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Unveiling the Superiority of Innovative Carbonated Self-Nanoemulsifying Drug Delivery Systems in Improving the Stability of Acid-Labile Drugs: Atorvastatin as a Model Drug

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Abstract: Atorvastatin (AT) is widely prescribed by physicians during the treatment of hyperlipidemia. The self-nanoemulsifying drug delivery system (SNEDDS) is used to overcome its low drug solubility and bioavailability. However, the presence of free fatty acids in SNEDDS formulation resulted in remarkable AT degradation. This study explores innovative carbonated SNEDDS to enhance the stability of AT within SNEDDS formulation. Various types of SNEDDS formulations were prepared and evaluated. In vitro dissolution was performed to examine the ability of SNEDDS formulation to enhance AT dissolution. The solidified SNEDDS formation was prepared using Syloid adsorbent (AT-SF6). In addition, sodium bicarbonate was loaded within the best formulation at various concentrations to prepare carbonated SNEDDS (AT-CF6). Kinetics of drug degradation were studied over 45 days to assess AT stability in SNEDDS formulations. It was found that the SNEDDS formulation was able to enhance the dissolution of AT by about 1.5-fold compared with the pure drug formulation. AT-SF6 did not reduce the degradation rate of the drug compared with AT-F6. However, AT-CF6 formulations showed that increasing the concentration of incorporated sodium bicarbonate significantly reduced the degradation rate of AT. It was found that sodium bicarbonate in AT-CF6 significantly reduced the degradation rate of AT (0.00019) six-fold compared with AT-F6 (0.00115). The obtained results show that carbonated SNEDDS is a promising approach to enhance the stability of acid-labile drugs and their pharmaceutical application.

Keywords: acid-labile drugs; carbonated SNEDDS; solid SNEDDS; kinetics; chemical degradation



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1. Introduction

Stability of drug molecules in prepared pharmaceutical formulations is a critical factor that directly influences their efficacy, safety, and shelf life. Instability issues could be attributed to incompatibility between drug molecules and the formulation excipients [1,2]. Consequently, understanding the definite reason for the instability factor could significantly facilitate the stabilization of drugs during the development of pharmaceutical formulations [3].

Oral pharmaceutical formulations are considered to be a superior route of administration due to their simplicity, safety, and ease of self-administration [4]. However, orally administered therapeutic molecules face various barriers that can negatively affect their bioavailability [5,6]. Therefore, various drug delivery systems have been invented to overcome this limitation. Interestingly, the development of innovative nano-based pharmaceutical formulations has received considerable attention due to their success in enhancing drug delivery and efficacy [7,8]. These include self-nanoemulsifying drug delivery systems (SNEDDS) [9], solid lipid nanoparticles [10], nanostructural lipid carriers [11], liposomes [12], and polymeric nanoparticles [13].

Among them, SNEDDS offers various advantages over other drug delivery systems. This is attributed to the ease of preparation by simply mixing its components (surfactant,

co-surfactant, and oil) and its applicability for large-scale production [14]. Furthermore, the anhydrous nature of SNEDDS formulations resulted in a significant enhancement in drug loading capacity for poorly water-soluble drugs [15]. In addition, this provides an additional advantage in terms of stability *in vitro*, where it only forms nanoemulsion droplets *in vivo* upon mild agitation produced by peristaltic movement [16].

The reported studies showed that direct contact between the drug and free fatty acid in SNEDDS formulation potentiates the chemical degradation of sensitive drug molecules [17–19]. Therefore, solidification of SNEDDS was performed to enhance the stability of loaded drugs [20]. However, numerous studies showed that the solidification of the SNEDDS formulation did not slow down the degradation rate of loaded drugs [17,21]. In light of this, there is a demand for innovative strategies to enhance the stability of acid-labile drugs in the SNEDDS formulation and protect them from chemical degradation.

Herein, sodium bicarbonate has been utilized for the first time as an alkalinizing agent to stabilize the loaded acid-labile drugs within the SNEDDS formulation. This is expected to increase the pH of the microenvironment in the SNEDDS formulation, which could enhance drug stability. Therefore, acid-labile drugs should be employed in the present study to compare the effect of traditional solidified SNEDDS and innovative carbonated SNEDDS on drug stability.

Atorvastatin (AT) is one of the most prescribed antihyperlipidemic agents as a result of its efficacy and safety [22]. It exerts its pharmacological effect by inhibiting hydroxy-3-methyl-glutaryl-coenzyme A, which prevents *de novo* cholesterol synthesis [23]. However, poor drug solubility limits its bioavailability [24]. Therefore, SNEDDS formulation was used in the literature with a remarkable increment in AT bioavailability and therapeutic activity [25–31]. Moreover, various studies have shown that AT suffers from chemical degradation when exposed to acid microenvironments [32,33]. Therefore, it is expected that the previously developed AT-loaded SNEDDS formulations in the literature could suffer from drug degradation during storage. This could be attributed to the presence of free fatty acids within the SNEDDS formulation [34]. The expected instability aligns with previously published data showing that an acidic microenvironment is responsible for the chemical degradation of acid-labile drugs [35].

Therefore, the present study aims to cover the existing gap in the stabilization of acid-labile drug (AT) using carbonated SNEDDS as a novel strategy. This is achieved through the preparation of carbonated liquid SNEDDS to increase the microenvironment pH of SNEDDS formulation, which in turn enhances drug stability. To achieve this objective, liquid, solid, and carbonated SNEDDS formulations were prepared and subjected to pharmaceutical characterization. The drug degradation rate was studied to investigate the impact of solidification and alkalinization on the stability of the acid-labile drug.

2. Materials and Methods

2.1. Materials

Atorvastatin calcium (AT) was generously supplied by Riyadh Pharma (Riyadh, Saudi Arabia). Tween-85 (T-85) was obtained from Merck-Schuchardt OHG (Hohenbrunn, Germany). Hydrogenated castor oil (HCO-30) was supplied by Nicole Chemical Co. (Tokyo, Japan). LauroglycolTM 90 (LG) and Labrasol ALF (LB) were provided by Gattefosse (Saint-Priest, France). Kolliphor-EL (K-EL), Kollisolv[®] PEG 400 (PEG-400), and Imwitor-308 (I-308) were purchased from BASF (Ludwigshafen, Germany). Span-80 (S-80) was supplied by Merck (Darmstadt, Germany). Black seed oil (BSO) was purchased from Wadi Al-Nahil Investment Group (Riyadh, Saudi Arabia). All other reagents were of analytical grade and used without further purification.

2.2. UPLC Method for Drug Quantification

DionexTM ultra-performance liquid chromatography (UPLC) system (Thermo Scientific, Bedford, MA, USA) was used to estimate AT concentration in the SNEDDS formulation. The mobile phase consisting of acetonitrile: 10mM, Ammonium formate buffer: 0.1%, Formic

acid solution (4.5:4.5:1) was eluted using a Dionex Pump System through Acquity® UPLC BEH C₁₈ column (2.1 × 50 mm, 1.7 µm) placed in a Dionex column oven chamber with a flow rate of 0.4 mL/min. The injection volume of samples was 2 µL, with a run time of 5 min. The Column temperature was kept at 30 ± 0.01 °C. The absorbance of AT was detected at 245 nm using Dionex Photodiode Array (PDA) detector. Peaks analysis was performed using the Chromeleon Client Program. Drug concentration was estimated using the injected calibration curve ranging from 5.0 to 50.0 µg/mL.

2.3. Preparation of SNEDDS Formulations

The drug-free SNEDDS formulation was prepared by mixing its components (surfactant, cosurfactant, and black seed oil) as listed in Table 1. SNEDDS components were flipped ten times and mixed using a vortex mixer for approximately 15 min at 800 rpm until a homogenous mixture was obtained. The prepared formulations were subjected to miscibility, emulsification, and AT solubility studies to select for the optimized formulation.

Table 1. Composition of prepared drug-free SNEDDS formulation.

Formulation Code	Surfactant (%)				Co-Surfactant (%)			Bioactive Oil (%)	
	T-85	HCO-30	LB	K-EL	PEG	I-308	S-80	LG	BSO
F1	40				30				
F2	40					30			
F3	40						30		
F4	40							30	
F5		40			30				
F6		40				30			30
F7		40					30		
F8		40						30	
F9			40		30				
F10			40			30			
F11			40				30		
F12			40					30	
F13				40	30				
F14				40		30			
F15				40			30		
F16				40				30	

The amounts of SNEDDS components are expressed as weight percentages. T-85; Tween-85, HCO-30; Hydrogenated castor oil, LB; Labrasol ALF, K-EL; Kolliphor-EL, PEG-400; Kollisolv® PEG 400, I-308; Imwitor-308, S-80; Span-80, LG; Lauroglycol™ 90, BSO; Black seed oil.

2.4. Preparation of Carbonated SNEDDS Formulation C-F6

The F6 was selected based on miscibility, globule size, and drug solubility, and was alkalinized by adding different concentrations of sodium bicarbonate. About 5 g of the SNEDDS formulation was placed in a 5 mL cylindrical beaker. After that, sodium bicarbonate was added in various amounts (5, 10, 25, and 37.5 mg) to prepare C-F6 (1.0 mg/g), C-F6 (2.0 mg/g), C-F6 (5.0 mg/g), C-F6 (7.5 mg/g) formulations, respectively. The dispersion was subjected to stirring using a magnetic stirrer at 1000 rpm for 24 h. Then, the prepared formulations were left for 24 h, and the bottom of the beaker was checked visually for any precipitated carbonate. The formulation showed that sodium bicarbonate precipitation was excluded from the study, and other formulations were subjected to further assessment.

2.5. Preparation of AT-Loaded SNEDDS Formulation

A solidified SNEDDS formulation was prepared as a counterpart to carbonated SNEDDS to compare the effect of both formulations on AT stability. To prepare AT-F6, 100 mg of the drug was mixed with 4900 mg of the SNEDDS formulation. This mixture was stirred using a magnetic stirrer at 1000 rpm at room temperature (22 ± 2.0 °C) until the AT was completely dissolved. The mixture was stirred until all the AT was completely dissolved. AT-loaded carbonated SNEDDS was prepared using a similar procedure. To prepare AT-loaded solidified formulation, the selected liquid SNEDDS formula was mixed with an equal amount of SYLOID 244 FP (1:1) in a plastic container. The resulting mixture was manually blended to ensure that the formulation was completely adsorbed by SYLOID. The prepared formulations were stored at room temperature (22 ± 2.0 °C) for further analysis.

2.6. Miscibility Study

To ensure a homogenous distribution of components, the prepared SNEDDS (F1–F16) formulations were subjected to a miscibility study, as previously described, with minor modifications [36]. The formulations were centrifuged at 10,000 rpm for 10 min to facilitate the separation of immiscible components. The miscibility of SNEDDS components was checked visually to confirm the presence or absence of phase separation. Only homogenous formulations were subjected to the subsequent studies.

2.7. Emulsification Study

Within a 25 mL plastic container, SNEDDS formulations were diluted with deionized water (1:1000) and mixed using a magnetic stirrer for 5 min at 250 rpm. The appearance of dispersed systems was checked visually to check the ability of SNEDDS formulation to form a homogenous dispersion system [37]. In addition, the surface of the dispersed system was checked to confirm the integration of BSO within the dispersed system [37].

2.8. Particle Size, PDI, and Zeta Potential Measurement

Physicochemical properties of dispersed formulation (1:1000) in distilled water were measured using Zetasizer. Particle size was measured using Dynamic Light Scattering mode, while zeta potential was evaluated using Laser Doppler Velocimetry mode. The value was represented as an average of three measurements for each formulation.

2.9. AT Solubility

The SNEDDS formulations that showed accepted miscibility and drug solubility, and the carbonated SNEDDS formulations were mixed with an excess amount of AT in a 4 mL glass vial. The mixtures were vigorously mixed to enhance the spreading of AT, then subjected to magnetic stirring at 1000 rpm. During the experiment, the mixture was monitored visually, and an additional amount of AT was added if the previous amount was completely dissolved. After 24 h, the mixture was centrifuged at 14,000 rpm for 10 min to precipitate the undissolved AT. Within 2 mL Epindorph, an accurately weighted amount of supernatant was diluted with 1.8 mL acetonitrile and sonicated for about 15 min to ensure complete drug extraction. AT concentration in the ACN was estimated using the developed UPLC method [38].

2.10. AT Content

AT content within the prepared SNEDDS formulation was measured as follows. An accurately weighed amount of SNEDDS formulation was placed in 2 mL Epindorph and diluted with 1.8 mL acetonitrile. Afterward, it was subjected to sonication for 15 min to ensure complete drug extraction from SNEDDS formulation. After that, drug concentration was measured using the UPLC method following appropriate dilution [36].

2.11. *In vitro* Dissolution Study

The *in vitro* dissolution study was performed to investigate the impact of SNEDDS formulation on the dissolution profile of Pure AT and TQ. Dissolution apparatus type II (LOGAN Inst. Corp., Franklin, NJ, USA) was utilized during the present study. Dissolution study was performed in 900 mL phosphate buffer (pH 6.8), and the temperature was set at 37.0 ± 0.5 °C. The test pure APIs and SNEDDS formulations were placed in hard gelatin capsules. During the experiment, the paddle stirrer was set at a speed of 50 rpm. At predetermined intervals (5, 10, 15, 30, 45, and 60 min), about 1.5 mL was withdrawn from the media, and the drug concentration was measured using the developed UPLC method. Dissolution efficiency was calculated to investigate the impact of SNEDDS on the dissolution profile of AT and TQ [39].

2.12. *Characterization of Solid SNEDDS Formulation*

2.12.1. FTIR

The FTIR spectrum of AT, adsorbent, and solidified SNEDDS was recorded using an FTIR spectrophotometer (FT-IR Nicolet 380; Thermo Fisher Scientific, Madison, WI, USA). This analysis assessed the possible chemical interaction between AT and Syloid adsorbent. The samples were prepared using the compressed disc technique, where a small amount of the sample was mixed with potassium bromide and pressed into a disc using a hydraulic press. The resulting disc was scanned over a range of 600 to 4000 cm^{-1} .

2.12.2. DSC

Thermal analysis of AT, adsorbent, and solidified SNEDDS was performed using the DSC–8000 Perkins Elmer (Waltham, MA, USA). The investigated sample was accurately weighed and sealed in a standard aluminum pan. Thermal scanning was performed over a range of 30–180 °C at a heating rate of 10 °C/min. Pyris Manager software version 10.1 (Pyris Elmer, Waltham, MA, USA) was used to access and estimate the solid state of the samples.

2.12.3. PXRD

The crystallinity of AT within the prepared solid SNEDDS formulation was performed using an X-ray diffractometer instrument (Ultima IV, Rigaku Inc., Tokyo, Japan). This was achieved through examining AT, adsorbent, and solidified SNEDDS using the PXRD instrument. Samples were analyzed at a scan rate of 1° per minute over a range of 3° to 40°. The X-ray diffractometer employed monochromatic radiation with a wavelength of 1.54 Å (Cu K α' 1) to collect the data. The system operated at a voltage of 40 kV and a current of 40 mA.

2.13. *pH Measurement*

pH measurements were conducted for each formulation to assess the effect of sodium bicarbonate on the pH of F6 formulations. A pH electrode was placed in a beaker containing the selected SNEDDS containing different concentrations of sodium bicarbonate compared to a carbonate-free one, and the pH value was recorded. Three measurements were taken for each formulation, and the average value was used to represent the pH of each formulation.

2.14. *Stability Study*

This experiment aimed to compare the impact of solidification and sodium bicarbonate on the chemical stability of AT within SNEDDS formulations. The thermal stability was examined under accelerated conditions. Formulations were placed in a 4 mL glass vial and were closed tightly using a screwed cap. The glass vials were placed in a stability chamber (Binder GmbH, Tuttlingen, Germany), and the temperature was maintained at 40 ± 2 °C and 15 ± 5 % relative humidity. Drug concentrations within the prepared formulations were determined using the developed UPLC method following 15, 30, and 45 days.

2.15. Degradation Kinetic Study

Zero-, first-, and second-order kinetic models were utilized to investigate the degradation rate of AT within the prepared formulations [40].

$$\text{Zero – order kinetic model : } C_t = C_0 - k t$$

$$\text{First – order kinetic model : } C_t = C_0 e^{-k t}$$

$$\text{Second – order kinetic model : } \frac{1}{C_t} = \frac{1}{C_0} + k_2 t$$

3. Results and Discussion

3.1. UPLC Method for Estimation of AT

Figure 1A,B shows the chromatogram obtained from the injected blank and AT solution, respectively. The detected retention time of AT was about 2.38 min. A forced degradation study revealed that AT was well-resolute from the detected degradation products of the drug, as shown in the chromatogram presented in Figure 1C–F. The main degradation product was detected in acidic conditions at 4.12 min with a resolution of 9.9 from the AT peak. However, oxidative stress shows a degradation peak at 4.1 min with a resolution of 9.8 from the AT peak. In addition, the initial peak at void volume corresponds to the presence of hydrogen peroxide. Therefore, it is clear from the chromatograms that the UPLC method was able to separate the drug from the main degradation products.

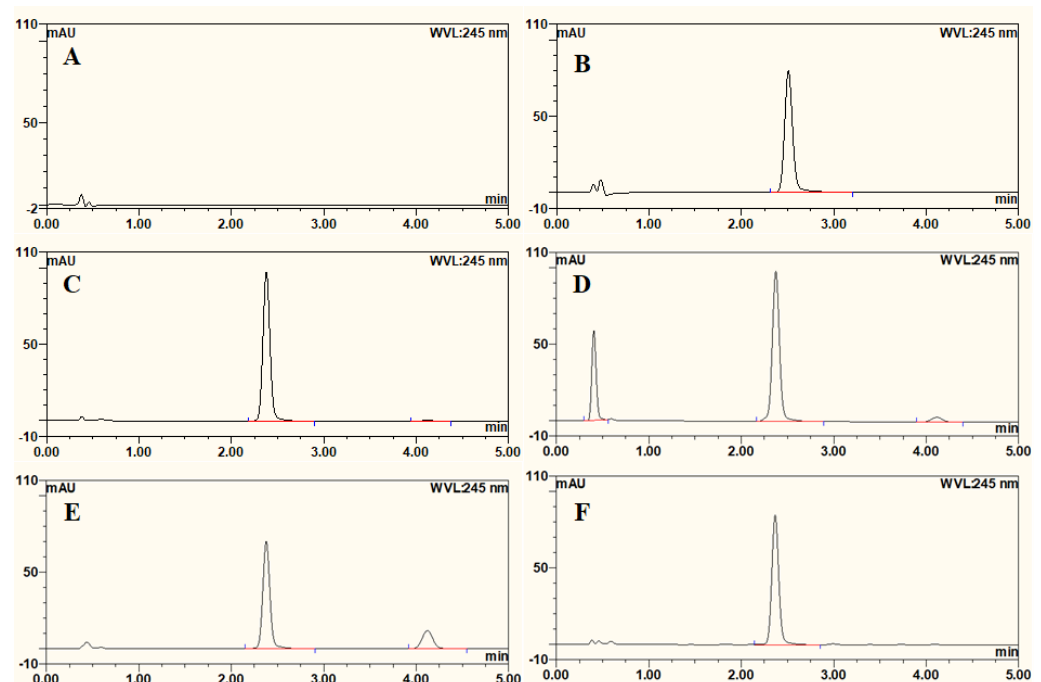


Figure 1. Chromatograms of (A) blank (B) standard solution, along with forced degradation obtained following exposure to (C) thermal-, (D) oxidative-, (E) acid-, and (F) base-stress conditions.

3.2. Evaluation of SNEDDS Formulations

Black seed oil (BSO) is a well-known bioactive ingredient used during the preparation of SNEDDS formulation to enhance its therapeutic activity [36,37]. Thymoquinone (TQ) is one of the phytochemical components present in BSO with antihyperlipidemic activity [41,42]. In addition, various studies showed that the administration of TQ significantly reduced cardiac damage induced by hyperlipidemia [43,44]. Therefore, BSO was utilized in the current study during the formulation of SNEDDS not only to enhance the bioavailability of AT, but also to augment AT therapeutic activity.

A previous study showed that BSO has low miscibility when mixed with different types of surfactants and cosurfactants [36]. Therefore, different types of SNEDDS components were used in the present study to prepare homogeneous SNEDDS with good emulsification properties. Table 2 shows the evaluation results of miscibility and emulsification in terms of physical appearance and system separation. Formulations (F1, F3, F5, F7, F9, and F13–15) were excluded from the study, owing to their inability to form homogeneous SNEDDS. In contrast, formulations (F2, F4, F6, F8, F10–12, and F16) were able to form homogeneous formulations. The immiscibility of the SNEDDS formulation could be attributed to the difference between mixed components [37].

Table 2. Evaluation results of prepared drug-free BIO-SNE DDS formulations.

Formulation Code	Miscibility	Physical Appearance	System Separation
F1	Immiscible	-----	-----
F2	Miscible	Turbid	Dispersible system with floating oil
F3	Immiscible	-----	-----
F4	Miscible	Turbid	Dispersible system with floating oil
F5	Immiscible	-----	-----
F6	Miscible	Clear solution	Uniform dispersion
F7	Immiscible	-----	-----
F8	Miscible	Suspended system	Indispensable system
F9	Immiscible	-----	-----
F10	Miscible	Semi-turbid solution	Dispersible system with floating oil
F11	Miscible	Semi-turbid solution	Dispersible system with floating oil
F12	Miscible	Semi-clear solution	Dispersible system with floating oil
F13	Immiscible	-----	-----
F14	Immiscible	-----	-----
F15	Immiscible	-----	-----
F16	Miscible	Bluish solution	Uniform dispersion

Homogeneous formulations were subjected to an emulsification study. It was found that F8 was unable to disperse the observation of aggregated particles in the media, as shown in Figure 2A. In addition, system separation was observed with F2, F4, and F10–12 formulations, which indicates the inability of surfactant and cosurfactant to incorporate oil within the dispersed system. Figure 2B,C show the turbid and semi-turbid appearance of the dispersed system, respectively, whereas Figure 2D shows oil floating on the surface during the experiment. On the contrary, Figure 2E,F shows that F6 and F16 were able to form homogeneous dispersion systems with clear and bluish appearance, respectively. Therefore, the F6 and F16 formulations were subjected to further assessment to select for the optimum formulation.

Table 3 shows that the particle size of the dispersed F6 and F16 formulations were in the nanosize scale with a particle size of 46.62 and 85.91 nm, respectively. The dispersed SNEDDS formulations exhibited a negative zeta potential value of -35.2 and -21.2 mV, respectively. The obtained nanoemulsions could significantly boost the bioavailability of administrated AT following oral administration [25]. This is attributed to the enhancement in drug solubility, which makes it available for absorption to systemic circulation. Moreover, it has been reported that the dispersed SNEDDS formulation is able to enhance permeability through the intestinal membrane [45]. In addition, the F6 and F16 were subjected to a solubility study, and AT solubility was found to be 58.21 and 7.88 mg/g, respectively. Therefore, F6 was selected as the optimized formulation, owing to its superiority in AT

solubility. This allows for the loading of AT within the minimal amount of SNEDDS formulation, and reduces the total dosage of administrated SNEDDS formulation [46].

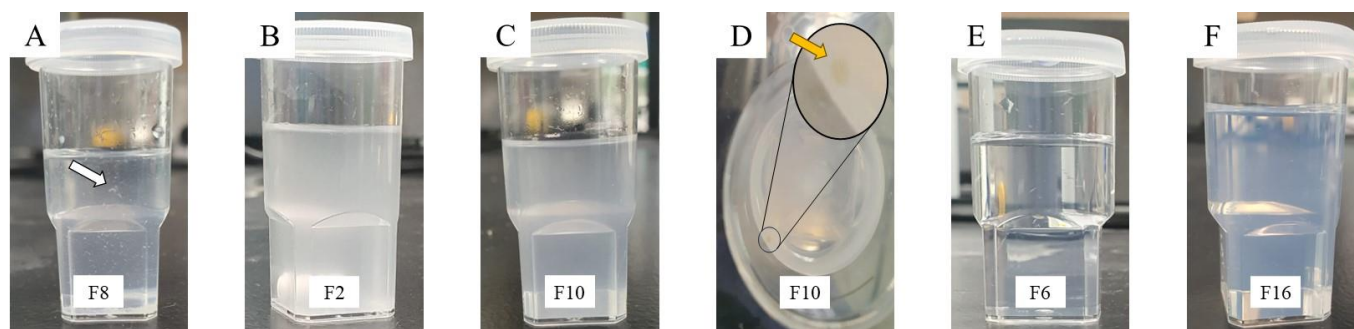


Figure 2. Physical appearance of dispersed SNEDDS formulations, whereas (A) shows the undispersed F8 formulation, (B) turbidity of dispersed F2, (C) semi-turbidity of dispersed F10, (D) floating oil on the surface of dispersed F10, (E) clarity of dispersed F6, and (F) bluish appearance of dispersed F16.

Table 3. Pharmaceutical assessment results of AT-loaded BIO-SNEDDS.

Formulation Code	Particle Size (nm)	Zeta Potential (mV)	AT Solubility (mg/mL)
F6	46.62 ± 0.12	−35.2 ± 3.6	58.21 ± 2.56
F16	85.91 ± 1.11	−21.2 ± 1.1	7.88 ± 0.19

The data were presented as the mean ± SD, with N = 3.

3.3. In vitro Dissolution Study

In vitro dissolution of pure AT and TQ was studied against the drug-loaded SNEDDS formulation (Figure 3). It was found that the selected formulation was able to increase the initial AT and TQ dissolved in the media within the first 5 min from 7.3 and 3.0 to 88.2 and 82.3%, respectively. In addition, the selected optimized formulation enhances the dissolution efficiency of AT and TQ by 1.5- and 7-fold, respectively. The obtained results agree with previously published studies that showed that the prepared SNEDDS formulations could significantly enhance the bioavailability of both drugs following oral administration [47,48].

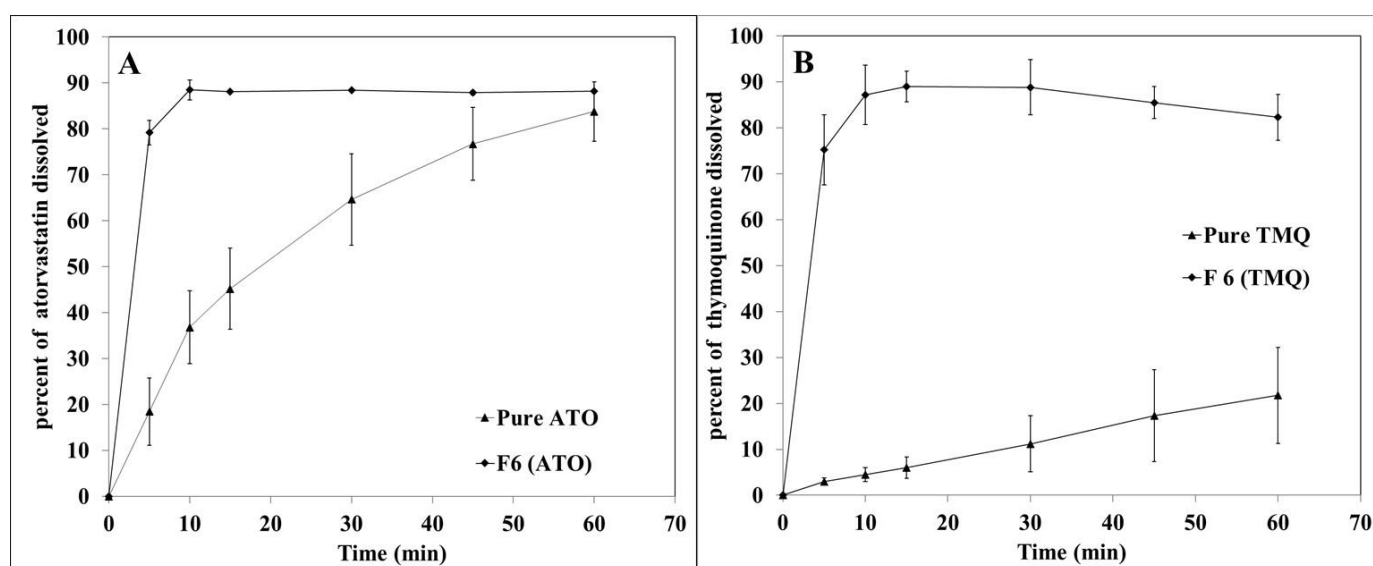


Figure 3. In vitro dissolution profile of (A) atorvastatin and (B) thymoquinone. The data were presented as the mean ± SD, with N = 3.

3.4. Evaluation of Solidified SNEDDS

3.4.1. FTIR

The FTIR analysis of AT, Syloid adsorbent, and solid SNEDDS (Figure 4) was performed to investigate the impact of solidification on the crystalline state of the drug. The FTIR spectrum of AT shows characteristic peaks at 3665.9 (hydroxyl group), 3361.53 (N-H stretch), 3235.56 (O-H stretching), two peaks between 3000 and 2900 (aromatic and alkyl C-H bond stretch), 1650 (C=O stretch), and 1581 cm^{-1} (N-H bend) that are in alignment with previously published data [49–51]. Syloid adsorbent has characteristic peaks at 1071 cm^{-1} and 799 cm^{-1} (Si-O-Si stretching vibrations), which agrees with previously published data and indicates its silicate structure [52]. The observed new peak in the solid SNEDDS spectrum at 1736 cm^{-1} (ester carbonyl stretching) could correspond to esterified glycerol present in the used oil during the preparation of SNEDDS formulation [53]. Furthermore, O-H peaks were significantly reduced and shifted, indicating hydrogen bonding formation between AT and SNEDDS components [23]. This could suggest that AT is present in the amorphous state and solubilized within the prepared solid SNEDDS formulation. Therefore, DSC and PXRD examinations were performed to check the crystalline state of AT within the prepared solid SNEDDS.

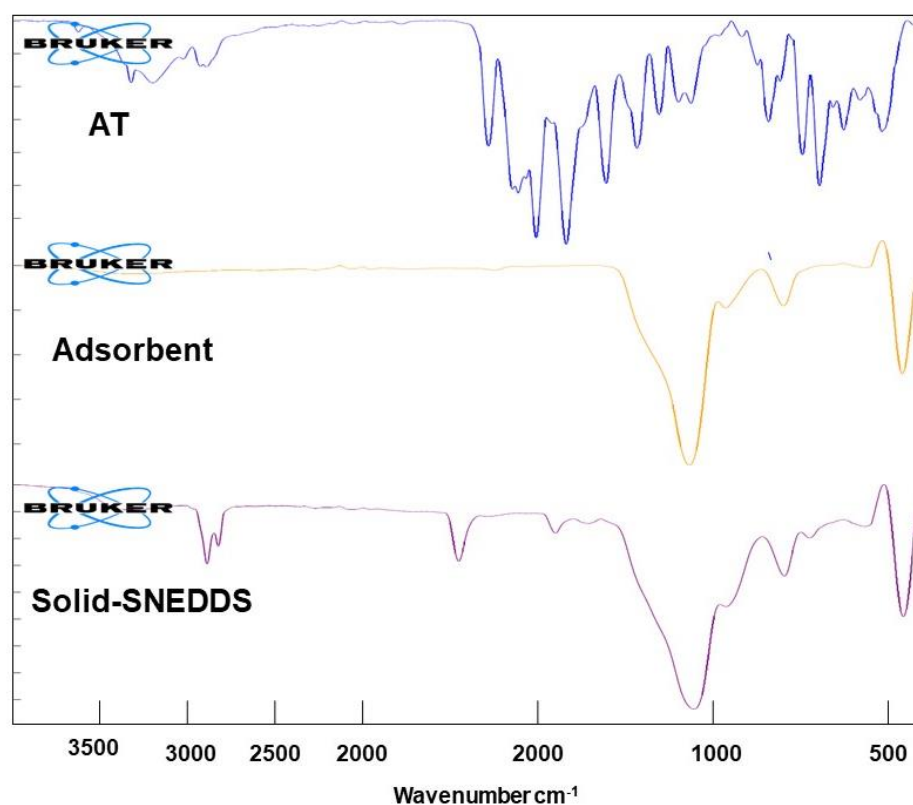


Figure 4. FTIR of AT, adsorbent, and solidified SNEDDS.

3.4.2. DSC

Figure 5 shows thermograms of AT, adsorbent, and solidified SNEDDS. It has been found that the AT thermogram has an endothermic peak at 132.8 °C, which could be attributed to water loss that is in harmony with previously published data [54,55]. In addition, the main endothermic peak was observed at 158.2 °C, which could be attributed to the melting of AT. In addition, endothermic peaks of AT were absent in the prepared solidified SNEDDS formulation. The obtained results agree with previously published studies by Tashish et al. [56], indicating that AT could be present in an amorphous state due to its solubilization. This is achieved through the adsorption of liquid SNEDDS containing solubilized AT by a solid adsorbent.

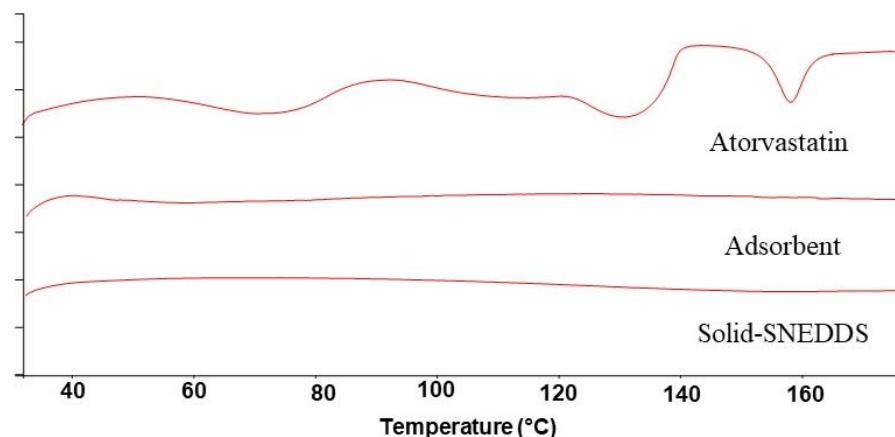


Figure 5. DSC of atorvastatin, adsorbent, and solidified SNEDDS.

3.4.3. PXRD

Powder X-ray diffractometry was performed to investigate the crystalline state of AT within the prepared solid SNEDDS formulation. Figure 6 shows that AT has characteristic crystalline peaks between 9.0 and 23.0° aligned with previous published studies [54,57]. However, it was found that AT peaks were completely absent in the chromatogram of solid SNEDDS formulation. The obtained results, in harmony with previously published data, showed that the solidification of drug-loaded SNEDDS formulation using an adsorbent resulted in a complete transformation of the drug from a crystalline to an amorphous state [19,56]. This finding is also in harmony with the DSC, indicating the presence of AT in the amorphous state.

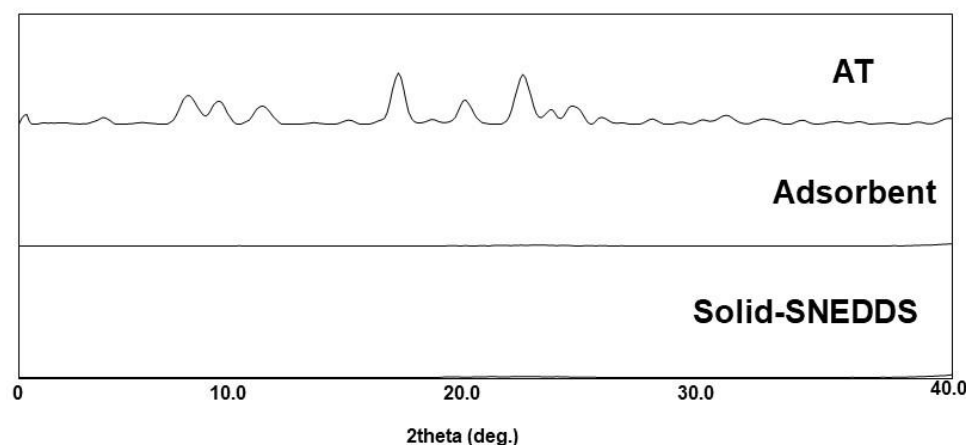


Figure 6. PXRD of atorvastatin, adsorbent, and solidified SNEDDS.

3.5. Kinetic Stability of AT in Liquid and Solid SNEDDS Formulation

AT is an acid-labile drug susceptible to chemical degradation when incorporated within SNEDDS formulation due to the presence of free fatty acids [32]. Therefore, several strategies have been employed to enhance the stability of drugs loaded in SNEDDS formulations. Among these strategies, solidification of SNEDDS through adsorption technology is commonly utilized due to its effectiveness, simplicity, and cost efficiency [58]. In this study, AT-loaded solid F6 formulation (AT-SF6) was prepared and subjected to a stability study to evaluate the efficacy of the traditional solidification method in protecting acid-labile drugs.

Figure 7 shows the percentage of intact AT remaining following incubation of AT-F6 and AT-SF6 formulations in the stability chamber. The percentage of drugs remaining at the end of the experiment was found to be 16.06 ± 1.74 and $18.60 \pm 2.17\%$ for AT-F6 and AT-SF6, respectively. Statistical analysis revealed that solidification did not significantly enhance AT protection. Regression analysis of obtained data was performed based on

three kinetic models (zero, first, and second order). Table 4 presents the coefficient of determination and slope values obtained from zero-, first-, and second-order equations. The coefficient of determination for both formulations was close to 1 when considering the second-order equation. This indicates that the degradation rate of AT in both formulations follows a second-order kinetics model. Figure 8 shows the observed linear trend in the case of the second-order equation for solid and liquid F6 formulation.

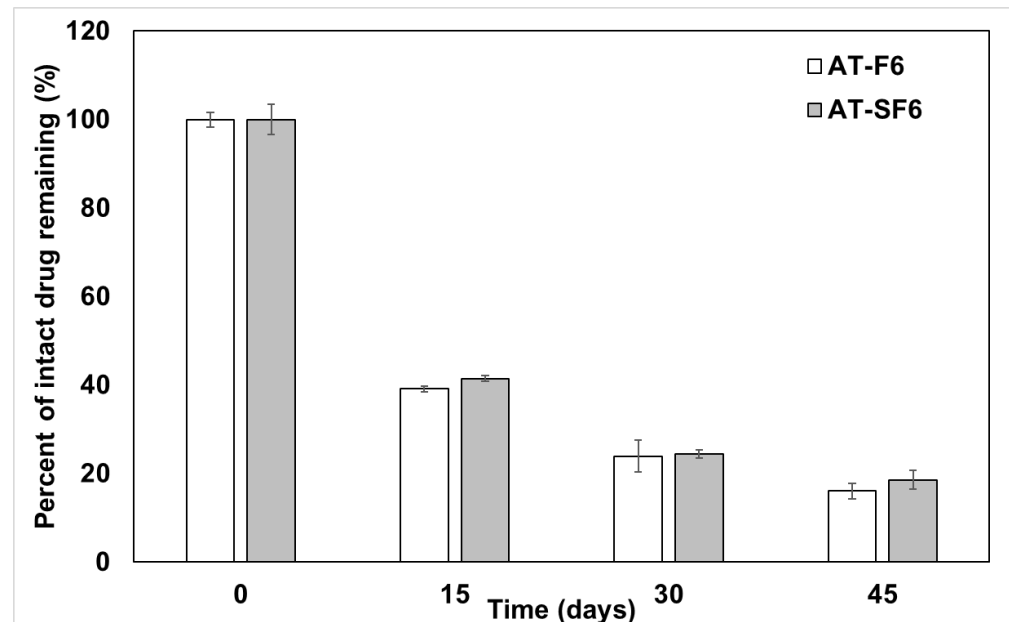


Figure 7. The histogram shows the percent of atorvastatin remaining within the liquid and solid SNEDDS formulation following incubation in the stability chamber. The data were presented as the mean $\pm$ SD, with N = 3.

Table 4. Comparison of the slope and coefficient of the determination values derived from fitting data of F6 and S-F6 formulations to zero-, first-, and second-order equations.

		F6	S-F6
Zero-order	Slope	−1.779926884	−1.741588269
	r2	0.821546666	0.821816861
First order	Slope	−0.039844248	−0.037165163
	r2	0.957916108	0.94371274
Second order	Slope	0.001153033	0.000987203
	r2	0.995319989	0.997570124

The obtained results indicate that solidification did not effectively slow down the degradation rate of the loaded acid-labile drug. This agrees with previously published data, where solidified formulations did not remarkably protect drugs from chemical degradation [17,21]. This could be attributed to the acidic microenvironment generated around AT (acid-labile drug). Therefore, an alternative strategy is required to resolve the instability issue of acid-labile drugs in SNEDDS formulations.

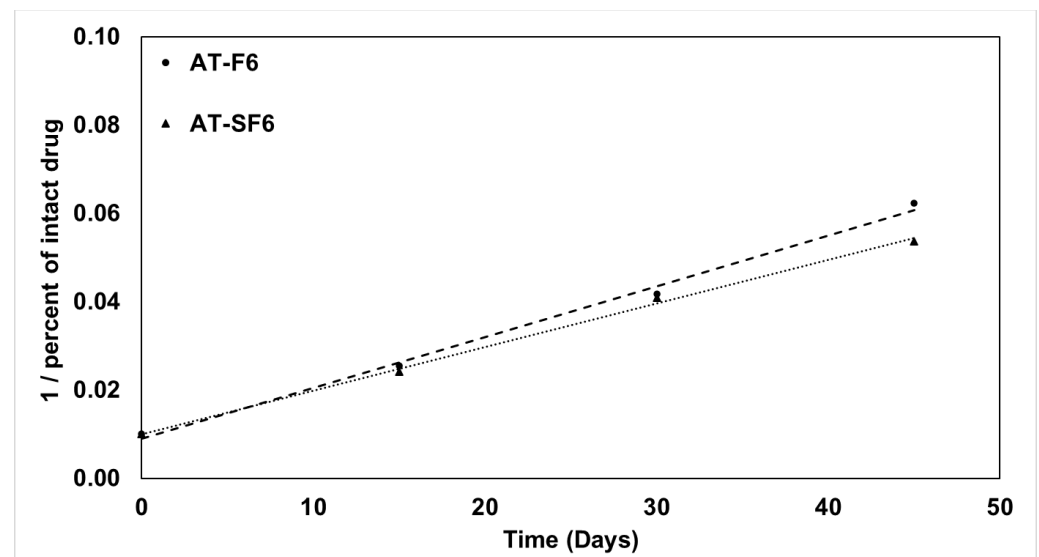


Figure 8. Second-order reaction kinetics for the degradation of atorvastatin in AT-F6 and AT-SF6 formulations.

3.6. Evaluation of Carbonated SNEDDS

To overcome the low pH microenvironment in the SNEDDS formulation, sodium bicarbonate was selected as an alkalization agent. Carbonated SNEDDS formulations with varying concentrations of sodium bicarbonate were prepared to assess their impact on the stability of the loaded drug. This could minimize the susceptibility of acid-labile drugs to chemical degradation, and provide a promising approach to utilize SNEDDS for these medications.

Table 5 illustrates the physicochemical characterization of CF6 formulations compared to F6 in terms of pH value, particle size, zeta potential, and AT solubility. The results showed that incorporating sodium bicarbonate in SNEDDS formulation significantly raises the pH of the F6 formulation. Additionally, an increase in the concentration of sodium bicarbonate leads to a reduction in the particle size of the dispersed SNEDDS formulation. Moreover, it was found that SNEDDS formulations containing sodium bicarbonate had a higher zeta potential value compared to the uncarbonated SNEDDS. Notably, there is a significant improvement in AT solubility with increasing sodium bicarbonate concentrations. This is in alignment with previous studies that highlight the pH-dependent nature of AT solubility [59]. Therefore, it is expected that carbonated SNEDDS formulation not only enhances AT's stability, but also increases AT's loading capacity within the carbonated SNEDDS formulation.

Table 5. Physicochemical characterization of F6 and C-F6 formulations.

Formulation Code	pH	PS (nm)	ZP (mV)	AT Solubility (mg/mL)
F6	4.8 ± 0.02	46.62 ± 0.12	−35.2 ± 3.6	58.21 ± 2.56
CF6 (1.0 mg)	5.9 ± 0.05	32.42 ± 0.20	−19.7 ± 1.4	73.89 ± 1.55
CF6 (2.0 mg)	6.1 ± 0.01	30.64 ± 0.38	−11.6 ± 2.7	87.01 ± 4.35
CF6 (5.0 mg)	6.9 ± 0.03	23.95 ± 0.11	−20.2 ± 2.3	91.85 ± 4.79

The data were presented as the mean ± SD, with N = 3.

3.7. Kinetic Stability of AT in Liquid and Carbonated SNEDDS Formulation

AT is an acid-labile drug, whereas free fatty acids are naturally present in oils, which could reduce the stability of AT within the proposed SNEDDS formulation [32,35]. It has been utilized in different types of solid dosage forms to enhance the stability of acid-labile drugs [60,61]. However, none of these studies evaluated the potential impact of sodium

bicarbonate as a stabilizing agent in SNEDDS formulations to protect acid-labile drugs from chemical degradation.

The solubility of sodium bicarbonate was studied within the optimized SNEDDS formulation, and maximum solubility was attained at 5 mg/g loading. Intact AT within CF6 was measured using the developed UPLC method at predetermined intervals (0, 15, 30, and 45 days). Figure 9 shows the percentage of AT remaining intact within F6 and CF6 formulations throughout the experiment. Statistical analysis revealed that sodium bicarbonate loading significantly enhances AT stability within the CF6 formulation compared to F6 formulation.

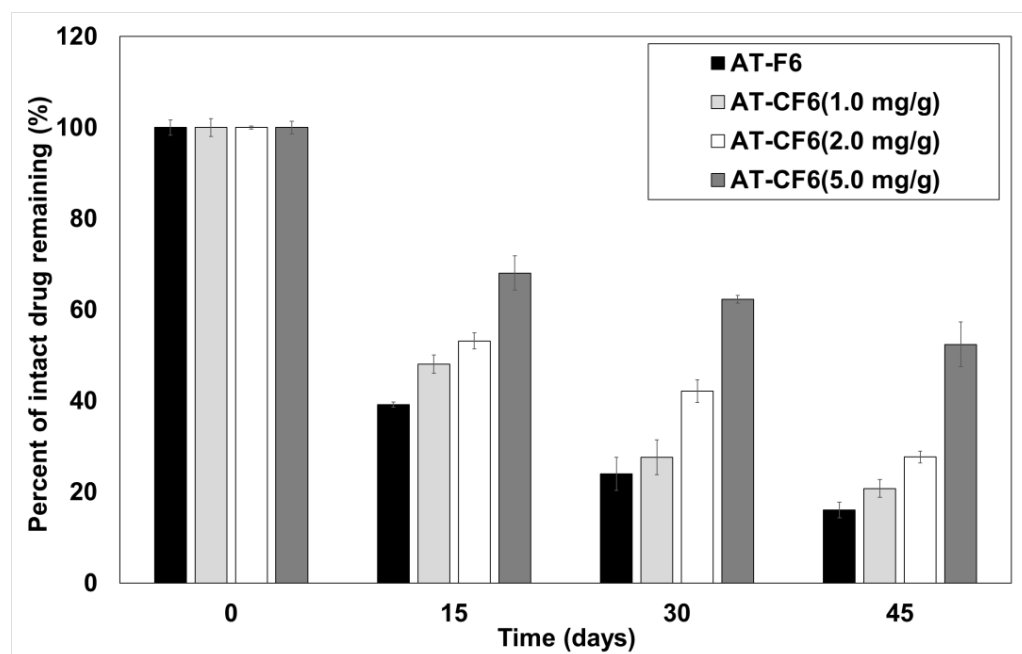


Figure 9. Histogram shows the percent of atorvastatin remaining within the liquid and the carbonated SNEDDS formulation following incubation in the stability chamber.

Figure 10A shows the chromatogram of the SNEDDS formulation at zero time. It shows the parent peak of AT at 2.3 min, along with an additional peak at 1.6 min. This peak corresponds to the bioactive thymoquinone present in the SNEDDS formulation. In addition, Figure 10B shows a chromatogram of the SNEDDS formulation following incubation in the stability chamber. AT's main acid degradation peak was observed along with two additional degradation peaks at 1.9 and 2.6 min. These two peaks could result from any incompatibility between AT and SNEDDS components.

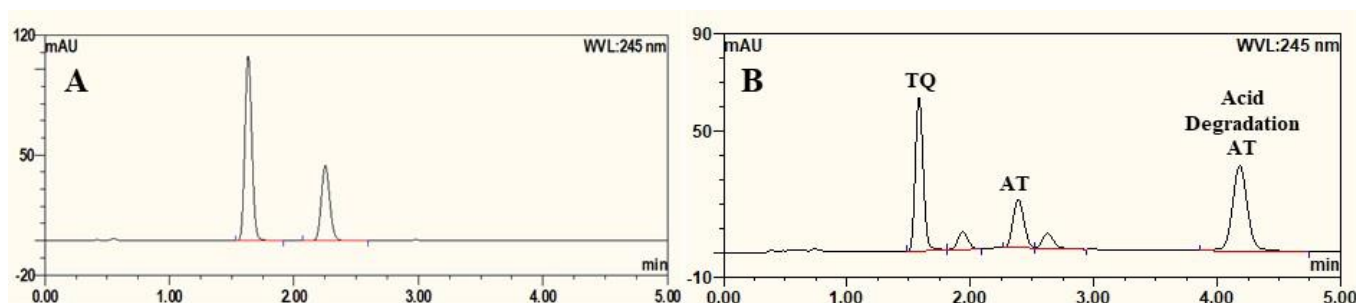


Figure 10. Chromatogram shows (A) atorvastatin and bioactive thymoquinone present in SNEDDS formulation, and (B) the resolution of parent atorvastatin peak from degradation peaks.

Kinetic analysis of drug degradation was performed using zero-, first-, and second-order equations. The coefficient of determination values indicates that AT degradation in all

formulations follows a second-order equation (Table 6). Figure 11 shows the plotting of time against the reciprocal of intact AT concentration remaining in the SNEDDS formulation. The linear trend of the plotted data indicated that AT degradation follows a second-order equation.

Table 6. Comparison of the slope and coefficient of the determination values derived from fitting data of AT-F6 and AT-CF6 formulations to zero-, first-, and second-order equations.

		AT-F6	AT-CF6 (1.0 mg/g)	AT-CF6 (2.0 mg/g)	AT-CF6 (5.0 mg/g)
Zero-order	Slope	−1.7799	−1.7213	−1.5198	−0.9907
	r ²	0.82155	0.86408	0.88434	0.86853
First order	Slope	−0.0398	−0.0351	−0.0272	−0.0135
	r ²	0.95792	0.96474	0.96702	0.91776
Second order	Slope	0.00115	0.00087	0.00056	0.00019
	r ²	0.99532	0.99565	0.97335	0.95475

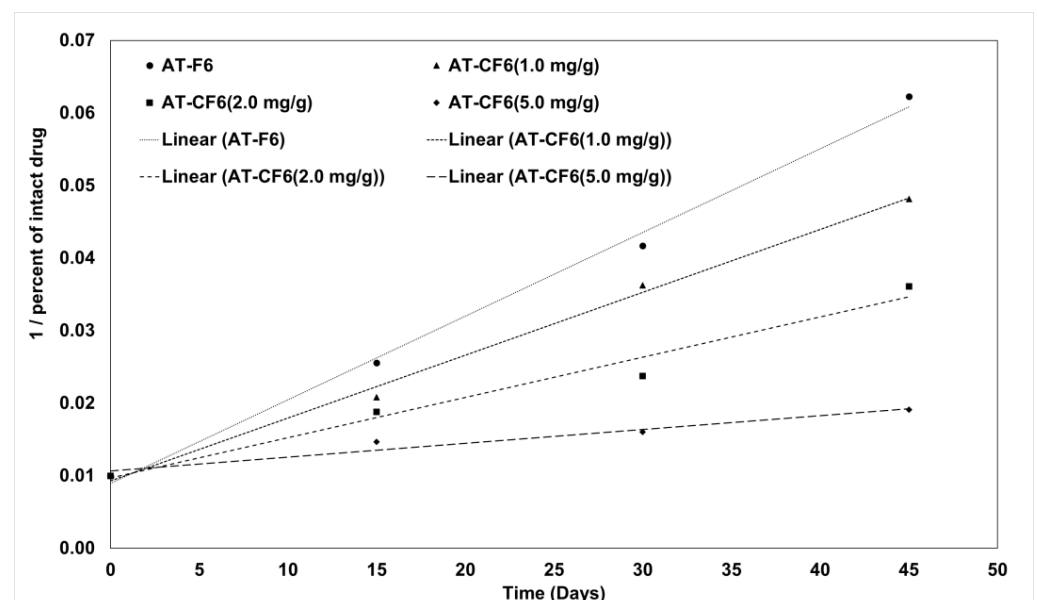


Figure 11. Second-order reaction kinetics for the degradation of atorvastatin in AT-F6 and AT-CF6 formulations.

The slope of the second-order equation was plotted against its corresponding sodium bicarbonate concentration, as shown in Figure 12. It was found that there was an inverse relationship between sodium bicarbonate concentration and the rate of drug degradation. Therefore, it could be concluded that sodium bicarbonate significantly increases the stability of acid-labile drugs in the SNEDDS formulation compared to the solidification approach. This aligns with the current results, which show that increasing sodium bicarbonate concentration increases the SNEDDS formulation's pH. This decreases the exposure of AT to acidic microenvironments and protects AT from chemical degradation [33,62]. The results of these studies provide valuable insights into the potential of sodium bicarbonate as a reliable stabilization strategy for acid-labile drugs within SNEDDS formulation.

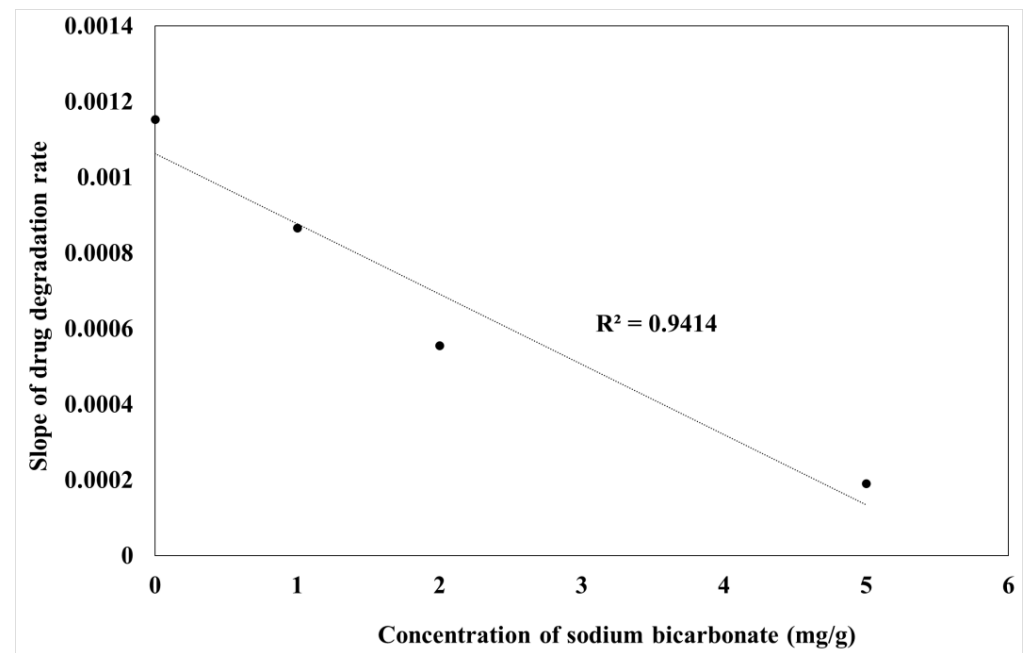


Figure 12. Correlation between concentration of sodium bicarbonate concentration and slope of AT degradation rate.

3.8. Future Prospective

In the present study, the alkalization agent was used for the first time, unlike in the literature, to enhance the stability of acid-labile drugs within the acidic microenvironment of the SNEDDS formulation. This could potentially allow for the usage of SNEDDS as a pharmaceutical formulation to enhance the therapeutic outcomes of poorly water-soluble drugs. However, limited sodium bicarbonate solubility in SNEDDS formulation (<7.5 mg/g) is the major challenge to increase its stabilization effect. Therefore, further studies are still required to address this issue. Moreover, further research and experimentation are necessary to fully evaluate the efficacy of sodium bicarbonate and its potential impact on the stability and performance of acid-labile drugs in SNEDDS formulations.

4. Conclusions

The self-nanoemulsifying drug delivery system (SNEDDS) showed outstanding results in enhancing the solubility and bioavailability of poorly water-soluble drugs. However, the presence of free fatty acids in SNEDDS formulation resulted in remarkable drug degradation. Therefore, innovative carbonated SNEDDS was utilized to overcome the instability issue of acid-labile drugs. The present study reveals that sodium bicarbonate has superior stabilization efficacy over the solidification approach for acid-labile drugs. Moreover, the stabilization effect produced by sodium bicarbonate was significantly enhanced by increasing its concentration. Further studies are required to explore the potential stabilization effect of sodium bicarbonate on different acid-labile drugs in SNEDDS formulations.

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