reaches systemic circulation in an active form [7]. These pharmacokinetic limitations necessitate the administration of higher doses to achieve the desired therapeutic effect, potentially leading to increased incidence of adverse effects. Enhancing the solubility and bioavailability of Atorvastatin Calcium is thus a focal point of ongoing pharmaceutical

research [8]-[10]. Strategies such as the development of novel drug delivery systems, use of solubilizing agents, and formulation modifications are being explored to overcome these challenges, aiming to maximize the drug's clinical efficacy while minimizing side effects.

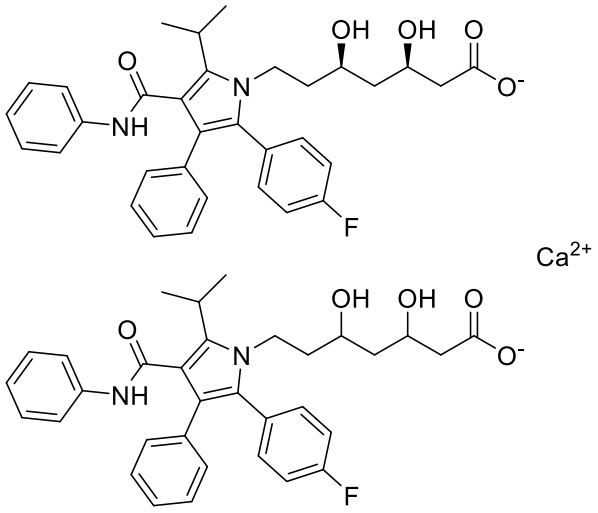

Figure 1: Atorvastatin Calcium structure

Deep Eutectic Solvents (DES) represent an innovative class of solvents, arising from the complexation of hydrogen bond donors (HBD) and acceptors (HBA) [11]. These solvents have garnered considerable attention for their potential to enhance the solubility and bioavailability of drugs with poor water solubility [12]-[14]. Characterised by their tunable physicochemical properties, biocompatibility, and environmentally benign nature, DES offer a versatile solution in pharmaceutical applications [15]. The unique ability of DES to improve drug solubility stems from their capacity to disrupt the crystalline lattice of hydrophobic drugs. This disruption facilitates an increase in the drug's dissolution rate, thereby enhancing its bioavailability. The molecular interactions within DES, primarily driven by hydrogen bonding, enable them to effectively solvate poorly soluble compounds, overcoming one of the major limitations in drug formulation [15]. Furthermore, deep eutectic solvents (DES) offer versatile physicochemical properties [16] that can be tailored to accommodate the specific requirements of diverse drug molecules, thereby optimizing their compatibility and therapeutic efficacy [17]. These solvents demonstrate excellent biocompatibility, ensuring minimal toxicity profiles that are crucial for pharmaceutical applications. Moreover, their environmentally benign nature aligns seamlessly with the escalating global emphasis on sustainable and eco-friendly practices in drug synthesis and formulation. DES thus represent a promising avenue in pharmaceutical research and development, facilitating both enhanced drug delivery systems and adherence to stringent environmental standards [18].

This study focuses on developing a deep eutectic solvent (DES) formulation of Atorvastatin Calcium to enhance its solubility and bioavailability for oral delivery. The key objectives encompass the careful selection and fine-tuning of DES constituents, thorough physicochemical characterization of the DES-Atorvastatin Calcium blend, and rigorous assessment of its in vitro performance. By systematically exploring these facets, we aim to establish a comprehensive understanding of how DES can optimize the formulation's efficacy, paving the way for improved therapeutic outcomes and potential advancements in pharmaceutical sciences. This study addresses the gap in existing solubility enhancement techniques for Atorvastatin Calcium by leveraging the unique properties of DES. Previous approaches such as solid dispersions [19], nanoparticle formulations [20], and cyclodextrin complexes [21] have shown limited

success due to complex manufacturing processes, stability issues, and potential toxicity. DES, on the other hand, provide a simpler and potentially more effective alternative.

Utilizing Deep Eutectic Solvents (DES) for the formulation of Atorvastatin Calcium represents a promising strategy to enhance therapeutic efficacy and promote patient adherence to treatment protocols. DES are known for their capacity to effectively dissolve poorly soluble drugs, thereby addressing a significant hurdle in pharmaceutical development. This research holds implications beyond Atorvastatin Calcium, potentially revolutionizing the formulation of other challenging pharmaceutical compounds by leveraging DES's ability to enhance solubility and therapeutic outcomes. Such advancements could significantly expand the utility of DES in pharmaceutical formulations, offering a versatile and sustainable solution to a prevalent industry challenge. Moreover, the development of DES-based formulations aligns with current pharmaceutical research efforts aimed at optimizing drug delivery systems to improve patient outcomes and compliance. By tackling solubility issues through innovative methodologies like DES, our work aim to advance pharmaceutical science and elevate patient healthcare experiences.

Material and Methods

Materials

The chemicals and reagents utilised in this study are of analytical grade and were sourced from reputable suppliers to ensure the accuracy and reliability of the experimental outcomes. Atorvastatin was procured from Anant Pharmaceuticals Pvt. Ltd., while choline bitartrate and choline chloride were obtained from Spectrum Chemical, United States. Urea was supplied by Fischer Scientific. Phenol and citric acid were purchased from Qualikems. Benzoic acid was sourced from Central Drug House. Thiourea and menthol were acquired from Lobachemie. All materials were used as received, without further purification, unless specified otherwise, to maintain the integrity of the experimental processes.

Equipment

The equipment used in this study was sourced from renowned manufacturers to ensure precision and reliability of the experimental results. The UV spectrophotometer used was from Shimadzu, Japan. A magnetic stirrer, melting point apparatus, vortex mixer, cooling centrifuge, and vortex shaker used were all from REMI Equipment, Mumbai. The pH meter used was from Ohaus, USA, and the digital balance used was also from Shimadzu, Japan. The FTIR spectrometer used was supplied by Perkin Elmer, and the water bath shaker used was from NSW India.

Methods

Pre-formulation studies

Pre-formulation studies constitute a critical phase in drug development, focusing on evaluating the chemical and physical properties of the drug prior to formulation. These investigations are pivotal as they assess the physicochemical attributes that can influence drug efficacy and guide the formulation process towards creating an optimal dosage form. Understanding these characteristics may also justify the need for molecular modifications if necessary. At its core, pre-formulation studies aim to ensure that the drug's development progresses smoothly without significant impediments. The protocol for developing a safe, effective, and stable dosage form hinges greatly on the outcomes derived from these studies. Analytical techniques such as melting point analysis, UV spectroscopy, and infrared spectroscopy were employed to characterize the drug sample thoroughly. These methods provided

essential insights into the drug's thermal behavior, spectral properties, and chemical structure, crucial for formulating a dosage form that meets stringent efficacy and safety standards.

Organoleptic Properties

The drug sample underwent physical characterization to assess its organoleptic properties. Firstly, the colour of the sample was visually inspected under standard lighting conditions. Secondly, the odour profile was evaluated. Finally, the physical form of the drug was determined through macroscopic examination, detailing its shape, texture, and any visible features.

Melting Point

The drug's melting point was determined using a melting point apparatus. A small quantity of the medication was placed into a capillary tube, which was 10–15 mm in length, 1 mm in diameter, and closed at one end. The sample in the capillary tube was heated gradually and uniformly, with the temperature measured using a thermometer. The melting point of the drug was identified by recording the temperature range at which the sample transitioned from solid to liquid phase.

UV absorption maxima of Atorvastatin

The UV absorption maxima (λmax) of Atorvastatin were determined using a double beam UV-visible spectrophotometer (Shimadzu UV-1800, Japan). A solution containing 20 µg/ml of Atorvastatin was prepared and scanned over the wavelength range of 200-400 nm. The absorbance spectrum was plotted against wavelength to identify the wavelength at which maximum absorption occurred, indicating the specific electronic transitions associated with the chromophoric part of the molecule in solution.

Estimation of Atorvastatin by UV-visible spectrophotometer

a. Preparation of Stock Solution

To prepare the standard stock solution of Atorvastatin, 10 milligrams (mg) of the compound were dissolved in 10 milliliters (ml) of methanol, resulting in a concentration of 1000 micrograms per milliliter (µg/ml). Subsequently, 10 ml of this stock solution was aliquoted and diluted to 100 ml with methanol to achieve a final concentration of 100 micrograms per milliliter (µg/ml). The resulting solution was then thoroughly mixed to ensure uniform distribution of the drug in the solvent.

b. Preparation of Working Solution

The standard stock solution of atorvastatin (100 micrograms per milliliter, µg/ml) was prepared using methanol as the solvent. Subsequent dilutions ranging from 4 to 20 µg/ml were prepared by diluting this stock solution with methanol. Each diluted solution's absorbance at 247 nm was measured using a UV-visible spectrophotometer, with methanol serving as the blank. A calibration curve was constructed by plotting the absorbance values against the corresponding concentrations. The intercept, slope, equation of the straight line, and correlation coefficient of the calibration curve were then determined to establish a quantitative relationship between absorbance and concentration.

Solubility Studies

Excess drug was placed in thoroughly cleaned culture tubes, each containing 2 ml of various solvents: methanol, ethanol, phosphate buffer pH 6.8 (PB pH 6.8), 0.1N hydrochloric acid (HCl), and water. These tubes were tightly sealed to ensure a quantitative solubility study. Over a period of twenty-four hours at room temperature (25°C), the culture tubes were agitated on a water bath shaker. Following agitation, each sample underwent centrifugation at 15,000 rpm for 24 hours, after which the supernatant was carefully decanted. The supernatant was subsequently filtered, and the resulting filtrates were

appropriately diluted and subjected to spectrophotometric analysis. This method enabled the determination of the drug's solubility in each solvent under standardized conditions.

Determination of Partition Coefficient (P)

The partition coefficient (P) of drugs, an essential indicator of their lipophilicity or hydrophilicity and their potential to traverse cellular membranes, was determined using a standard method involving n-octanol and water phases. The partition coefficient was defined as the ratio of the drug's concentration in n-octanol ($C_{n\text{-octanol}}$) to its concentration in water (C_{water}), denoted as $P_{o/w}$:

$$P_{o/w} = \frac{C_{n-octanol}}{C_{water}}$$

where $C_{n-octanol}$ and C_{water} represent the equilibrium concentrations of the drug in n-octanol and water phases, respectively. Drugs with partition coefficient values significantly greater than 1 are classified as lipophilic, indicating a strong affinity for lipid-rich environments, whereas values significantly less than 1 indicate hydrophilicity, suggesting a preference for aqueous environments. To facilitate quantitative comparisons across different drugs, the partition coefficient ($P_{o/w}$) was further expressed as the logarithm to base 10 (log P), providing a standardized measure of lipophilicity or hydrophilicity. This methodological approach ensures consistency and accuracy in evaluating the physicochemical properties of drugs relevant to their pharmacokinetic behavior.

FTIR of Atorvastatin and Excipients

Fourier Transform Infrared (FT-IR) spectroscopy was employed to analyze the structural composition of atorvastatin in combination with various excipients. This analytical technique enables the identification of functional groups present in the sample based on their characteristic absorption bands. Approximately 4-5 mg of atorvastatin was placed in the FT-IR chamber and subjected to infrared radiation over a wavenumber range of 4000 to 400 cm^{-1}. This spectral range encompasses vibrations associated with different molecular bonds, allowing for comprehensive analysis of chemical structures and potential interactions between atorvastatin and excipients. The FT-IR spectra obtained were scrutinized to detect any shifts, new peaks, or alterations in absorption patterns, indicating possible interactions between atorvastatin and the excipients present in the formulation. This methodological approach provides valuable insights into the compatibility and stability of atorvastatin in pharmaceutical formulations, aiding in the assessment of potential drug-excipient interactions crucial for formulation development and quality control.

Drug-excipients Compatibility Study by FTIR

The study aimed to detect any physical or chemical interactions between the drug and excipients by analyzing their respective spectra. The drug was uniformly blended with different excipients in a 1:1 ratio. Spectral scans were conducted using FTIR over the range of 400–4000 cm^-1. Comparative analysis of the spectra of the pure drug and the drug-excipient mixtures was performed to identify any signs of incompatibility or physical alterations. This methodological approach allowed for a comprehensive investigation into the drug-excipient compatibility using FTIR spectroscopy.

Preparation of Deep Eutectic Solvent Mixture of Atorvastatin

To prepare Deep Eutectic Solvent Derivatives (DESDs) containing Atorvastatin, choline chloride and choline bitartrate were combined in varying ratios with aqueous media (5 mL). Selected carboxylic acids

were introduced into the mixtures, resulting in uniform solutions which were then sealed in vials. The vials were heated to 75°C in an oven and subsequently cooled to room temperature, with only solutions remaining in liquid form being considered viable room-temperature solvents for poorly soluble model drugs. Atorvastatin, chosen due to its low aqueous solubility, was utilized as the model drug for the solubility experiments. A measured quantity of Atorvastatin was added to blank DESD solvents, followed by vigorous vortexing until no excess solid remained undissolved. The resulting solution, containing the additional medication, was allowed to stand for 24 hours to achieve equilibrium and determine the drug's solubility in the DESDs. This method ensured thorough evaluation of Atorvastatin's solubility enhancement potential within the prepared DESD formulations.

Table 1: Screening of Carboxylic Acid for Deep Eutectic Solvent Mixture of Atorvastatin.

Sr. No	Formulation Code	Carboxylic Acid Used	Molar Ratio	Drug Atorvastatin	Choline Chloride	Choline Bitartrate	Carboxylic Acid amount
1.	F1	Benzoic acid	1:1:1	100 mg	-	22 mg	1.06mg
2.	F2	Benzoic acid	1:1:1	100 mg	12 mg	-	1.06mg
3.	F3	Citric acid	1:1:1	100 mg	-	22 mg	1.68 mg
4.	F4	Citric acid	1:1:1	100 mg	12 mg	-	1.68 mg
5.	F5	Thiourea	1:1:1	100 mg	-	22 mg	0.66 mg
6.	F6	Thiourea	1:1:1	100 mg	12 mg	-	0.66 mg
7.	F7	Urea	1:1:1	100 mg	-	22 mg	0.52 mg
8.	F8	Urea	1:1:1	100 mg	12 mg	-	0.52 mg
9.	F9	Menthol	1:1:1	100 mg	-	22 mg	1.36 mg
10.	F10	Menthol	1:1:1	100 mg	12 mg	-	1.36 mg

Table 2: Different Composition of Benzoic Acid and Choline chloride in Deep Eutectic Mixture of Atorvastatin

Sr. No.	Formulation Code	Molar Ratio	Drug Atorvastatin	Choline Chloride	Benzoic Acid
1.	F11	1:1:2	100 mg	12 mg	2.12 mg
2.	F12	1:1:3	100 mg	12 mg	3.18 mg
3.	F13	1:1:4	100 mg	12 mg	4.42 mg
4.	F14	1:1:5	100 mg	12 mg	5.3 mg
5.	F15	1:2:3	100 mg	24 mg	3.18 mg
6.	F16	1:3:3	100 mg	36 mg	3.18 mg
7.	F17	1:4:3	100 mg	48 mg	3.18 mg
8.	F18	1:5:3	100 mg	60 mg	3.18 mg

Evaluation of Deep Eutectic Solvent Mixture of Atorvastatin

a. pH of the Solution

For the determination of pH, freshly prepared solutions were maintained at a temperature of 25 ± 2 °C for 30 minutes. Following this equilibration period, the pH of each solution was measured at this initial temperature. Subsequently, the solutions were transferred to a water bath and incrementally heated to 60 °C. The pH measurements were conducted using a digital pH meter.

b. Drug Solubility

The shake-flask method, renowned for its wide spread application, was employed to evaluate drug solubility. The pharmaceutical compound was initially dissolved in each deep eutectic solvent derivative (DESD) through stirring at ambient temperature. To ensure equilibrium was reached, the mixture was maintained in a temperature-controlled water bath for a duration of 24 hours. Subsequently, the solutions were filtered prior to attaining the maximum solubility of the drug in the DESDs. The absorbance spectra of the resulting clear solutions were then measured using a double beam spectrophotometer, providing data on the drug's solubilization.

c. Drug Content

After determining solubility, the mixture containing the two pharmaceuticals was passed through a 0.22 µm membrane-equipped Millex 69 PES syringe filter unit. The concentration of the drug in the filtered DESD supernatant was subsequently determined using a UV-Vis spectrophotometer. To measure the absorbance of atorvastatin, the filtered aliquots were diluted with methanol.

d. Visual inspection

Atorvastatin was evaluated for its physical characteristics, including appearance and colour, through visual inspection.

e. Particle Size and Polydispersity Index

The particle size of the formulated dispersions was determined by diluting the samples with water and analysing them using a particle size analyser. The polydispersity index (PDI) was calculated to evaluate the homogeneity of the dispersions. A PDI value of less than 0.1 indicates a highly homogeneous population, whereas values greater than 0.3 suggest significant heterogeneity.

f. Zeta Potential Measurement

The zeta potential of the nanoparticles was measured using dynamic light scattering (DLS). Prior to measurement, the silver nanoparticles were subjected to sonication using an ultrasonicator for thirty minutes. The DLS analysis was then performed, and the resulting graph was used to determine the zeta potential of the nanoparticles.

g. FTIR Spectra of final formulation of deep eutectic mixture of Atorvastatin

The FTIR spectrum of the optimized formulation F12 was obtained using FTIR spectroscopy.

h. In vitro dissolution studies

Atorvastatin proniosomes were evaluated for dissolution using a USP Type II dissolution apparatus with a paddle stirrer. The dissolution tests were performed in vessels containing 900 ml of pH 6.8 phosphate buffer and 0.1N HCl (pH 1.2), with the paddle speed set to 50 rpm and the temperature maintained at 37 ± 0.5°C. To compensate for the volume removed at each sampling interval, five milliliters of fresh dissolution medium were added. The cumulative percentage of drug released was plotted against time, with measurements conducted in triplicate.

i. Drug release kinetics

Model-dependent methods, which rely on various mathematical functions, were utilised to describe the

release profile of the drug. Once an appropriate function was selected, the release profiles were assessed based on the derived model parameters. Data obtained from ex vivo permeation studies were analysed using the following models:

Zero Order Model: Zero order kinetics is applicable for describing the drug dissolution profiles of various modified release pharmaceutical dosage forms, including transdermal systems, matrix tablets containing poorly soluble drugs in coated forms, and osmotic systems. In its simplest form, zero order release can be mathematically expressed as:

$$Q_t = Q_0 - K_0 t$$

Where:

Q_t is the amount of drug dissolved at time t,

Q_0 is the initial amount of drug in the solution (often $Q_0=0$),

K_0 is the zero order release constant, expressed in units of concentration per time.

To study release kinetics, data from in vitro drug permeation studies were plotted as cumulative amount of drug released versus time.

First Order Model: First order kinetics is employed to describe the drug dissolution in pharmaceutical dosage forms containing water-soluble drugs within porous matrices. The release of the drug, which follows first order kinetics, is mathematically expressed by the equation:

$$\log C = \log C_0 - \frac{Kt}{2.303}$$

Here, C_0 represents the initial drug concentration, K denotes the first-order rate constant, and t signifies time. The experimental data are typically depicted through plotting the logarithm of cumulative percentage of drug remaining against time. This graphical representation yields a linear relationship, where the slope corresponds to $\frac{K}{2.303}$. This method provides a robust means to analyze and interpret the release kinetics of drugs from these dosage forms.

Higuchi's Model: Higuchi's model is widely utilized to elucidate the drug release dynamics from matrix systems, originally formulated for planar configurations and subsequently extended to various geometries and porous structures. The model operates under several fundamental assumptions: (i) the initial drug concentration within the matrix significantly exceeds its solubility; (ii) drug diffusion occurs predominantly in one dimension with minimal edge effects; (iii) drug particles are substantially smaller than the thickness of the system; (iv) negligible matrix swelling and dissolution occur during the release process; (v) drug diffusivity remains constant; and (vi) the release environment consistently maintains perfect sink conditions.

Higuchi initially derived an equation describing drug release from an insoluble matrix based on Fickian diffusion, proposing the simplified form:

$$Q_t = K_H \sqrt{t}$$

where Q_t represents the amount of drug released at time t, and K_H denotes the release rate constant specific to the Higuchi model. Experimental data are conventionally plotted as cumulative drug release against the square root of time, yielding a linear relationship that confirms diffusion-controlled drug release. The slope of this line corresponds to K_H, providing a quantitative measure of the release rate constant under the model's assumptions.

Korsmeyer-Peppas Model: The Korsmeyer-Peppas model provides a fundamental framework for understanding drug release kinetics from polymeric systems. Originally conceptualized by Korsmeyer, it offers a mathematical description of drug release rates from controlled-release matrices. The model is expressed by the equation:

$$Q = Kt^n$$

Where Q represents the percentage of drug released at time t, K denotes the kinetic constant encompassing the tablet's structural and geometric attributes, and n signifies the diffusional exponent that characterizes the release mechanism.

For different types of drug release mechanisms, n assumes distinct values: n = 0.45, corresponds to Fickian diffusion, indicating uniform drug release over time due to molecular diffusion. In contrast, non-Fickian transport, denoting anomalous release behavior, encompasses a broader range for n between 0.45 and 0.89. Specifically, when n = 0.89, the release mechanism follows zero-order kinetics, where drug release occurs at a constant rate independent of concentration. To evaluate the applicability of the Korsmeyer-Peppas model, log-transformed cumulative percentage drug release versus log-transformed time is plotted. This logarithmic relationship aids in assessing the consistency of the model with experimental data, providing insights into the release dynamics and mechanism of the polymeric matrices under investigation.

Table 3: Interpretation of diffusional release mechanisms

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.45 < n = 0.89	Non-Fickian transport	t^{n-1}
0.89	Case II transport	*Zero order release*
Higher than 0.89	Super case II transport	t^{n-1}

Result and Discussion

Organoleptic properties

The organoleptic properties of atorvastatin, a crucial factor in its pharmaceutical formulation and acceptance, were evaluated as follows. Atorvastatin exhibited a characteristic appearance of a white to off-white crystalline powder, consistent with its known physical properties. The absence of any discernible odour further confirms its suitability for oral administration, as it ensures patient compliance by minimizing any potential unpleasant sensory experiences during ingestion. These findings highlights the importance of organoleptic evaluation in pharmaceutical development, where characteristics such as colour and odour play pivotal roles in formulation design and patient acceptance. Such attributes contribute significantly to the overall quality and therapeutic efficacy of atorvastatin formulations intended for clinical use.

Melting Point

The melting point (M.P.) determination of atorvastatin was conducted and compared against the drug reference values. The observed melting point of atorvastatin was found to be 158 ± 1.00°C, closely aligning with the established range of 159-161°C as documented in drug references. This consistency indicates the reliability and purity of the sample tested, crucial for ensuring the quality and efficacy of atorvastatin formulations in pharmaceutical applications. The slight deviation from the reference range

falls within acceptable limits and reaffirms the robustness of the analytical methodology employed. Such precise characterization of the melting point supports the accurate identification and assessment of atorvastatin in pharmaceutical research and development contexts.

Determination of absorption maxima of atorvastatin

A quantitative analysis of atorvastatin in methanol was performed using a double beam UV-visible spectrophotometer, scanning a solution with a concentration of 20 µg/ml over the wavelength range of 200-400 nm. The absorption maxima of atorvastatin were determined to occur at 247 nm (shown in Figure 2), consistent with literature references. This characteristic wavelength corresponds to the point of maximum absorbance for atorvastatin in the UV-visible spectrum, providing a specific and identifiable marker for its quantitative analysis.

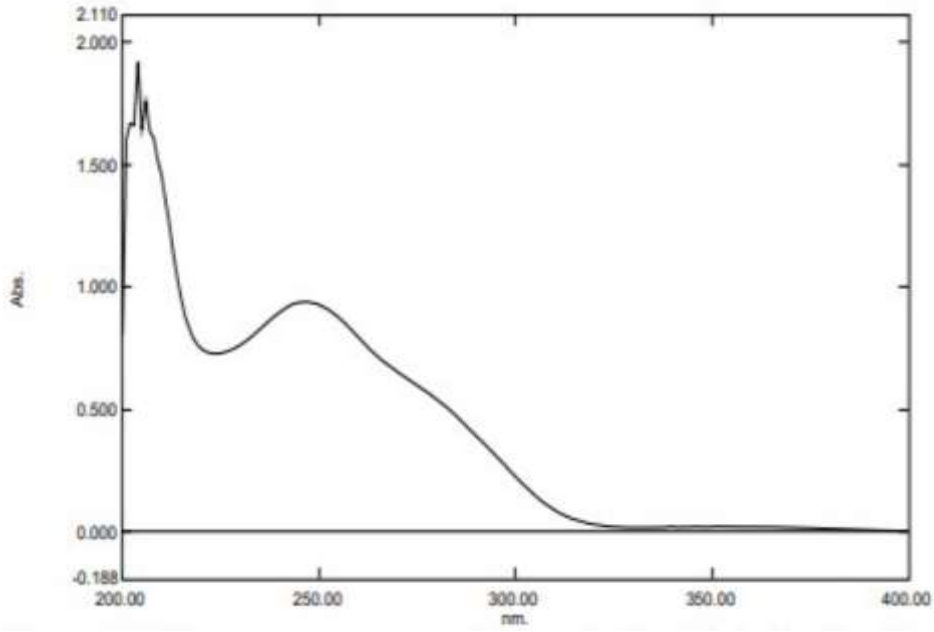

Figure 2: The absorption maxima of atorvastatin.

Preparation of standard curve of Atorvastatin

In this study, we constructed a calibration curve for Atorvastatin calcium at its maximum absorbance wavelength of 247 nm. The calibration curve was generated using a series of standard solutions ranging from 4 µg/ml to 20 µg/ml, and their corresponding absorbance values were recorded with a UV-Vis spectrophotometer. The absorbance values for each concentration were as follows: 4 µg/ml yielded an absorbance of 0.154 ± 0.002, 8 µg/ml resulted in 0.349 ± 0.001, 12 µg/ml exhibited an absorbance of 0.527 ± 0.002, 16 µg/ml showed 0.715 ± 0.003 absorbance, and 20 µg/ml displayed an absorbance of 0.939 ± 0.001. Each concentration point was measured in triplicate, and the mean absorbance values were calculated to ensure accuracy. The relationship between the concentration of Atorvastatin and its absorbance was found to be linear over the specified range, with a correlation coefficient (R^2) of 0.999 indicating excellent linearity (shown in Figure 3). The standard deviation values for the absorbance measurements were consistently low, demonstrating the precision of the experimental method. This calibration curve will serve as a reliable reference for the quantification of Atorvastatin in subsequent

analyses, ensuring accurate determination of its concentration in pharmaceutical formulations or biological samples using UV-Vis spectroscopy.

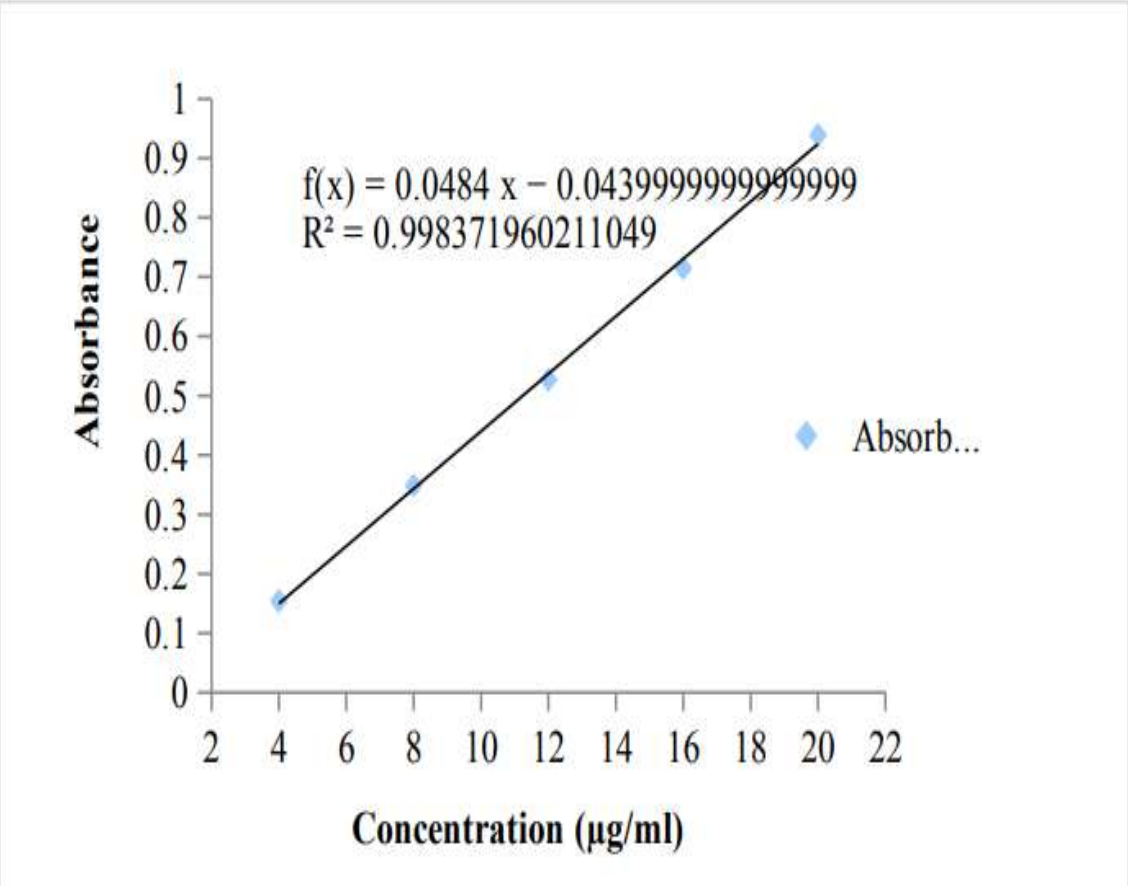

Figure 3: Calibration curve for Atorvastatin calcium.

Solubility studies

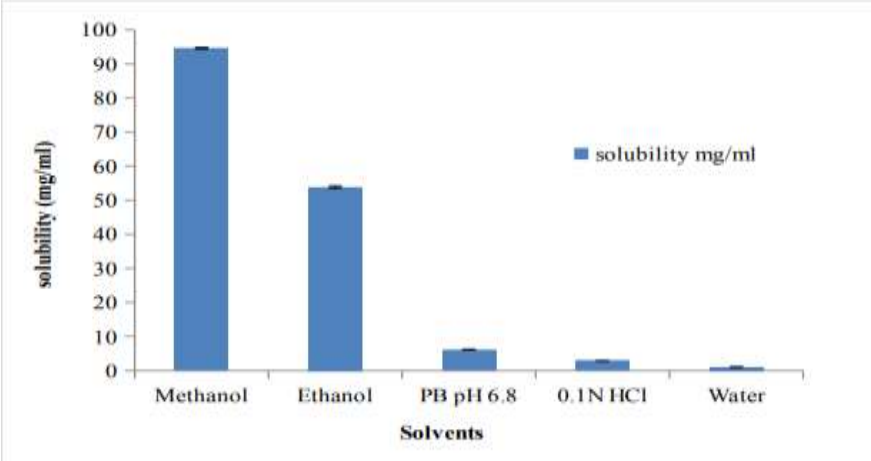

Figure 4: Solubility studies of Atorvastatin.

Solubility studies of Atorvastatin were conducted using various solvents to assess its dissolution characteristics under different conditions (Shown in Figure 4). Methanol exhibited the highest solubility among the tested solvents, with a mean solubility of 94.835 mg/ml ± 0.413. This finding aligns with the

polar nature of methanol, which facilitates effective dissolution of polar compounds like Atorvastatin. Ethanol, another polar solvent, showed a lower but still significant solubility of 53.857 mg/ml ± 0.316. Phosphate buffer (PB) at pH 6.8, which simulates physiological conditions, resulted in a substantially reduced solubility of 6.129 mg/ml ± 0.032, indicating pH-dependent solubility properties of Atorvastatin. In acidic conditions represented by 0.1N HCl, the solubility further decreased to 2.851 mg/ml ± 0.055, underscoring the sensitivity of Atorvastatin's solubility to pH variations. Water exhibited the lowest solubility among all solvents tested, with a mean value of 1.033 mg/ml ± 0.021. These results emphasize the importance of solvent selection in pharmaceutical formulations of Atorvastatin, particularly in optimizing its bioavailability and dissolution profiles for therapeutic efficacy. Future studies could explore solvent combinations or cosolvency techniques to enhance Atorvastatin's solubility in aqueous and physiological conditions.

Partition coefficient determination

In this study, the partition coefficient (log P) of Atorvastatin was determined using the n-octanol solvent system. The calculated log P value was found to be 6.35 ± 0.052 (mean ± SD, n = 3). The log P value indicates the lipophilicity of Atorvastatin, with higher values suggesting greater affinity for lipid-rich environments over aqueous media. This characteristic is crucial for understanding the drug's pharmacokinetic properties, such as absorption, distribution, metabolism, and excretion (ADME). The precision of the measurement, indicated by the low standard deviation (0.052), underscores the reliability of the experimental methodology employed. Such information is vital for predicting the drug's behavior in biological systems and optimizing its formulation for therapeutic efficacy.

FTIR of pure Drug and excipient

FTIR of Atorvastatin

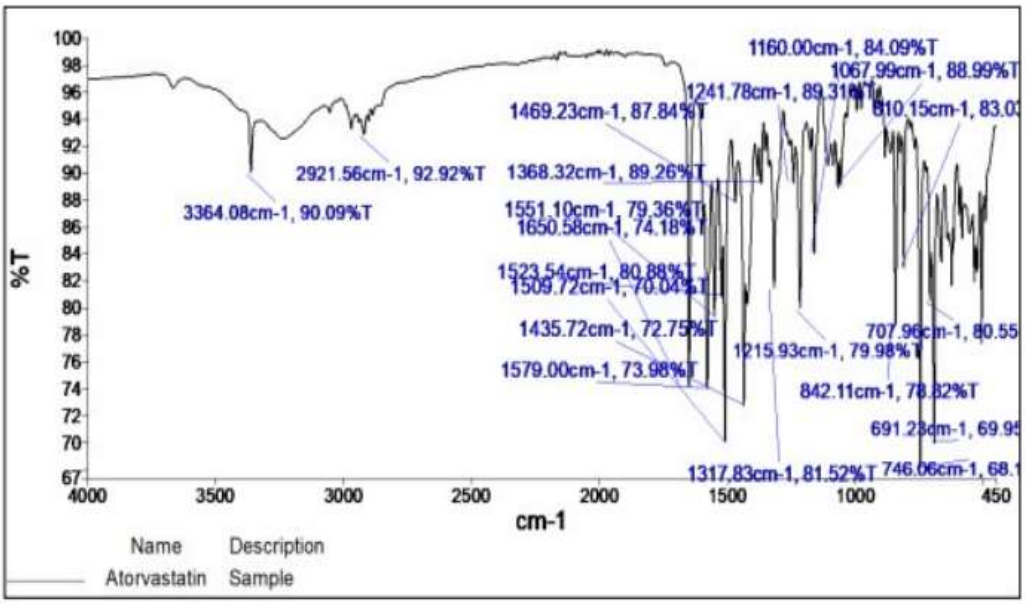

Figure 5: FTIR spectrum of Atorvastatin.

The Fourier-transform infrared (FTIR) spectrum of Atorvastatin reveals distinctive absorption bands corresponding to its molecular functional groups (shown in Figure 5). The observed peak at approximately 3363.92 cm^{-1} corresponds to the hydroxyl (-OH) group, slightly shifted from the reported

literature value of 3365.5 cm^{-1}. This band signifies the presence of the alcohol functional group within the molecule. The peak observed at 1650.53 cm^{-1} aligns with the reported literature value of 1651.7 cm^{-1} and is attributed to the carbonyl (C=O) stretching vibration of the amide group (-CONH-) in Atorvastatin, indicating its presence and structural integrity. Notably, the aromatic C=C stretching vibration, typically observed around 1595.1 cm^{-1}, appears at 1578.54 cm^{-1} in the spectrum of Atorvastatin, demonstrating a characteristic shift. This alteration could be indicative of local molecular environment effects or intermolecular interactions within the sample under analysis.

FTIR of Choline Chloride

The FTIR spectrum analysis of Choline Chloride (shown in Figure 6) was conducted to identify and confirm the presence of specific functional groups within the compound. The experimental results closely aligned with the reported literature values, validating the structure and composition of the sample. Firstly, the C–H stretching vibrations were observed at 3025.98 cm^{-1}, closely matching the reported peak at 3025.82 cm^{-1}. This concordance affirms the presence of aliphatic C–H bonds within the choline chloride structure. Additionally, the spectrum displayed a peak at 1672.06 cm^{-1}, which is slightly shifted from the reported value of 1634.16 cm^{-1}. This peak is indicative of halogenated organic compounds and suggests minor variations potentially due to the molecular environment or interactions within the sample matrix. Furthermore, the O–H stretching vibration was detected at 1423.94 cm^{-1}, differing from the reported 1441.65 cm^{-1}. This slight deviation could be attributed to hydrogen bonding or other intermolecular forces present in the sample. Lastly, the characteristic C=O vibration appeared at 1348.95 cm^{-1}, which is in close agreement with the literature value of 1348.26 cm^{-1}, confirming the presence of carbonyl groups. Overall, the FTIR spectral data corroborate the expected functional groups in choline chloride, with minor shifts potentially attributable to sample-specific interactions or slight experimental variations.

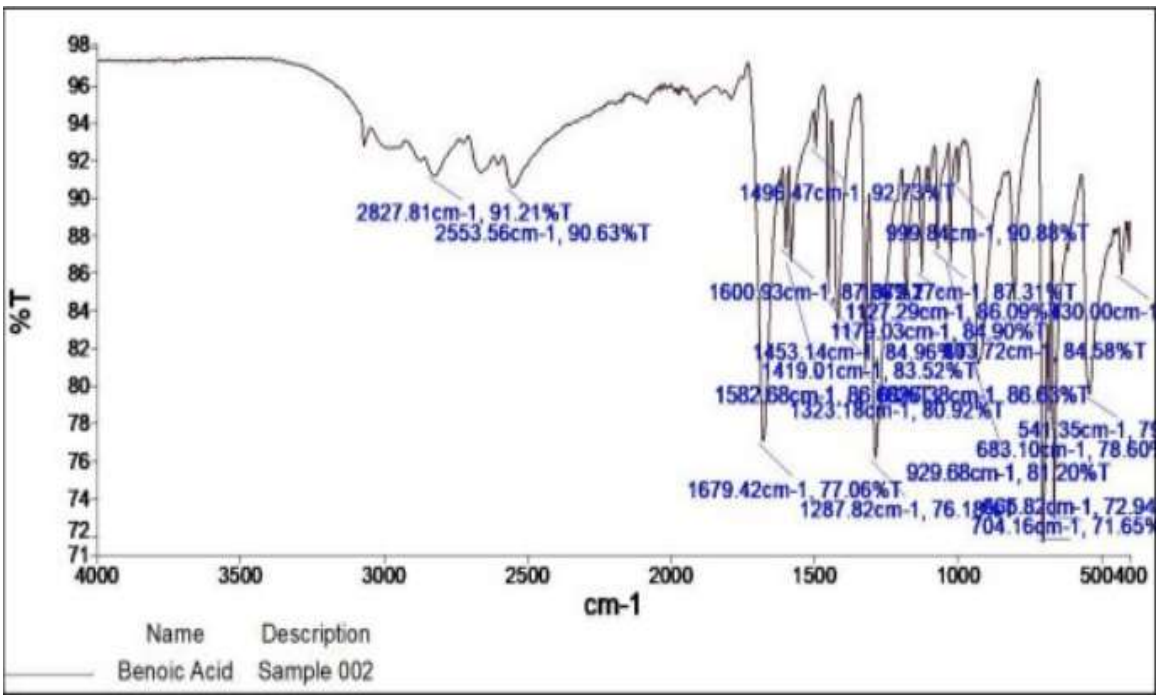

Figure 6: FTIR spectrum of Choline Chloride.

FTIR of Benzoic acid

The FTIR spectrum of benzoic acid was analyzed to elucidate its structural composition by identifying its characteristic functional groups (shown in Figure 7). The spectral data revealed peaks that closely matched the reported literature values, thereby confirming the integrity of the sample. The aliphatic C-H stretching vibration was observed at 2827.81 cm^{-1}, in close alignment with the reported peak at 2830 cm^{-1}. This consistency indicates the presence of aliphatic C-H bonds within the benzoic acid structure. The O-H stretching vibration appeared at 2553.56 cm^{-1}, nearly identical to the reported value of 2550 cm^{-1}. This minor shift suggests robust O-H bonding, likely influenced by the intermolecular hydrogen bonding typical of carboxylic acids. The spectrum also exhibited a peak at 1679.42 cm^{-1}, corresponding to the C=C aromatic bending vibration, which is in near perfect agreement with the reported 1680 cm^{-1}. This validates the presence of aromatic ring structures within the benzoic acid molecule. Additionally, the C=C stretching in the benzene ring was detected at 1600.93 cm^{-1}, matching precisely with the reported value of 1600 cm^{-1}. This further confirms the aromatic nature of benzoic acid.

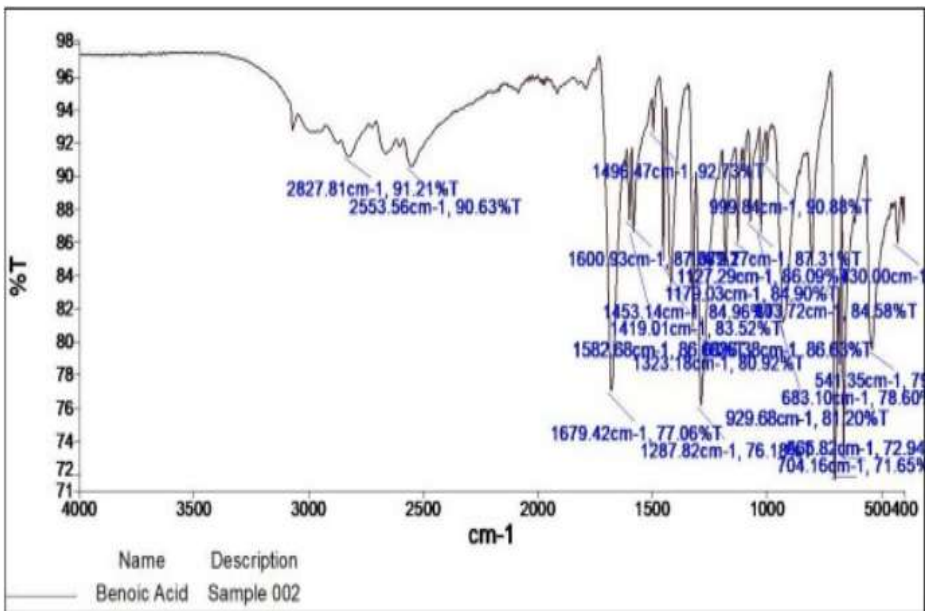

Figure 7: FTIR spectrum of Benzoic acid.

Evaluation of Deep Eutectic Solvent Mixture of Atorvastatin

Appearance and pH of the DESM of Atorvastatin

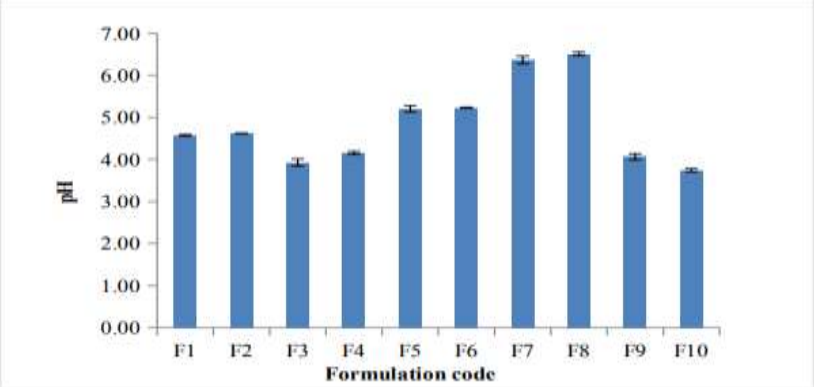

Figure 8: pH data of DESM of Atorvastatin.

The various DESM formulations of Atorvastatin were meticulously analysed for their appearance and pH to ascertain their physicochemical properties (shown in Figure 9). Formulations F1 and F2 were yellowish transparent solutions with pH values of 4.57 ± 0.021 and 4.62 ± 0.006, respectively, indicating a slightly acidic environment and good solubility. In contrast, F3 and F4 presented as white turbid solutions, with pH values of 3.92 ± 0.085 and 4.15 ± 0.040, respectively, suggesting partial dissolution or the presence of undissolved particles that could influence bioavailability. Formulations F5 and F6 were white crystalline solutions, with near-neutral pH values of 5.20 ± 0.074 and 5.23 ± 0.012, respectively, hinting at potential solubility issues despite their stable pH. F7 and F8, appearing as white transparent solutions, had higher pH values of 6.36 ± 0.090 and 6.50 ± 0.047, respectively, suggesting improved solubility and stability in a slightly basic medium. Lastly, formulations F9 and F10, both white transparent solutions, exhibited pH values of 4.07 ± 0.072 and 3.74 ± 0.036, respectively, indicating a more acidic environment. These observations provide critical insights into the formulation characteristics of Atorvastatin, essential for optimizing its efficacy and stability.

Appearance and pH of the DESM of DESM of Atorvastatin with Benzoic acid and Choline Chloride

The analysis of additional DESM formulations of Atorvastatin, designated as F11 through F18, was conducted to evaluate their appearance and pH levels, providing further insights into their physicochemical properties (Figure 9). Formulations F11 to F14 were characterised as yellowish transparent solutions, with pH values ranging from 4.46 ± 0.030 to 3.55 ± 0.026. Specifically, F11 had a pH of 4.46 ± 0.030, F12 had a pH of 3.84 ± 0.031, F13 had a pH of 3.83 ± 0.015, and F14 had a pH of 3.55 ± 0.026. These formulations exhibited slight acidity, which may influence the solubility and stability of Atorvastatin within the DESM. Formulations F15 to F18, described as transparent solutions, presented pH values ranging from 4.85 ± 0.032 to 5.66 ± 0.031. Specifically, F15 had a pH of 4.85 ± 0.032, F16 had a pH of 4.96 ± 0.038, F17 had a pH of 5.16 ± 0.036, and F18 had a pH of 5.66 ± 0.031. These transparent solutions, with their near-neutral to slightly basic pH, suggest a more stable environment for the drug, potentially enhancing its solubility and bioavailability.

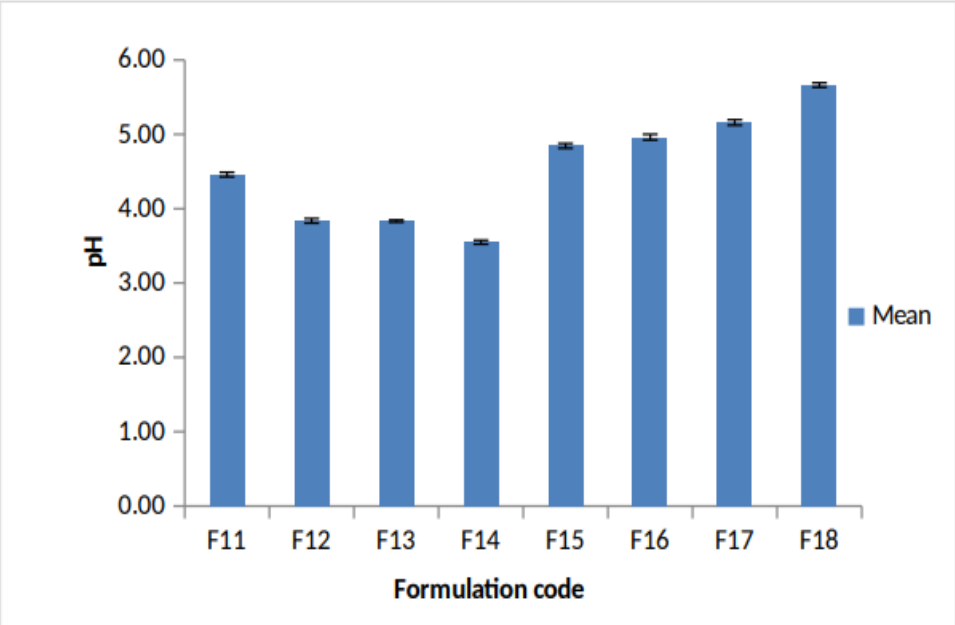

Figure 9: pH data of DESM of Atorvastatin with Benzoic acid and Choline Chloride.

Drug Content analysis of Atorvastatin DESM formulations

The drug content analysis of Atorvastatin DESM formulations, incorporating various carboxylic acids, revealed significant variations in the percentage of drug content across the different formulations (Figure 9). Formulation F1 displayed a drug content of 57.851 ± 0.207%, indicating moderate drug incorporation efficiency. Formulation F2 demonstrated the highest drug content at 85.64 ± 0.273%, suggesting superior compatibility and incorporation of Atorvastatin with the respective carboxylic acid used. In contrast, F3 had a notably lower drug content of 37.948 ± 0.215%, indicating less efficient drug incorporation. Formulations F4 and F5 showed drug contents of 46.178 ± 0.207% and 45.558 ± 0.103%, respectively, while F6 exhibited a slightly lower drug content of 42.114 ± 0.215%. These moderate values reflect variable but generally adequate incorporation efficiencies. F7 and F8 demonstrated higher drug contents of 63.74 ± 0.207% and 75.482 ± 0.215%, respectively, suggesting better drug-carboxylic acid interactions. On the other hand, formulations F9 and F10 had the lowest drug contents at 40.289 ± 0.103% and 35.916 ± 0.26%, respectively, indicating suboptimal drug incorporation.

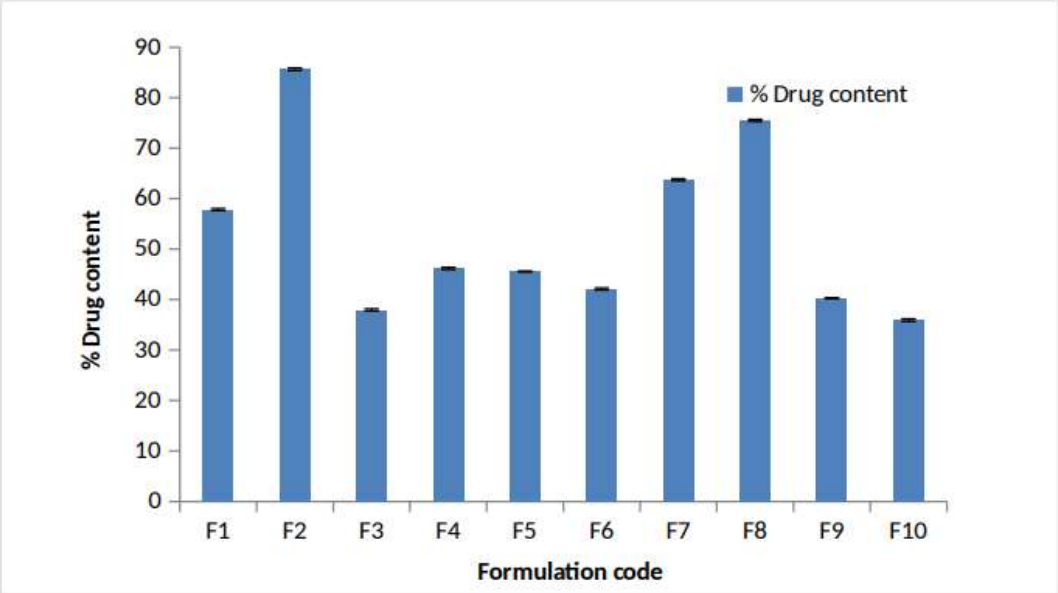

Figure 9: Drug Content of Atorvastatin DESM with different carboxylic acid.

Drug Content of Atorvastatin DESM with different concentration of Benzoic acid and Choline Chloride

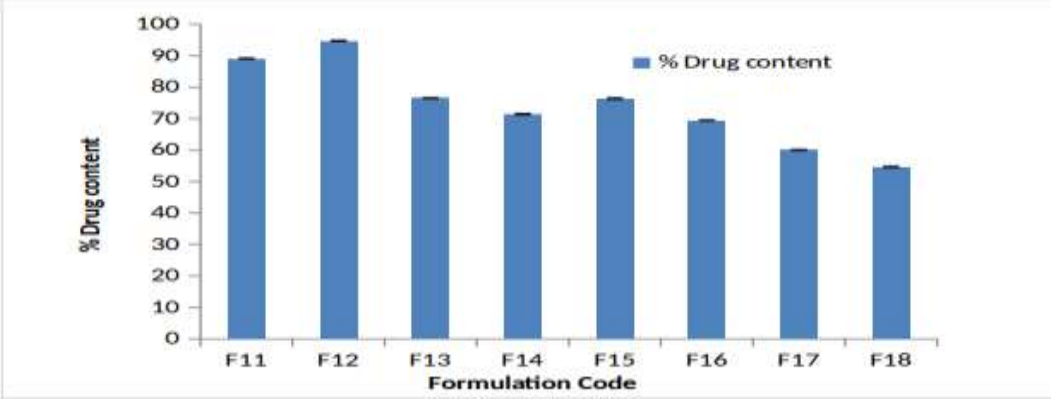

Figure 10: Drug Content of Atorvastatin DESM with different concentration of Benzoic acid and Choline Chloride

The drug content analysis of the additional DESM formulations of Atorvastatin (F11 to F18) revealed significant differences in drug incorporation efficiencies (Figure 10). Formulation F11 exhibited a drug content of 89.118 ± 0.158%, indicating a high level of drug incorporation. Formulation F12 achieved the highest drug content among the group, with an impressive 94.731 ± 0.207%, suggesting excellent compatibility and incorporation of Atorvastatin. In comparison, F13 and F14 demonstrated lower drug contents of 76.515 ± 0.158% and 71.419 ± 0.215%, respectively, while F15 also showed a similar level of drug content at 76.412 ± 0.215%. These values indicate moderate efficiency in drug incorporation for these formulations. Formulations F16, F17, and F18 exhibited progressively lower drug contents, with F16 at 69.456 ± 0.119%, F17 at 60.55 ± 0.158%, and F18 at 54.683 ± 0.215%. These results reflect a decreasing trend in drug incorporation efficiency, potentially due to varying interactions between Atorvastatin and the specific carboxylic acids used in these formulations.

Appearance of Deep eutectic mixture of Atorvastatin (F12)

The deep eutectic mixture (DESM) formulation F12 of Atorvastatin exhibited a yellowish transparent appearance (Figure 11). The yellowish hue in the transparent solution could indicate specific interactions between Atorvastatin and the carboxylic acids used, possibly due to the formation of specific complexes

Figure 11: Appearance of Deep eutectic mixture of Atorvastatin (F12).

Particle Size & Zeta Potential

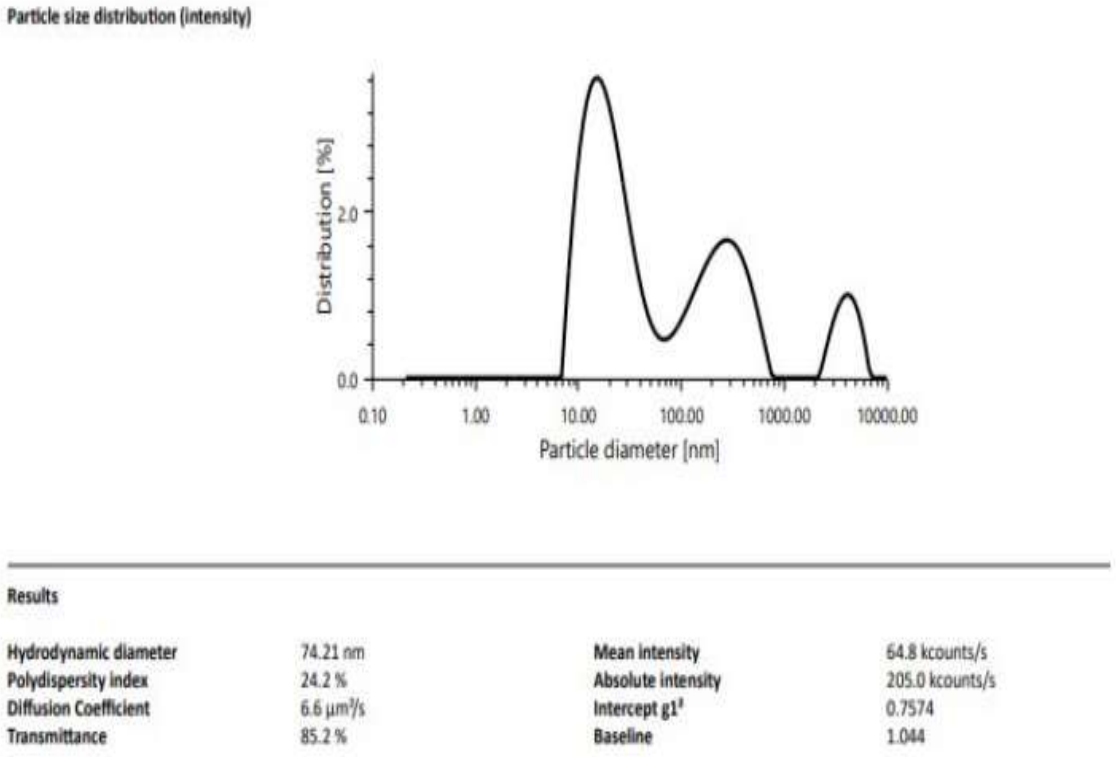

Figure 12: Particle size peak of F12 formulation.

The particle size analysis of the F12 formulation of Atorvastatin, as demonstrated in Figure 12, revealed a mean particle size of 74.21 nm. This nanoscale particle size is indicative of the formulation's potential for enhanced solubility and bioavailability. The polydispersity index (PDI) of 24.2% reflects the size distribution of the particles within the formulation. A PDI value below 30% generally indicates a relatively narrow size distribution, suggesting a homogeneous formulation with consistent particle sizes. The combination of a small particle size and a low PDI signifies the stability of the F12 formulation. Such stability is crucial for the consistent performance of the drug delivery system, ensuring that Atorvastatin is effectively delivered and maintained within therapeutic levels. The stable particle size distribution also implies that the formulation is less likely to aggregate or precipitate over time, which is essential for maintaining its efficacy and safety.

Zeta Potential

Figure 13 illustrated that the zeta potential of the F12 formulation of Atorvastatin was measured at -22.3 mV. The zeta potential is a key indicator of the surface charge of particles within a colloidal system and plays a crucial role in predicting the stability of the formulation. A zeta potential value of -22.3 mV indicates a moderately negative surface charge, which is typically associated with sufficient repulsion between particles to prevent aggregation. This repulsive force contributes to the colloidal stability of the formulation, ensuring that the nanoparticles remain well-dispersed and stable over time. The stability imparted by this zeta potential is essential for maintaining the homogeneity of the F12 formulation, preventing particle settling or clumping, which can adversely affect the drug's bioavailability and therapeutic efficacy. Therefore, the zeta potential value of -22.3 mV supports the stability of the F12 formulation, corroborating its potential as an effective drug delivery system for Atorvastatin.

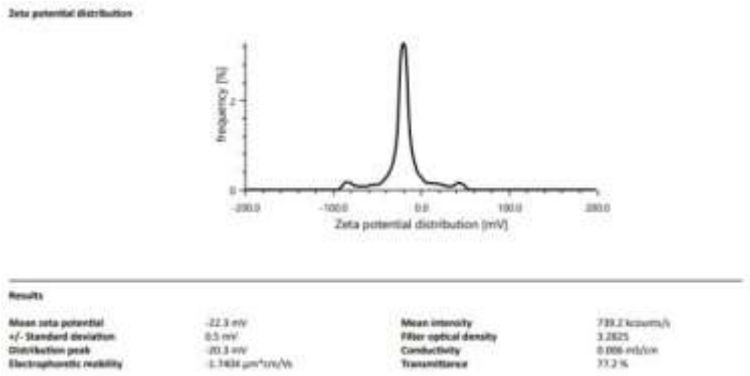

Figure 13: zeta potential peak of F12 formulation.

FTIR of Deep eutectic mixture of Atorvastatin

The FTIR spectrum of the final formulation indicated significant changes in the characteristic peaks of the drug, suggesting its encapsulation within the drug DESM formulation (shown in Figure 14). Specifically, observed peaks either disappeared or showed slight shifts with reduced intensity compared to the pure drug spectrum. This alteration in the FTIR spectrum is indicative of interactions between the drug molecules and the components of the DESM formulation, such as carboxylic acids or other constituents used in the formulation. These interactions can lead to changes in the vibrational frequencies of functional groups within the drug molecule, resulting in peak shifts or intensity reductions.

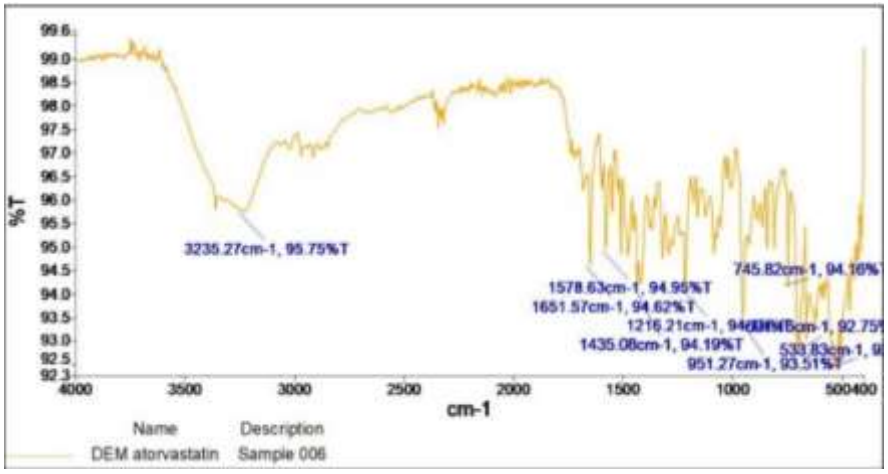

Figure 14: FTIR of Deep eutectic mixture of Atorvastatin.

In-vitro Drug release study of deep eutectic mixture of Atorvastatin

The in-vitro drug release profiles of Atorvastatin from both the pure drug and the formulated Deep Eutectic Mixture (DEM) represented by formulation F12 were evaluated over a 24-hour period (Figure 15). The study revealed distinct differences in the release kinetics between the two formulations. Initially, at the 0.25-hour mark, the pure drug exhibited a significantly higher percentage of drug release (33.41%) compared to the formulation F12 (12.58%). This trend continued at subsequent time points: at 0.5 hours, the pure drug showed 45.93% release whereas formulation F12 exhibited 15.06%. The disparity persisted up to 2 hours, with the pure drug releasing 92.6% of the drug compared to 37.38% from formulation F12. As the study progressed beyond 2 hours, the differences in drug release between the pure drug and formulation F12 diminished. Notably, at the 24-hour mark, both formulations

approached near-complete drug release, with the pure drug achieving 97.62% and formulation F12 reaching 97.73%. These findings suggest that the formulation F12, based on a Deep Eutectic Mixture, alters the release profile of Atorvastatin compared to the pure drug. The delayed release observed in formulation F12 could potentially offer advantages in terms of prolonged therapeutic effect and reduced dosing frequency, which are critical considerations in pharmaceutical formulations aimed at enhancing patient compliance and efficacy. Further studies are warranted to elucidate the underlying mechanisms governing the drug release behavior observed in this study.

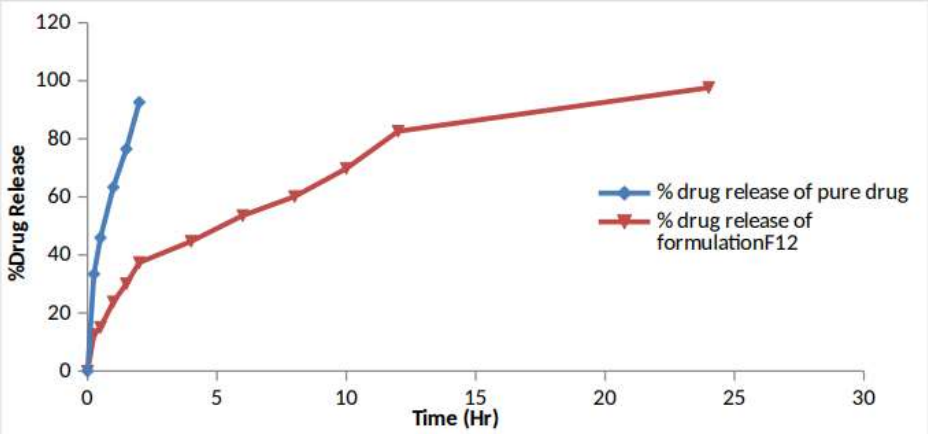

Figure 15: In-Vitro Drug release of deep eutectic mixture of Atorvastatin.

In vitro release Kinetics

Formulation F12, a Deep Eutectic Mixture (DEM) containing Atorvastatin, was analyzed using various kinetic models to elucidate its drug release behavior. The study employed zero-order (Figure 16), first-order (Figure 17), Higuchi (Figure 18), and Korsmeyer-Peppas (Figure 19) models to characterize the release kinetics over time. The zero-order model yielded a rate constant (K_0) of 3.959, indicating a constant release rate independent of drug concentration. In contrast, the first-order model provided a rate constant (R) of 0.854, suggesting a release mechanism where the drug concentration decreases exponentially over time. The Higuchi model, with a rate constant (K_0) of 20.895 and an R^2 value of 0.986, revealed a square root of time-dependent release pattern, implying a diffusion-controlled mechanism or dissolution from a matrix system. Finally, the Korsmeyer-Peppas model, characterized by a rate constant (K_0) of 0.475 and an R^2 value of 0.9929, further underscored the formulation's release mechanism, likely influenced by both drug diffusion and polymer relaxation. These kinetic parameters provide comprehensive insights into the controlled release behavior of Atorvastatin from formulation F12, crucial for optimizing its therapeutic efficacy and ensuring predictable drug delivery profiles in pharmaceutical applications. Future studies could explore additional factors such as formulation composition and environmental conditions to further refine and validate these findings.

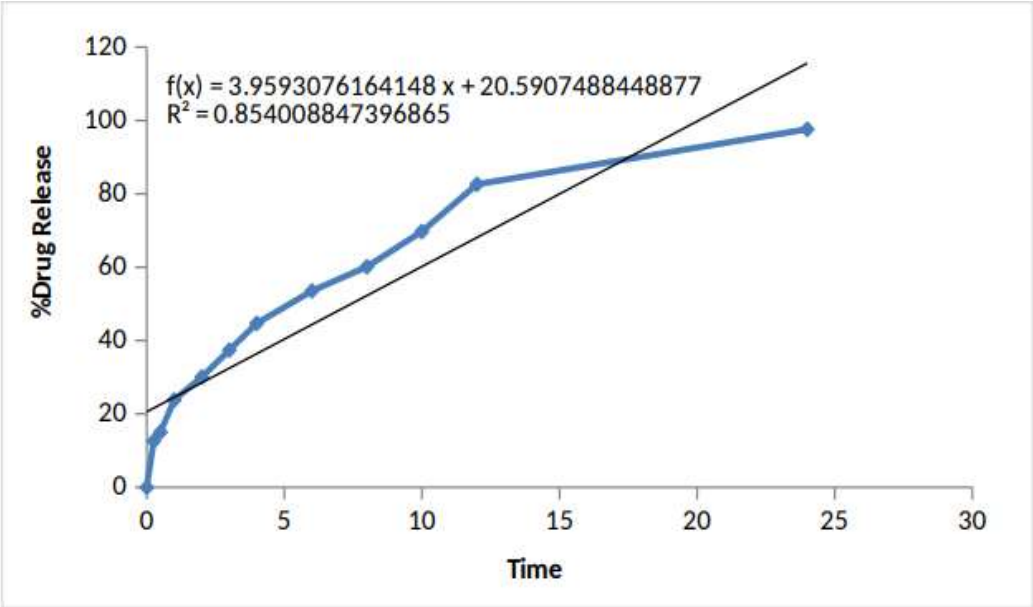

Figure 16: Zero order graph of formulation F12.

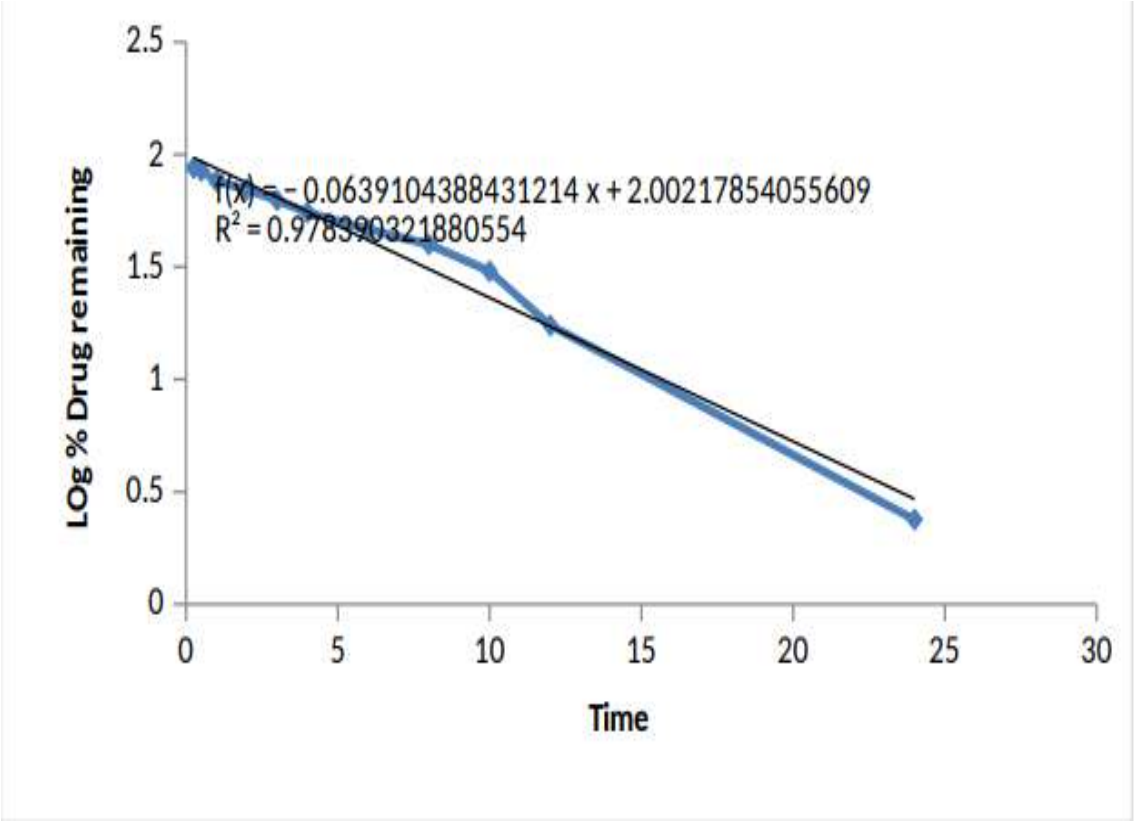

Figure 17: First order graph of formulation F12.

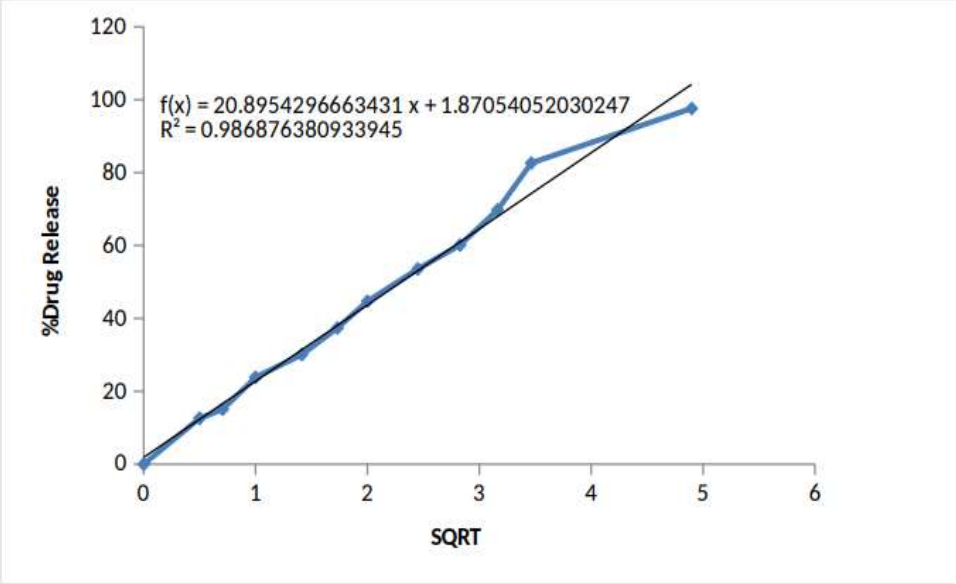

Figure 18: Higuchi order graph of formulation F12

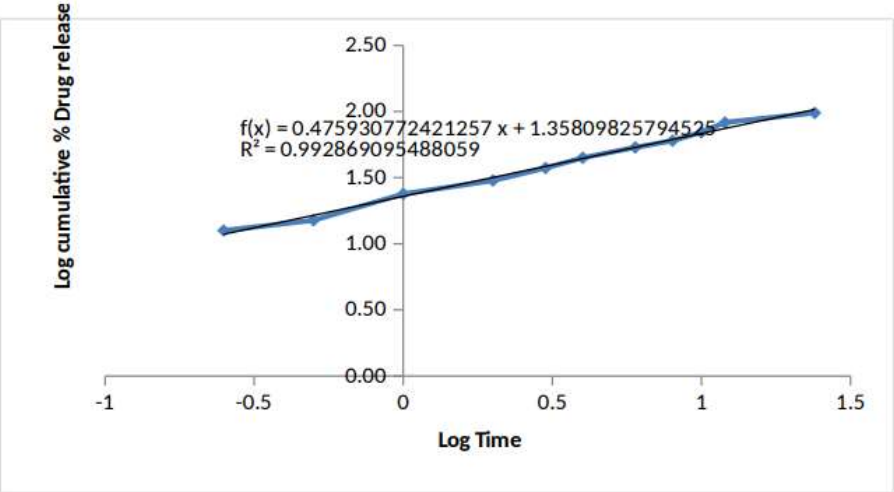

Figure 19: Korsmeyerpeppas order graph of formulation F12.

Conclusion

The development of innovative formulations to enhance drug solubility and bioavailability is a critical challenge in pharmaceutics. This study demonstrated the promising potential of DES for improving the delivery of the poorly soluble drug atorvastatin calcium. Formulation F12, comprising atorvastatin, choline chloride, and benzoic acid, exhibited favorable properties like nanoscale particle size, colloidal stability, and controlled release kinetics governed by diffusion and polymer relaxation mechanisms. The controlled release profile could lead to prolonged therapeutic effects, reduced dosing frequency, and improved patient adherence. While establishing proof-of-concept for DES-based atorvastatin formulations, further research is needed to optimize composition, evaluate stability, and conduct in vivo studies. The versatility of DES formulations extends beyond atorvastatin, offering a promising avenue for enhancing solubility and bioavailability of other poorly soluble drugs. This study contributes to pharmaceutical formulation development by exploring innovative strategies to overcome poor drug

solubility, potentially unlocking avenues for improved therapeutic outcomes and patient compliance across various medical conditions.

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