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Solubility Enhancement of Atorvastatin Calcium Using Cyclodextrin

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SOLUBILITY ENHANCEMENT OF ATORVASTATIN CALCIUM USING CYCLODEXTRIN

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ABSTRACT

The present study is intended to increase the solubility and dissolution rate of Atorvastatin calcium (ATN Ca) by formulating the drug- hydroxy-propyl-beta cyclodextrin (HPBCD) complexes using solvent-assisted extrusion (SAE) using a twin-screw extruder. Various studies on ATN Ca saturation solubility in water and other pH mediums revealed a pH-dependent solubility with a difference. Thus, the saturation solubility for ATN Ca and Solvent Assisted Extruded complex was conducted in water and different pH mediums in this study. Phase solubility studies between ATN Ca and CDs revealed an A_L-type solubility profile exhibiting a linear increase with HPBCD. Drug-HPBCD complexes were made using the solvent-assisted extrusion (Twin Screw Extruder) procedure and conventional techniques, including physical mixing and the kneading method. The solubility of the SAE complexes, as compared to normal ATN Ca (0.0167 mg/mL), was 0.294 mg/mL, respectively. Differential scanning calorimetry and Fourier transform infrared spectroscopy analyses were used to confirm the formation of drug-cyclodextrin inclusion complexes. The formulated products were filled into #2 clear gelatin capsules for better dissolution. According to tests on drug release at 5 min, 10 min, 15 min, and 30 min time intervals, ATN Ca was released at the highest rate from solvent-assisted extruded complex (103 %) compared to other samples. When compared to the drug release from complexes formed by kneading was much lower (89 %). All these studies show promising results to conclude that the complex formed through solvent-assisted extrusion (SAE) has enhanced the solubility and dissolution rate of the Atorvastatin Calcium.

DEDICATION

This thesis is dedicated to everybody who has given constant support throughout and taken my back all the time, especially my parents, Umamaheswara Rao Abburi and Sridevi Abburi.

LIST OF ABBREVIATIONS

ATN Ca	Atorvastatin Calcium
A-CD	Alpha Cyclodextrin
B-CD	Beta Cyclodextrin
G-CD	Gamma Cyclodextrin
HPBCD	Hydroxy-propyl-beta cyclodextrin
HPGCD	Hydroxy-propyl-gamma-cyclodextrin
SAE	Solvent-Assisted Extrusion
USP	United States Pharmacopeia
HPLC	High-Performance Liquid Chromatography
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy

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I would like to convey special thanks to Sivaram Munnangi and Nagarjuna Narala for always keeping me motivated and helping me throughout the project and to all my fellow lab mates and seniors for helping me stand here for what I am today.

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1. INTRODUCTION

One of the most known challenges during drug development and delivery is associated with poorly soluble drugs resulting in low bioavailability. The commonly used methods to enhance solubility, such as physical modifications of the entities, tend to increase solubility to a limited extent. However, when it comes to high-dose formulations with low hydrophilicity and high melting points, these conventional methods don't fulfill the objectives. In recent times many methods have been established to improve the water solubility of drugs apart from the conventional techniques. Different techniques to improve the solubility are adding surfactants, polymorphism, salt formation, micronization, etc. But they also have their own limitations for each technique.

Atorvastatin calcium, as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme. An (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, is an early rate-limiting step in cholesterol biosynthesis, and it is currently used as calcium salt for the treatment of hypercholesterolemia (1). It also plays a significant role in the treatment of osteoporosis, benign prostatic hyperplasia, and Alzheimer's disease. It is absorbed quickly after oral administration but leads to first-pass metabolism in the liver and gut wall which justifies its low oral bioavailability. The absolute bioavailability of atorvastatin (parent drug) is approximately 14 % and the systemic bioavailability of HMG CoA reductase inhibitory activity is approximately 30 %. It undergoes extensive lactonization, oxidation, and glucuronidation; the metabolites are then excreted through biliary secretion and direct blood-to-intestine secretion (2). The molecular weight of pure atorvastatin calcium is 1153 g/mol, the

melting point ranges between 160 ^{0}C – 180 ^{0}C and the pKa of the pure drug is 4.31 and logP is 5.39 (3). Pure API is insoluble in aqueous solutions of pH 4 and below, very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

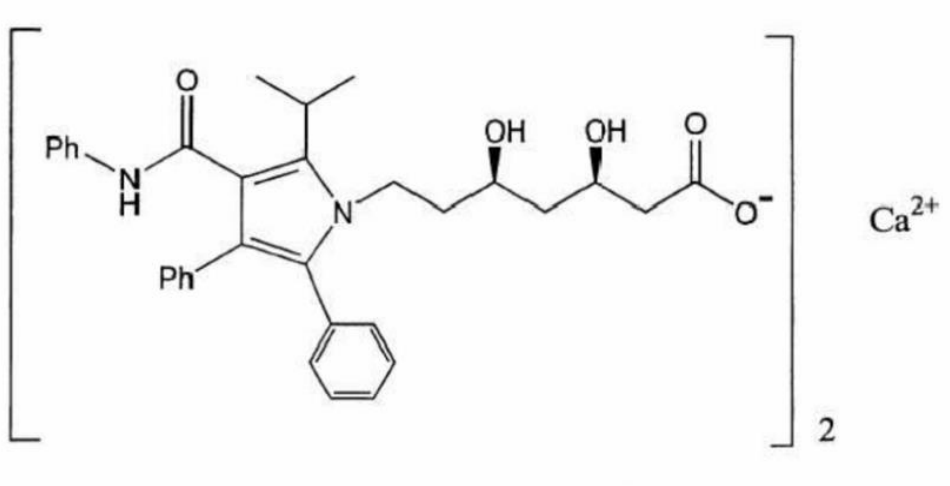

Figure 1 Structure of Atorvastatin Calcium

Cyclodextrins are oligosaccharides comprised of sugars called glucopyranose units attached by alpha 1-4 bonds. They have been used to complex drugs having low hydrophilicity by forming inclusion complexes. Their main purpose is to enhance solubility, dissolution rate, and stability. In general, cyclodextrins are classified into 3 types based on the number of glucopyranose units present. Alpha-cyclodextrin consists of 6 units, beta-cyclodextrin consists of 7 units and gamma-cyclodextrin consists of 8 glucopyranose units. With the increase in the number of units, there is an increase in their molecular weight and cavity size. Among these 3 cyclodextrins, beta-cyclodextrin has the least solubility (1.85 mg/mL) and gamma-cyclodextrin has the highest solubility (23.2 mg/mL) (4). Beta-cyclodextrins are broadly used due to their sensible cavity size which can suit a wide assortment of drugs and their prepared accessibility (5). But having low

solubility results in limiting their application as a drug carrier and solubility enhancer. Subsequently, chemically adjusted β -CD derivatives have been synthesized resulting in increased water solubility and expanded inclusion capacity. A few such modified beta cyclodextrins include methyl-β-CD, 2,6-di-o- methyl-β-CD, 2-hydroxypropyl-β-CD (HP-β-CD), sulfo butyl ether β-CDs, and maltosyl and glucosyl β-CDs (6), (7).

When drug molecules interact with CDs they result in the formation of inclusion complexes, with the lipophilic cavity of the CDs (host molecule) providing a benefit for the entrapment of drugs that aren't very soluble in water. Aqueous solutions containing CDs cause polar water molecules to enter the chamber of the CDs. However, the relatively less polar drug molecules quickly replace these (8). The main mechanisms that cause inclusion complexes to develop include hydrogen bonding between drugs and CDs, Van der Waals interactions, charge transfer reactions, and substituting polar water entities with less polar guest entities (9). Though conventional methods such as spray drying, solvent evaporation, kneading, and freeze-drying have shown improved bioavailability and solubility of the drugs, they are difficult to scale up and are long processes that include the addition of various excipients and other materials, and at times it is not guaranteed that the complexes are formed through these techniques.

Hot melt extrusion has become an efficient way to make pharmaceutical products like granules, pellets, sustained-release tablets, and implants over time. Using a hot melt extruder, raw materials are fed into a device with one or two rotating screws that are heated to various temperatures. The processed material then goes through a die to create the desired shape and size of the material. This molecular mixing of different entities is changed into an amorphous result with a consistent shape and density, improving the drug's weakly water-soluble dissolution profile (11).

The Twin Screw Extruder has two agitator assemblies positioned on parallel shafts. In all the zones of the extruder, from the feeding of the material via the hopper to the rotating screw and

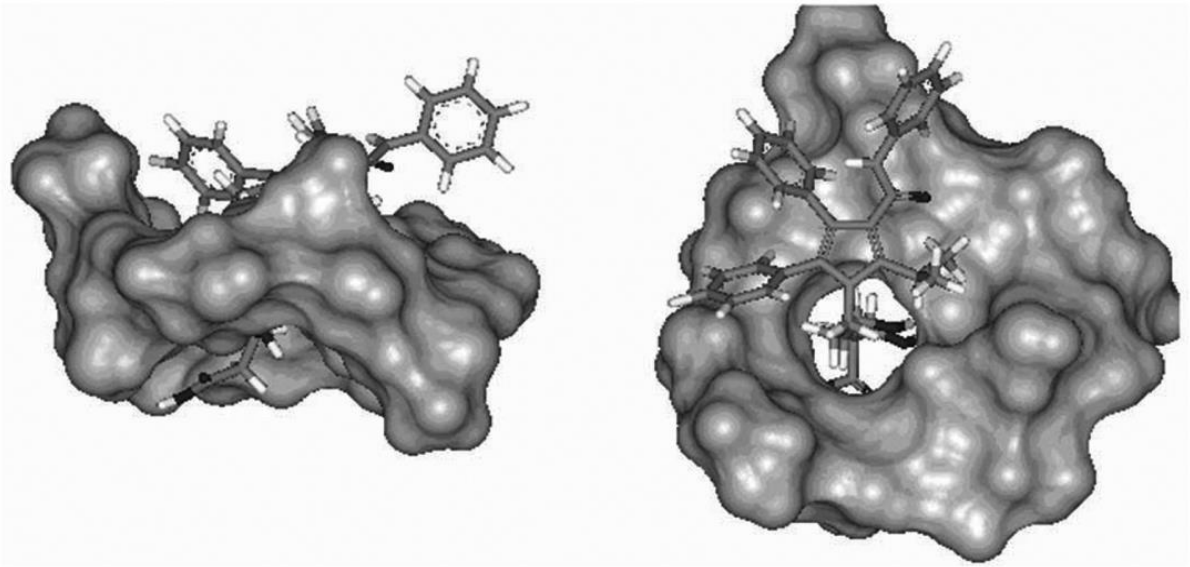

Figure 2 Molecular diagram of complexing between Cyclodextrin and Atorvastatin Calcium (10)

ultimately to conveying the material, the use of two screws allows various configurations applies different conditions. It produces a homogenous product by consistently mixing the excipients and the drug (12). This equipment is categorized into two types in which the rotation of the screws can be co-rotating (same) or counter-rotating (opposite). The most preferred type is the co-rotating type due to its approaching advantages like easy feeding of the mixture, lower approach to overheating, enhanced productivity, and flexibility with the process parameters (13).

Solvent Assisted Extrusion (SAE) is a continuous process and allows uniform mixing and an enhanced intermolecular interface compared to conventional complexing methods (14). This study aimed to evaluate the utility of SAE compared to the conventional method in improving the solubility and bioavailability of Atorvastatin Calcium. A parallel (11 mm) twin-screw, co-rotating Process 11 Twin-screw Extruder (Process 11 Thermo Fisher Scientific, Waltham, DE, USA) was

used. The complexes were prepared by the twin screw extruder combined with solvent addition while the entities were mixed inside the extruder. The complex was also prepared by the conventional method (Kneading), and the solubility profiles were compared with the complex prepared by the SAE process. All complexes were evaluated for saturation solubility, dissolution rate, DSC, and FTIR.

The primary goal of this study was to combine cyclodextrin with atorvastatin calcium to increase its solubility and rate of dissolution. Various studies were done to enhance the solubility of ATN Ca through conventional methods but in this study, solvent-assisted extrusion is a different technique considered to enhance the solubility and dissolution rate of the pure drug. Different complexes were prepared through the physical mixture, kneading method, and solvent-assisted extrusion technique. The characteristic analysis such as the phase solubility study, Differential scanning calorimetry (DSC), and Fourier transform infrared spectroscopy (FTIR) supported the formation of such complexes. The final formulation was evaluated for solubility and dissolution rate.

2. MATERIALS AND METHODS

2.1. MATERIALS

Atorvastatin calcium was purchased from RIA International LLC (Melanie Ln, East Hanover, NJ, USA). Alpha-cyclodextrin (A-CD), Beta-cyclodextrin (B-CD), Gamma cyclodextrin (G-CD), Hydroxypropyl-beta-cyclodextrin (HPBCD), and Hydroxypropyl-gamma-cyclodextrin (HPGCD) were donated by Ashland. All other chemicals and solvents for analysis were procured from Fischer Scientific.

2.2. METHODS

2.2.1. QUANTIFICATION OF ATORVASTATIN CALCIUM USING HPLC

The amount of ATN Ca present was determined by the HPLC method described in the USP(15). The HPLC equipment used was Waters 2790 Separations Module which comprised an autosampler, UV-visible detector, and EMpower software. Analysis was performed using a C18 column (Cogent 4 Bidentate, 15 cm x 4.5 mm) maintained at room temperature. 0.05 M Ammonium Citrate buffer was prepared by adding 9.6gm of anhydrous citric acid to 950 mL of water and the pH was adjusted to 4.0 using ammonium hydroxide and was diluted with water to 1000 mL(15). The mobile phase comprised Acetonitrile, Tetrahydrofuran, and ammonium citrate buffer in 27:20:53 ratios, and the flow rate was maintained at 1.00 mL/min. The mobile phase prior to use was degassed in an ultrasonic bath sonicator for 15 minutes. The injection volume of the sample was 20 μL, the analysis was evaluated at 244 nm, and the run time was set for 10 minutes for each sample.

2.2.2. STANDARD CALIBRATION CURVE

The standard calibration curve of Atorvastatin Calcium was obtained by plotting the average of the area under the curve vs concentration. A 100 g/mL stock solution was prepared, and samples were diluted to concentrations ranging from 0.75 g/mL to 40 μg/mL.

2.2.3. SATURATION SOLUBILITY

As atorvastatin calcium is insoluble in aqueous solutions of pH 4 and below, very slightly soluble in distilled water, pH 7.4 phosphate buffer, the solubility of pure drug, physical mixture, and inclusion complex in water and pure drug and SAE complex solubility in 0.1N HCl, pH4.5 acetate buffer, and pH 6.8 phosphate buffer were evaluated to check for pH-dependent solubility. Excess amounts of the entities were added to vials containing 10 mL solvent and were placed in an orbital Shaker for 48hrs. This was done to make sure that the solutions reached the saturation level. The solutions were then filtered through 0.45 μM filters and analyzed the concentrations using HPLC as per the USP mentioned method for Atorvastatin Calcium.

2.2.4. PHASE SOLUBILITY STUDIES

Different concentrations varying from 0-25 mM of A-CD, B-CD, G-CD, HPBCD, and HPGCD in water were prepared in vials. An excess amount of Atorvastatin Calcium was added to the prepared solutions and placed in an orbital shaker for 48 hrs at 25 ^{0}C Later, the samples were filtered through 0.45 μM filters and were analyzed for atorvastatin calcium using HPLC at 254 nm. The phase solubility graphs were plotted using the molar concentrations of the solubilized drug versus the molar concentration of the cyclodextrins. The cyclodextrin used further for complexation was decided based on the results from this study.

2.2.5. PREPARATION AND OPTIMIZATION OF DRUG-CYCLODEXTRIN COMPLEXES

2.2.5.1.PHYSICAL MIXTURE

A physical mixture weighing 29.99 gm was prepared by mixing atorvastatin calcium and HPBCD in a plastic bag and the mixture was sieved through USP sieve No. 35. The powder was stored in an air-tight container and was evaluated for characteristic analysis, solubility, and dissolution.

2.2.5.2.KNEADING METHOD

Atorvastatin calcium (1.2 gm) and HPBCD (1.8 gm) were taken comprising the total mixture weight to 3gm in a mortar to form a complex. Methanol was used as a solvent, added to the mixture, and mixed thoroughly with a pestle until a uniform slurry formed. The resulting formulation was kept in an oven at 40 ^{0}C for 24 hrs to dry. The resulting dry product was then made into powder, sieved through USP sieve 35, stored in an air-tight container, and evaluated for content, solubility, and dissolution.

2.2.5.3.SOLVENT-ASSISTED EXTRUSION (TWIN SCREW EXTRUDER)

Using a parallel (11 mm) twin-screw, co-rotating Process 11 Twin-screw Extruder (Process 11 Thermo Fisher Scientific, Waltham, DE, USA), six batches of drug-cyclodextrin complexes (ATN Ca-HPBCD) were formulated using different solvents. In summary, the co-rotating twin-screw processor consists of two heated, temperature-controlled screws that rotate in the same direction. The hopper receives the feed, which is then sent to the barrel. Six batches of mixtures of ATN Ca-HPCD mixtures were prepared at 1:1 molar ratio weight and were placed into the feeder using a hopper with a screw. The configuration of the screws was aligned according to the standard screw configuration given by Thermo Fischer as shown in figure 3. The solvent is injected into the

zone adjacent to the feeder through a jacket simultaneously as the mixture enters the screws through the hopper. The solvent composition was used as a parameter to optimize the process of the extrusion. The resulting thick slurry-like product was collected at the other end of the extruder. The temperature of the barrel was kept constant at 25 ^{0}C. Different solvents that were used in this technique were water + methanol, pH 6.8 phosphate buffer + methanol, and pH 4.3 acetate buffer + methanol and methanol. Prepared formulations were tested for solubility and dissolution, and based on the solubility results, dissolution was planned. The feed rate (1600 mg/min) and solvent injection (0.1 mL/min) rates were kept constant for each formulation.

Table 1 Optimization of solvent composition for ATN Ca-HPBCD Mixture

Mixture	Molar Ratio	Screw speed(rpm)	Solvent Composition	Temperature ^{0}C
ATN Ca – HPBCD	1:1	50	Water	25
ATN Ca – HPBCD	1:1	50	Water + Methanol	25
ATN Ca – HPBCD	1:1	50	0.1N HCl	25
ATN Ca – HPBCD	1:1	50	pH4.3 Acetate buffer + Methanol	25
ATN Ca – HPBCD	1:1	50	pH6.8 Phosphate buffer + Methanol	25
ATN Ca – HPBCD	1:1	50	Methanol	25

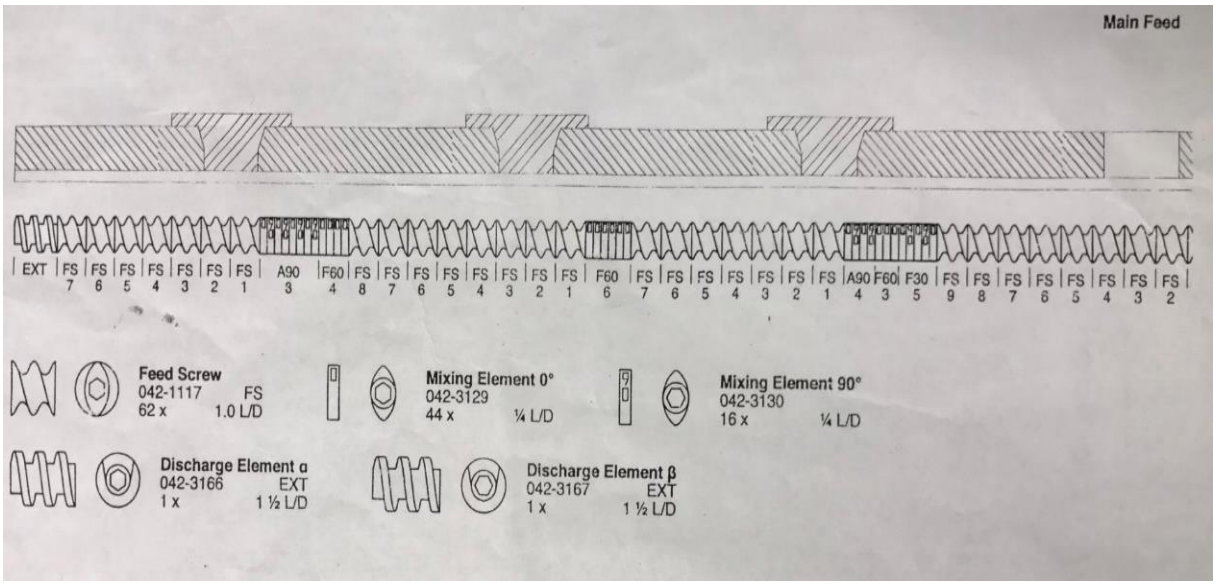

Figure 3 Standard screw configuration

2.2.6. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Using differential scanning calorimetry (DSC), the thermal characteristics of pure excipients, physical mixtures, and hot liquid extrudates were assessed. (TA Instruments, New Castle, DE, USA). Utilizing standards for temperature and heat capacity made of indium and sapphire, the instrument was calibrated. A sample weighing containing equivalent to 3 mg of ATN Ca was enclosed in an aluminum pan and allowed to equilibrate at 25 °C for one minute. Using ultra-purified nitrogen flowing at a 50 ml/min rate, the samples were heated from 10 to 250 °C at a rate of 10 °C/min. Using Trio's software, the difference in heat flow between the test sample and reference pan was tracked and plotted along with the change in temperature.

2.2.7. FOURIER TRANSFORMS INFRARED (FTIR)

The FTIR study is done to detect the spectral bands and interactions of pure API, HPBCD, physical mixture, kneaded complex, and solvent-assisted extruded complex. Investigations were made using an Agilent Cary 660 Fourier transform infrared spectrophotometer (Agilent

Technologies, Santa Clara, CA, USA) to examine interactions between the API and cyclodextrin over a range of 1000-4000 cm^{-1} through transmittance. The spectra for each were overlayed and evaluated for interactions to form a promising inclusion complex between ATN Ca-HPBCD.

2.2.8. IN-VITRO DISSOLUTION STUDY

The products that were to be analyzed for dissolution rate were filled into No. #2 hard gelatin capsules. Table 2 provides the details of the capsule formulations. Each capsule consists of 40 mg of the API as the marketed formulation (LIPTOR) dose is 40 mg.

Table 2 Composition of capsules used for dissolution study.

Capsule	Ingredients	Weight(mg)	Complex preparation method	Solvent used for complex preparation
1	Pure ATN Ca	40	N/A	N/A
2	ATN Ca & HPBCD	93.23	Physical Mixture	N/A
3	ATN Ca & HPBCD	93.23	Kneading	Methanol
4	ATN Ca & HPBCD	93.23	Solvent Assisted Extrusion	Methanol

As per USP (16), Using a dissolution apparatus II (Hanson SR8-plusTM; Hanson Research, Chatsworth, CA, USA), the dissolution profiles of pure ATN Ca, a Physical mixture of ATN Ca-HPCD, a kneaded mixture of ATN Ca-HPBCD, and hot liquid extrudate of ATN Ca-HPBCD were evaluated. The dissolution device was continuously kept at 37.5 °C, and the paddle speed was maintained at 75 rpm, respectively. In vessels containing 900 mL of pH 6.8 phosphate buffer dissolving medium was added. Samples of 2 mL were withdrawn at 5,10,15,30 min time intervals, filtered through a 0.45 μM filter, and 2 mL of dissolution media was added each time to replace the volume. The samples were evaluated using HPLC.

3. RESULTS AND DISCUSSION

3.1. CALIBRATION CURVE

The plotted graph in Figure 3 shows the standard calibration curve of ATN Ca. The graph showed a slope of 35216 and a correlation coefficient of 0.9982. The equation from the graph was used in further studies to determine the concentration of ATN.

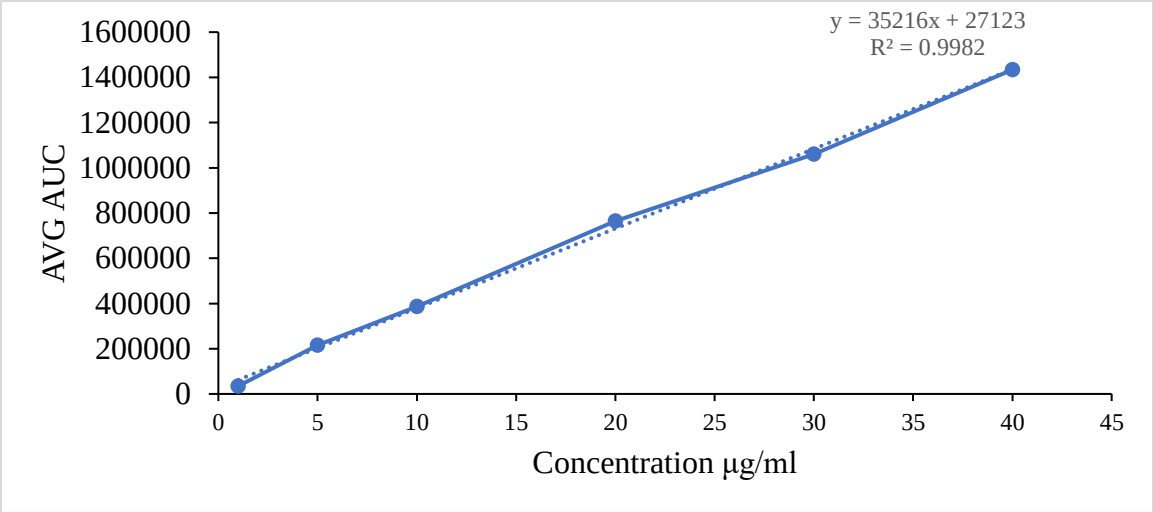

Figure 4 Calibration curve

3.2. SATURATION SOLUBILITY

The saturation solubility was conducted in water for pure ATN and was evaluated using the HPLC discussed above. The solubility of ATN was found to be very low (0.0167 mg/mL) in water as it is poorly soluble in water. Saturation solubility for API and SAE complex in different solvents is shown in Figure 4. The SAE complex has shown the highest solubility increase in water (0.294 mg/mL) and pH 6.8 phosphate buffer (0.328 mg/mL). Whereas there was only a

slight increase in solubility of the SAE complex in 0.1N HCl (0.1023 mg/mL) and in pH 4.5 acetate buffer (0.177 mg/mL). This might be due to the pH-dependent solubility of atorvastatin calcium.

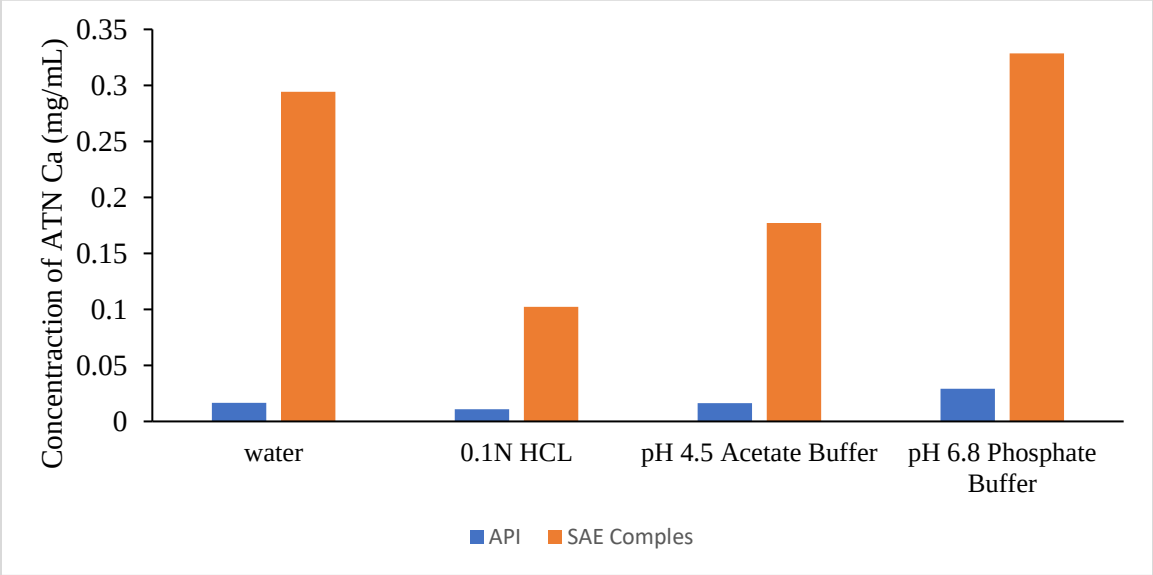

Figure 5 Saturation solubility for API and SAE complex in different solvents

From this study it can be stated that cyclodextrin has enhanced the solubility of the pure drug in various solvents with different pH.

3.3. PHASE SOLUBILITY STUDIES

This study is performed to determine the inclusion complexation between the poorly soluble drug and different cyclodextrins in water. The samples were analyzed using HPLC and the results are shown in Figure 5. The curves were distinguished to be of A_L type from the Higuchi and Connors (4). The solubility of ATN Ca was apparently increased linearly when combined with HPBCD than other cyclodextrins which shows that a water-soluble complex is formed with HPBCD. The inclusion complex between the drug and cyclodextrin molar ratio was concluded to be 1:1 as the slope values obtained were less than 1 (r^2=0.988). Further studies were performed only with the inclusion complex formed between ATN Ca-HPBCD.

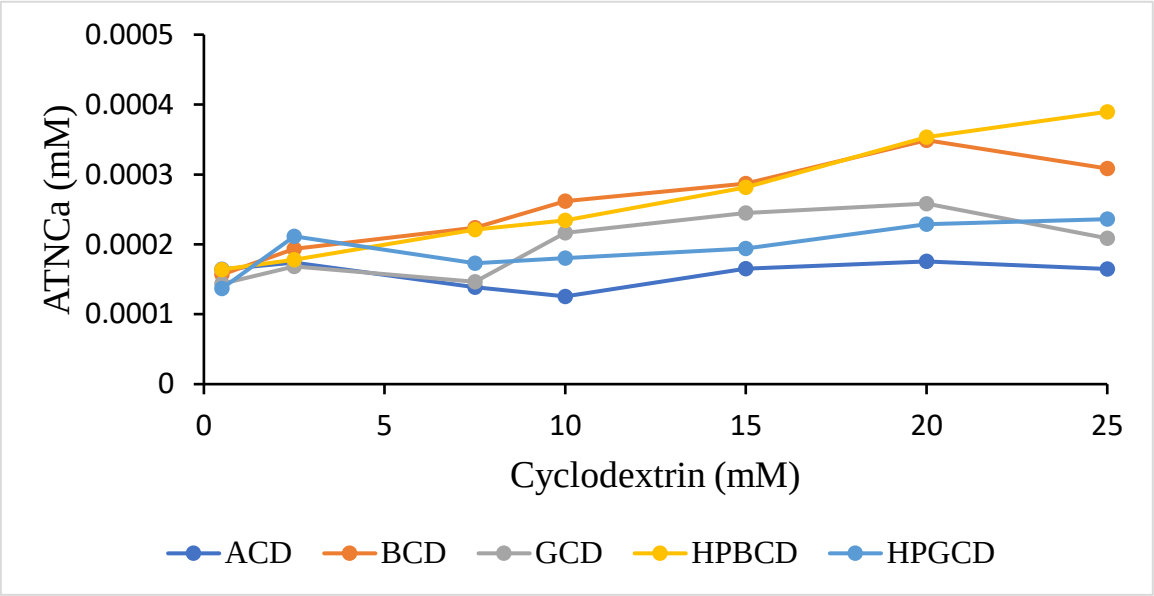

Figure 6 Phase solubility study between ATN Ca and Cyclodextrins

3.4. PREPARATION OF DRUG-CYCLODEXTRIN COMPLEXES

3.4.1. PHYSICAL MIXTURE

The mixture was prepared with ATN Ca and HPBCD and was evaluated for solubility in water. The samples were filtered through a 0.45 μM filter and were analyzed using HPLC. The solubility of this mixture was determined to be 0.236 mg/mL.

3.4.2. KNEADING METHOD

The dried product obtained from this technique was subjected to a solubility study. The solubility of this complex was found to be 0.251 mg/mL which is quite similar to that of the physical mixture. This shows that any complexation is formed between Atorvastatin Calcium and HPBCD, resulting in a slight increase in solubility over the physical mixture.

3.4.3. SOLVENT-ASSISTED EXTRUSION (TWIN SCREW EXTRUDER)

The SAE complexes that were formed through this technique were evaluated for their solubility in water. The complexes prepared using different compositions of the solvents were compared evaluating the solubility. The presence of methanol in the formation of complexes increased the ability to form enhanced complexation as ATN Ca and HPBCD are soluble in methanol. The superior interaction between ATN Ca and HPBCD during the hot liquid extrusion process results in higher hydrophilicity and wetting properties, which may be responsible for the increased solubility of the complex compared to those prepared by other techniques. Therefore, the SAE process is considered a more effective method than the kneading method or other conventional methods (14). Based on these findings, the complex obtained from this technique was used for further evaluation of studies.

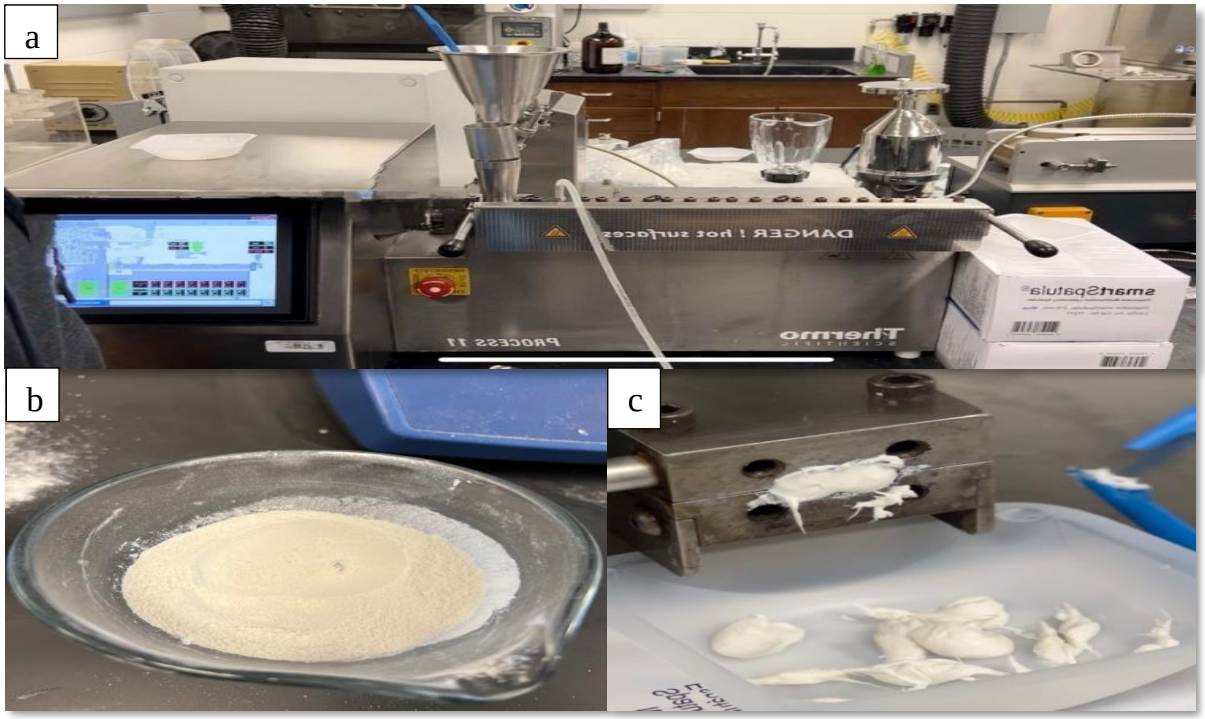

Figure 7 a) Solvent Assisted Extrusion b) Kneaded product c) SAE product.

3.5. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

This study shows the confirmation of prominent complex formation between ATN Ca – HPBCD based on the enthalpy of the peaks. The DSC thermogram of pure ATN Ca showed a peak with a melting point of 164.43 ^{0}C (Enthalpy=64.003 J/g) which shows its crystalline nature. The DSC thermogram of the complex prepared through SAE using water as a solvent showed a peak with a melting point of 166.46 ^{0}C (Enthalpy=30.918 J/g) and the complex prepared using (water + methanol) as a solvent showed a peak with a melting point of 166.82 ^{0}C (Enthalpy=30.118 J/g). The DSC thermogram of the complex prepared through SAE using pH (4.3 Acetate Buffer + Methanol) as a solvent showed a peak with a melting point of 159.11 ^{0}C (Enthalpy=20.11 J/g) and the complex prepared using (pH 6.8 Phosphate buffer + methanol) showed a peak with a melting point of 166.37 ^{0}C (Enthalpy=22.348 J/g). These thermograms tend to show the conversion of the pure API from crystalline to amorphous but not completely as the peaks and enthalpy still exist. From this, it can be concluded that the tendency to form an inclusion complex between ATN Ca – HPBCD is minimal with these solvents. The DSC thermogram of the complex prepared through the Kneading method using methanol as a solvent showed a peak with a melting point of 158.64^{0}C (Enthalpy=3.7855 J/g) and the complex prepared through SAE using methanol as a solvent showed a peak with a melting point of 152.09 ^{0}C (Enthalpy=2.688 J/g). Complexation of the drug and cyclodextrin results in the shifting of the peak to less temperature or complete loss of the peak depicting the change from crystalline to amorphous nature. Additionally, it is intriguing to observe that the complex prepared to utilize the SAE approach was more amorphous than the complex prepared using the kneading method. The relative drug crystallinity of all the formulations is displayed in Table 3.

The RDC was calculated based on the below equation.

$$\%RDC = \frac{Enthalpy\ of\ Pure\ Drug * 100}{Enthalpy\ of\ formulation}$$

Table 3 Relative drug crystallinity of all the formulations

SAMPLE	ENTHALPY (J/G)	%RDC
Pure ATN Ca	30.91	100
Inclusion complex with water as solvent	30.10	47.3
Inclusion complex with 4.3 acetate buffer and methanol as solvent	20.10	31.4
Inclusion complex with 6.8 phosphate buffer and methanol as solvent	22.30	34.84
Inclusion complex with Kneading method and methanol as solvent	3.7	5.78
Inclusion complex with SAE method and methanol as solvent	2.6	4.06

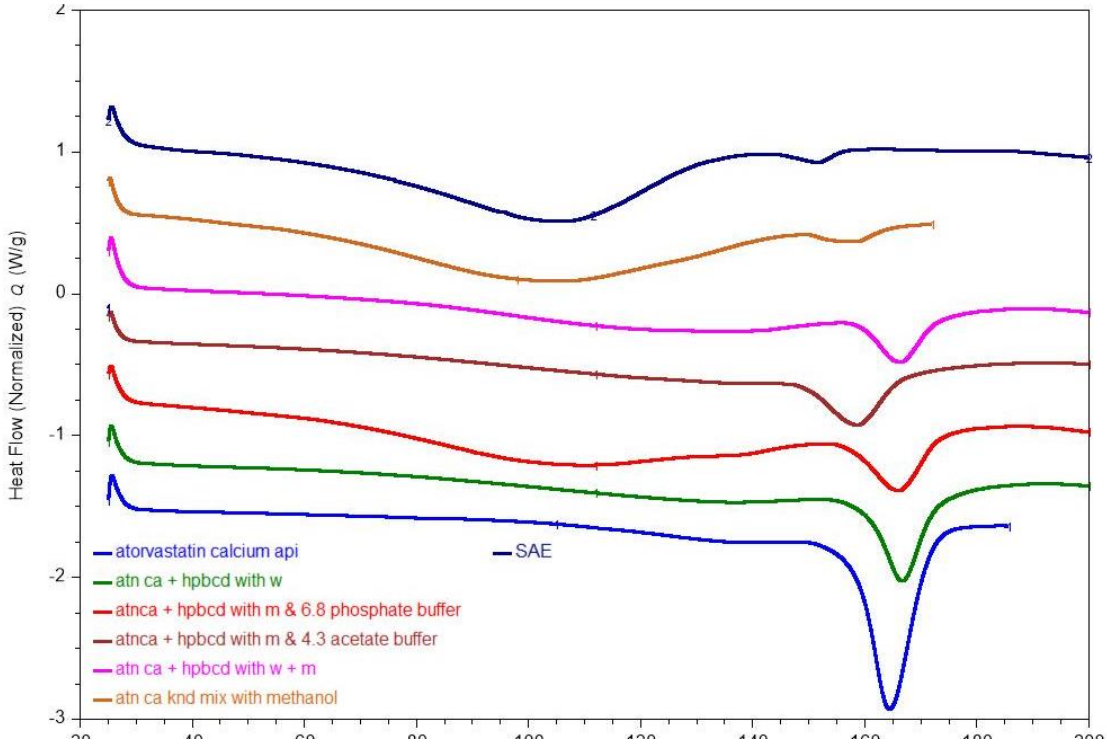

Figure 8 DSC profiles of pure API and prepared mixtures with solvents by SAE and kneading method

3.6. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

This study evaluates the drug cyclodextrin interactions to confirm the formation of an inclusion complex. The spectrum of Atorvastatin Calcium exhibited a strong band at 3408 cm^{-1} which could be attributed to N-H stretching vibration. Also, characteristic C-C stretching vibrations are observed for an aromatic ring of atorvastatin calcium between 1700–1400 cm^{-1} (10). A wide O-H peak was observed for the FTIR spectrum of HPBCD at the band of 3000–3500 cm^{-1} (17). A significant difference was observed in the spectrum of the physical mixture and inclusion complex of atorvastatin ca and HPBCS prepared by SAE Method and Kneaded method. All the characteristic bands for N-H starching, aromatic C-C stretching of ATN Ca, and wide band for HPBCD were clearly seen in the physical mixture spectrum without alteration of bands. However, C-C aromatic stretching bands disappeared in the inclusion complex spectrum prepared

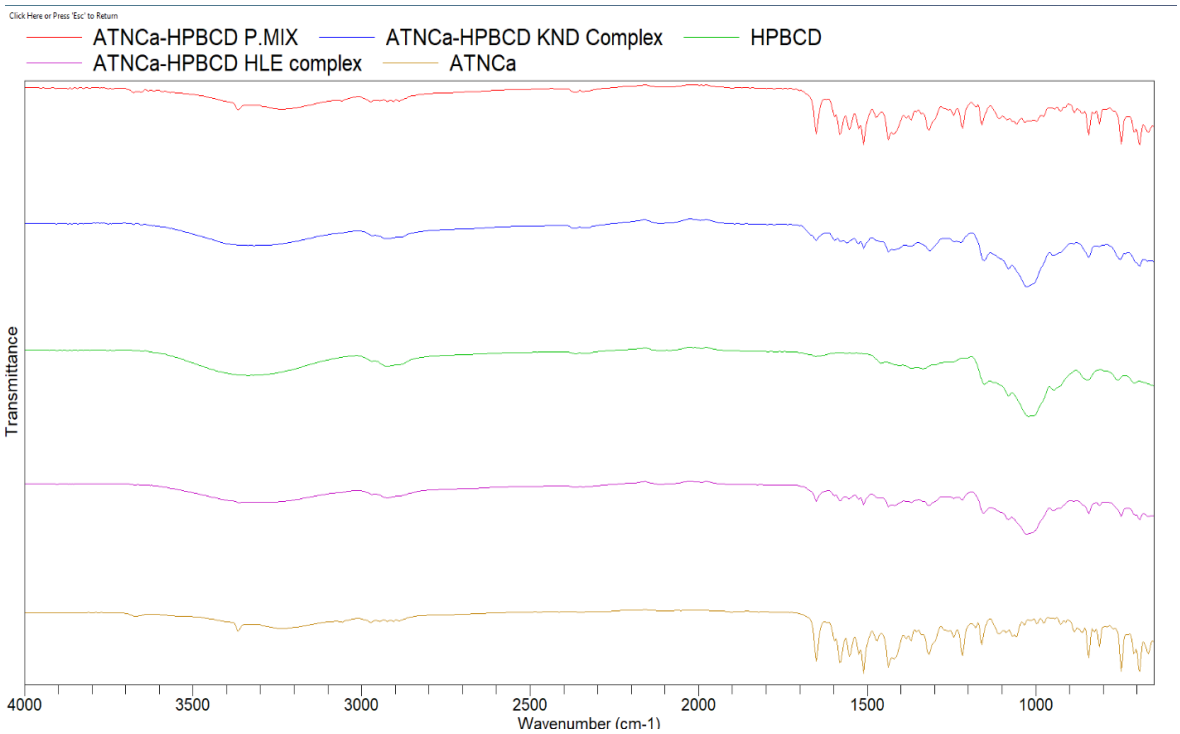

Figure 9 FT-IR of ATN Ca, HPBCD, physical mixture, and inclusion complex Prepared by Kneading and SAE method

by both methods. In the final formulation spectrum intensity of N-H stretching vibration was significantly reduced due to the restriction of vibration of ATN Ca and not clearly seen due to the wide 3000–3500 cm^{-1} band of HPBCD suggesting inclusion in the HPBCD. No new bands were observed, indicating no chemical interaction between ATN ca and HPBCD.

3.7. IN-VITRO DISSOLUTION STUDY

Figure 10 shows the drug release profiles of pure ATN Ca, the physical mixture of ATN Ca-HPBCD, and complexes prepared using kneading and solvent-assisted techniques (SAE complex) using the USP method for 40 mg ATN tablets (USP apparatus 2, stirred at 75 rpms with pH 6.8 phosphate buffer as the dissolution medium (16). The USP limits for drug release of atorvastatin calcium is 80 % of drug release in 15 minutes for 40 mg tablets. Pure ATN Ca showed a drug release of 57 % at 15 min, whereas other samples combined with cyclodextrin showed a linear increase in drug release at 15 min compared to pure ATN Ca. This could have been due to

the hydrophilic characteristic of cyclodextrin to enhance the solubility of the drug. The physical mixture with no solvent has shown a drug release of 61 % at 15 min. In contrast, complexes prepared through kneading and SAE showed higher drug release rates comparatively. The complex prepared through the kneading technique has shown 84 % of drug release at 15 min, and the complex prepared through the SAE technique has shown 99 % of drug release at 15 min which is quite promising for good complex formation between ATN Ca and HPBCD. The drug release for pure API and physical mixture are almost the same where neither of them meets the USP limit whereas the complexes prepared by kneading and SAE method meet the USP limits. Thd complex prepared by kneading had better dissolution than the physical mixture although the solubility of kneaded complex and physical mixture were similar. This could have been due to the hydrophylic carrier. Thus, further characteristic analysis was performed for the SAE complex formed using methanol as a solvent. This dissolution study has revealed the formation of an inclusion complex between ATN Ca and HPBCD using SAE, resulting in superior dissolution rates.

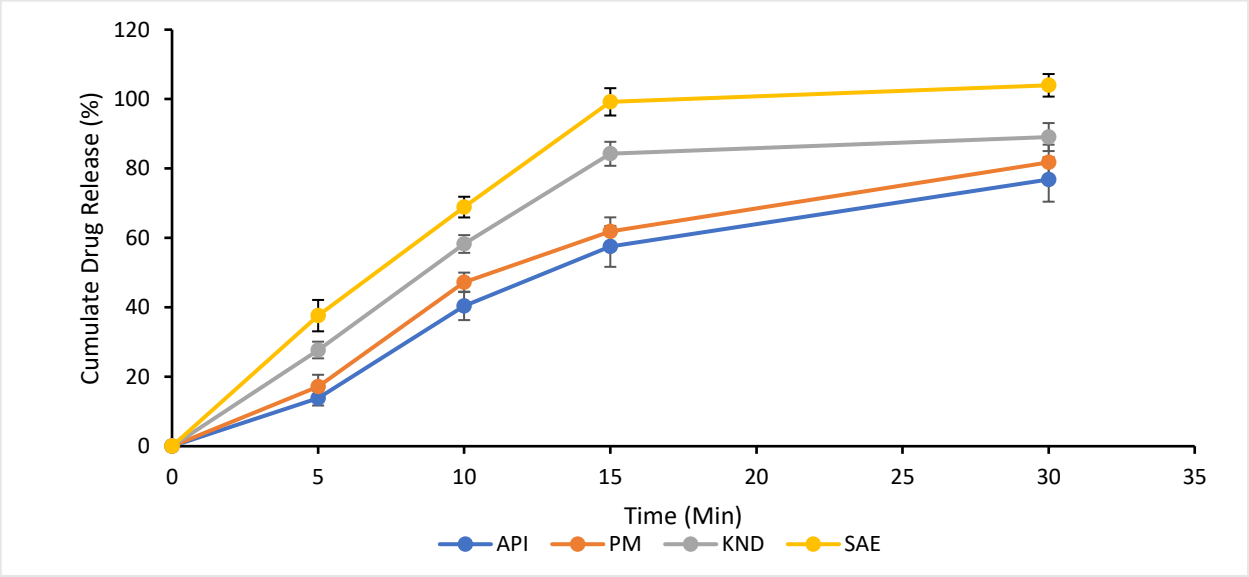

Figure 10 Drug release profiles of pure ATN Ca, a physical mixture of ATN Ca-HPBCD, and complexes prepared using kneading and SAE method

This could have been due to the mechanical force generated due to the rotating screws ensuring uniform distribution of ATN Ca in HPBCD leading to the transformation of the crystalline drug to an amorphous state, thus enhancing the solubility and dissolution profile of the drug. Therefore, SAE can be considered a promising technique for forming drug-CD inclusion complexes.

4. CONCLUSION

Complexes were formulated using the kneading method and solvent-assisted extrusion technique using a twin-screw extruder. ATN Ca-HPBCD complex, one of the complexes created using the SAE procedure, showed a significantly increased solubility compared to the plain drug, physical mixture, and complex created using a traditional technique, kneading. The advantage of the SAE procedure in forming ATN Ca-CD complexes was also amply demonstrated by in vitro drug release tests, which showed a more significant release of ATN Ca from complexes prepared by SAE compared to pure drug and kneading technique. Due to its superior solubility, the inclusion complex prepared by the SAE technique also demonstrated faster drug release compared to the complex prepared by the kneading technique and the pure drug alone. Altogether an increase in solubility and dissolution rate of ATN Ca was seen with the SAE technique, which supports the objective of this study that SAE is a successful method for formulating drug-cyclodextrin complexes.

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VITA

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