Effect of Polymers Dispersions on the Inclusion Complexe Formation Between β-Cyclodextrin and Insoluble Drugs. Case Study: Atorvastatin Solubility Enhancement in the Biological Conditions of the Blood Medium (In-Vitro Dissolution Study)

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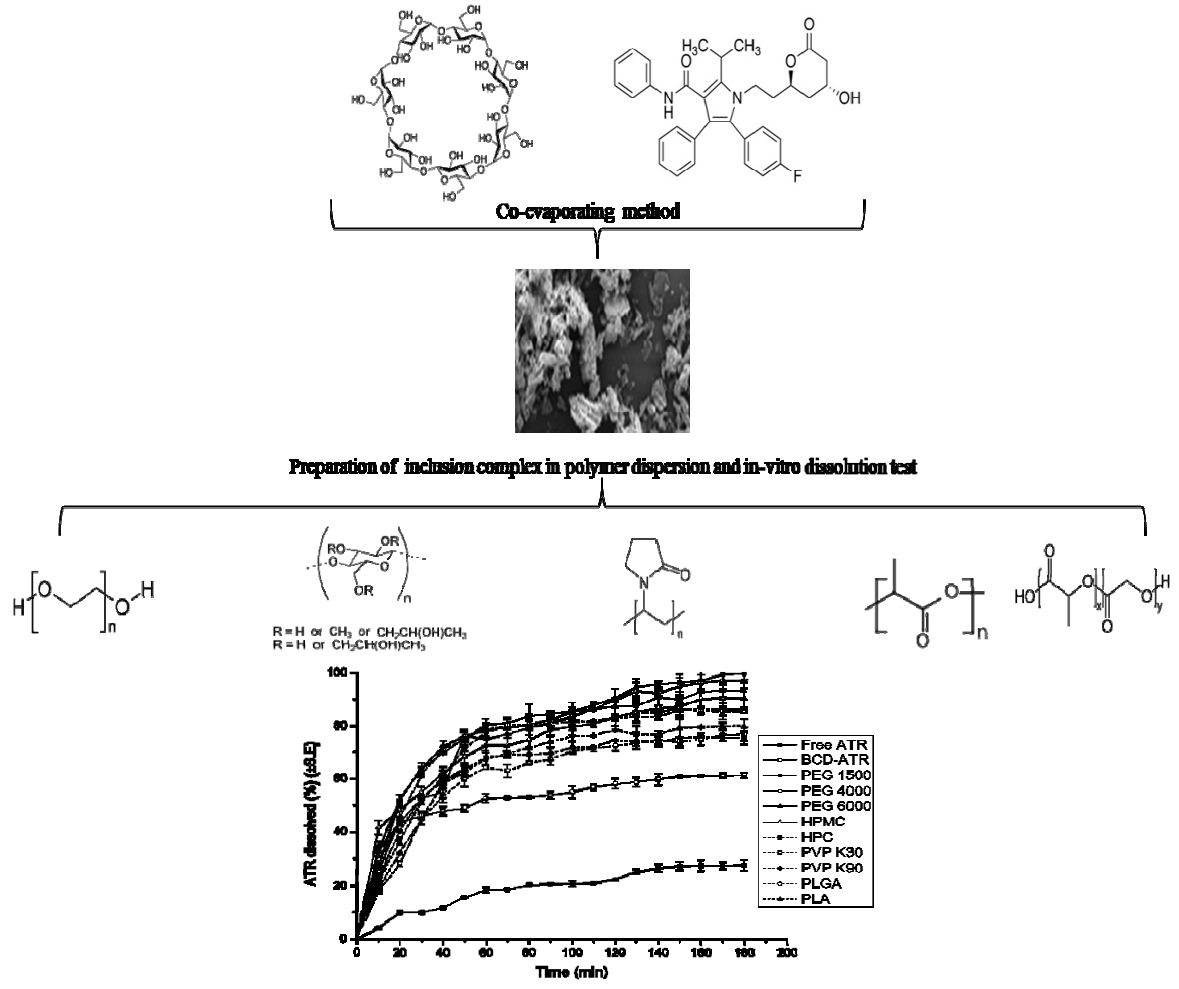
Graphical abstract:



Abstract: Through the years, cyclodextrins have been used in order to increase the solubility of insoluble drugs in aqueous solutions. The aim of the present work was to improve the solubility of Atorvastatin, as a lipid-lowering agent. Inclusion complex of Atorvastatine in β-cyclodextrin was prepared in polymer dispersion using four different methods: physical mixture, kneading method, Freeze-drying and co-evaporating method with stoichiometric molar ratio 1:1 and 5% (W/W) polymers; drug content estimation found that co-evaporating is the best one of them. Formulations with different polymers concentrations 2.5%, 5%, 7.5%, 10%, 15% and 20% (W/W) are prepared with co-evaporating method and in-vitro dissolution study of pure drug, β-cyclodextrin–Atorvastatine inclusion complex and all formulations have been released, the formulation which presents the best dissolution rate was 5% of PEG1500, the dissolution rate has attended more than 99.70%.

Keywords: Polymer Dispersion, Inclusion Complex, β-cyclodextrin, Insoluble Drug, in-vitro dissolution.

1. Introduction:

Cyclodextrins (CDs) are cyclic oligosaccharides composed of 6–12 glucose units degraded from starch macromolecules by enzymatic conversion. The α, β and γ CDs are natural forms, consisting of six, seven and eight glucose units, respectively. The hydrogen atoms connected with C-3, C-5 of glucopyranose in the cavity cover the oxygen atom, making the interior of cavity a hydrophobic space. To the contrast, the exterior of cavity is hydrophilic because of the presence of hydroxyl groups, which is one of the significant characteristics of cyclodextrin [1]. Hence, it can form complex by introducing micromolecules, polymers and macromolecules. Cyclodextrin is widely applied and developed in the fields of supermolecule, molecular capsule, nano-biomaterial, etc. in the recent years. It also becomes an indispensable functional material in the pharmaceutical design and formulation in pharmaceutical science. Cyclodextrin and biopolymers, which can serve as solubilization and stabilization agents of drug, is very significant in improving pharmacological effects, bioavailability and solubility of drugs [2,3] and natural bioactive substances [4,5].

Atorvastatin(ATR), as a lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG CoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, an early rate-limiting step in cholesterol biosynthesis [6]. The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH. Atorvastatin is a molecule which allows a very low solubility in water, the formulation of inclusion complexes with B-cyclodextrin (BCD) has enabled the increase in its solubility much as 60% approximately [7-9]. Studies previously made by the researchers show clearly formation inclusion complex between BCD and ATR, The formation of the inclusion complexation was identified by molecular modeling [7], and characterized by X-ray powder diffractometry [7-9], differential scanning calorimetry [7,9], Scanning Electron Microscope [8,9], Particle Size Analyzer (PSA) [7] and FTIR. But the complete information on the behavior of ATR inside the cavity of βCD is study using 1H, 13C and fluorine NMR analysis [10].

Therefore, in order to improve the solubility of ATR we had added a water soluble polymers, polyethylene glycol (macrogol® 1500, 4000 and 6000), polyvinylpyrrolidone (K30 and K90), hydroxypropyl methylcellulose (HPMC) hydroxypropyl cellulose (HPC), PLGA poly(lactic-co-glycolic acid) and PLA Poly(lactic acid).

2. Materials and Methods:

2.1. Materials:

Atorvastatin was a gift sample obtained from Pr. Guermouche's laboratory (USTHB, Algiers, Algeria). poly(lactic-*co*-glycolic acid) (lactide:glycolide 50:50, M_w 7,000-17,000) and Poly(lactic acid) (M_w ~60,000) was a gift sample obtained from laboratory of polymer chemistry (USTHB, Algiers, Algeria). β-cyclodextrin (98% purity), hydroxypropyl methylcellulose and hydroxypropyl cellulose were procured from Sigma-Aldrich, biochim algeria. Polyethylene glycol (macrogol® 1500, 4000 and 6000), polyvinylpyrrolidone (K30 and K90) were sample gifts obtained from El kendi pharmaceutical industry (Algiers, Algeria). All other materials used are of pharmacopeial grade.

2.2. Preparation of BCD-ATR inclusion complex in polymer dispersions:

2.2.1. Physical mixture:

ATR with BCD in 1:1 molar ratio and 5% W/W water soluble polymers were mixed in a mortar for about one hour with constant trituration, passed through sieve N°. 80 and stored in desiccators over fused calcium chloride [11], the formulation codes are given in table 1.

2.2.2. Kneading method:

ATR with BCD in 1:1 molar ratios and 5% W/W water soluble polymers were taken. First cyclodextrin is added to the mortar; small quantity of 50% methanol is added, while triturating to get slurry like consistency. Then slowly drug and polymers is incorporated into the slurry and trituration was further continued for one hour. Slurry was then air dried at 25 °C for 24 hours, pulverized and passed through sieve N°. 80 and stored in desiccators over fused calcium chloride [13], the formulation codes are given in table 1.

2.2.3. Freeze-drying (Lyophilisation):

ATR with BCD in 1:1 molar ratios and 5% W/W water soluble polymers were dissolved in distilled water, and the solution was frozen by immersion in liquid nitrogen and freeze-dried over 24 h in a Lyph-lock 6 apparatus [12], the formulation codes are given in table 1.

2.2.4. Co-evaporating technics:

ATR with BCD in 1:1 molar ratios and 5% W/W water soluble polymers were taken . ATR was dissolved in methanol, BCD and polymers in distilled water, the two solutions were mixed and the new solution obtained was shaken for 1 hour at room temperature on a rotary flask shaker, the solvents were evaporated using a boiling bath and the solid inclusion complexes were recuperated [14]. The formulation codes were given in table 1.

Table 1. Composition, codes and drug content of all formulations obtained by different methods.

Formulation Codes	Formulation Technics	ATR	BCD	5% Polymers	Drug Content (%)
F1	Co-evaporating	+	+	PEG 1500	99.38
F2		+	+	PEG 4000	99.74
F3		+	+	PEG 6000	99.65
F4		+	+	HPMC	99.32
F5		+	+	HPC	99.42
F6		+	+	PVP K30	99.07
F7		+	+	PVP K60	99.35
F8		+	+	PLGA	99.98
F9		+	+	PLA	99.37
F10	Kneading method	+	+	PEG 1500	98.74
F11		+	+	PEG 4000	98.41
F12		+	+	PEG 6000	98.07
F13		+	+	HPMC	98.71
F14		+	+	HPC	99.11
F15		+	+	PVP K30	98.37
F16		+	+	PVP K60	98. 07
F17		+	+	PLGA	99.27
F18		+	+	PLA	98.03
F19	Freeze-drying	+	+	PEG 1500	98. 41
F20		+	+	PEG 4000	98.60
F21		+	+	PEG 6000	98.33
F22		+	+	HPMC	98.19
F23		+	+	HPC	99.1
F24		+	+	PVP K30	98.01
F25		+	+	PVP K60	97.62
F26		+	+	PLGA	99.09
F27		+	+	PLA	98.15
F28	Physical mixture	+	+	PEG 1500	97.99
F29		+	+	PEG 4000	96.84
F30		+	+	PEG 6000	98.01
F31		+	+	HPMC	97.62
F32		+	+	HPC	97.32
F33		+	+	PVP K30	97.78
F34		+	+	PVP K60	97.66
F35		+	+	PLGA	96.52
F36		+	+	PLA	98.02
BCD-ATR	Co-evaporating	+	+	-	99.92
FREE ATR		+	-	-	99.99

2.3. Drug Content Estimation:

50 mg of formulation was accurately weighed and transferred to 50 ml of volumetric flask and volume was made up to the mark with methanol. From this 1ml was taken in 10 ml volumetric flask and the volume is adjusted up to the mark with same solvent. The absorbance of the solution was measured at 249 nm. The drug content of ATR was calculated using calibration curve data [15]. The measurements were performed in triplicate and the data is given in table 1.

2.4. Effect of polymers concentrations on the formation of BCD-ATR inclusion complex:

The method used in formulation is Co-evaporating, before cited. ATR with BCD in 1:1 molar ratios and varying quantities of water soluble polymers were taken, 2.5, 5, 7.5, 10, 15 and 20 % W/W [16, 17].

2.5. In-vitro dissolution test of pure drug and all formulations:

In-vitro dissolution of all formulations was studied in Electrolab TDT- 08L dissolution apparatus employing a paddle stirrer. 900 ml of phosphate buffer of pH 7.4 was used as dissolution medium at 50 rpm, the temperature of 37 ± 0.5°C was maintained throughout the experiment. Formulation equivalent to 50 mg of ATR was used in each test. 5ml of sample of dissolution medium were withdrawn by means of syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 245 nm after suitable dilution with phosphate buffer [18]. The measurements were performed in triplicate.

3. Results and discussion:

3.1. Drug content estimation:

The formulation and Drug Content Estimation data was given in table 1. The formulation prepared by co-evaporating method showed maximal drug content. But the formulations prepared by Freeze-drying, Kneading and Physical mixture methods were found to be slightly less shown in table 1.

3.2. In-vitro dissolution study of pure ATR and all formulations:

3.2.1. Effect of polymers dispersion (polymers types) on the formation of BCD-ATR inclusion complex:

The dissolution characteristics of ATR (pure drug), BCD-ATR (inclusion complex) and BCD-ATR-5% polymers systems are shown in figure 1, 2, 3 and 4. Shows that inclusion complexation of ATR in BCD increased the dissolution rate of the drug. This increase is favorised in the adition of hydro soluble polymers follows the order: PEG 1500 > PEG 4000 > PEG 6000 > HPMC > HPC > PLGA > PLA > PVP K30 > PVP K90. This increase in the dissolution rate of the drug can be attributed to both improvements in drug wettability and formation of readily soluble complexes in the dissolution medium.

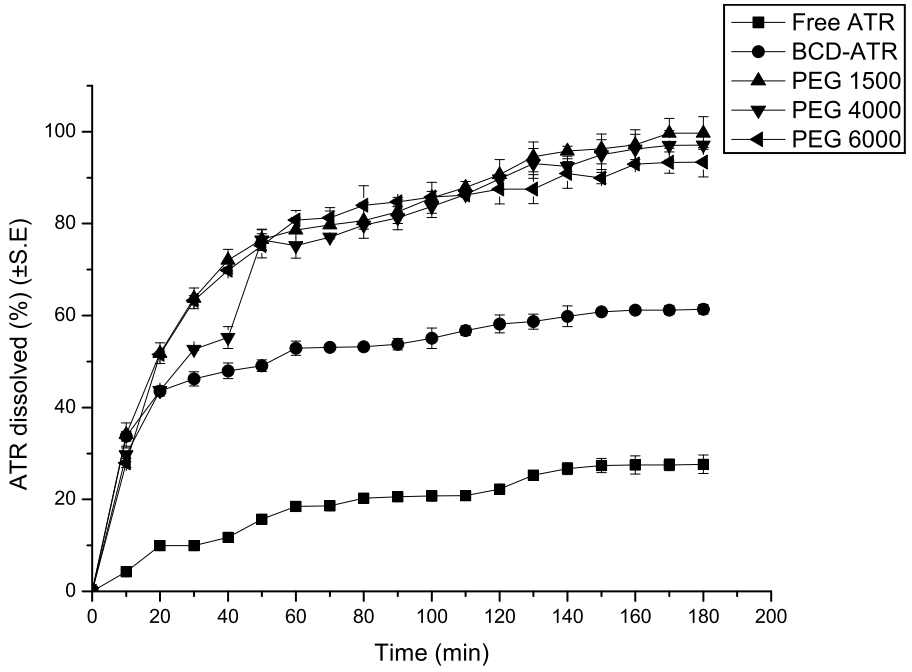


Fig 1: Effect of polymers (Polyethylene glycol) on the dissolution rate of BCD-ATR inclusion complex.

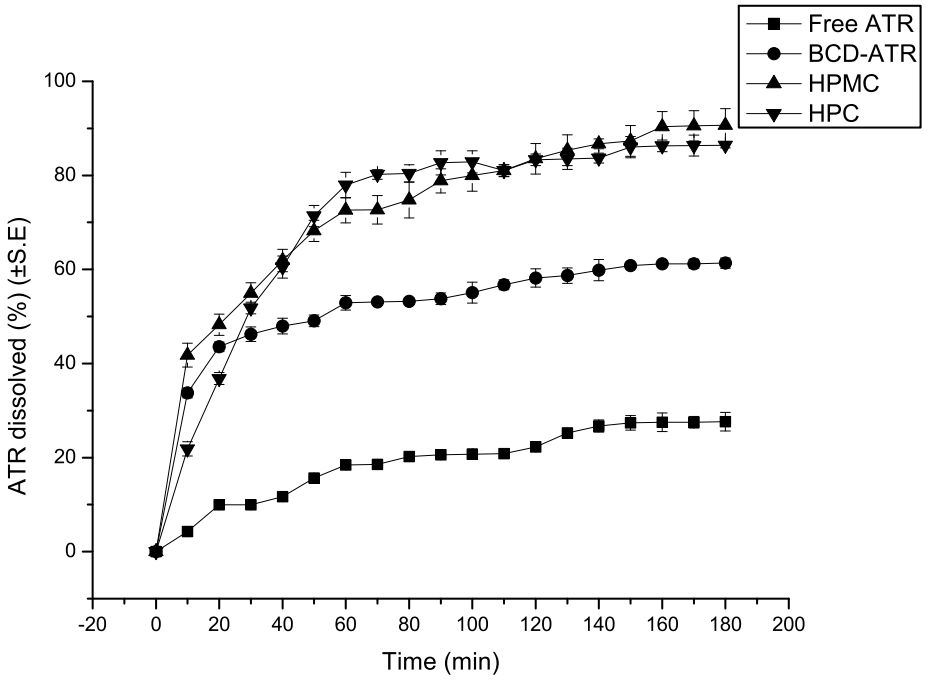


Fig 2: Effect of polymers (cellulose derivatives) on the dissolution rate of BCD-ATR inclusion complex.

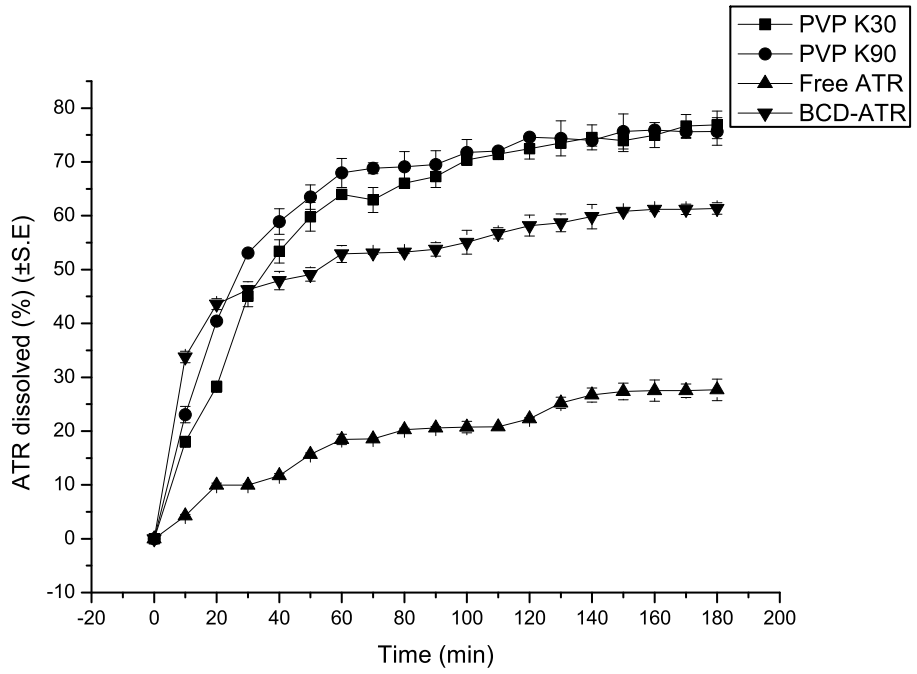


Fig 3: Effect of polymers (polyvinylpyrrolidone) on the dissolution rate of BCD-ATR inclusion complex.

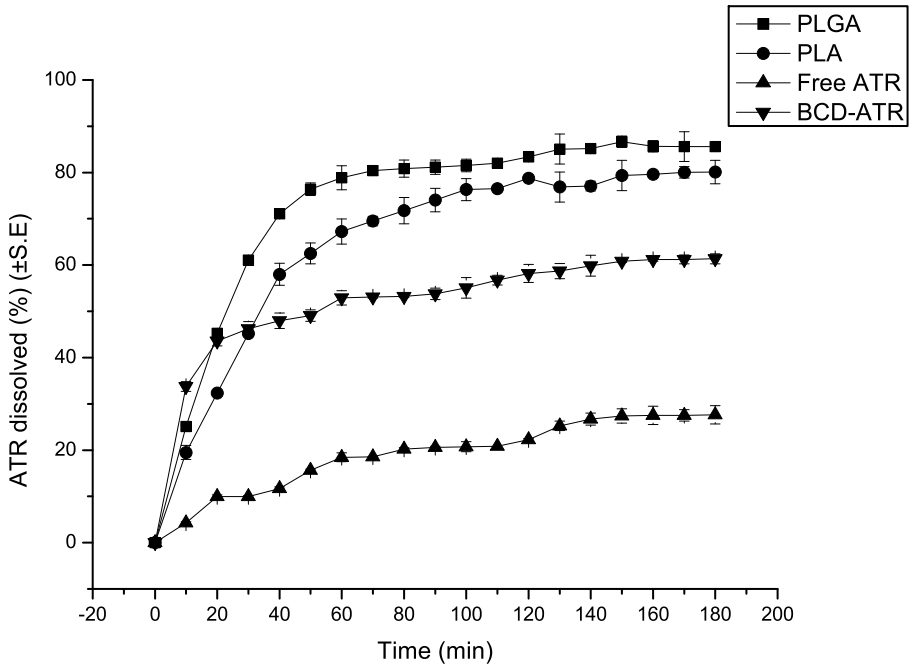


Fig 4: Effect of polymers (poly(lactic-*co*-glycolic acid) and Poly(lactic acid)) on the dissolution rate of BCD-ATR inclusion complex.

3.2.2. Effect of polymers dispersion (concentration of each polymer) on the formation of BCD-ATR inclusion complex:

The dissolution ATR-BCD in PEG polymer dispersion was increased irrespective of polymer concentration (Figure 5). For PEG 1500, PEG 2000 and PEG 6000, the dissolution rate of the drug showed optimum increase at 5% polymer concentration. However, concentration of PEG 6000 showed no effect on the dissolution rate of the drug.

The dissolution of ATR-BCD with HPMC, HPC or PVP dispersion, the enhancement of dissolution rate was found to increase with increasing polymer concentration (Figures 6 and 7). Previous studies showed that HPMC and PVP increase complexation of glimepiride, hydrocortisone, dexamethasone and naproxone with BCD [13, 19-22].

In presence of PLGA or PLA polymer dispersion was increased irrespective of polymer concentration (Figure 8). For PLGA and PLA the dissolution rate of the drug showed optimum increase at 7.5 and 15% polymer concentration, respectively.

We attributed the changing formation of the inclusion complex BCD-ATR in the different polymer dispersions by the existence of different interactions between polymers, BCD and drug molecules, such as hydrophobic bonds, interactions of van der Waals dispersion forces and hydrogen bonds [23].

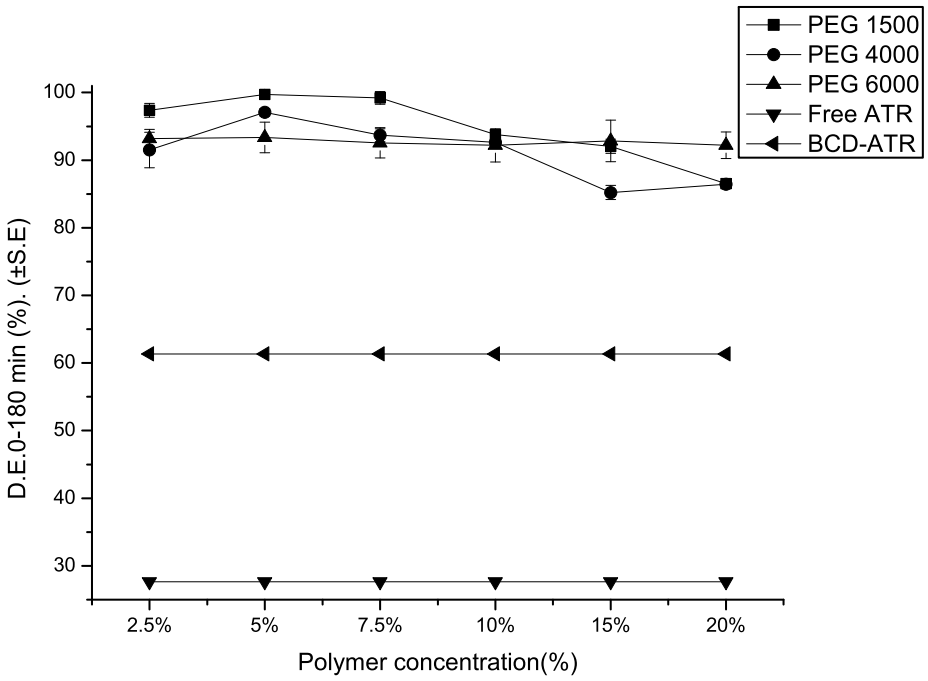


Fig 5: Effect of PEG 1500, PEG 4000 and PEG 6000 concentration on the D.E. of ATR-BCD inclusion complex.

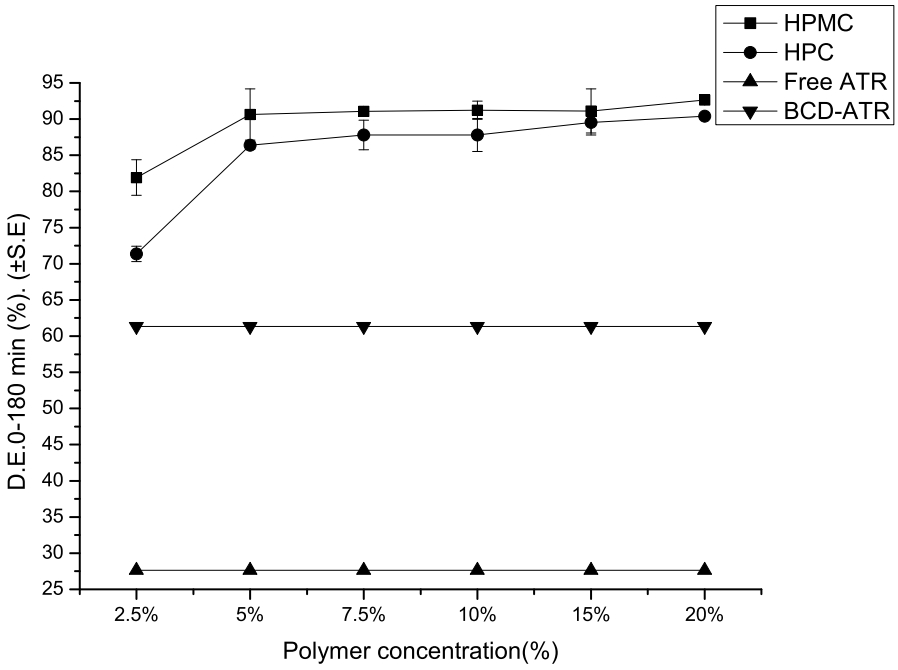


Fig 6: Effect of HPMC and HPC concentration on the D.E. of ATR-BCD inclusion complex.

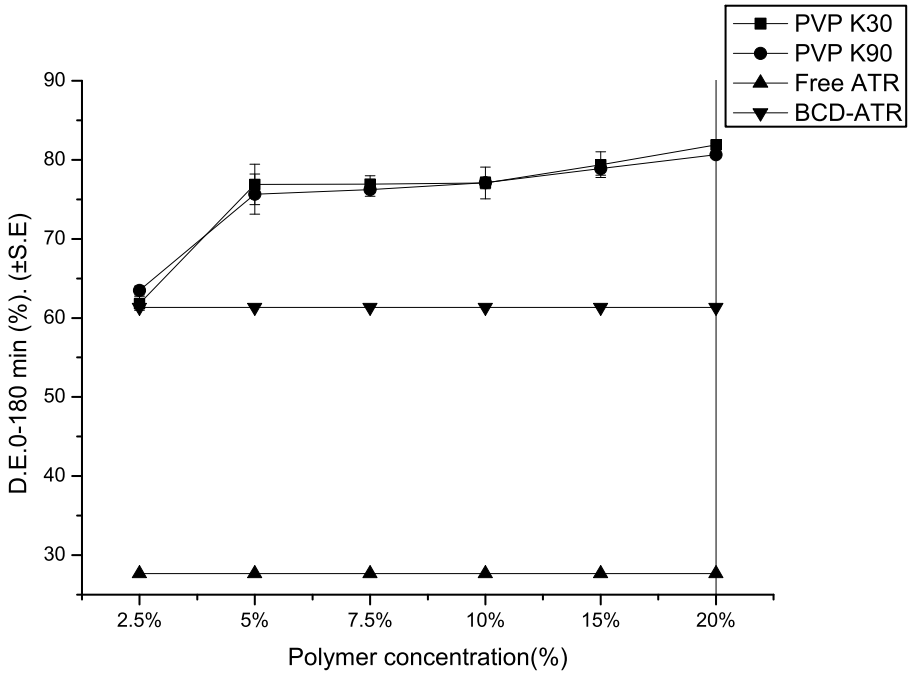


Fig 7: Effect of PVP K30 and PVP K60 concentration on the D.E. of ATR-BCD inclusion complex.

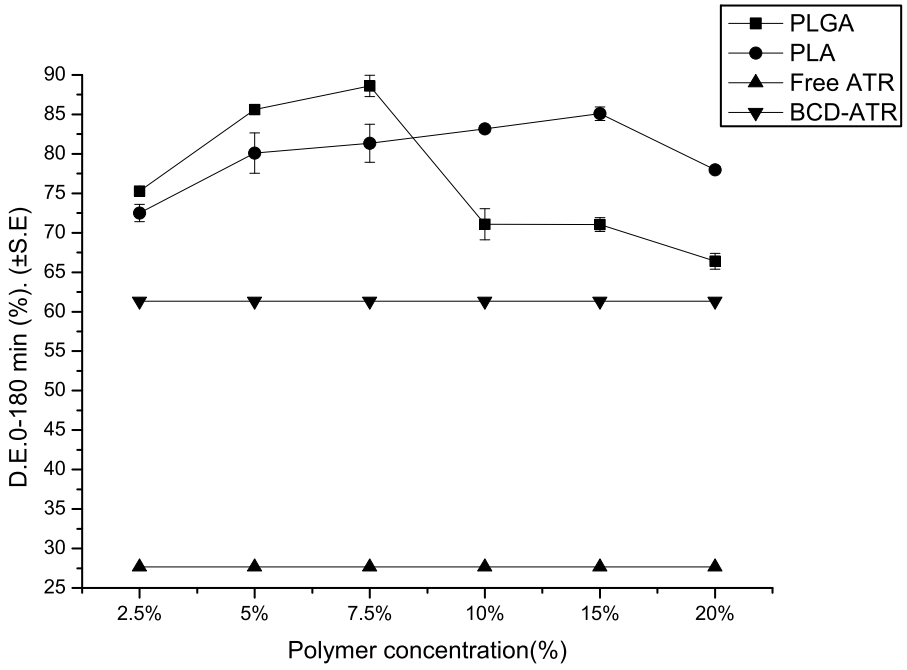


Fig 8. Effect of PLGA and PLA concentration on the D.E. of ATR-BCD inclusion complex.

4. Conclusion:

The Cyclodextrins like β-cyclodextrin can be used to prepare inclusion complexe with insoluble molecules like ATR in the aime to improve its solubility in water using different methods, physical mixture, kneading method, Freeze-drying and co-evaporating method. Co-evaporating method is the best of them; this solubility can be ameliorated by adding water soluble polymers like PEG, PVP, PLGA, PLA and cellulose derivative. This increase is favorised by the addition of hydro soluble polymers follows the order: PEG 1500 > PEG 4000 > PEG 6000 > HPMC > HPC > PLGA > PLA > PVP K30 > PVP K90. Effect of concentration polymers in the formation of BCD-ATR inclusion complex showed maximal dissolution rats at 5% for Polyethylene glycol (PEG 1500, PEG 4000, PEG 6000), 20% for cellulose derivatives and polyvinylpyrrolidone (HPMC, HPC, PVP K30 and PVP K90), 7.5% and 15% for PLGA and PLA, respectively. The inclusion complex prepared with

BCD-ATR in 5% PEG 1500 (15%PEG1500) showed the highest solubility and enhancement in dissolution profile.

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