

Preparation and Characterization of Atorvastatin Calcium Trihydrate -cyclodextrin Inclusion Complex

Basma M. Hadi^{*1}, Eman B. H. Al-Khedairy²

¹*Baghdad Health Directorate, Al-Karkh, Ministry of Health, Baghdad, Iraq*

²*Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq*

Received: 09th June, 2022; Revised: 27th July, 2022; Accepted: 26th August, 2022; Available Online: 25th September, 2022

ABSTRACT

Atorvastatin calcium (ATR) is an antihyperlipidemic agent used for lowering blood cholesterol levels. However, it is very slightly soluble in water with poor oral bioavailability, which interferes with its therapeutic action. It is classified as a class II drug according to Biopharmaceutical Classification System (low solubility and high permeability). The present study aimed to enhance the solubility and dissolution rate of ATR by complexation technique using beta-cyclodextrin (β -CD) in comparison with other β -CD derivatives which are hydroxy propyl beta cyclodextrin (HP- β -CD), methyl beta cyclodextrin (M - β -CD) and sulfobutyl ether beta-cyclodextrin (SBE- β -CD) as binary and ternary complex with Soluplus® using co-grinding, kneading, solvent evaporation and microwave methods. Differential scanning calorimetry (DSC), X-ray diffraction (XRD), Fourier-transform spectroscopy (FT-IR) characterized the resultant complex. The results showed that the ternary complex of ATR/SBE- β -CD/Soluplus (1-mole/ 1-mole/ 10% w/w) prepared by microwave method had the highest solubility, highest percentage yield, and faster release rate, which was greater than 90% (in the first 15–20 minutes). The FT-IR results showed that ATR was partially included in the SBE- β -CD cavity, while the DSC, XRD results confirmed the amorphous nature of the resultant complex.

Keywords: Atorvastatin calcium trihydrate, Cyclodextrin, SBE- β -CD, Soluplus®, Binary complex, Ternary complex.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.3.41

How to cite this article: Hadi, BM, Al-Khedairy, EBH. Preparation and Characterization of Atorvastatin Calcium Trihydrate -cyclodextrin Inclusion Complex. International Journal of Drug Delivery Technology. 2022;12(3):1171-1179.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Solubility is defined quantitatively as the solute concentration in a saturated solution at a certain temperature. A saturated solution is one in which the solute is in equilibrium with the solvent. Absorption of drugs from the GIT is limited by many factors, including poor aqueous solubility and poor membrane permeability of the drug molecule. When an active pharmaceutical ingredient is administered orally, it should dissolve in gastric and/or intestinal fluids then permeate the membranes of the GIT to the systemic circulation. So, there are two areas of pharmaceutical research that focus on enhancing the oral bioavailability of drugs; either by; improving the solubility and dissolution rate of poorly water-soluble drugs or improving the permeability of poorly permeable drugs and sometimes both areas may be employed. The Biopharmaceutical Classification System (BCS) is a scientific system for classifying a drug substance according to its aqueous solubility and intestinal permeability. As for BCS class II and IV drugs,

their solubility and release in GIT fluid are considered the rate limiting step, enhancing the solubility in turn improve their bioavailability.¹

Atorvastatin calcium (ATR) is an antihyperlipidemic agent used to lower blood cholesterol levels by reversible inhibition of HMG-CoA reductase, considered a rate-limiting step in cholesterol biosynthesis. ATR belongs to BCS class II drug (low solubility with high permeability).²

The drug is very slightly soluble in water with the pKa value of 4.46 and Log p values of 6.36 in octanol/water. It has an absolute oral bioavailability of 12%. The poor oral bioavailability is caused by pre-systemic clearance in the gastrointestinal mucosa and high hepatic first-pass metabolism.³

Many trials were used to enhance its solubility such as by cocrystallization technique⁴ and by solid dispersion adsorbate technique,² in addition, complexation may be another promising method.

*Author for Correspondence: basma.mudhafar2@gmail.com

Cyclodextrins (CD) are cyclic oligosaccharides composed of glucopyranose units.

The α , β , and γ cyclodextrins are extensively known, which contain 6, 7, and 8 units, respectively. Glucopyranose units form a conical cylinder with a hydrophobic inner cavity and a hydrophilic outer surface. Their structures allow them to form inclusion complexes with hydrophobic drugs and molecules.⁵

When CDs are substituted, the solubility may be increased or decreased. Modification of the 2- or 3-hydroxyl group results in distraction of the intramolecular hydrogen bonding. This distraction permits more interactions of these hydroxyl groups with water molecules, resulting in changing the solubility. Complexation of molecules to CDs happens by a non-covalent interaction between the molecule and the CD cavity.⁵

The addition of ternary materials like hydrophilic polymers to the drug: CD system, lead to formation of co-complexes, by interaction with outer surface of CD that may offer better stability constant, complexation efficiency, physicochemical properties, and drug transport in comparison to binary complexes.⁶

Soluplus[®] is a polymeric solubilizer with an amphiphilic nature. It is soluble in water and show better solubility in many organic solvents. Researchers confirmed that the use of Soluplus[®] (as ternary substance) with CDs improved the solubility and *in-vivo* drug absorption of insoluble drugs.⁶

The aim of this study was to enhance the solubility and dissolution rate of ATR by cyclodextrin inclusion complex technique using Beta cyclodextrin (β -CD) in comparison with other β -CD derivatives which are hydroxy propyl beta cyclodextrin (HP- β -CD), methyl beta cyclodextrin (M- β -CD) and sulfobutyl ether beta cyclodextrin (SBE- β -CD) as binary and ternary complex with Soluplus[®] using different methods.

MATERIALS AND METHODS

MATERIALS

Atorvastatin calcium trihydrate (ATR) was supplied by Pioneer pharmaceutical company, Iraq as a gift sample. Beta cyclodextrin (β -CD) and Hydroxy propyl beta cyclodextrin (HP- β -CD) were purchased from Wuhan Senwayer Century Chemical CO., LTD, China. Methyl beta cyclodextrin (M- β -CD) and sulfobutyl ether beta cyclodextrin (SBE- β -CD) were purchased from Shanghai Ruizheng chemical Tech Co., Ltd, China, and Soluplus[®] was Supplied by BASF SE, Germany.

METHODS

Phase Solubility Diagram

Binary Complex

The phase solubility diagram for binary complex was carried out according to the procedures first described by Higuchi and Connor.⁷

Excess amount of ATR was added separately to 10 mL distilled water containing different concentrations (2–10 mM) of β -CD, HP- β -CD, M- β -CD and SBE- β -CD in 10 mL plane

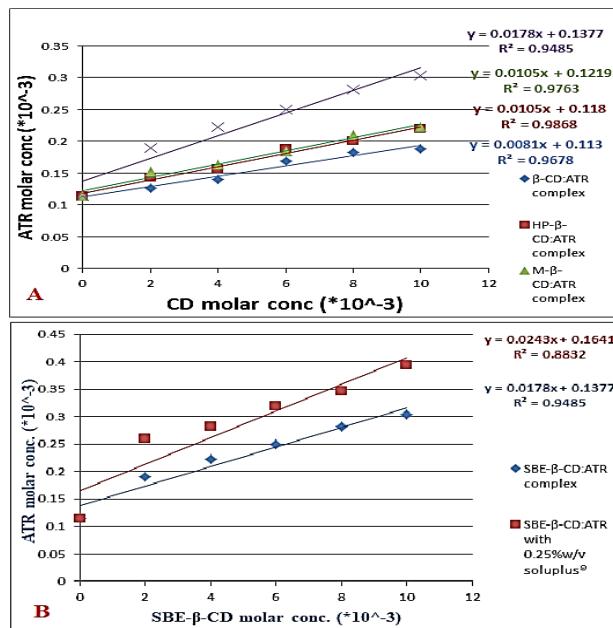


Figure 1: Phase solubility diagram of (A) binary complex of ATR with different types of CD at 25°C, (B) binary complex and ternary complex at 25°C.

Table 1: Solubility of ATR in different concentrations of Soluplus[®] with 2 mM SBE- β -CD.

Soluplus [®] concentration w/w%	ATR solubility μ g/mL
0%	230
1%	246
5%	324
10%	336
15%	350

tubes. These tubes were placed in a thermally controlled water bath shaker at 25°C for 48 hours. The samples were filtered using filter syringe of 0.45 μ m, the filtrates were analyzed spectrophotometrically at λ_{max} of 242 nm against suitable blanks prepared with the same concentrations of CDs.⁶

The stability constant (K_c) of the complex and the molar ratio of the complex were calculated using the following equation:⁶

$$\text{Slope}/\text{So} (1-\text{Slope}).$$

The slope is obtained from the initial straight-line portion of the plot of ATR concentration against CD concentration, and So is the solubility of ATR in water, in absence of CD.

A slope of less than 1 indicate formation of 1:1 molar ratio of drug: CD complex while if the slope is greater than 1 (but less than 2) indicate formation of higher order complexes in respect to CD (e.g., formation of 1:2 drug/CD complex).⁵

Ternary Complex

In order to determine the effect of addition of hydrophilic polymer on the formation of the inclusion complex, another phase solubility diagram was performed by adding excess amount of ATR to 10 mL of aqueous solutions containing

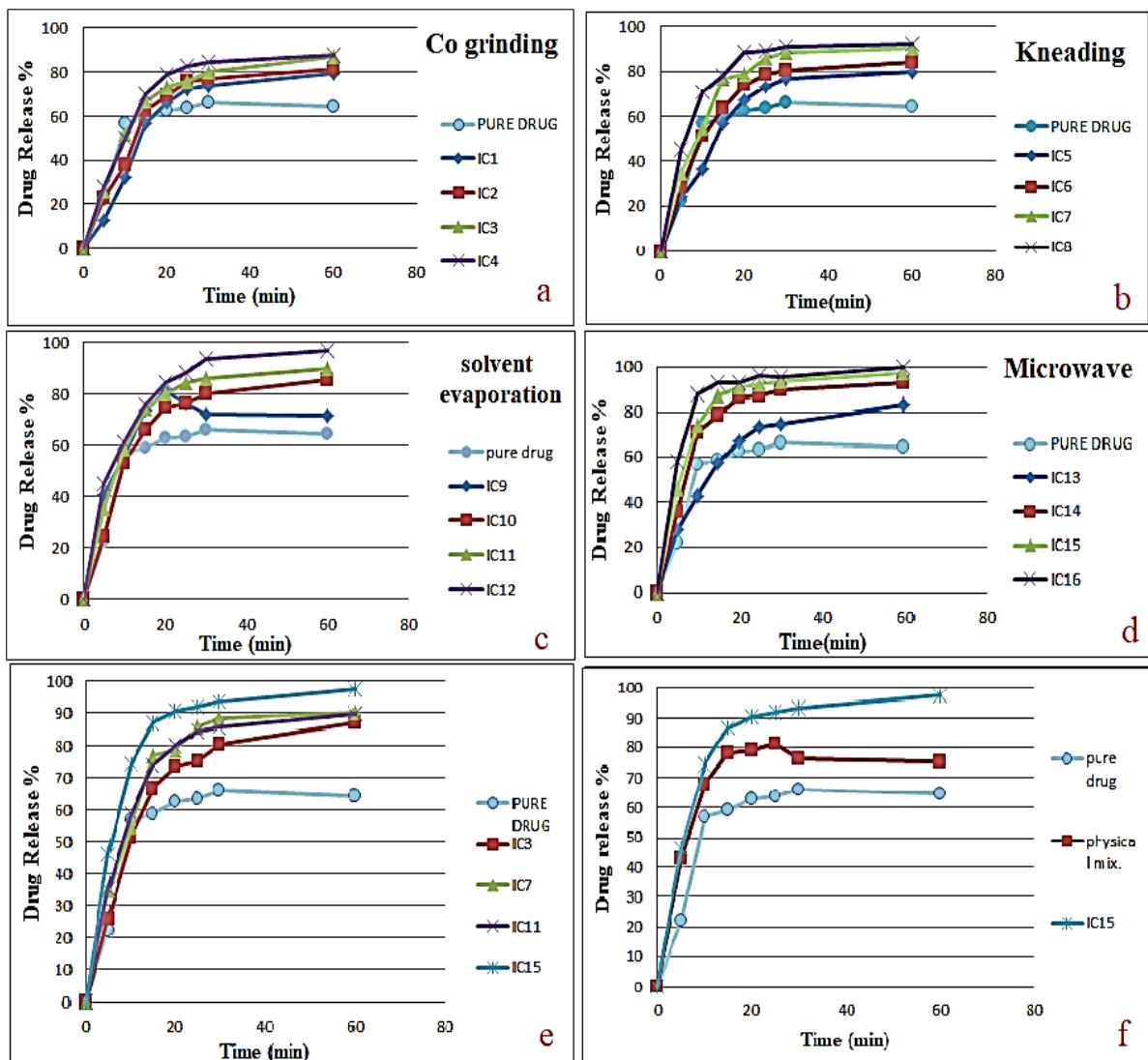


Figure 2: The dissolution profile of pure ATR, physical mix., ICs prepared by different methods at pH 6.8 and $37 \pm ^\circ\text{C}$

increasing concentrations of selected CD (2–10 mM) in the presence of Soluplus® (0.25% w/v).⁸

Determining the Effect of Soluplus® Concentration on Solubility

In order to evaluate the effect of souplus® concentration on solubility of ATR, saturation solubility study was performed by adding excess amount of the drug separately to 10 mL aqueous solutions containing fixed concentration (2 mM) of CD containing increasing concentrations (1, 5, 10 and 15%,w/w relative to the total weight of ATR and selected CD mixture. The samples were placed in a thermally controlled water bath shaker at 25°C for 48 hours. Then samples were filtered using filter syringe of 0.45 µm, the filtrates were analyzed spectrophotometrically at λ_{max} of 242 nm against suitable blanks.⁹ (Table 1)

Preparation of Inclusion Complex (IC)

Physical Mixture

A physical mixture was prepared by mixing the drug, with the required amount of the selected CD and soluplus® in a porcelain mortar for few minutes to get homogenous mixture and then sieved with sieve no 60.¹⁰

Co-Grinding

A co-ground mixture of ATR, the selected CD and soluplus® was prepared using porcelain mortar and pestle for 1-hours and sieved through sieve no. 60. Then the product was stored for further study.¹¹

Kneading

Weigh accurately the specified quantity of ATR, selected CD and soluplus® then mixed them in a mortar for 5 min. Small amount of water-methanol mixture (1:1 v/v) was added drop by

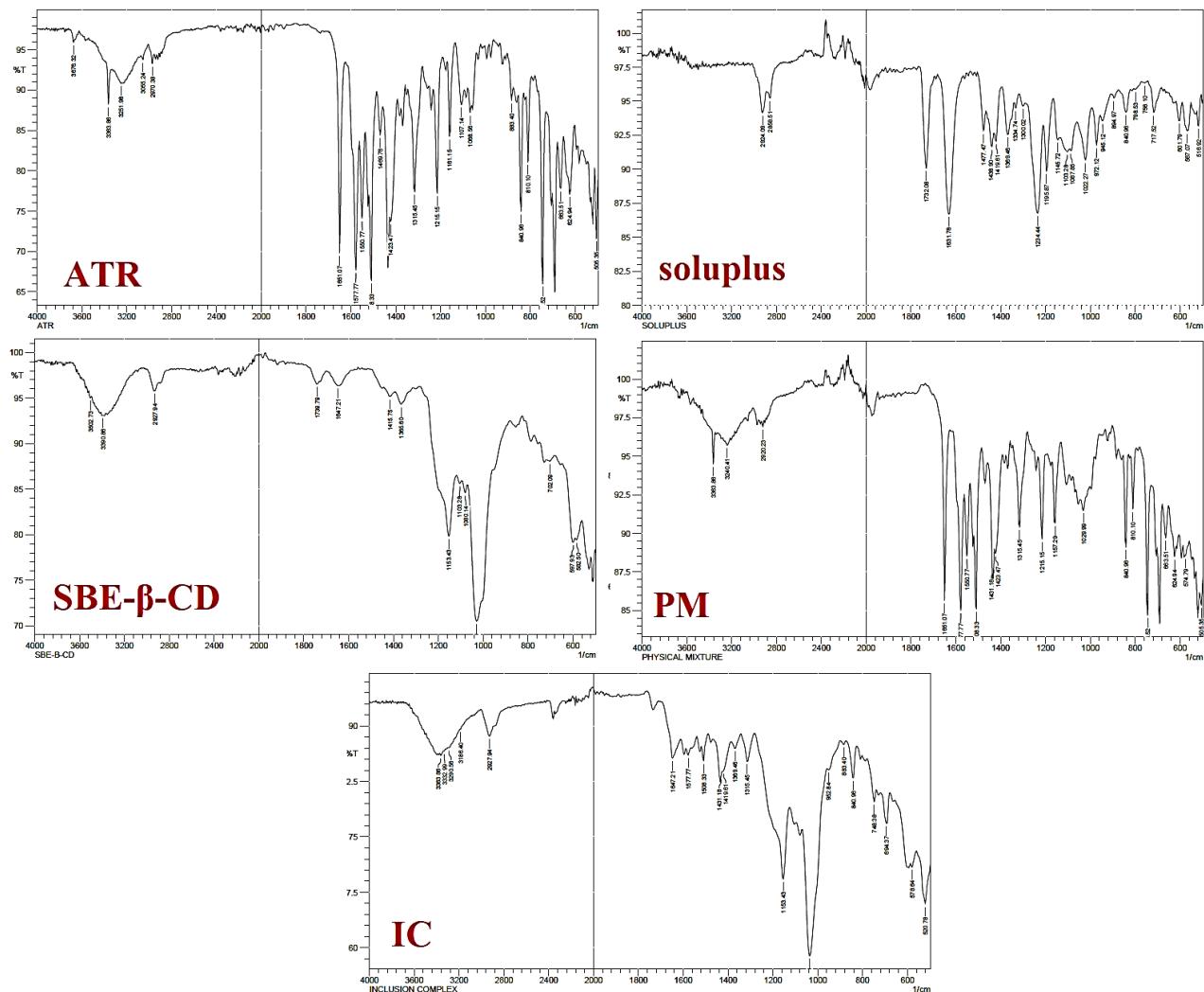


Figure 3: FTIR spectrum of ATR, SBE- β -CD, soluplus®, PM and IC15.

drop until the mixture became as slurry and this slurry mixture was kneaded for 30 minute. The dried mass was pulverized and sieved through sieve no. 60. and stored for further study.¹²

Solvent Evaporation Method

The aqueous solution of the required amounts of selected CD and soluplus® was added to the methanolic solution of ATR. The resulting mixture was stirred for 1-hours. Evaporation of the solvent was done at a temperature of 45°C until dried. The dried mass was pulverized and sieved through sieve no. 60. and stored for further study.⁶

Microwave

For the preparation of ATR- CD-soluplus® IC by microwave irradiation, a physical mixture of the required amounts of drug, selected CD and soluplus® was suspended in water-methanol mixture (1:1 v/v). The mixture was then subjected to microwave irradiation in a domestic microwave oven (kenwood) at a power of 450 W for 120s. The product was then washed with water-methanol solvent mixture to remove the residual components and let it dry. A control experiment

was carried out to check the properties of ATR (melting point, UV-vis absorption characteristics) upon exposure to microwave irradiation under the same conditions as that of preparation the IC.¹²

Evaluation of Inclusion Complex

Determination of Saturation Solubility

The saturated solubility of ATR, ICs was determined in distilled water. An excess amount of drug was added to 10 mL distilled water in stoppered tube. These tubes were incubated in shaking water bath for 48 hours at a temperature around $25 \pm 0.5^\circ\text{C}$. Then the resulting samples were filtered using filter syringe with pore size of 0.45 μm . The filtrate after suitable dilutions was analyzed for ATR content by UV-visible Spectrophotometer at λ_{max} 242 nm. The study was done in triplicate.¹³

Determination of Percentage Yield (PY%)

The yield was calculated as a percentage of the actual weight of ICs obtained to the total weights of starting materials (drug, CD and polymer) introduced into the system which

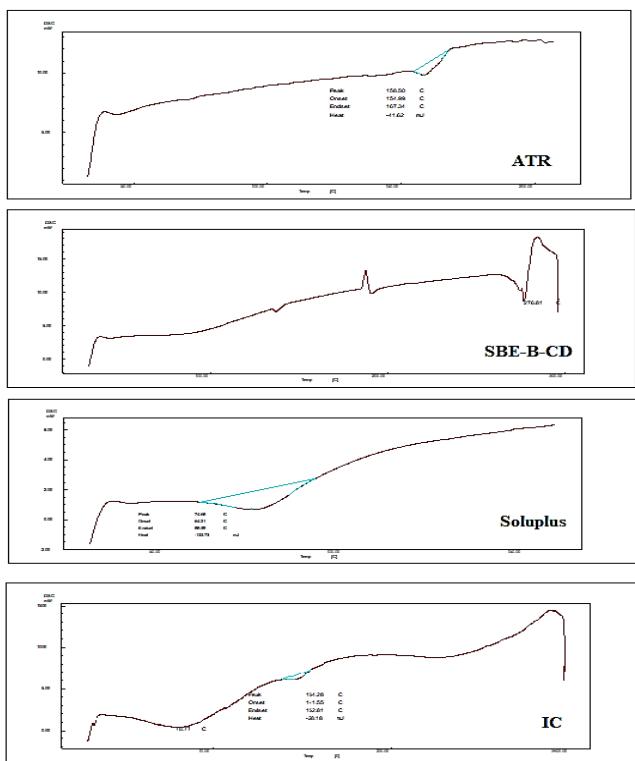


Figure 4: DSC thermogram of ATR, SBE- β -CD, soluplus® and IC15. represents the theoretical weight of IC. The percent yield was calculated using the following equation:¹³

$$\% \text{Yield} = (\text{Actual weight of IC gained}) / (\text{Theoretical weight of ICs}) \times 100$$

Determination of Drug Content

Complexes equivalent to 10 mg of ATR was accurately weighed and added into 50 mL methanol. The resultant solution was stirred for 30 minutes, till the entire drug dissolved. The solution was filtered and suitably diluted with methanol. ATR content was estimated by UV spectrophotometrically at 247 nm.²

In-vitro Dissolution Studies

An IC equivalent to 10 mg of drug was used to perform dissolution study using USP Type II dissolution test apparatus (Pharma test, Germany). The dissolution test was carried out at $37 \pm 0.5^\circ\text{C}$ in 900 mL 0.05 M phosphate buffer (pH 6.8) at 75 rpm. Samples of 5 mL were withdrawn and replaced by fresh dissolution media periodically, filtered using filter syringe with pore size of 0.45 μm and the amount of dissolved drug was determined by UV spectrophotometer at 242 nm. The resultant dissolution profile was compared with that of pure drug and with the physical mixture.²⁴

Characterization of the Best Complex

Fourier Transform Infrared (FT-IR)

FT-IR is commonly used in the study of CDs inclusion complex formation .Samples included in this test were; pure ATR, selected CD, soluplus®, physical mixtures and the prepared

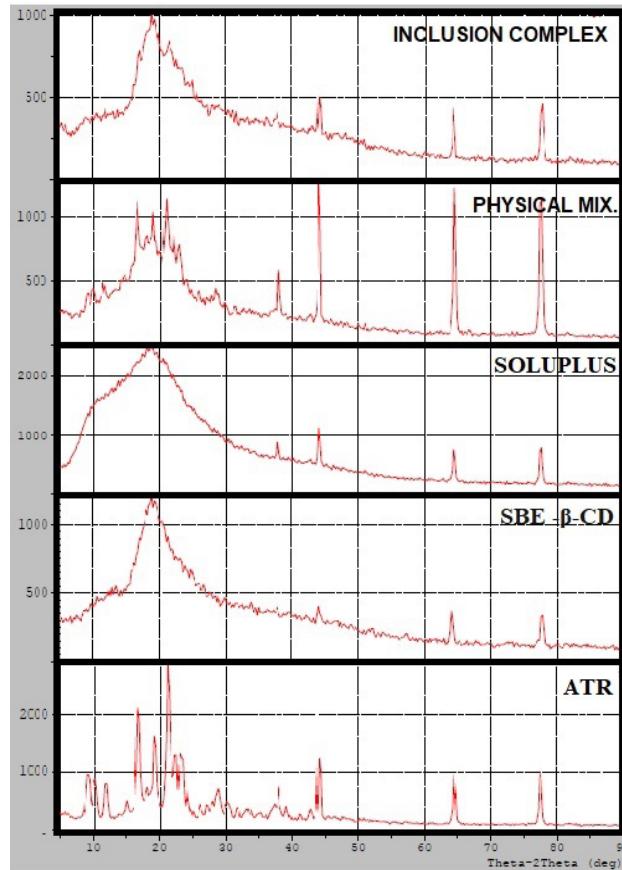


Figure 5: XRD diffractograms of ATR, SBE- β -CD, soluplus®, PM and IC15.

IC which scanned from 4000 to 600 cm^{-1} using FTIR (IRAffinity-1, Shimadzu, Japan).¹⁵

Differential Scanning Calorimetry (DSC)

DSC is the main thermal method used for studying the solid-state interactions among drugs and CDs. The DSC studies of ATR, the selected CD, soluplus®, and IC were recorded on DSC-60 plus (Shimadzu, Japan) calibrated using Indium. The weighed quantity of samples were hermetically sealed in aluminium pans and heated at a rate of 10°C/min between 30 and 300°C.¹⁰

X-ray Powder Diffraction (XRD)

Lattice nature and drug–polymers possible interactions characterized using XRD diffractometer (XRD-6000, Shimadzu, Japan) operated at voltage 40 kV, current 30 Ma, Cu-K α radiation at $\lambda = 1.5406$ nm, scanning speed of 10°/min and 20 range of 5–90 degree for pure ATR, selected CD, soluplus®, physical mixture and IC each separately.¹⁶

Statistical Analysis

The results of the experiments were analyzed according to the one-way variance analysis (ANOVA) and (t-test) and the level of significance was set at a p-value of 0.05

A $p > 0.05$ was considered to be non-significant.

A $p > 0.05$ was considered to be significant.

Table 2: Saturation solubility, percentage yield and percent drug content of ICs.

Formula name	ATR (molar ratio)	SBE - β -CD (molar ratio)	Soluplus (w/w %)	Method of preparation	Saturation solubility $\mu\text{g}/\text{mL}$ Mean \pm STD (n=3)	Percentage yield (PY %)	Drug content (w/w) (%) Mean \pm STD (n=3)
Pure ATR	138.2 \pm 3.6		
IC1	1	1		210 \pm 12.4	78.2	90.3 \pm 0.5
IC2	1	1	5		277 \pm 9.9	75.4	90.8 \pm 0.2
IC3	1	1	10	Co grinding	314 \pm 8.2	75.2	92.1 \pm 0.3
IC4	1	1	15		355 \pm 9	76.9	91.5 \pm 0.4
IC5	1	1		215 \pm 7.9	80	92 \pm 0.7
IC6	1	1	5	Kneading	303 \pm 14.7	77	94.1 \pm 0.6
IC7	1	1	10		384 \pm 3.6	76.5	91.8 \pm 0.2
IC8	1	1	15		460 \pm 11.9	80.1	91.3 \pm 0.3
IC9	1	1		237 \pm 6.1	90.5	97.3 \pm 0.5
IC10	1	1	5	Solvent evaporation	372 \pm 8.8	88	92 \pm 0.4
IC11	1	1	10		440 \pm 19.9	91	96.7 \pm 0.5
IC12	1	1	15		504 \pm 24.8	87.7	91.1 \pm 0.5
IC13	1	1		224 \pm 11.6	98.5	96 \pm 0.5
IC14	1	1	5	Microwave	321 \pm 30.5	94.5	99 \pm 0.5
IC15	1	1	10		436 \pm 17.3	94	96.4 \pm 0.4
IC16	1	1	15		509 \pm 7.6	96.4	93.5 \pm 0.7

Table 3: The similarity (f2 value) among the ICs formulas.

Formula	F2	Method
Pure drug & IC2	46	
Pure drug & IC3	45	
Pure drug & IC4	40	Co grinding
IC3 & IC4	69	
Pure drug & IC6	45	
Pure drug & IC7	36	
Pure drug & IC8	32	kneading
IC7&IC8	50	
Pure drug & IC10	46	
Pure drug & IC11	37	
Pure drug & IC12	32	Solvent evaporation
IC11 & IC12	61	
Pure drug & IC14	33	
Pure drug & IC15	28	
Pure drug & IC16	24	Microwave
IC15 & IC16	No need for calculations*	
IC3 & IC7	56	
IC7 & IC11	79	10% soluplus of all methods
IC11 & IC15	47	

*Most of countries mention that when the drug for both the test and reference is dissolved more than 85% within 15 min, dissolution profiles can be considered similar without further mathematical evaluation.²¹

RESULTS AND DISCUSSION

Phase Solubility Diagram

Binary Complex

The phase solubility diagram (Figure 1) shows linear increase in the solubility of ATR as the molar ratio of CDs increase,

indicating there was a direct relationship between the solubility and concentration of CD. with slope less than 1 for all types of CD suggesting the formation of 1:1 molar ratio of drug:CD complex.^{5,7}

The stability constant of the complexes of ATR with β -CD, M- β -CD, HP- β -CD and SBE- β -CD were 70, 91, 91 and

153 M⁻¹ respectively, all the stability constants found to be between 50 to 5000 M⁻¹ which considered to be suitable for the formation of stable and soluble complex as reported by Gabriel O K L *et al.*¹⁷

The highest stability constant of ATR with SBE-β-CD with the highest solubility than other types of CDs, could be explained to be due to the presence of the four carbon butyl chain coupled with repulsion of the end group negative charges which allows for an “extension” of the hydrophobic region of the CD cavity and thus increase its affinity towards ATR. Therefore, SBE-β-CD was selected to continue this research.¹⁸

Ternary Complex

The use of soluplus® with ATR and SBE-β-CD increases the solubility (Figure 1) and stability constant from 153 to 208 M⁻¹ by externally adhering to the SBE-β-CD surface, which ensures the formation of a co-complex (ternary complex).⁶

Determining the Effect of Soluplus® Concentration on Solubility

Results of saturation solubility studies of ATR, ICs are shown in Table 2 All the ICs showed significant solubility enhancement over the pure ATR ($p < 0.05$).

This enhancement in ATR solubility can be explained to be due to the inclusion of its lipophilic part inside the CD cavity with an enhancement of its wettability by the hydrophilic outer part of CD.¹⁹

The solubility of drug was directly increased when soluplus® concentration was raised from 1, 5, 10 and 15% w/w, in comparison with that of pure drug and SBE-β-CD:ATR alone As soluplus® enhance the stability of the complex, and wettability of the drug due its hydrophilic nature, hence it increases the solubility of the drug.⁶ The preparation of SBE-β-CD :ATR: soluplus® (1% w/w) complex was canceled from further studies because it produces the lowest solubility enhancement than other soluplus® concentrations, as seen in Table 1.

Evaluation of Inclusion Complex

Determination of Saturation Solubility

The highest aqueous solubility of the drug was observed in the solvent evaporation and microwave method (with no significant difference between them ($p > 0.05$)) compared to co-grinding and kneading methods. These results can be attributed to low energy input and hence weak interaction with lowest drug solubility values in the manual grinding method.¹¹

While in the kneading method, there is a minimum amount of solvent used, making it of lower effectivity than solvent evaporation and microwave methods.

The microwave irradiation possess the ability to penetrate into any substance, making the rotation of water molecules with an electric dipole that stimulates the interaction of drug and CDs, favoring the exit of the water molecules from the CDs cavity, which gave a greater chance for a drug to be incorporated inside the CD cavity resulting in high solubility.²⁰

In the microwave method no change was observed in ATR properties such as melting point, UV-vis absorption characteristics, thereby suggesting no adverse effect of microwave radiation on ATR under the experimental conditions.

Determination of Percentage Yield (PY %) and Drug Content

The percentage yield and percent drug content of ATR /SBE-β-CD/Soluplus® complex are shown in Table 2. The prepared ICs formulas showed percentage yield ranged between 75.2-98.5% depending on the method of preparation. Co grinding method had the lowest value which may be due to sticking of powder during long mixing and grinding operation, while the microwave method had the highest value which could be due to direct mixing of all components and lower sticking during the scrunching process. The percent drug content of all methods was within the acceptable range 90–110% according to USP.¹⁴

In-vitro Dissolution Studies

This test was done to study the effect of complex formation, effect of concentration of soluplus® and the preparation method on the drug release. The similarity factor have been used for comparing the *in-vitro* dissolution profiles. The similarity factor (f_2) is calculated by the following equation :

$$f_2=50 \times \log \{ [1 + 1/n \sum_{t=1}^n |R_t - T_t|^2]^{-0.5} \} \times 100$$

Where (n) is the number of dissolution time points. (R_t) and (T_t) is the reference and test dissolution values at time t.

The two dissolution profiles are similar when f_2 values are higher than 50 (50–100); otherwise, the profiles are not similar.²¹ It can be concluded from the dissolution profiles (Figure 2) that as the soluplus® concentration increases, the dissolution rate increases as a result of enhancing the solubility.

Depending on f_2 factors in table 3 it can be concluded that there was no similarity in dissolution profile of pure drug with the dissolution profile of ICs containing 5,10,15 % w/w soluplus® for all methods. On the other hand, there was similarity between dissolution profiles of ICs containing 10 and 15 % w/w soluplus®; therefore, from an economic viewpoint, the ICs with 10% w/w soluplus® was selected for further studies . Figures 2-e and Table 3 show that IC prepared by microwave (IC15) had fastest non-similar dissolution compared to the other methods, which were all similar to each other. Moreover, IC15 showed the fastest dissolution compared to its physical mixture (Figure 2-f).

Therefore from all the previous results, it can be concluded that IC15 is the best formula as it produce highest solubility, highest PY and faster release rate, so it was subjected to further studies.

Fourier Transform Infrared (FT-IR)

FT-IR absorption spectrum for ATR, SBE-β-CD, soluplus®, physical mixtures and IC formula (IC15) are presented in Figure 3, respectively. ATR showed its typical peaks for free OH stretching (3676 cm⁻¹), N-H stretching (3363 cm⁻¹), asymmetric and symmetric OH stretching (3251 and 3055 cm⁻¹, respectively), C-H stretching (2970 cm⁻¹), asymmetric and symmetric C = O stretching (1651 and 1577 cm⁻¹, respectively),

C-C stretching (1508 and 1431 cm⁻¹), -CH₃ and -CH₂ deformation (1315 cm⁻¹), C-N stretching (1215 cm⁻¹), C-O stretching (1161 cm⁻¹), aromatic C-H in plan bending (1107 and 1068 cm⁻¹), aromatic C-H out plane bending (840, 810 and 744 cm⁻¹) and C-H deformation bending (690, 663 and 624 cm⁻¹). which are in agreement with previous study.¹⁶

The IR spectra of SBE7-β-CD (Fig 5) showed strong absorption bands for OH stretching (3390 cm⁻¹), C-H stretching (2927 cm⁻¹), δ-HOH bending of water molecules attached to CD (1647 cm⁻¹), CH vibrations (1153) and C-O stretching (1029 cm⁻¹). These are in accordance with findings from Das SK *et al.*¹⁵

IR absorption bands for pure Soluplus are listed as follows: asymmetric and symmetric CH stretching (around 2924 and 2858 cm⁻¹), ester carbonyl stretching (1732 cm⁻¹), tertiary amide C = O stretching (1631 cm⁻¹), C-O-C stretching (1477 cm⁻¹), CH₃ bending (1438 cm⁻¹), ester C–O stretching (1234 and 1103 cm⁻¹). Nearly the same results were obtained by Lin HL *et al.*²²

The physical mixture IR spectra shows most of ATR peaks with lower intensity attributed to dilution, however some bands of ATR are shifted to lower wavelength including (O-H) stretch peak (from 3251 to 3240 cm⁻¹) and (C-O) stretch (from 1161 to 1157 cm⁻¹) that indicate even in physical mixture there was some signs of interaction.

In the IC IR spectra, the N-H stretching (3363 cm⁻¹) of ATR masked by the broad intense band corresponding to the OH vibration of SBE7-β-CD, C = O stretching bands shifted (from 1651 to 1647 cm⁻¹), aromatic C-H bendings shifted (from 744, 690 to 784, 694 cm⁻¹) respectively.

For SBE7-β-CD there were shifting of OH (from 3390 to 3363 cm⁻¹) and CO (from 1029 to 1037 cm⁻¹). The shifts in the IR absorption band of the hydrogens of the aromatic ring in ATR and the OH group of SBE7 β-CD were signposts of interaction between ATR and SBE7 β-CD.²³ Some diminution and disappearance of some peaks of ATR in inclusion complex may be due to encapsulation of drug into the cyclodextrin.²⁴ Analysis of the structure of ATR indicated that pyrrole-heptanoic acid part was embedded into the cavity and the phenylamino carbonyl remained outside the cavity.

In both physical mixture and IC15, the absorption bands of soluplus® didn't appear maybe because of its very low concentration (10% w/w).

Differential Scanning Calorimetry (DSC)

The DSC curve of ATR (Figure 4) gives an endothermic peak corresponding to its melting point at 158.5°C; the result indicated the purity and crystallinity of the used ATR.²⁵ The DSC curve of SBE7 β-CD (Figure 4) showed a very broad endothermic peak between 50 -100 because of the dehydration phenomena and endotherm peak around 276°C was due to the beginning of decomposition events as reported in previous studies.^{23,26}

While the thermograms of pure soluplus® (Figure 4)

displayed a broad endothermic peak at 74°C due to the glass transition of amorphous soluplus.²⁷

In the thermogram of IC15 (Figure 4), the endothermic peak at 158°C of ATR was shifted to 154 with lower intensity, and the decomposition peak of SBE-β-CD was also shifted to a lower temp with broadening. These changes are mostly related to the conversion of the drug to amorphous form due to formation of complex.^{10,26,28}

Substitution of the water molecules in the SBE-β-CD cavity from IC altered the energy states of these preparations.⁶

X-ray Powder Diffraction (XRD)

The XRD diffractogram of ATR, SBE-β-CD, soluplus®, physical mixtures and IC(IC15) are shown in Figure 5. The diffractogram for ATR shows its distinctive crystalline diagram with tapered and intensive peaks at diffraction angles (2θ) of 9.06°, 10.08°, 11.69°, 14.9°, 16.7°, 18.06°, 19.12°, 21.30°, 22.25° and 23.25° as well as dull peaks at various diffraction angles which are in accordance with the reported results in the previous studies.¹⁶ (Figure 5)

Both pure SBE-β-CD and soluplus® showed a halo-pattern demonstrating their amorphous states as evidenced from the absence of diffraction peaks.

The diffraction patterns of physical mixtures of ATR with SBE-β-CD and soluplus® correspond to the superposition of those of the pure components, with lower intensities. Some interaction could explain this (or maybe dilution) between ATR and SBE-β-CD/soluplus, resulting in a reduction in the crystalline nature of ATR. On the other hand, the diffraction pattern of the IC (IC15) showed a greater reduction in crystallinity than that of physical mixture evidenced by complete disappearance of intense peaks of ATR and formation of amorphous inclusion complexe in solid state as previously confirmed by DSC study. The peak of high intensity at all/ome diffraction angles (2θ) of 38°, 44°, 64°, 77° appearing in the diffractograms in is probably due to diffraction from the planes of the aluminum sample holder. This is a common error incurred in the recording of XRD patterns and is not an indication of potential crystalline characteristics of a compound.²⁹

CONCLUSION

An enhancement in solubility and dissolution of ATR with good production yield was obtained by preparing it as IC by microwave method using SBE-β-CD as complexing agent and soluplus® as a hydrophilic polymer which improve the stability constant of the complex.

Characterization studies suggested a partial inclusion of ATR in the SBE-β-CD cavity, also, the crystallinity behavior of ATR was found to be decreased, which led to enhancement in solubility as well as dissolution rate.

REFERENCES

1. Kumar S., Singh P. Various techniques for solubility enhancement: An overview. *The Pharma Innovation*, 2016, 5.1, Part A: 23.
2. Ali SK, Al-Khedairy EB. Solubility and Dissolution Enhancement

- of Atorvastatin Calcium using Solid Dispersion Adsorbate Technique. *Iraqi J Pharm Sci.* 2019;28(2):105-14.
3. Moffat A, Osselton M, Widdop B. Clarke's analysis of drug and poisons . 4th edition. The Pharmaceutical Press 2011: 930 p.
 4. Naqvi A, Ahmad M, Minhas MU, Khan KU, Batool F, Rizwan A. Preparation and evaluation of pharmaceutical co-crystals for solubility enhancement of atorvastatin calcium. *Polymer Bulletin.* 2020;77(12):6191-211.
 5. Saokham P, Muankaew C, Jansook P, Loftsson T. Solubility of cyclodextrins and drug/cyclodextrin complexes. *Molecules.* 2018;23(5):1161.
 6. Alshehri S, Imam SS, Altamimi MA, Hussain A, Shakeel F, Alshehri A. Stimulatory effects of Soluplus® on flufenamic acid β -cyclodextrin supramolecular complex: physicochemical characterization and pre-clinical anti-inflammatory assessment. *AAPS PharmSciTech.* 2020;21:1-3.
 7. Higuchi, T.; Connors, K.A. Phase solubility techniques. *Adv. Anal. Chem. Instrum.* 1965, 4, 117–212.
 8. Borghetti GS, Pinto AP, Lula IS, Sinisterra RD, Teixeira HF, Bassani VL. Daidzein/cyclodextrin/hydrophilic polymer ternary systems. *Drug development and industrial pharmacy.* 2011;37(8):886-93.
 9. Vieira AC, Fontes DA, Chaves LL, Alves LD, de Freitas Neto JL, Soares MF, Soares-Sobrinho JL, Rolim LA, Rolim-Neto PJ. Multicomponent systems with cyclodextrins and hydrophilic polymers for the delivery of Efavirenz. *Carbohydrate polymers.* 2015;130:133-40.
 10. Xu J, Zhang Y, Li X, Zheng Y. Inclusion complex of nateglinide with sulfobutyl ether β -cyclodextrin: Preparation, characterization and water solubility. *Journal of Molecular Structure.* 2017;1141:328-34.
 11. Jug M, Mura PA. Grinding as solvent-free green chemistry approach for cyclodextrin inclusion complex preparation in the solid state. *Pharmaceutics.* 2018;10(4):189.
 12. Das S, Mohanty S, Maharanja J, Jena SR, Nayak J, Subuddhi U. Microwave-assisted β -cyclodextrin/chrysin inclusion complexation: An economical and green strategy for enhanced hemocompatibility and chemosensitivity in vitro. *Journal of Molecular Liquids.* 2020;310:113257.
 13. Mohammed Ihsan A.; Ghareeb Mowafaq M. Investigation of solubility enhancement approaches of ticagrelor. *Iraqi J Pharm Sci.* 2018;27(1): 8-19.
 14. The United State Pharmacopeia (USP) 41, NF36. Convention Inc.Rockville, MD. 2018; P 391-396.
 15. Das SK, Kahali N, Bose A, Khanam J. Physicochemical characterization and in vitro dissolution performance of ibuprofen-Captisol®(sulfobutylether sodium salt of β -CD) inclusion complexes. *Journal of molecular liquids.* 2018;261:239-49.
 16. Shaker MA. Dissolution and bioavailability enhancement of Atorvastatin: Gelucire semi-solid binary system. *Journal of Drug Delivery Science and Technology.* 2018;43:178-84.
 17. Gabriel O K L, Fung Tan Y T, Khiang Peh K, Enhancement of norfloxacin solubility via inclusion complexation with β -cyclodextrin and its derivative hydroxypropyl- β -cyclodextrin, *Asian journal of pharmaceutical sciences.* 2016;11:536-546.
 18. Semcheddine F, Guissi NE, Liu X, Wu Z, Wang B. Effects of the preparation method on the formation of true nimodipine SBE- β -CD/HP- β -CD inclusion complexes and their dissolution rates enhancement. *AAPS PharmSciTech.* 2015;16(3):704-15.
 19. Ain S, Singh R, Ain Q. Characterization and intrinsic dissolution rate study of microwave assisted cyclodextrin inclusion complexes of gemfibrozil. *Int J. Pharm. Sci.* 2016;8(10):160-3.
 20. Rodríguez-López MI, Mercader-Ros MT, López-Miranda S, Pellicer JA, Pérez-Garrido A, Pérez-Sánchez H, Núñez-Delicado E, Gabaldón JA. Thorough characterization and stability of HP- β -cyclodextrin thymol inclusion complexes prepared by microwave technology: A required approach to a successful application in food industry. *J. Sci. Food Agric.* 2019;99(3):1322-33.
 21. Diaz DA, Colgan ST, Langer CS, Bandi NT, Likar MD, Van Alstine L. Dissolution similarity requirements: how similar or dissimilar are the global regulatory expectations. *The AAPS journal.* 2016;18(1):15-22.
 22. Lin HL, Chi YT, Huang YT, Kao CY, Lin SY. DSC-FTIR Combined approaches used to simultaneously prepare/determine the amorphous solid dispersions of indomethacin/soluplus in real-time. *EC Pharmaceutical Science.* 2015;2(1):183-93.
 23. Jain AS, Date AA, Pissurlenkar RR, Coutinho EC, Nagarsenker MS. Sulfobutyl ether 7 β -cyclodextrin (SBE 7 β -CD) carbamazepine complex: preparation, characterization, molecular modeling, and evaluation of in vivo anti-epileptic activity. *Aaps Pharmscitech.* 2011;12(4):1163-75.
 24. Mahajan HS, Bhalkar KG. Development and evaluation of rutin- β CD inclusion complex based mouth dissolving tablets. *Int J Pharm Sci Dev Res.* 2017;3(1):001-6.
 25. Sonje VM. Profiles of drug substances, excipients, and related methodology, Volume 35. Elsevier Inc.; 2010. 1–69 p.
 26. Badilli U, Amasya G, Şen T, Tarimci N. Topical emulgel formulation containing inclusion complex of calcipotriol with cyclodextrin. *J Incl Phenom Macrocycl Chem.* 2014;78(1-4):249-55.
 27. Ramesh KV. Characterization of sulfobutyl ether beta-cyclodextrin binary and ternary inclusion complexes of loratadine. *Asian J Pharm.* 2020;14(4).
 28. Spamer E, Müller DG, Wessels PL, Venter JP. Characterization of the complexes of furosemide with 2-hydroxypropyl- β -cyclodextrin and sulfobutyl ether-7- β -cyclodextrin. *European journal of pharmaceutical sciences.* 2002;16(4-5):247-53.
 29. Yildiz ZI, Celebioglu A, Uyar T. Polymer-free electrospun nanofibers from sulfobutyl ether7-beta-cyclodextrin (SBE7- β -CD) inclusion complex with sulfisoxazole: Fast-dissolving and enhanced water-solubility of sulfisoxazole. *International journal of pharmaceutics.* 2017;531(2):550-8.