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Development of water-soluble ternary system for enhancing biological activities of mefenamic acid

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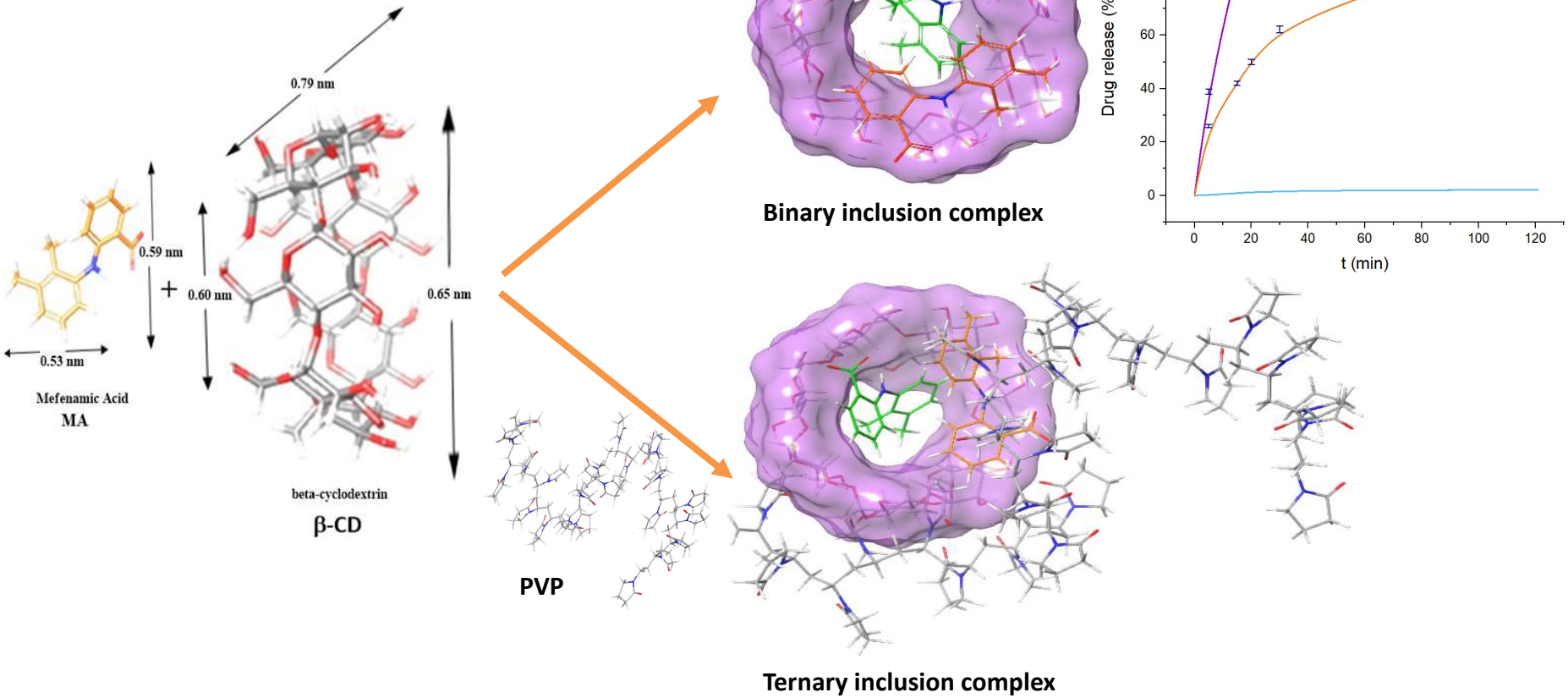
Development of water-soluble ternary system for enhancing biological activities of mefenamic acid

Graphical Abstract



Abstract:

The aim of this work is to investigate the solubility enhancement of mefenamic acid (MA), a non-steroidal anti-inflammatory agent, by formation of stable amorphous ternary system (MA, polyvinylpyrrolidone (PVP), β-cyclodextrin (β-CD)) compared to the binary system (MA, β-CD). Firstly, on the basis of the molecular docking simulation and job's plot results, three methods were adopted for the preparation of the binary inclusion complexes at the ratio of 2:1 of MA:β-CD, namely solvent co-evaporation (CE), kneading (KN) and physical mixture (PM). However, in order to decrease tendency to self-assembly of cyclodextrins and form aggregates in aqueous media, each binary system was co-milled at ambient temperature in presence of different ratios of a highly water-soluble polymer (PVP). These complexes were characterized using Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectroscopy and scanning electron microscopy (SEM) techniques. The release of the drug from the diverse formulations was also investigated by means of UV-VIS spectroscopy. Finally anti-inflammatory and anti-nociceptive activities were performed. The results showed that the solubility of MA in water from ternary complexes was significantly improved.

Keywords: Mefenamic acid; β-cyclodextrin; polyvinylpyrrolidone; solubility; *in vivo*.

Introduction

- ✓ **Physicochemical properties** and **ADME** (Absorption, Distribution, Metabolism, Elimination) **parameters** of a drug substance are essential to ensure the accessibility to its target(s).

- ✓ Only the **free part of the drug substance** diffuses in tissues from the blood and then can access to the active binding site of the desired target.

- ✓ Among all parameters to control for a successful treatment, an **acceptable level of drug substance solubility** is required.

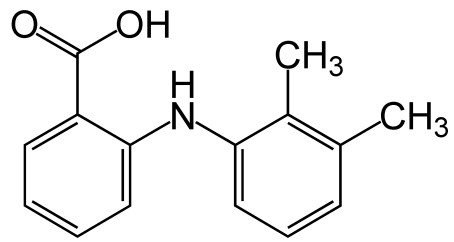
- ✓ In the case of low solubility drug substances, **inclusion complexes** with β-CD can be an interesting alternative to facilitate drug administration.

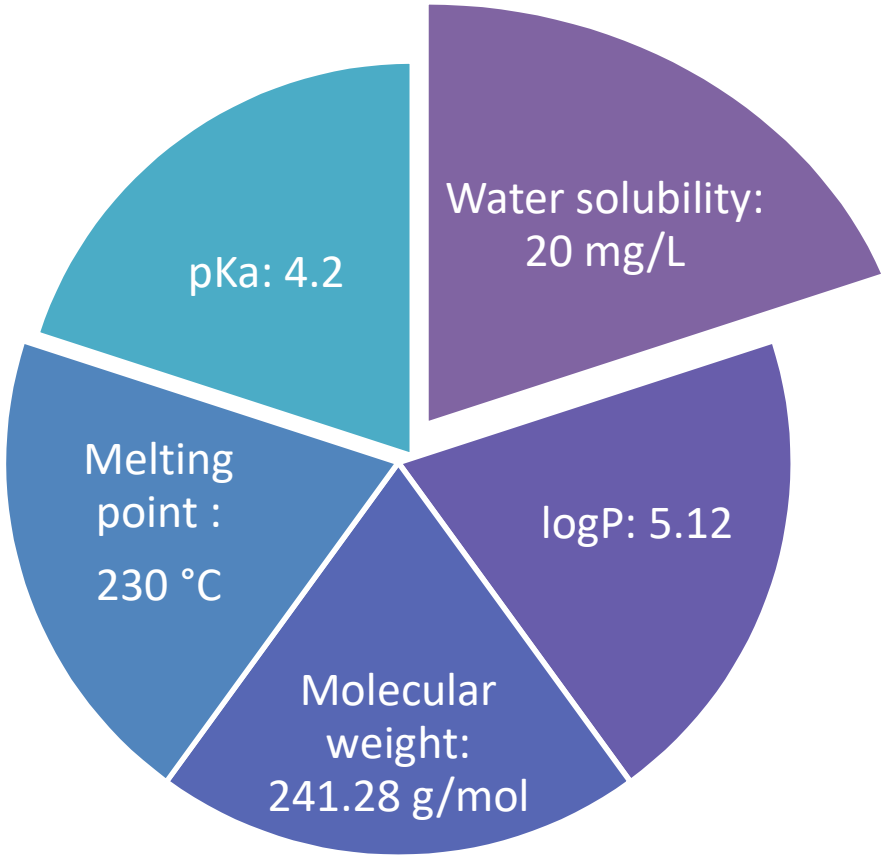
- ✓ **To increase complexation and to optimize solubilization**, addition of small quantities of a suitable hydrophilic polymer to a drug:β-CD system is very favorable.

Introduction

- ✓ **Mefenamic acid (MA)** and its physicochemistry

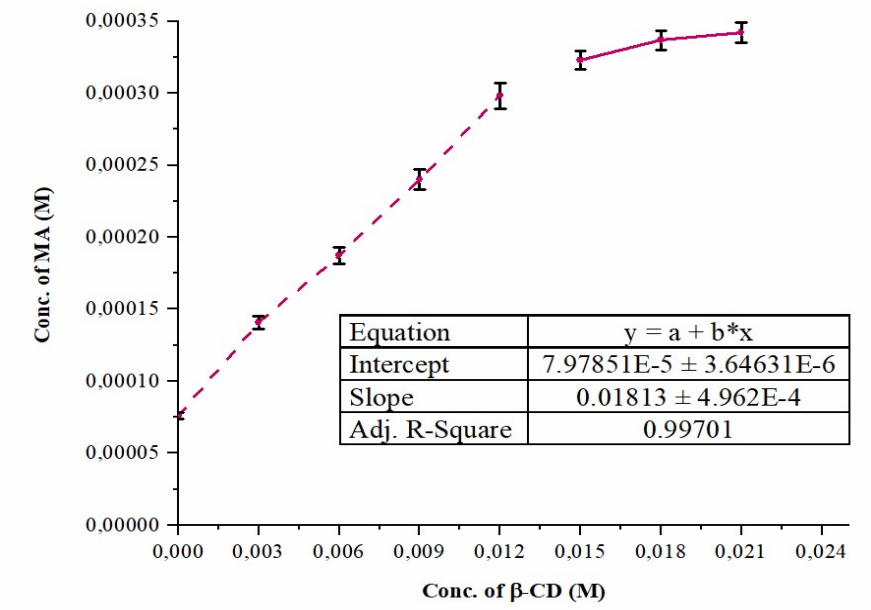
- ✓ 2-[(2,3-dimethylphenyl)amino]benzoic acid.
- ✓ A potent non-steroidal anti-inflammatory drug (NSAID).



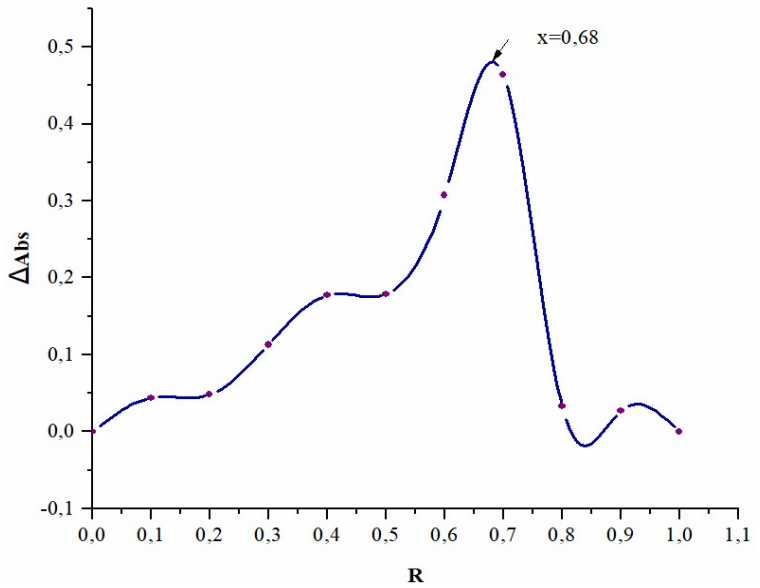


Results and discussion – *Determination of complex stoichiometry*

Phase solubility diagram



Continuous variation plot (Job's plot)



✓ β-CD formed A_N-subtype complexes with MA. This fact implies that β-CD is proportionally less effective at higher concentrations.

✓ The maximum Δ_{Abs} variation was observed at mole fraction value of 0.67. This suggests a 2:1 stochiometric ratio of MA:β-CD.

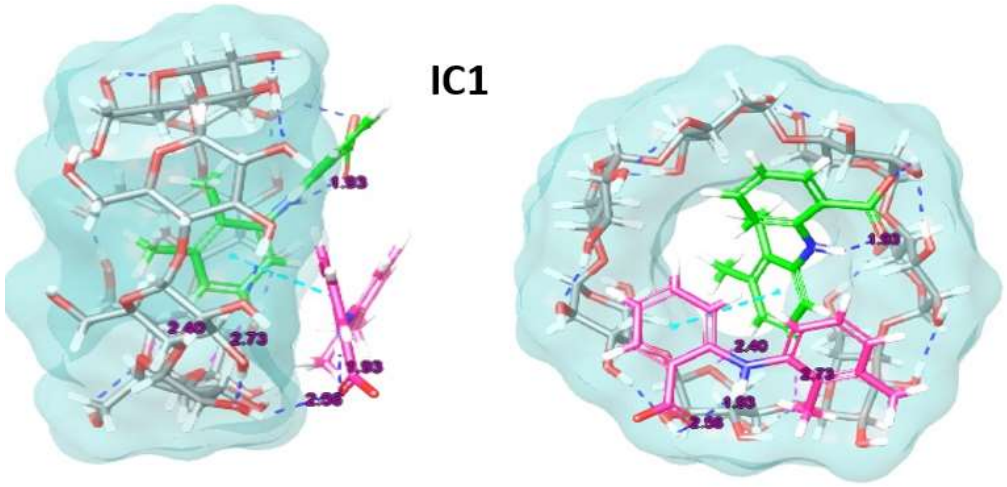
Results and discussion – *In silico molecular modeling studies*

ΔG values in Kcal.mol^{-1}						
Ratio (MA:β-CD)	Bind[a]	Docking score	Glide Lipo[b]	Glide vdw[c]	Glide Hbond[d]	Glide Emodel
1:1 (IC2)	-21.468	-4.575	-2.179	-21.123	-0.042	-26.612
2:1 (IC1)	-37.698	-7.890	-3.454	-37.387	-0.202	-46.262
1:2 (IC3)	-35.995	-7.375	-3.139	-28.990	-0.160	-50.976

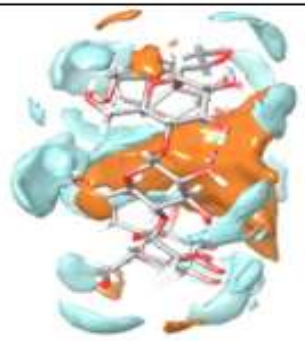
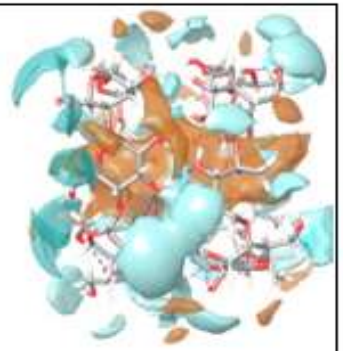
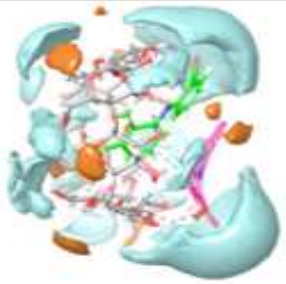
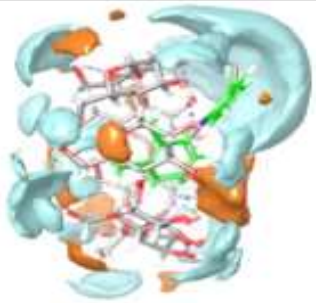
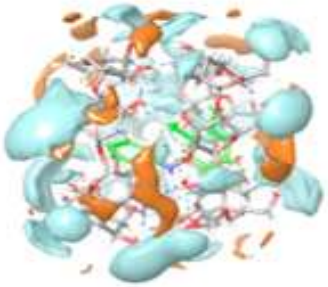
[a] free energy of binding (Glide energy); [b] free energy of binding from lipophilic binding;[c] free energy of binding from van der Waals energy; [d] free energy of binding from hydrogen bonding.

- ✓ **IC1** - (2:1) MA:β-CD - is the most stable complex (highest docking score and glide energy).
- ✓ **Contribution of Van der Waals** is the most important.

- ✓ **Introduction of a second MA molecule** in the primary complex enhances the stability (further hydrogen electrostatic interaction and improved filling of the β-CD cavity)



Results and discussion – *In silico molecular modeling studies*

		
MA Phobic 5.28 CÅ Phillic: 234.95 CÅ	**β-CD** Phobic: 264.75 CÅ Phillic: 348.44 CÅ	**2 β-CD** Phobic: 371.46 CÅ Phillic: 610.83 CÅ
		
IC1 Phobic: 89.80 CÅ Phillic: 692.85 CÅ Dielectric (solvation) energy: -118.67 Kcal.mol^{-1}	**IC2** Phobic: 137.09 CÅ Phillic: 525.07 CÅ Dielectric (solvation) energy: -115.89 Kcal.mol^{-1}	**IC3** Phobic: 552.53 CÅ Phillic: 727.03 CÅ Dielectric (solvation) energy: -162.72 Kcal.mol^{-1}

- ✓ **Hydrophobic** (brown) and **hydrophilic** (blue) **surface areas** of MA, β-CD and MA:β-CD binary inclusion complexes (CÅ, cubic Ångströms).

- ✓ **Hydrophilic area** increases upon formation of IC1 ((2:1) MA:β-CD).
- ✓ **Higher polar surface area** of the supramolecular inclusion complex improved the MA solubility.

Results and discussion – *Preparation of solid complexes and loading*

✓ According to job's plot results and the molecular docking observations, **(2:1) MA:β-CD complexes** were prepared by three methods

- Physical mixture (PM)
- Kneading (KN)
- Co-evaporation (CE)

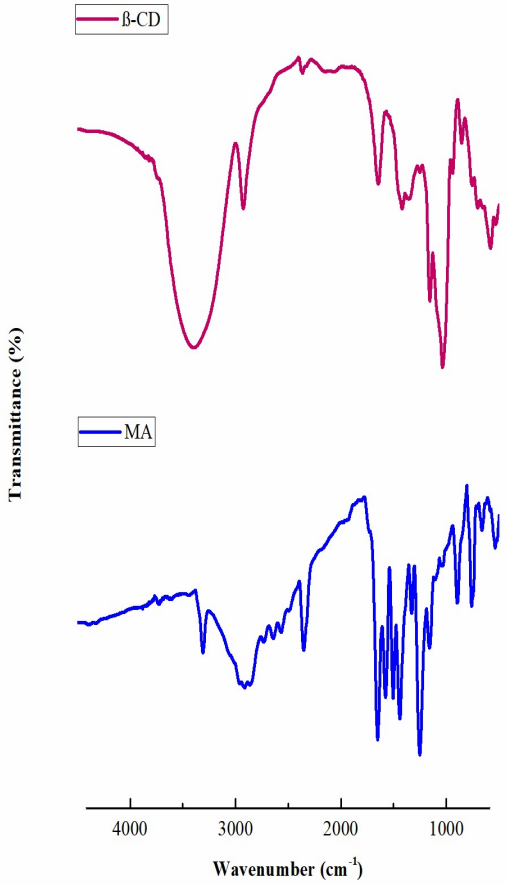
Complex	CE
Experimental yield (%)	90.1
Drug content (%)	28.96 ± 0.39
Experimental molar ratio MA:β-CD	1.9:1

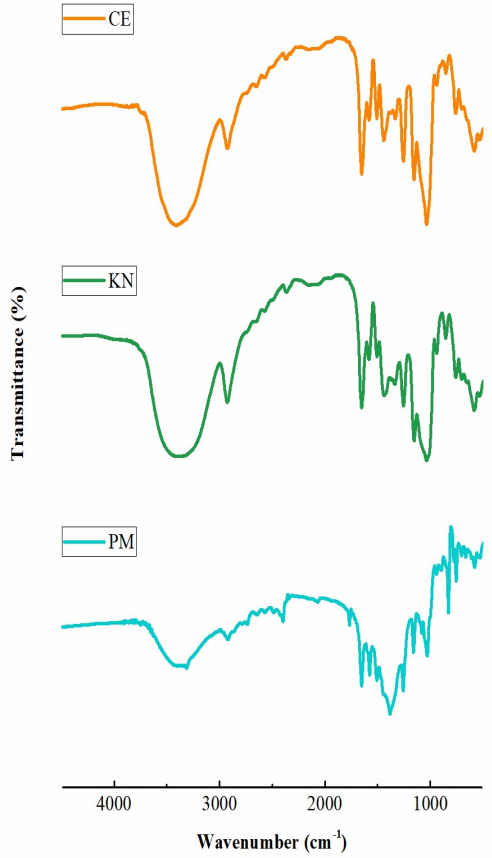
✓ With an experimental loading value of 28.96%, the ratio of (2:1) MA:β-CD complex was also confirmed.

Results and discussion – *Characterization of the ingredients and their complexes*

FTIR

- ✓ The presence of chemical incompatibility among pure MA and β-CD is ruled out.
- ✓ A good indication of the inclusion complex formation in the FTIR spectra for the KN and CE complexes.
- ✓ No new chemical bonds are formed with the obtained complexes.

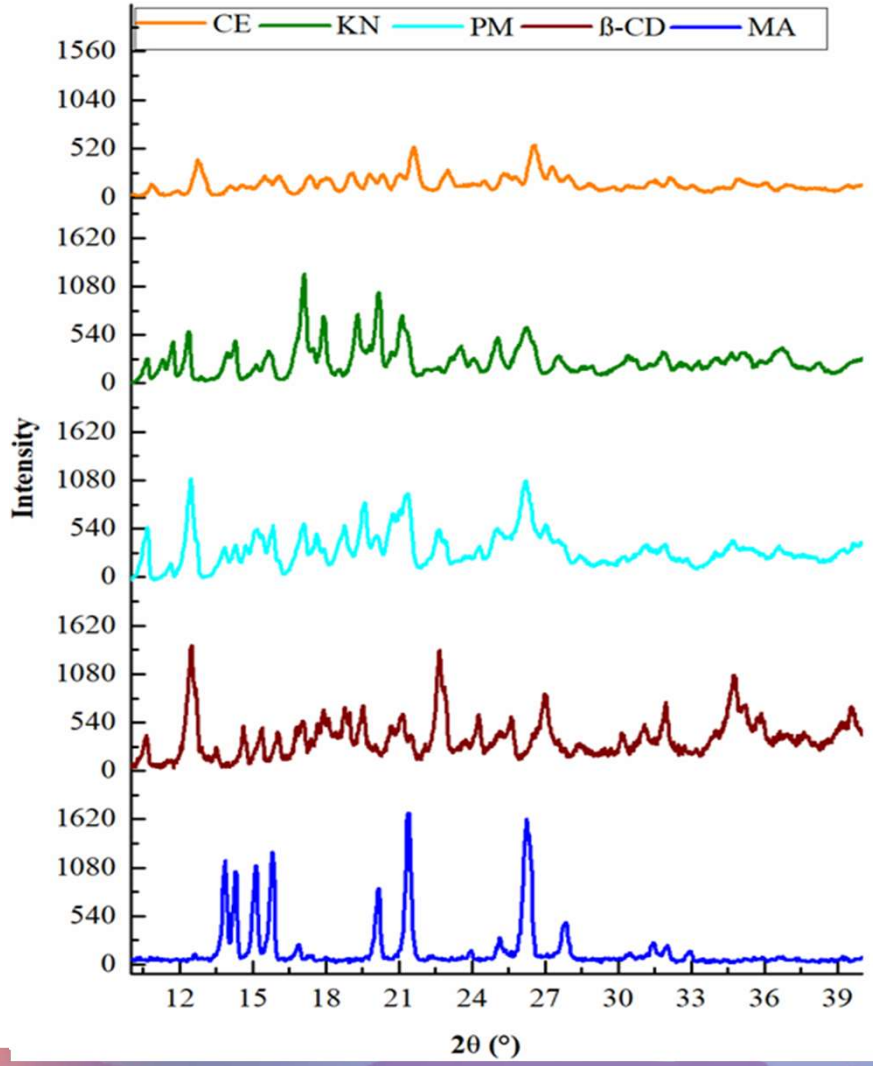




Results and discussion – *Characterization of the ingredients and their complexes*

PXRD

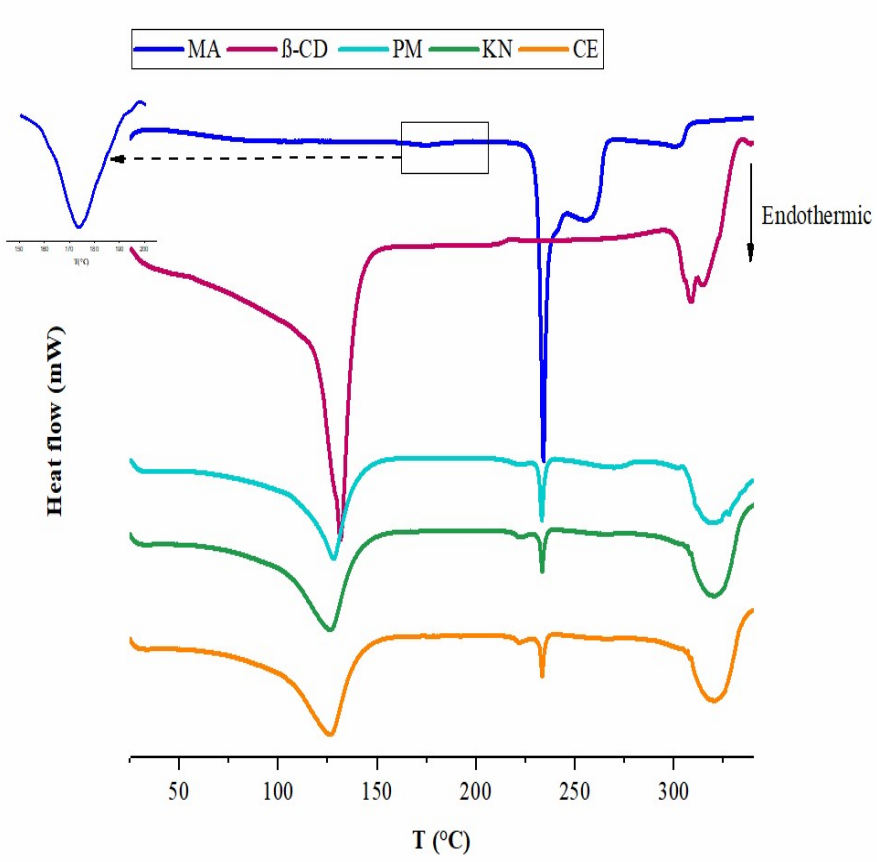
- ✓ The **same diffraction peaks** of MA and β-CD clearly appear **in PM**, which indicates the absence of interaction between them.
- ✓ **Loss of the crystalline nature of MA** in the KN and CE complexes suggests partial inclusion of MA in the β-CD cavity.



Results and discussion – *Characterization of the ingredients and their complexes*

DSC

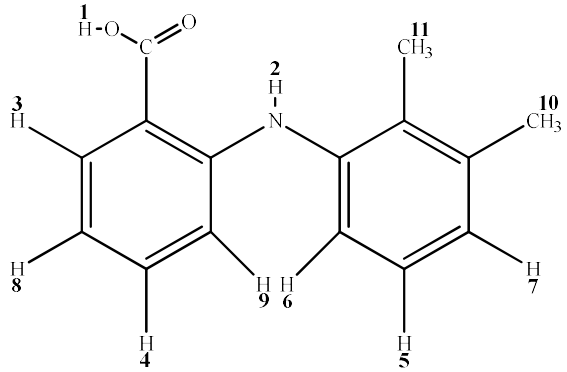
- ✓ The **presence of the endothermic peaks** of the two individual components at their corresponding temperatures indicates the absence of chemical interaction between them.
- ✓ The **thermogram of binary inclusion complexes** illustrates the characteristic endothermic peak of the drug with reduced sharpness and intensity as compared to the pure drug, indicating an incomplete inclusion of the drug in the β-CD cavity.



Results and discussion – *Characterization of the ingredients and their complexes*

^{1}H NMR

MA Protons	δ_{guest}	CIS (CE)	CIS (KN)	CIS (PM)
H-1	12.98	-2.23	-	0
H-2	9.45	1.30	0.72	0
H-3	7.89	0	0	0
H-4	7.31	-0.18	-0.09	0
H-5	7.12	-0.10	0.01	0
H-6	7.03	-0.21	0.05	0
H-7	6.72	-0.09	-0.05	0
H-8	6.70	-0.09	-0.05	0
H-9	6.68	-0.08	-0.05	0
H-10	2.29	-0.03	-0.01	0
H-11	2.10	0.01	0.01	0

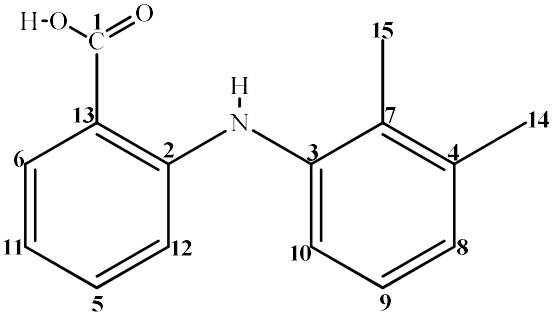


- ✓ **^{1}H NMR Chemical shifts** for CH protons of MA alone (δ_{guest}) and their complexation induced shifts (CIS = $\delta_{complex} - \delta_{guest}$) in DMSO-$d_6$ at 25 °C.
- ✓ The **OH and NH functions** of guest participate in the formation of the inclusion complex leading to a more stable complex (CE).

Results and discussion – *Characterization of the ingredients and their complexes*

^{13}C NMR

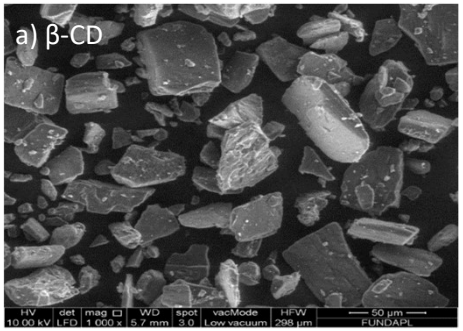
MA Carbons	δ_{guest}	CIS (CE)	CIS (KN)	CIS (PM)
C-1	170.66	-	0.34	0
C-2	149.22	-2.2	-1.43	-0.02
C-3	138.81	1.4	0.57	0
C-4	138.35	-0.93	-0.72	0
C-5	134.66	-2.76	-2.22	0
C-6	132.17	-1.16	-0.35	0
C-7	131.71	-2.64	-1.68	-0.02
C-8	129.89	-1.26	-1.08	-0.01
C-9	126.49	-2.22	-1.28	0.01
C-10	122.66	-	-2.31	-0.02
C-11	116.71	2.16	-0.6	0.01
C-12	113.55	2.43	-0.62	-0.01
C-13	111.69	1.11	-	-0.03
C-14	14.14	-0.45	-0.45	0
C-15	20.69	-0.31	-0.38	0.01

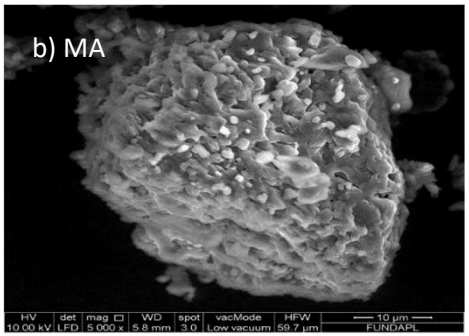


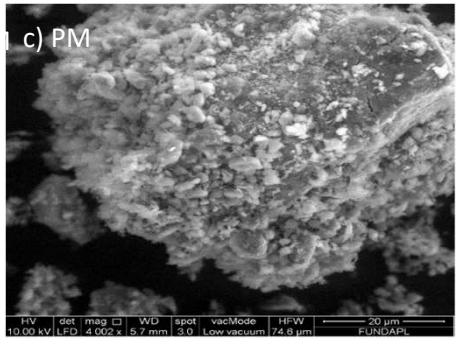
- ✓ **^{13}C NMR Chemical shifts** for carbons of MA alone (δ_{guest}) and their complexation induced shifts (CIS = $\delta_{complex} - \delta_{guest}$) in DMSO-$d_6$ at 25 °C.
- ✓ **All the carbon atoms of MA** show an upshift, the maximum corresponds to C1 of guest which confirms that C=O is involved in the interaction.
- ✓ By **CE method**, the most stable complex is obtained.

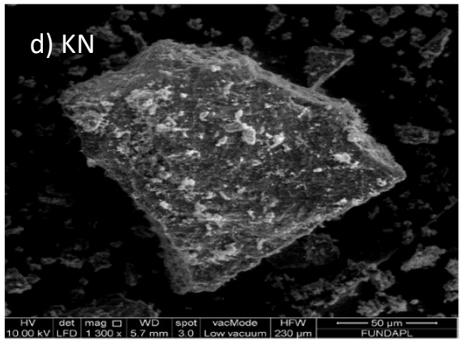
Results and discussion – *Characterization of the ingredients and their complexes*

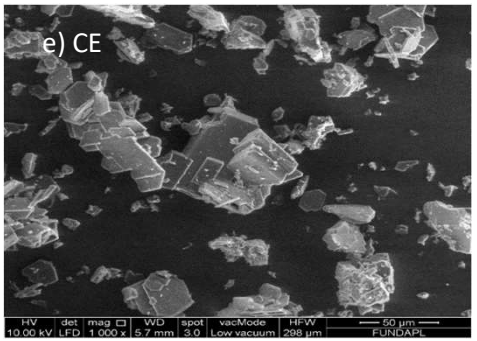
SEM





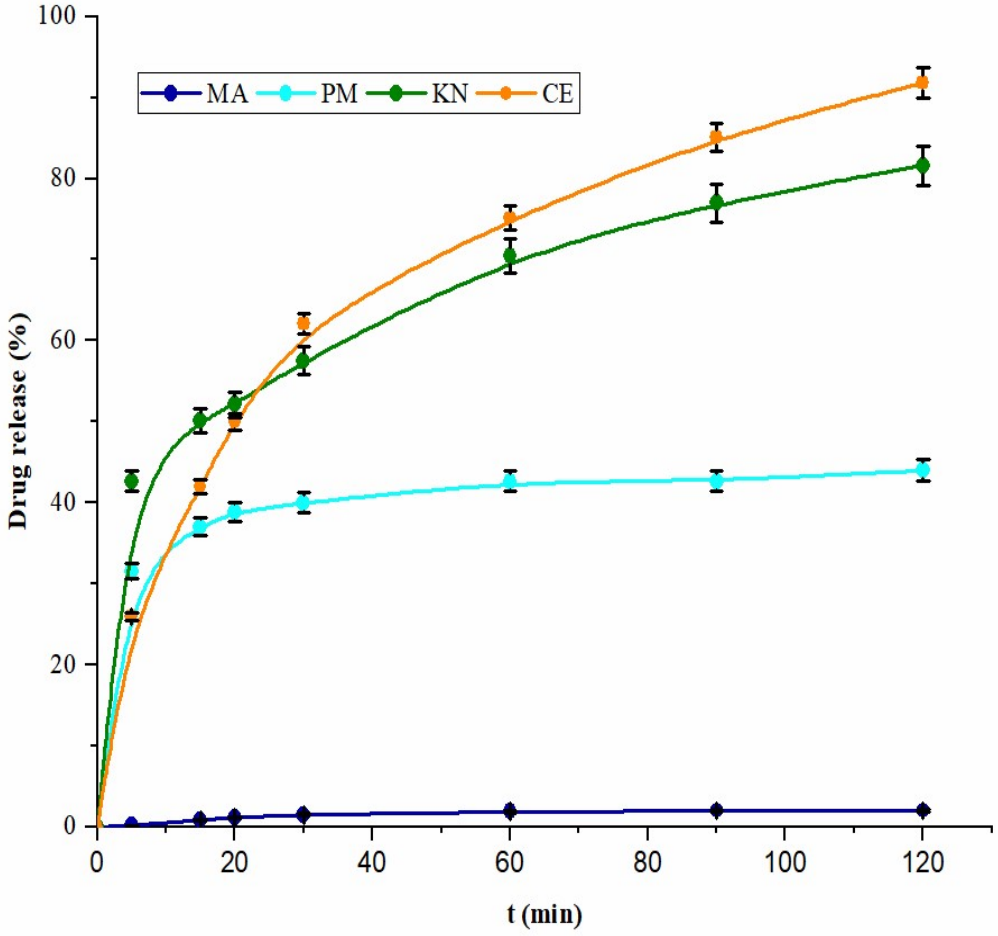






- ✓ **MA crystals** are adhered to smooth surface of β-CD (**in PM**).
- ✓ **In KN complex**, a reduction in the agglomerated drug on the surface of β-CD is observed.
- ✓ **In CE complex**, the crystal nature of MA disappeared. Micrographs show small, more agglomerated, and amorphous smooth structures, which suggest that MA is well dispersed in the β-CD cavities.

Results and discussion – *In vitro drug release test*



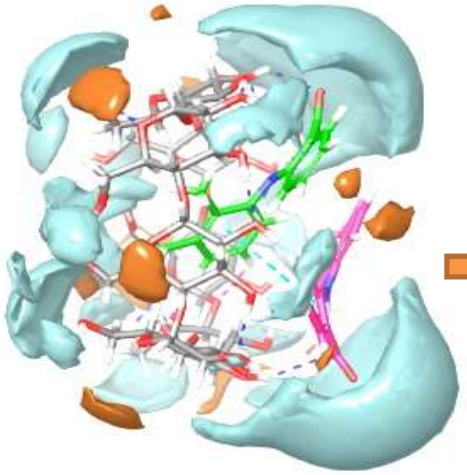
- ✓ The **improved MA dissolution characteristics of the PM complex** may be explained by the drug wettability enhancement.
- ✓ **The greater enhancement of MA dissolution in KN and CE complexes** is due to partial trapping of the drug in β-CD verified by molecular modeling, FTIR, DSC, XRPD and SEM.

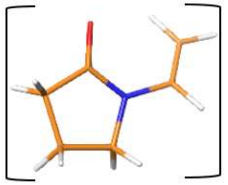
Results and discussion – *Mathematical models of drug release kinetics*

MA:β-CD	First order		Higuchi			Kosmeyer-Peppas		
	R^2	K_1 (min^{-1})	R^2	K_H x100 ($min^{-1/2}$)	a	R^2	K_{KP}	n
PM	0.6553	0.0022	0.8363	1.2367	31.7150	0.9424	3.5851	0.0997
KN	0.9113	0.0054	0.9934	4.6504	32.2330	0.9784	3.4922	0.2154
CE	0.7551	0.0090	0.9688	7.4297	14.7730	0.9832	6.8865	0.3980

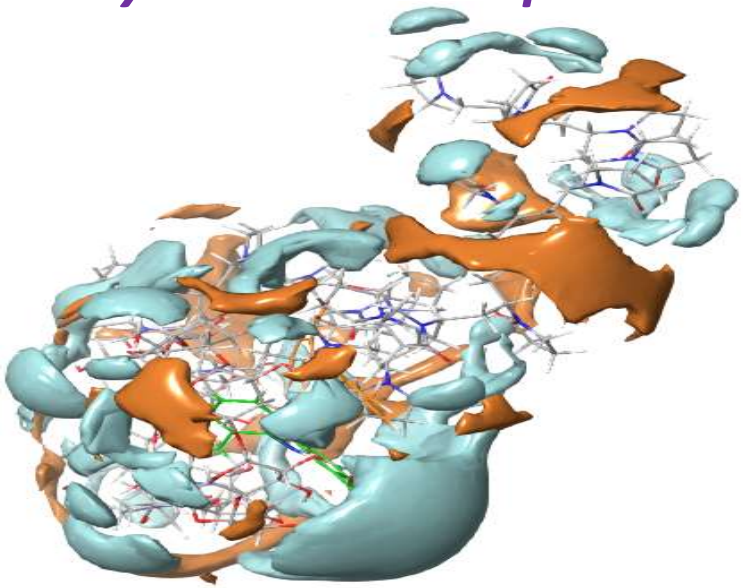
- ✓ **CE formulation presented a higher value of K_H** which indicated the complex formation and the enhancement of drug dissolution compared to other formulations.
- ✓ The **exponent (n)** of the Korsmeyer–Peppas model indicated that the drug release is related to a quasi-Fickian diffusion (n < 0.5).

Results and discussion – *Preparation of ternary inclusion complexes (TIC)*





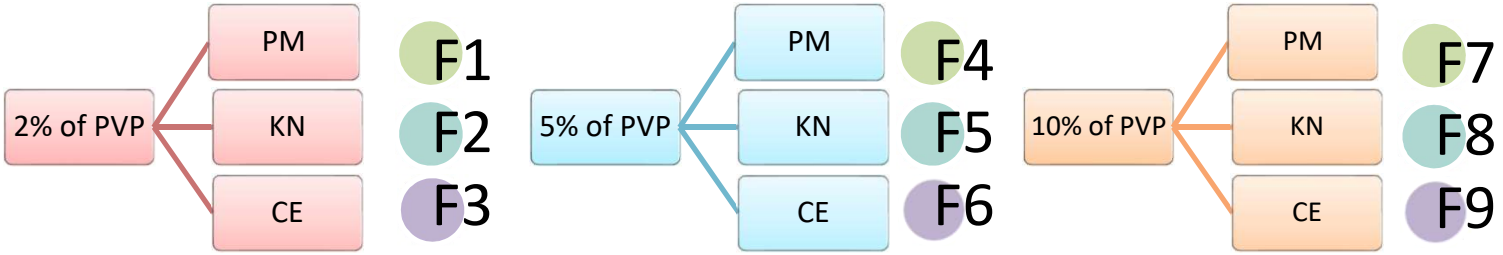
Polyvinylpyrrolidone (PVP)



Binary inclusion complex (BIC)

Ternary inclusion complex (TIC)

✓ Each binary system was co-milled at ambient temperature in presence of different ratios of a highly water-soluble polymer (PVP).

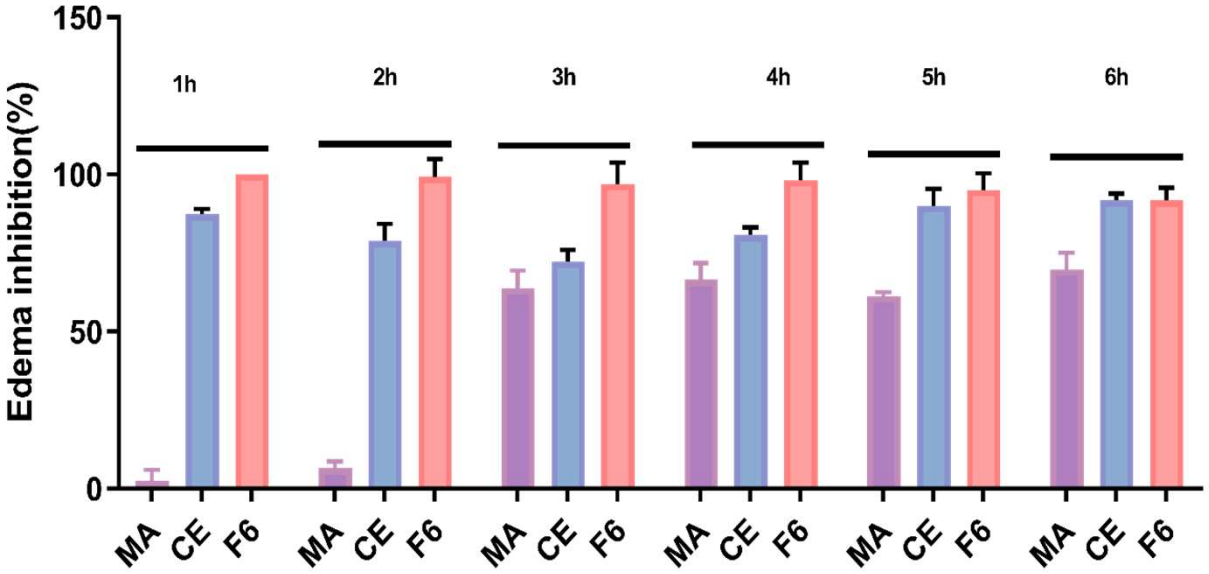


Results and discussion – *In vitro release of MA from ternary inclusion complexes*

% of PVP	2%			5%			10%		
TIC	F1(PM)	F2(KN)	F3(CE)	F4(PM)	F5(KN)	F6(CE)	F7(PM)	F8(KN)	F9(CE)
Time	120 min	120 min	120 min	90 min	30 min	20 min	120 min	120 min	90 min
% of drug release	50	90	99	100	100	100	60	92	99

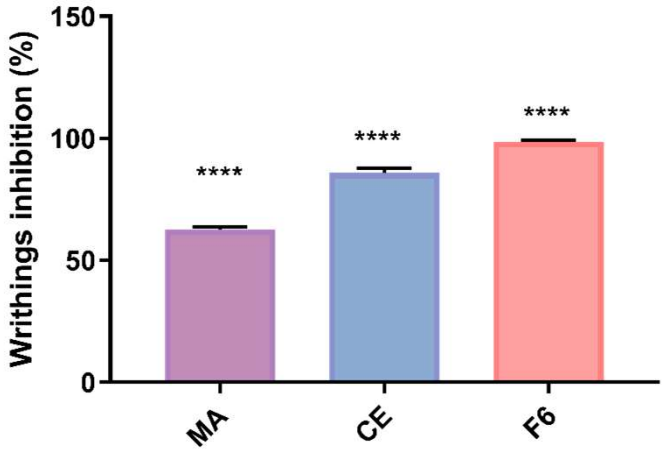
- ✓ The **CE method** is the most appropriate method to get improved MA dissolution properties of (2:1) MA:β-CD binary complex.
- ✓ The **add of 5% of PVP** to the CE binary system (F6), the dissolution of MA was significantly improved. This ternary inclusion complex constitutes the best dosage form.

Results and discussion – *In vivo evaluation*



- ✓ To **assess the *in vivo* anti-inflammatory effectiveness of the formulations** (CE and F6) in comparison with the pure drug, Carrageenan-induced rat paw edema test was used.
- ✓ The **strongest inhibitory effect** on the total edema response was observed for F6.

- ✓ **Acetic acid-induced writhing test** was uses to evaluate the analgesic activity of the formulations (CE and F6) in comparison with the pure drug.
- ✓ The **treatment with ternary inclusion complex** (F6) by oral administration remove writhing and enhancing the anti-nociceptive effect.



Conclusions

- ✓ **Phase solubility diagram** and **job's plot experiment** were used to determine the stoichiometry of the MA:β-CD complex. Molecular modeling approach helps to select the most stable inclusion complex (2:1).
- ✓ **Inclusion complexes of MA:β-CD** in the 2:1 molar ratio were prepared using PM, KN and CE methods.
- ✓ **Characterization of BIC** showed no evidence of chemical reactions between MA and β-CD.
- ✓ **Add of 5% of PVP** to the CE binary inclusion complex exhibited the highest enhancement in MA dissolution properties.
- ✓ **Ternary inclusion complex** (F6) constitutes the best dosage form. In vivo anti-inflammatory and analgesic assays confirmed the therapeutic benefits of MA when its solubility is improved.

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