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Preparation and comparison of binary and ternary inclusion complexes of aripiprazole using L-arginine/lysine and MβCD/HPβCD by using different molar ratios

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Abstract: Aripiprazole (ARI), an antipsychotic having low solubility and stability. To overcome this, formation of binary and ternary using inclusion complexes of Methyl-β-cyclodextrin (MβCD) /Hydroxy propyl beta cyclodextrin (HPβCD) and L-Arginine (ARG)/ Lysine (LYS) are analyzed by dissolution testing and phase stability study along with their complexation efficacy and solubility constants made by physical mixing. Inclusion complexes with ARG were better than LYS and prepared by solvent evaporation and lyophilization method as well. They are characterized by Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (AT-FTIR), X-ray powder diffractometry (XRD), Differential Scanning Calorimetry (DSC), Scanning electron microscopy (SEM) and Thermal gravimetric analysis (TGA). The bond shifting in AT-FTIR confirmed the molecular interactions between host and guest molecules. The SEM images also confirmed a complete change of drug morphology in case of ternary inclusion complexes prepared by lyophilization method for both the polymers. ARI: MβCD: ARG when used in the specific molar ratio of 1:1:0.27 by prepared by lyophilization method has 18 times best solubility while ARI:HPβCD:ARG was 7 times best solubility than pure drug making MβCD a better choice than HPβCD. Change in the molar ratio will cause loss of stability or solubility. Solvent evaporation gave significant level of solubility but less stability.

Keywords: Aripiprazole, L-arginine, methyl-β-cyclodextrin, hydroxy propyl-β-cyclodextrin.

INTRODUCTION

Aripiprazole (ARI), is an antipsychotic drug for the treatment of with schizophrenia, bipolar disorders including mania, seizures, anger management. It has very low water solubility. It follows Lipinski's rule of 5, making it a BCS class II drug (Awais *et al*., 2023). For improving the therapeutic efficacy of the API's, one of the techniques used to improve solubility along with stability is making binary and ternary complexes of active pharmaceutical agent (API) with amino acids and suitable type of cyclodextrin (Awais *et al*., 2023). There are different types of method of preparation which can be compared for best candidate for the preparation of inclusion complexes. Cyclodextrin (CDs) have a hydrophobic internal cavity and capable of forming non-covalent host guest complex with API and Arginine (ARG) (Jiwanti *et al*., 2022). CD are known to alter their molecular arrangements of API but only gave stability by weak intermolecular interactions. This is reported to enhance the Physico-chemical properties like drug permeation and solubility etc. Awais *et al*., 2022) (Suvarna *et al*., 2022). CDs have some drawbacks as well, like, bulky formulations, raised costs and high molecular weight so it is prepared to used them in minimum amounts (Singh *et al*., 2022). Factors that should be considered for making inclusion of guest molecules in cyclodextrin molecule are; size of guest molecule and thermodynamic interactions of different parts of system (Degreef, 2022), (Tilborg *et al*., 2014). The amino acids which are initially screened for better complex formation are ARG and LYS. The arginine proved itself a better agent for increasing solubility and stability. The arginine is also reported in many other studies as best linker when aripiprazole is attached with the hydrophilic surface of the CD. In this study along with increased solubility a better stability profile was also obtained with MβCD. Thus in this study a comparative study was done to conclude whether HPβCD or MβCD has the better inclusion capacity as well as the selected amino acid to provide the best linkage.

MATERIALS AND METHODS

This experiment was conducted in the research laboratory of the Department of Pharmacy at University of Lahore in

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the year 2018-19 after obtaining ethical approval from the institution. The chemicals used in this study included ARI (Global pharmaceuticals, Islamabad), MβCD (Vision Pharmaceuticals Islamabad), Pepsin, Ethanol L-Arginine (Sigma-Aldrich, located in St. Louis, MO, USA.) and Hydrochloric acid (HCl) Merck (Germany). Only analytical grade solvents are used in this study.

The instruments and equipment used included Electrical Analytical Balance, Magnetic Stirrer, Vortex Mixture, MS2-Minishaker, Dissolution Apparatus (II), UV-Visible Spectrophotometer (UV1700, Shimadzu Kyoto, Japan), FTIR Spectrophotometer (7600 spectrometer by Lambda, Bruker Tensor 27 Series), Thermal Gravimetric Analysis / DSC Analyzer made by PerkinElmer STA 6000, X-Rays JDX-3532 Diffractometer, JEOL, Japan, and Scanning Electron Microscope by Perkin Elmer. Pipettes Pyrex, Measuring Cylinder, Drying Oven Memet, Powder X-ray Diffractometer, HPLC Agilent Technologies, Measuring Flask Pyrex, Beaker Pyrex, Centrifuge Machine.

In this study, the drug to polymer ratios in binary and ternary complexes were selected by quality by design software (QBD) in the ratio of 1:1, 1:2.5, 1:4 and 1:9, 1:1:1, 1:1:0.27, 1:2.5:0.27, 1:3.6:3.6, 1:4:1 and 1:9:1 (Abreu *et al*., 1995).

Physical mixing

The components of the mixture were mixed gently in glass mortar and pestle for 5-7 minutes with gentle grinding. After passing through a sieve of suitable size (180 µm), samples are stored in previously washed, dried amber colored glass bottles and labeled. These bottles are kept in desiccators having silica *(Sherje et al., 2018, Awais et al., 2022).*

Solvent evaporation method

The ARI, ARG/LYS and polymer were dissolved in absolute methanol to make crystal clear solution separately. The clear solutions are mixed in a single glass beaker with the help of magnetic stirrer and finally orbital shaker (150rpm for 24 hr.) is used to suspend all the three components of the mixture in each other. Rotary evaporator is used to recover the sample. The sample was dried in oven at 27ºC (not exceeding 29ºC) until completely dried. Grinded and dried sample is passed through a sieve no. 180 µm and stored in previously washed ambered colored glass bottles that are tightly closed. These bottles are kept in desiccators having silica (Suvarna *et al*., 2017, Awais *et al*., 2022).

Lyophilization method

The ARI, ARG and polymer were dissolved in absolute methanol to make crystal clear solution separately. The clear solutions are mixed in a single glass beaker with the help of magnetic stirrer and finally orbital shaker (150rpm for 24hr.) is used to suspend all the three components of the mixture in each other. Rotary evaporator is used to recover the sample flask by using minimum amount of solvent and was placed overnight in a scientific freezer at -60ºC. Lyophilizer was used to freeze dry the samples and the dried powder was sieved through a diameter 80 µm. The final product was then transferred to amber colored glass bottles (Awais S, *et al*, 2023).

Preparation of capsules

The exactly measured 15mg of all samples as well as pure ARP was packed in a hard gelatin capsule. Each sample was made in triplicate. All the tests were performed in triplicate (Awais S, *et al*, 2023).

Phase stability study

ARI is practically insoluble is water but soluble in methanol (500µg/ml). It follows Lipinski rule (Kusherova *et al*., 2024). Solubility study was done with regards to the Higuchi plus Conners, 1965 (table 2, 3 and 4). Precisely weight samples (0.4 g) were taken to a glass test tube having 10 ml of deionized water 0.4g of sample is added and placed in a vortex mixture for 2 min at 1400 rpm. After mixing on shaking incubator (37°C for 3 days at 150 rpm), they are centrifuged at 6000 rpm for about 30 min. After decanting the supernatant liquid, using a micro syringe filter (0.45µm), 5ml of deionized water was used to dilute the 1ml upper clear layer. These samples were analyzed in triplicate for ARI peaks at 219 nm by using UV-Vis 1600 Spectrophotometer (Shimadzu Spectrophotometer, from Tokyo, Japan). Control testing was performed to authenticate the results. Results are authenticated by control testing. The apparent stability constants (K_s) along with complexation efficiency of the samples were calculated by

$$Ks = \frac{Slope}{So(I - Slope)}$$

Where S_o is said to be the equilibrium solubility of ARI in water.

$$C.E = \frac{Slope}{(I - Slope)}$$

In vitro dissolution study

The capsule shells are checked for dissolution in SGF (USP 41 Dissolution apparatus I, 2018) (Kalmer *et al*., 2024). 10ml of sample after certain time intervals collected by a syringe filter (0.45µm) were tested. The level of SGF is maintained after each withdrawal. Finally, the withdrawn samples at certain time frame were evaluated for the concentration of ARI at 219 nm on UV spectrophotometer. For the calculation of the percentage Dissolution Efficiency (abbreviated as DE) as follows.

$$DE\% = \frac{foyXdt}{y100Xt} \times 100$$

Where, y is the % of dissolved drug; area under the dissolution curve is DE, between time points t1 and t2 which is expressed as a % of the curve at maximum dissolution, y100, over the same period.

Table 1: Sample codes with ratios.

Code	Composition		
ARI	Pure drug		
MPM2	ARI:MβCD (1:1)		
MPM3	ARI:MβCD (1:2.5)		
MPM4	ARI:MβCD (1:4)		
MPM5	ARI:MβCD (1:9)		
MPM6	ARI:MβCD:ARG (1:1:1)	MPML1	ARI:MβCD:LYS (1:1:1)
MPM7	ARI:MβCD:ARG (1:1:0.27)	MPML2	ARI:MβCD:LYS (1:1:0.27)
MPM8	ARI:MβCD:ARG(1:2.5:0.27)	MPML3	ARI:MβCD:LYS (1:2.5:0.27)
MPM9	ARI:MβCD:ARG (1:3.6:3.6)	MPML4	ARI:MβCD:LYS (1:3.6:3.6)
MPM10	ARI:MβCD:ARG(1:4:1)	MPML5	ARI:MβCD:LYS (1:4:1)
MPM11	ARI:MβCD:ARG (1:9:1)	MPML6	ARI:MβCD:LYS (1:9:1)
MLY12	ARI:MβCD (1:1)		
MLY13	ARI:MβCD (1:2.5)		
MLY14	ARI:MβCD (1:4)		
MLY15	ARI:MβCD (1:9)		
MLY16	ARI:MβCD: ARG (1:1:1)		
MLY17	ARI:MβCD: ARG (1:1:0.27)		
MLY18	ARI:MβCD:ARG(1:2.5:0.27)		
MLY19	ARI:MβCD:ARG (1:3.6:3.6)		
MLY20	ARI:MβCD: ARG (1:4:1)		
MLY21	ARI:MβCD: ARG (1:9:1)		
MSE22	ARI:MβCD (1:1)		
MSE23	ARI:MβCD (1:2.5)		
MSE24	ARI:MβCD (1:4)		
MSE25	ARI:MβCD (1:9)		
MSE26	ARI:MβCD: ARG (1:1:1)		
MSE27	ARI:MβCD: ARG (1:1:0.27)		
MSE28	ARI:MβCD:ARG(1:2.5:0.27)		
MSE29	ARI:MβCD: ARG (1:3.6:3.6)		
MSE30	ARI:MβCD: ARG (1:4:1)		
MSE31	ARI:MβCD: ARG (1:9:1)		
HPM32	ARI:HPβCD (1:1)		
HPM33	ARI:HPβCD (1:2.5)		
HPM34	ARI:HPβCD (1:4)		
HPM35	ARI:HPβCD (1:9)		
HPM36	ARI:HPβCD:ARG (1:1:1)	HPML1	ARI:HPβCD:LYS (1:1:1)
HPM37	ARI:HPβCD:ARG (1:1:0.27)	HPML2	ARI:HPβCD:LYS (1:1:0.27)
HPM38	ARI:HPβCD:ARG (1:2.5:0.27)	HPML3	ARI:HPβCD:LYS (1:2.5:0.27)
HPM39	ARI:HPβCD:ARG (1:3.6:3.6)	HPML4	ARI:HPβCD:LYS (1:3.6:3.6)
HPM40	ARI:HPβCD:ARG (1:4:1)	HPML5	ARI:HPβCD:LYS (1:4:1)
HPM41	ARI:HPβCD:ARG (1:9:1)	HPML6	ARI:HPβCD:LYS (1:9:1)
HLY42	ARI: HPβCD (1:1)		
HLY43	ARI: HPβCD (1:2.5)		
HLY44	ARI: HPβCD (1:4)		
HLY45	ARI: HPβCD (1:9)		
HLY46	ARI: HPβCD: ARG (1:1:1)		
HLY47	ARI: HPβCD: ARG (1:1:0.27)		
HLY48	ARI: HPβCD: ARG (1:2.5:0.27)		
HLY49	ARI: HPβCD: ARG (1:3.6:3.6)		
HLY50	ARI: HPβCD: ARG (1:4:1)		
HLY51	ARI: HPβCD: ARG (1:9:1)		

Continue…

HSE52	ARI: HPβCD (1:1)
HSE53	ARI: HPβCD (1:2.5)
HSE54	ARI: HPβCD (1:4)
HSE55	ARI: HPβCD (1:9)
HSE56	ARI: HPβCD: ARG (1:1:1)
HSE57	ARI: HPβCD: ARG (1:1:0.27)
HSE58	ARI: HPβCD: ARG (1:2.5:0.27)
HSE59	ARI: HPβCD: ARG (1:3.6:3.6)
HSE60	ARI: HPβCD: ARG (1:4:1)
HSE61	ARI: HPβCD: ARG (1:9:1)

Table 2: Comparison of Saturation profile of ARI, MβCD and LYS and ARP, HPβCD and LYS by physical mixing

Code	Solubility (µg/ml)					
	1:1:1	1:1:0.27	1:2.5:0.27	1:3.6:3.6	1:4:1	1:9:1
Aripiprazole: MβCD: Lysine	7.076	8.359	13.94	8.74	9.926	11.667
Aripiprazole: MβCD: Arginine	16.07	17.35	20.94	18.74	13.92	18.66
Aripiprazole: HPβCD: Lysine	7.49	1.286	0.482	0.9028	1.468	1.963
Aripiprazole: MβCD: Arginine	9.8	8.34	11.01	20.33	13.88	17.07

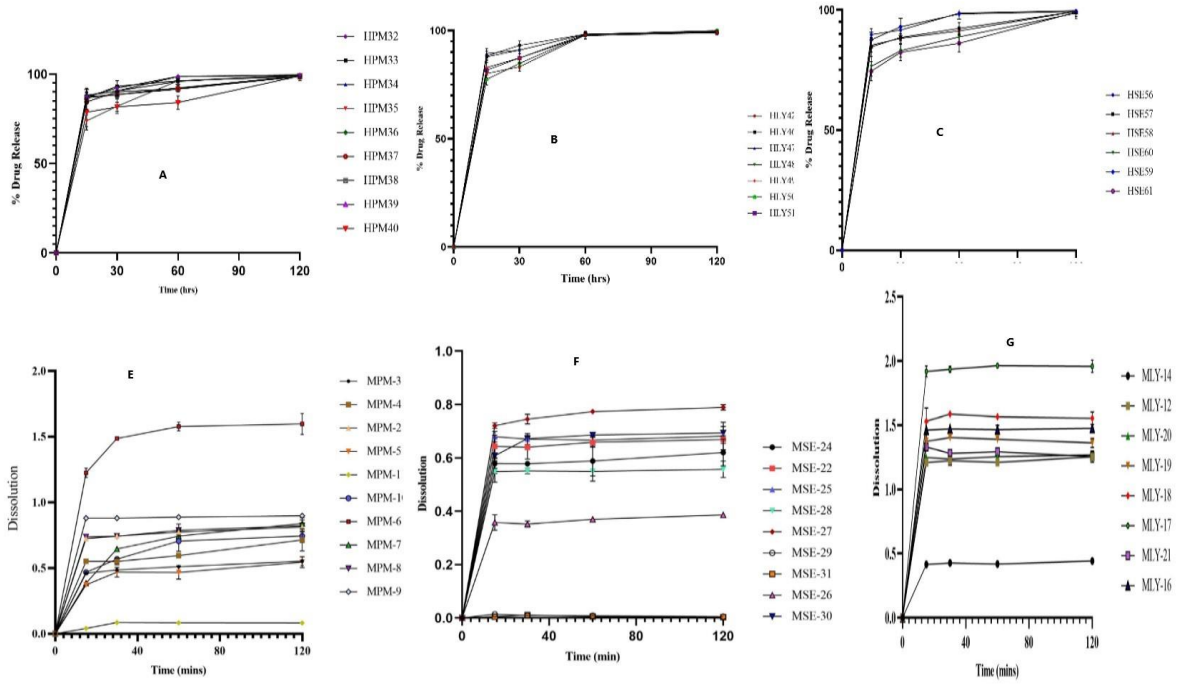

Fig. 1: *In vitro* drug percentage drug release of ARI: MβCD: ARG binary and ternary complexes by (A) PM (B) LY (C) SE. Showing Lyophilization (B) is the best technique and highest solubility values are obtained both in case of binary and ternary making MLY-14 the highest. In vitro drug percentage drug release of ARI:HPβCD:ARG binary and ternary complexes by (D) PM (E) LY (F) SE. Showing that lyophilization is best techniques for complexation with HPβCD as well but MBβCD showed better result.

It was then computed to compare the performance of the binary as well as ternary formulations (Sodeifian *et al.*, 2024). The drug dissolution profile gave time taken for 50% drug release (T50%).

Dissolution studies

The dissolution testing was performed on all the samples. The samples showed different behavior depending upon the composition and methods of preparation. (Mahdi *et al*., 2023)(fig. 1).

Attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR)

The AT-FTIR spectra of all the new inclusion complexes reflect the blue shift and red shift for all the bond angles. Brief comparison is given in the table 5.

Table 3: Phase stability of ARP, MβCD/HPβCD and LA by different methods.

Drug Code	Solubility (µg/ml)	Code	Solubility (µg/ml)
ARI	0.04		
MPM2	3.72	HPM32	1.72
MPM3	4.68	HPM33	2.99
MPM4	6.54	HPM34	5.32
MPM5	13.81	HPM35	9.81
MPM6	16.07	HPM36	9.88
MPM7	17.35	HPM37	8.34
MPM8	20.94	HPM38	11.012
MPM9	18.74	HPM39	20.33
MPM10	13.92	HPM40	13.88
MPM11	18.66	HPM41	17.067
MLY12	11.63	HLY42	1.12
MLY13	13.99	HLY43	3.7
MLY14	14.56	HLY44	11.029
MLY15	16.66	HLY45	13.786
MLY16	15	HLY46	12.04
MLY17	32.4	HLY47	28.45
MLY18	24.32	HLY48	23.6
MLY19	30.94	HLY49	24.2
MLY20	17.64	HLY50	18.6
MLY21	19.633	HLY51	21.73
MSE22	6.86	HSE52	1.38
MSE23	8.3	HSE53	6.6
MSE24	11.71	HSE54	8.9
MSE25	12.16	HSE55	10.89
MSE26	18.5	HSE56	20.91
MSE27	23.94	HSE57	21.63
MSE28	25.72	HSE58	19.42
MSE29	25.5	HSE59	20.9
MSE30	21.19	HSE60	15.1
MSE31	23.6	HSE61	12.63

AT-FTIR graph results

All the samples gave a small shift of peak intensity either blue shift or red shift (1674 cm-1 and 1675 cm-1). The characteristic peaks are visible in the finger print region (Oliinyk *et al*., 2023). Only a small shifting of peak intensity is observed in case of all the inclusion complexes. The detail of all the bonds shifting is represented in the table. 6.

Scanning electron microscopy (SEM)

The aripiprazole appeared as crystals, MβCD and HPβCD are amorphous with spherical particle while ARG represents as non-linear crystalline appearance (Kumar *et al*., 2020).

Thermo gravimetric analysis (TGA)

The thermo grams can detect either energy is produced or required. Sample is heated from 30.00°C to 500.00°C at 10°C per min under a dry nitrogen gas having a flow rate of 20ml per min by using PerkinElmer STA 6000 simultaneous TGA/DSC analyzer. Sample MPM2, MPM6, MPM9, MLY14, MLY16 and MLY19 were evaluated by using TGA thermo gram fig. 7.

XRD

The amorphous or crystalline nature of the complexes can be checked by powder XRD patterns. ARI, MβCD, HPβCD and ARG, were studied for their binary and ternary complexes.

Differential scanning calorimetry (DSC)

ARI, MβCD, LA and ARI, HPβCD, LA were evaluated by DSC and proved to be amorphous with decrease or disappearance of crystalline peaks. The amount of amorphousness and crystallinity was also studied.

STATISTICAL ANALYSIS

Results were analysed by using one-way ANOVA**,** Graph Pad instant software (San Diego, CA, USA). $p < 0.05$ was considered as a statistically significant value. t-test was also applied to evaluate the data.

RESULTS

In this study, we have highlighted the problem of Aripiprazole's low solubility which is considerably enhanced along with stability in the form of inclusion complexes. The MβCD proved to be better choice while ARG is superior to LYS. We compared three different methods of physical mixing, solvent evaporation and lyophilization.

Initially, it was concluded from the results that ARG is a better candidate for inclusion complexes than LYS due to high solubility levels in phase solubility studies (table 2). Inclusion complexes' complexation efficiency and solubility constant were better (tables 4 and 6). The MβCD provide better results if compared with HPβCD (Yao *et al*., 2023). The SC values indicate that ternary complex MLY17 and HLY 48 have the highest levels (tables 4 and 6).

When checked in triplicate for dissolution profile, the accurately weighed 15mg of the drug; it was concluded that the MβCD enhanced the dissolution extensively compared to binary complexes of HPβCD. But when the ternary component is introduced, the behavior of the drug is changed (Abdelquader *et al*., 2023). The LYS had shown a lesser increase in dissolution, but ARG has a synergistic effect with MβCD and HPβCD (Sakurama *et al*., 2018). The lowest levels of dissolution obtained from the lyophilization method were much more significant than the pure drug as shown in fig. 1.

Table 4: Table for stability constant and complexation efficiency of MβCD/HPβCD and ARG

Code	SC	CE	Code	SC	CE
MPM2	226±2	0.072	HPM32	241±8	0.081
MPM3	271±1	0.144	HPM33	292±2	0.141
MPM4	316±4	0.211	HPM34	356±1	0.219
MPM5	361±5	0.355	HPM35	391±4	0.385
MPM6	296±6	0.241	HPM36	406±2	0.281
MPM7	437±3	0.312	HPM37	437±8	0.319
MPM8	326±1	0.398	HPM38	456±2	0.318
MPM9	377±9	0.471	HPM39	477±7	0.441
MPM10	391±1	0.355	HPM40	489±1	0.335
MPM11	396±11	0.389	HPM41	492±4	0.379
MLY12	243±2	0.175	HLY42	193±2	0.179
MLY13	283±3	0.266	HLY43	303±1	0.286
MLY14	344±1	0.311	HLY44	344±1	0.319
MLY15	401±15	0.366	HLY45	388±1	0.326
MLY16	206±16	0.303	HLY46	276±6	0.323
MLY17	471±17	0.506	HLY47	468±7	0.497
MLY18	416±18	0.476	HLY48	440±1	0.485
MLY19	451±19	0.411	HLY49	457±9	0.409
MLY20	416±20	0.455	HLY50	419±10	0.461
MLY21	406±2	0.488	HLY51	411±2	0.481
MSE22	208±2	0.142	HSE52	213±8	0.182
MSE23	281±3	0.174	HSE53	291±1	0.184
MSE24	323±1	0.287	HSE54	343±1	0.294
MSE25	399±5	0.312	HSE55	409±7	0.331
MSE26	311±6	0.317	HSE56	361±1	0.345
MSE27	356±2	0.319	HSE57	396±2	0.321
MSE28	422±8	0.441	HSE58	442±2	0.454
MSE29	401±19	0.398	HSE59	461±1	0.376
MSE30	386±03	0.408	HSE60	418±04	0.414
			HSE61	401±1	0.402

Table 5: Comparison of AT-FTIR confirming the red and blue shift in the data.

	CD	MβCD	HPβCD	LA	MPM11	MSE24	MLY17	HPM34	HLY42	HSE57
C-H stretching	2936	2919	2919	2960	2923	2924	2883	2934	2941	2943
C-H bending	1442	1452	1452	1424	1450	1451	1446	1446	1447	1448
C-0-C strecthing	1027	1019	1019	1123	1018	1016	1029	1008	1030	1014
C-Cl PEAK	763	753	753	788	756	756	785	838	784	797
Carbonyl Peak	1672	1641	1641	1563	1630	1674	1669	1672	1674	1673
Oh bending	1366	1362	1362	1318	1363	1364	1354	1362	1332	1353
Presence of amide	1577			1563	1540	1522	1577	1578	1582	1592

AT-FTIR studies revealed that all the characteristic peaks in the fingerprint region are visible with a slight bond shift. They either show a red shift or a blue shift without disappearing the peak. However, peak intensity was little changed. As the CD and ARG have no bond formation, as the previous literature shows (Wang *et al.*, 2024), there are only weak molecular interactions between the guest and the host molecule as shown in fig. 2. The comparison is shown in table 6. The FTIR graphs for comparison are shown in fig. 3. The bonds shifting (either blue or red shift) were observed clearly in the graphs. In the SEM images, the polymer groups behave in the same manner as far as the preparation method is considered. Dispersed crystals can be seen in case of solvent evaporation. (Khan *et al*., 2023). In the case of lyophilized ternary inclusion complexes, the drug is incorporated inside the cyclodextrin cavity and flake crystals were not visible. The same behavior was observed from using both polymers, but MβCD ternary complexes were better than the corresponding HPβCD and ARG ternary complexes as shown in fig. 4 (Suvarna *et al.*, 2017).

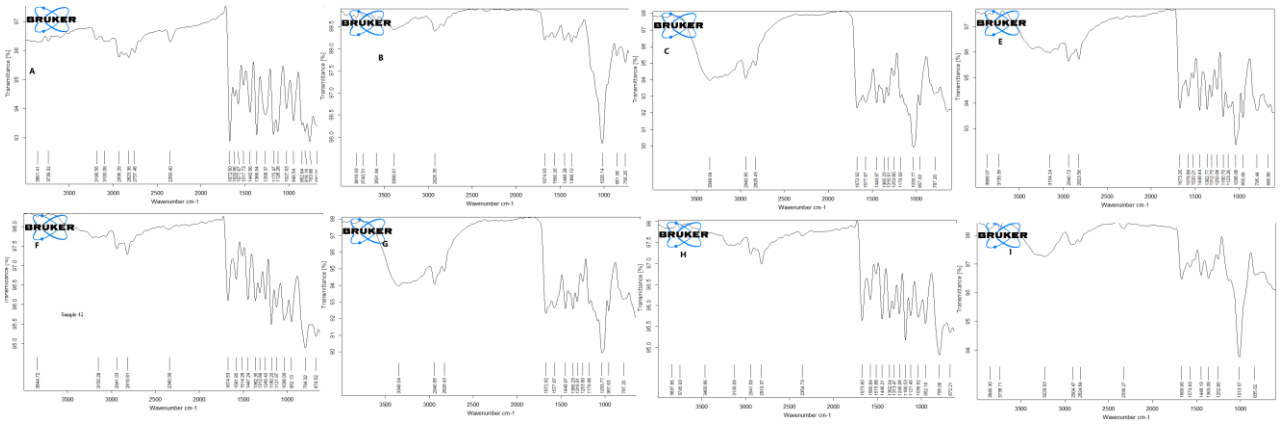

Fig. 2: FTIR scans of inclusion complex with ARI (A), inclusion complex with MβCD and ARG in the molar ratio of 1:1:1 (B) and 1:9:1 (C) by solvent evaporation (D), 1:1:0.27 by lyophilization. Each FTIR graph shows a red or blue shift. FTIR scans of complex with ARI with HPβCD and ARG in the molar ratio of 1:1 SE (E) and 1:1:1 (F) by solvent evaporation (G), 1:1:0.27 and 1:9:1 (H) by lyophilization. A clear bond shifting is obvious from the graphs.

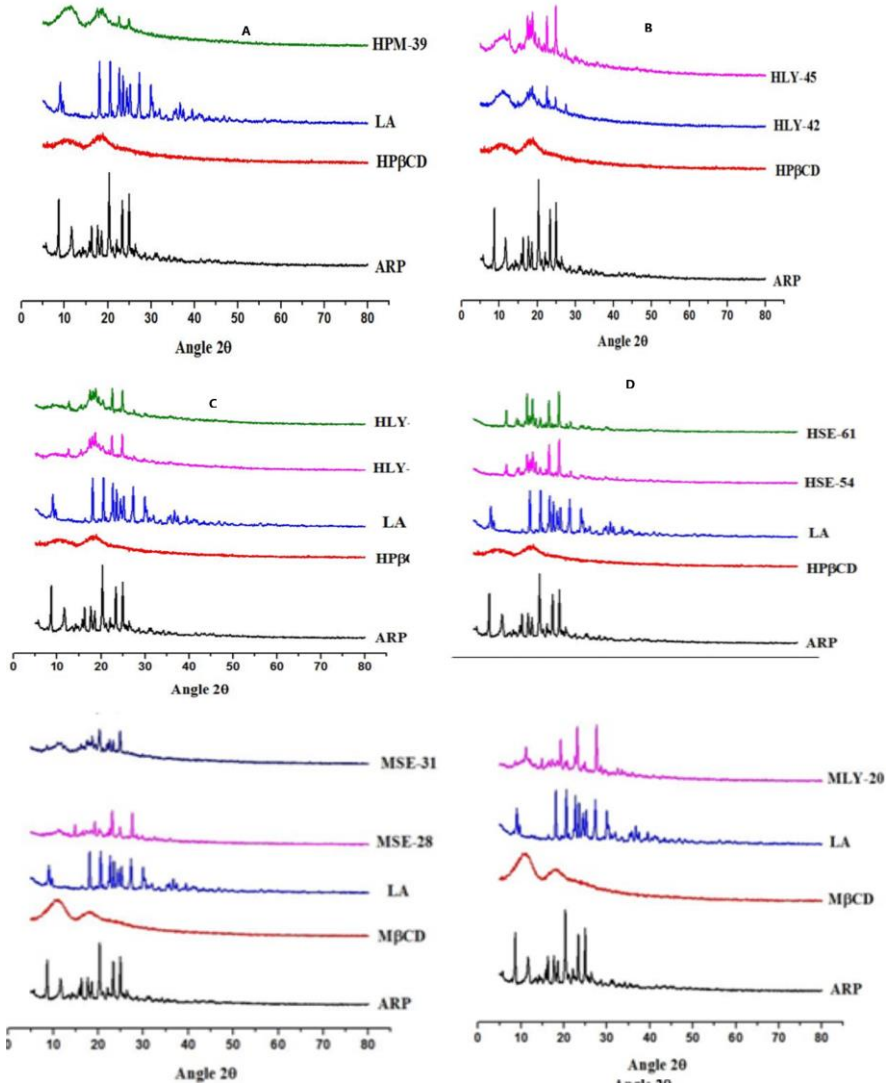

Fig. 3: Comparison of XRD graphs showing the change in bond angles showing that the corresponding peaks of aripiprazole, amino acid and CDs are present with minor bond shifting. This may leads to the conclusion that the inclusion complexes are successfully made.

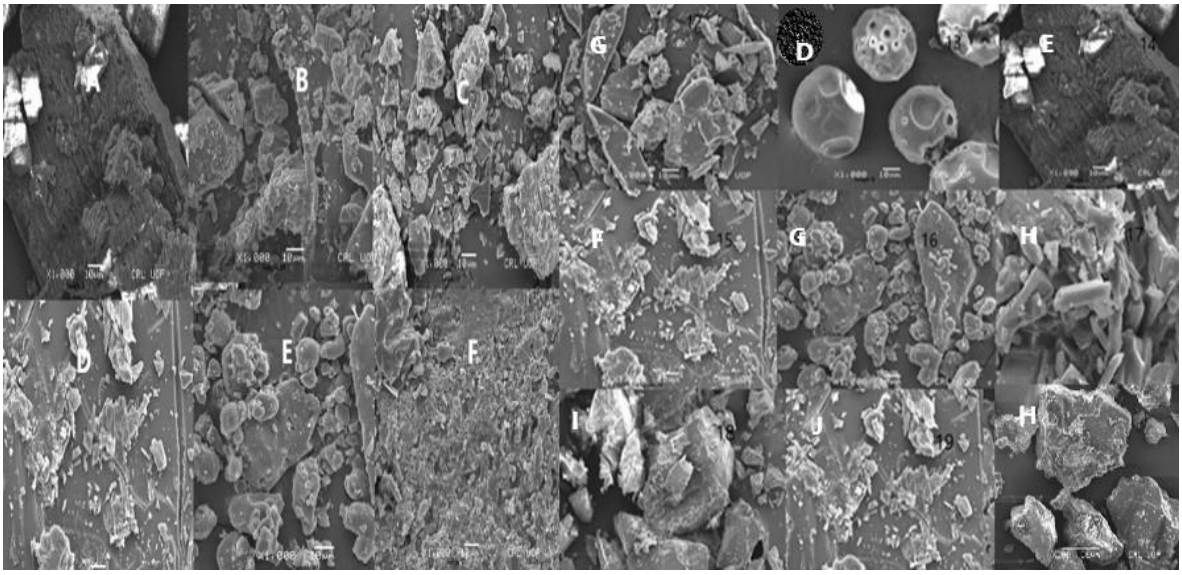

Fig. 4: (A) ARI (B) ternary inclusion complex by PM (C) LY binary complex (D) LY ternary complex (E) binary inclusion complex by SE (F) ternary inclusion complex by SE and (A) ARI (B) HPβCD (C) LA (D) HPM35 (E) HSE55, (F) HLY42 (G) HPM40 (H) HSE57 (I) HLY47. Complete change of drug morphology occurred after the formulation of inclusion complexes (Santos *et al.,* 2023). Hexagonal shape of ARI cannot be seen. Some of the complex is filled in the CD molecule while some are adsorbed on the surface as shown in fig. 5 and 6. All the formulations are optimized.

In case of inclusion complexes prepared by MβCD and ARG, the weight loss of unstable compounds was illustrated in a 3-step fashion in an inert atmosphere containing N2.

Initially, 10% was observed at T10%, which was gradually raised to 277±4°C for MPM1; 123±5°C for MPM6; 143±3°C for MPM9, 47±1.5°C for MLY13 and 246±7°C for MLY17, respectively as shown in fig. 5. After moisture loss, second weight loss (endothermic peak) was monitored at T max (335±4.5°C), which was found to be 50±2.5% for MPM1; 55±3.3%, for MPM6; 51±1.4% for MPM9; 71±4.2% for MLY13 and 66±2.2% for MLY17, respectively as shown in fig. 5. The measurement of residual weight at 490°C showed no significant differences among all the samples. As the ratio of LA increased from 0.27 to 3.6, there was a gradual reduction in peak intensity throughout the range, with an exception being a notable increase at 11.55. The sequence of increasing thermal stability of ARI with MβCD and ARG is 16>17>2>9>6>12.

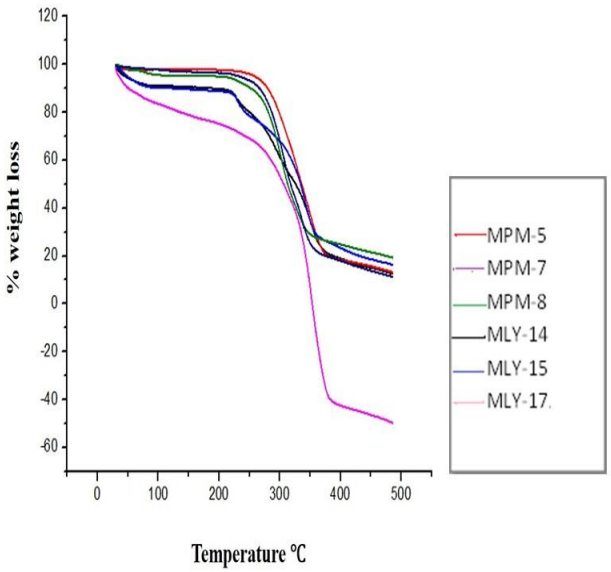

Fig. 5: Thermo grams of ARI with MβCD along with ARG which shows optimized preparation in terms of thermal stability. This makes MLY-17 the best candidate for the enhanced solubility along with stability. The lyophilization is hence the best method for the preparation of inclusion complexes. Even MLY-14 is also better in terms of stability although its solubility is less than MLY-17. The stability of binary and ternary inclusion complexes prepared by physical mixing are better than the drug alone but are comparable to lyophilized complexes.

DISCUSSION

In the context of HPβCD, we observed a consistent stability pattern similar to that of MβCD and ARG samples. In fig. 5, we generated TGA curves representing various combinations of pure constituents. DSC analysis confirmed that ARP was in a crystalline, anhydrous state, and TGA analysis indicated its initial decomposition starting at 220°C (Hamidon *et al*., 2024).

We employed TGA to delve deeper into the thermal behavior of both the ARP-HPβCD and ARP-HPβCD-LA

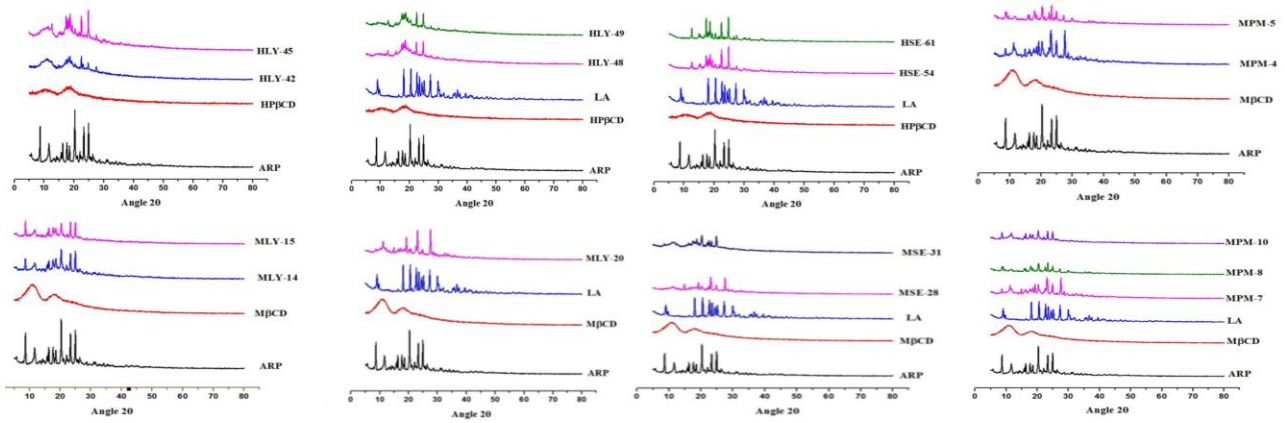

Fig. 6: Comparison of XRD graph of inclusion complexes prepared by HPβCD and MβCD with ARG. The HLY-42 and HLY-47 are the most optimized preparations. The peaks demonstrate that the peak intensity of the complexes is generally reduced for all the complexes, due to the expected effect of cyclodextrin. The HLY samples are superior but when further a ternary agent is added the amorphous nature is reduced to a greater extent.

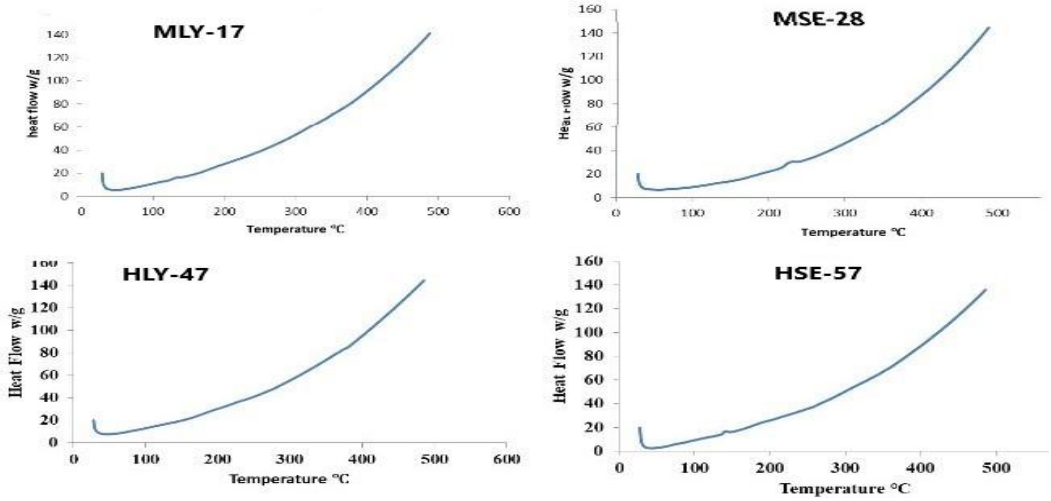

Fig. 7: DSC Graphs of selected inclusion complexes MLY17 (A), MSE28 (B), HLY47 (C) and HSE57 (D) showing optimized preparations. HLY 47 has no crystalline peak making it completely amorphous.

ternary systems, which had previously been characterized through DSC analysis. In the case of the ARP-HPβCD physical mixture, we observed a mass loss occurring between 35°C and 100°C due to the release of amino acid H2O and a Tonset of 208°C attributed to thermal decomposition. However, this dehydration effect was absent in the evaporated inclusion complexes or the lyophilized inclusion complexes obtained after treating the sample. The TGA curves of these evaporated or lyophilized inclusion complexes exhibited a similar behavior, as depicted in fig. 9. Conversely, despite the temperature at which the medication melts, no mass losses were observed.

The TGA curves of all the lyophilized samples reflected the substantial increase in the onset temperature of ARI, supporting increased thermal stability. ARI completely encapsulated by polymer and amino acid had hydrogen bonding with the polymer changes from crystalline to amorphous forms, with lyophilized samples showing superior properties to other preparation methods (Liu *et al*., 2024). Binary complexes exhibited reduced peaks for ARI at specific angles, but lyophilization led to merged peaks. Ternary complexes displayed higher amorphousness, especially those with increased LA ratios (Awais *et al*., 2023). The physical mixture of the binary complexes of HPβCD with the ARI demonstrated reduced peaks for the ARI at 8.65, 23.35 and 24.90. (Liu *et al*., 2024). However, when employing the lyophilization method, the binary complexes formed between MβCD and ARI exhibited combined peaks for the drug component at 8.65, 10.95 and 11.2. In contrast to the physical mixtures, the peaks at 14.8 and 27.55 remained present without disappearing. Nonetheless, the overall distinct peak intensities of the drug were diminished, indicating the amorphous nature of the binary system. Binary complexes exhibited reduced peaks for ARI at specific angles, but lyophilization led to merged peaks.

Ternary complexes displayed higher amorphousness, especially those with increased LA ratios. The physical mixture of the binary complexes of MβCD with the ARI demonstrated reduced peaks for the drug at 8.65, 23.35 and 24.90. The higher degree of amorphous nature of MβCD caused the crystalline peak of the drug to be reduced in their binary inclusion complex. (fig. 8) But by the lyophilization method, the binary complexes of MβCD with the ARI had demonstrated merged peaks for the ARI at 8.65, 10.95 and 11.2. The peaks at 14.8 and 27.55 have not vanished compared to the physical mixtures as shown in fig 5. But the distinct peak intensities of the drug are reduced, demonstrating the binary system amorphousness.

On the other hand, the peak at 24.90 exhibited a higher intensity compared to the physical mixtures. When the AR ratio was increased from 0.27 to 3.6, there was a gradual reduction in peak intensity, although there was a notable spike at 11.55 (Awais *et al*., 2022). The ratio of 1:3.6:3.6 showcased the most pronounced amorphous characteristics when contrasted with the physical mixtures of the same ratio for both polymers, albeit at varying intensities with coordinated water removal at higher temperatures, leading to non-isolable complexes (Sweed *et al.*, 2024).

The XRD and DSC analyses demonstrated the transformation of crystalline to amorphous forms in inclusion complexes as shown in fig. 6 and 7. Lyophilization showed improved results and ternary complexes with specific ratios exhibited enhanced amorphousness and solubility (Garbiec *et al*., 2023). DSC analyses supported complex formation and water removal, leading to non-isolable complexes in fig. 9 and 10. The complexation with MβCD was better than HPβCD. Arginine enhances the solubilization capacity synergistically with MβCD making 1:1:0.27 lyophilized products a promising candidate for drug designing.

CONCLUSION

The successful formation of inclusion complexes involving ARP, MβCD/HPβCD and LA demonstrated a synergistic effect in various preparation methods. The choice of preparation method played a pivotal role (Liu *et al*., 2024), with solvent evaporation yielding better results than physical mixing. Inclusion complexes specifically prepared from lyophilization method significantly enhances the stability and dissolution rate that could lead to a more soluble drug withy good stability. Adding ARG as an auxiliary substance led to a noteworthy 80% increase in dissolution rate, reducing ARI and MβCD/HPβCD amounts for enhanced cost-effectiveness and reduced side effects. The optimal complex ratio was 1:1:0.27 and any deviations led to decreased solubility and stability.

At the end of the research, we could find the inclusion complex having any of the two polymers (MβCD, HPβCD) gave better solubility and stability than the parent drug. Ternary complexes with ARG gave better results than the binary complexes of both with either HPβCD or MβCD in a minimum ratio (1:1:0.27), and superior amino acid is Arginine, not Lysine. The best method of preparation is lyophilization that gave maximum solubility and stability. So, ternary inclusion complexes made with HPβCD or MβCD along with arginine are good candidate for the drug designing in the future. The MβCD is superior to HPβCD, when arginine is used. This will make the drug cost effective and with less side effect as the pure aripiprazole.

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