Supporting Information for:

Structural diversity in native cyclodextrins/folic acid complexes – from [2]-rotaxane to exclusion compound

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1. Materials

Folic acid was commercially available product (obtained from Sigma Aldrich) and was used without further purification. α, β and γ cyclodextrins were obtained from Cyclolab and used as received. Distilled water was used for aqueous solutions. D_2O (99.9% D) was purchased from Cambridge Isotope Laboratories.

2. Preparation of the folic acid complexes

To the solution of folic acid sodium salt (66.2 mg, 1 eq. in 5ml H_2O) a solution of α CD (145 mg, 1 eq. in 1.2 mL H_2O) was added and obtained solution was stirred at RT for 48 h. The procedure was repeated for β and γ CDs (amounts used: 92.5 mg of βCD in 5.6 mL H_2O, 232 mg of g CD in 1.2 mL H_2O). The solvent was evaporated and the resulting solid was dried under vacuum and re-dissolved in D_2O for NMR experiments.

3. NMR experiments

NMR spectra were recorded on a Bruker Avance II 300 spectrometer operating at 300.17 MHz for ^1H. Standard pulse sequences were used for recording proton spectra, phase sensitive 2D ROESY with cw spinlock for mixing time (roesyph). Mixing time was set approximately

to the value of the shortest proton T_1 in a measured sample, i.e. 200 ms in the cases of β and γ CDs and 300 ms in the case of α CD.

Typical parameters for the 2D ROESY experimets were: at least 3 times of relaxation of the signal with the longest T_1 value (4.5 – 8.5 s), number of scans 128 (in the case of β CD – 32). All experiments were performed at 298 K and the sample concentration was 0.02 M/dm^3

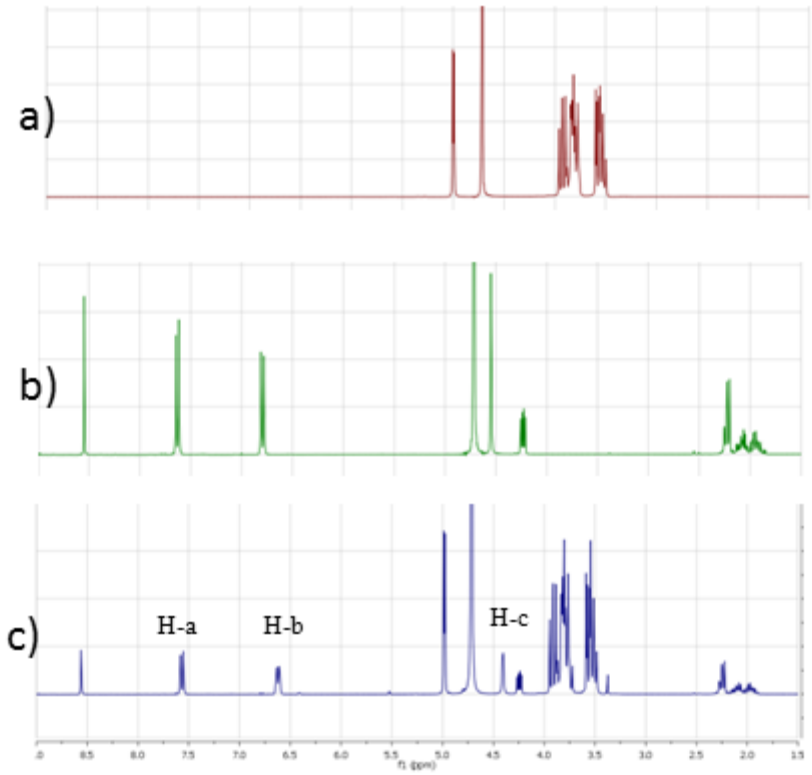

Figure 1. ^1H NMR spectra of the resultant solid dried from a) α-CD b) folic acid c) α-CD +folic acid

Protons H-1 to H-6 belong to cyclodextrin (numbering according to the well-established rules) and protons H-a-H-c belong to the guest compound (as seen in the Figure 1 in the main text)

Table 1. The proton chemical shifts (δ) and the longitudinal relaxation times (T_1) of α-cyclodextrin, folic acid, their mixture and the corresponding changes

	M	α–cyclodextrin		folic acid		α–cyclodextrin + folic acid		
		δ /ppm	T_1 /s	δ /ppm	T_1 /s	δ /ppm	T_1 /s	Δδ /ppm
H-1	D	5.00	0.46	-	-	4.98	0.44	-0.02
H-2	dd	3.58	0.78	-	-	3.56	0.75	-0.02
H-3	M	3.92	0.81	-	-	3.90	0.83	-0.02
H-4	dd	3.54	0.46	-	-	3.52	0.45	-0.02
H-5	M	3.85	0.28*	-	-	3.82	0.29*	-0.03
H-6	dd	3.81	0.28*	-	-	3.75	0.29*	-0.06
H-a	M	-	-	7.63	1.49	7.58	1.49	-0.05
H-b	D	-	-	6.79	0.90	6.62	0.90	-0.17
H-c	S	-	-	4.55	0.36	4.40	0.33	-0.15

* not resolved signals

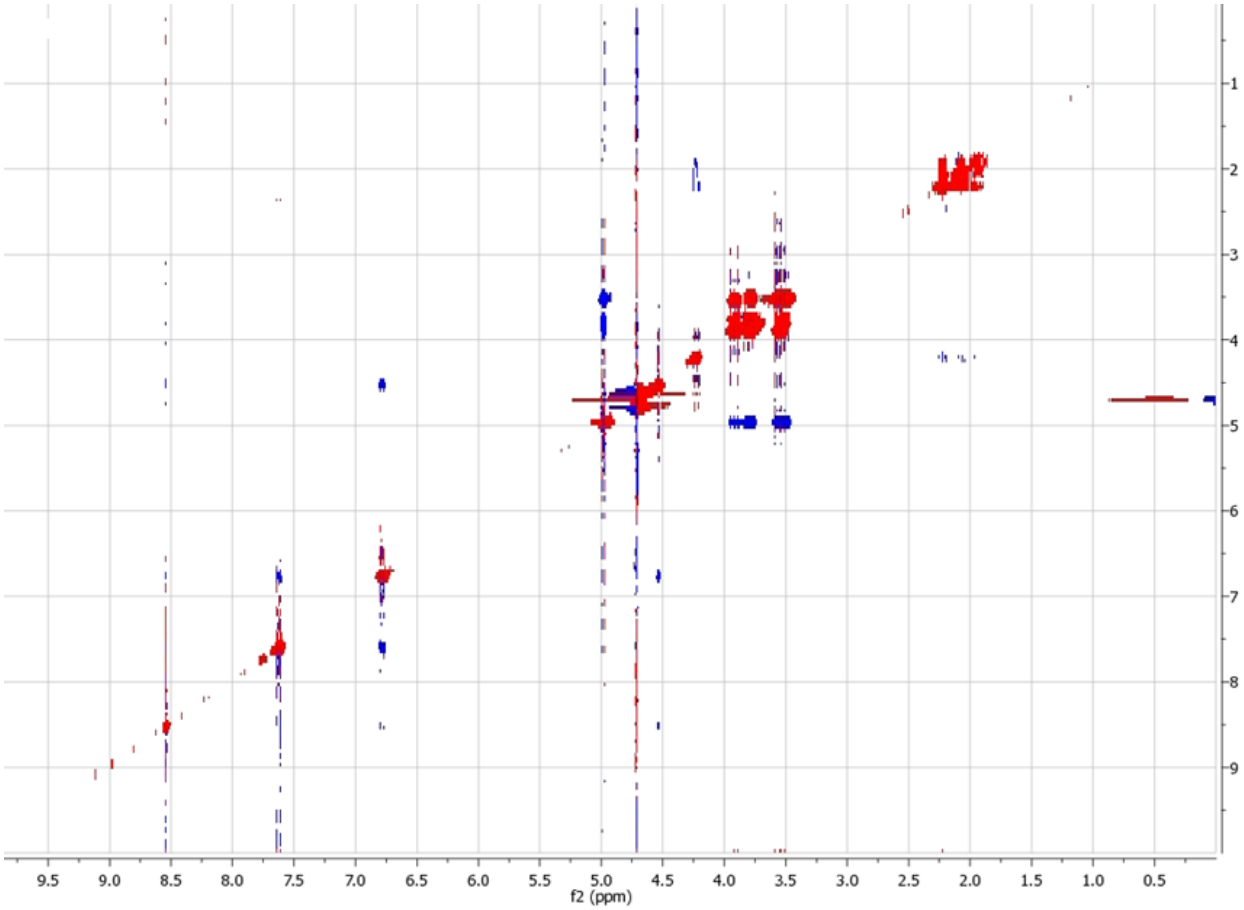

Fig. 2. ^{1}H NMR-ROESY spectrum of α-CD/folic acid complex in D_2O at 298K after the mixing time of 300 ms.

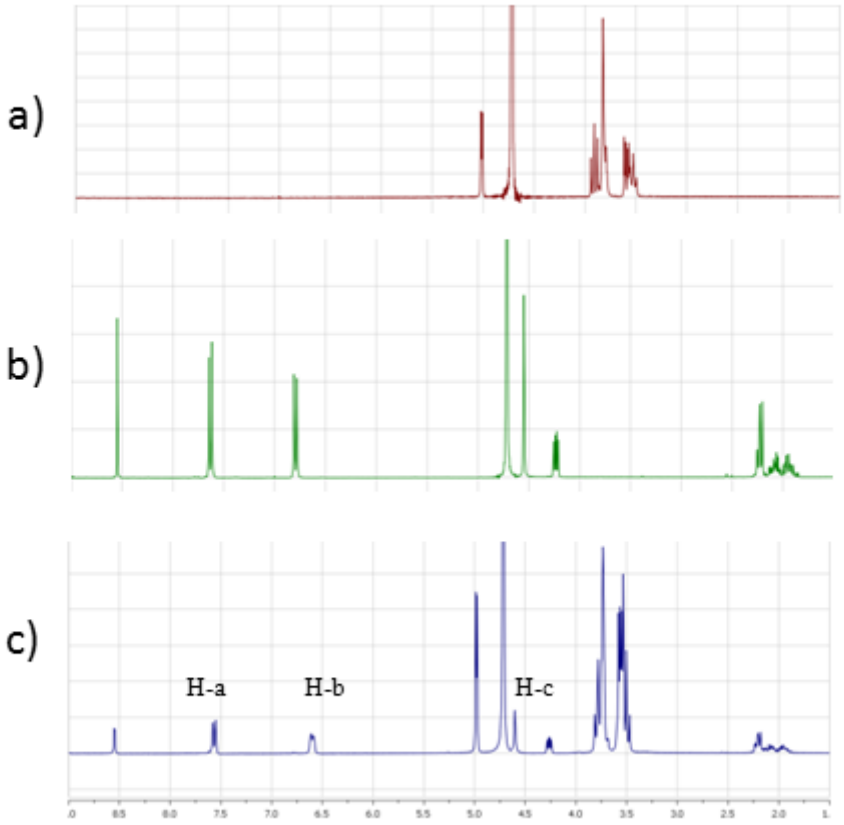

Fig.3 ^{1}H NMR spectra of the resultant solid dried from a) β-CD, b) folic acid and c) β-CD +folic acid

Table 2. The proton chemical shifts (δ) and the longitudinal relaxation times (T_1) of β-cyclodextrin, folic acid, their mixture and the corresponding changes

	m	β–cyclodextrin		folic acid		β–cyclodextrin + folic acid		
		δ /ppm	T_1 /s	δ /ppm	T_1 /s	δ /ppm	T_1 /s	Δδ /ppm
H-1	d	5.01	0.53	-	-	4.98	0.51	-0.03
H-2	dd	3.59	0.86	-	-	3.57	0.61	-0.02
H-3	m	3.90	1.01	-	-	3.78	0.81	-0.12
H-4	dd	3.53	0.49	-	-	3.54	0.46	-0.01
H-5	m	3.84	0.31*	-	-	3.75	0.26*	-0.09
H-6	dd	3.83	0.31*	-	-	3.77	0.26*	-0.06
H-a	m	-	-	7.63	1.49	7.58	0.73	-0.05
H-b	d	-	-	6.79	0.90	6.60	0.51	-0.19
H-c	s	-	-	4.55	0.36	4.60	0.39	+0.05

* not resolved signals

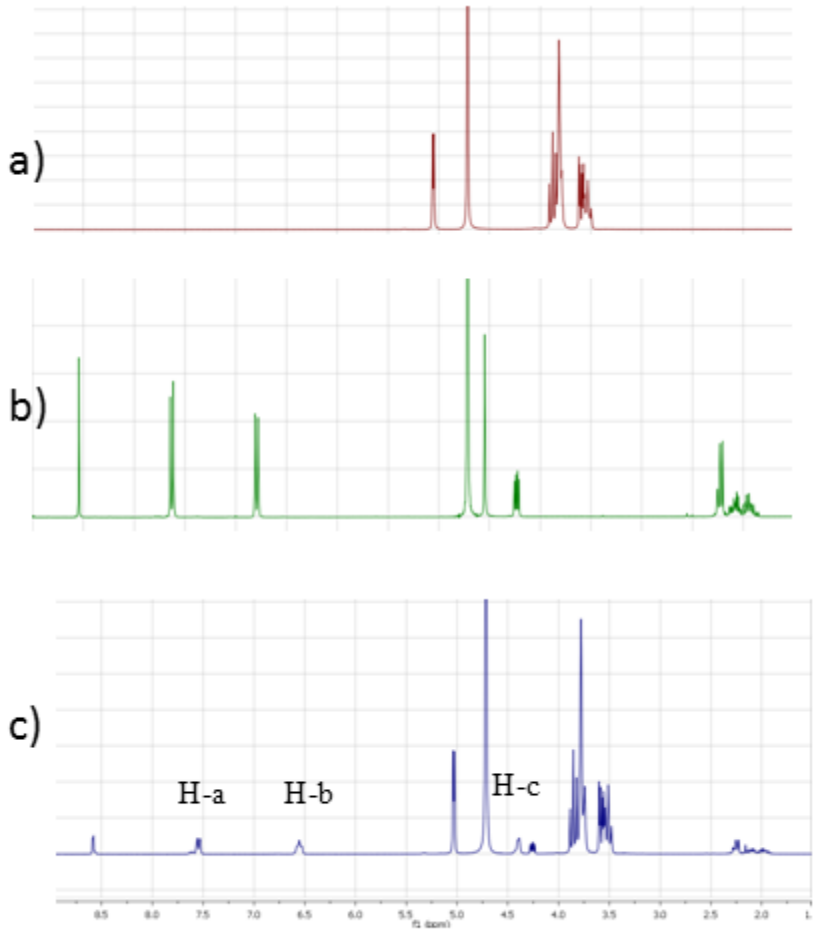

Fig. 4 ^{1}HNMR spectra of the resultant solid dried from a) γ-CD, b) folic acid and c) γ-CD +folic acid

Table 3. The proton chemical shifts (δ) and the longitudinal relaxation times (T_1) of γ-cyclodextrin, folic acid, their mixture and the corresponding changes

	m	γ–cyclodextrin		Folic acid		γ–cyclodextrin + folic acid		
		δ /ppm	T_1 /s	δ /ppm	T_1 /s	δ /ppm	T_1 /s	Δδ /ppm
H-1	d	5.05	0.56	-	-	5.03	0.55	-0.02
H-2	dd	3.60	0.86	-	-	3.58	0.89	-0.02
H-3	m	3.88	1.09	-	-	3.84	1.00	-0.04
H-4	dd	3.52	0.56	-	-	3.52	0.54	0
H-5	m	3.83	0.34*	-	-	3.78	0.35*	-0.05
H-6	dd	3.82	0.34*	-	-	3.76	0.35*	-0.06
H-a	m	-	-	7.63	1.49	7.56	1.15	-0.07
H-b	d	-	-	6.79	0.90	6.54	0.72	-0.25
H-c	s	-	-	4.55	0.36	4.39	0.28	-0.16

* not resolved signals

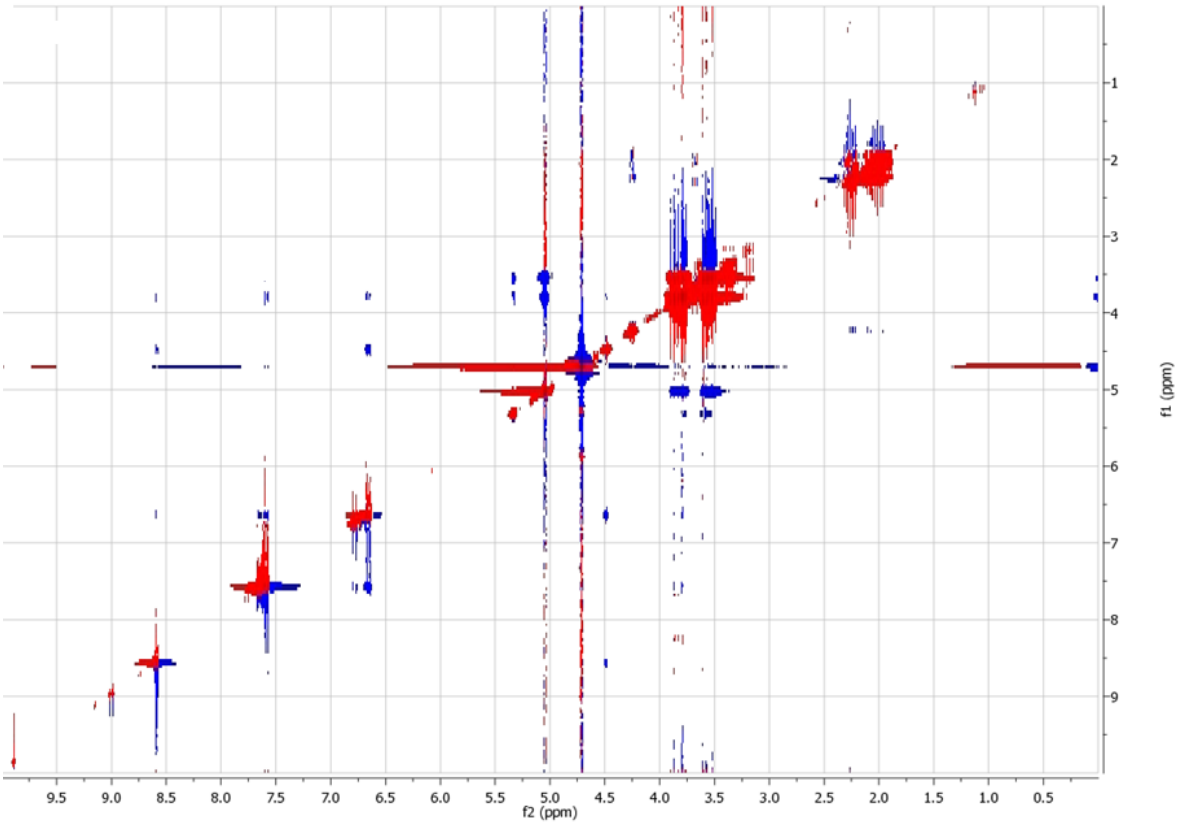

Fig.e 5. ^{1}H NMR-ROESY spectrum of γ-CD/folic acid complex in D_2O at 298K after the mixing time of 200 ms.

4. Mass Spectrometric Experiments

Electrospray mass spectra and collisional dissociation spectra were recorded on an API 365 triple quadrupole mass spectrometer (Applied Biosystems). The mass spectrometer is equipped with a TurboIonSpray^{TM} electrospray ion source operated in the standard negative ESI mode, i.e. without additional drying gas. Typically, water/methanol (1:1) solution served as the spray solvent and ca. 0.3 mM solutions of the studied complexes were used. Analyte solutions were introduced into the ion source with a syringine pump at flow rate of 10 µl/min. The ion source parameters were optimized to obtain the highest possible abundance of the complex anion and were adjusted as follows: ionspray voltage (IS) -4 kV, declustering potential (DP) -30, focusing potential (FP) -180. In CID experiments, nitrogen was used as the collision gas and the collision energy was varied in the range -5 eV to -40 eV.

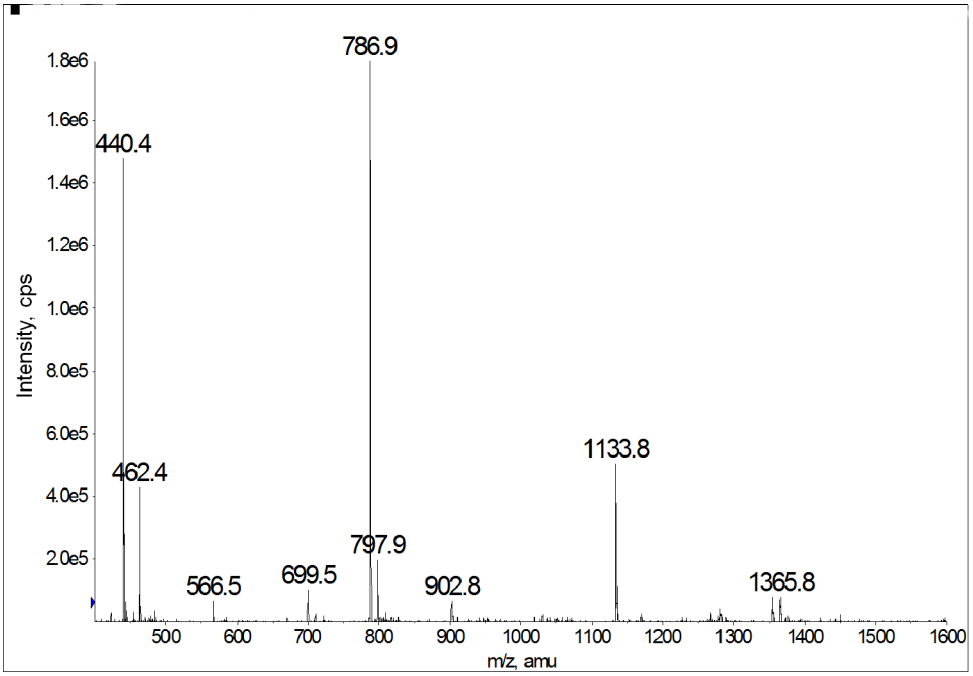

Fig. 6. Negative-ESI mass spectrum of solution of β CDKF.

5. Thermogravimetry (TG) and Differential Scanning Calorimetry (DSC)

Thermal analysis of solid cyclodextrins, folic acid sodium salt and cyclodextrin complexes were examined using 9900A Thermal Analyzer (Du Pont, USA) equipped with TGA 951 module and 910 DSC cell. TG and DSC experiments were performed in the range 25-300^{o}C at heating rate 5^{o}C/min. The sample size was in the range 3-7 mg. Measurements were made using flow of argon (flow rate 6 dm^{3} /h).

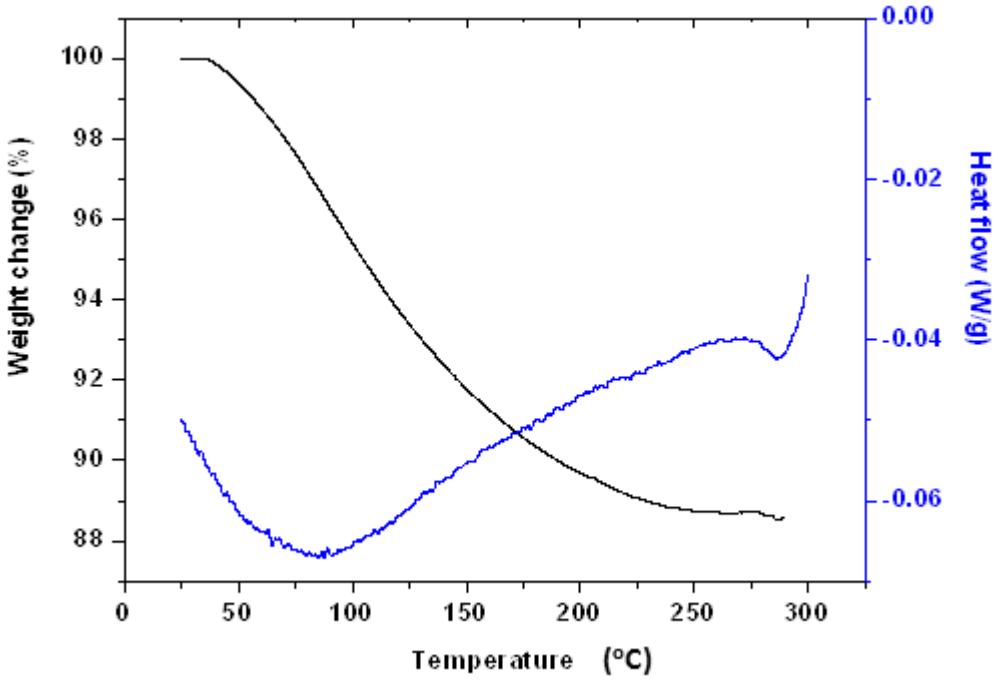

Fig 8. TG –DSC Thermograms of sodium salt of folic acid.

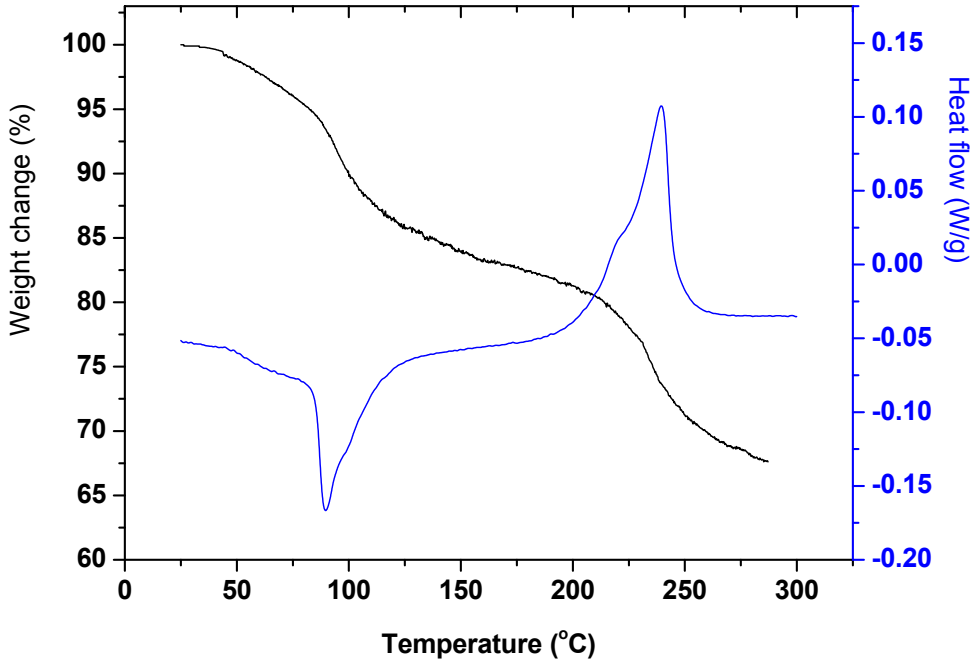

Fig 9. TG-DSC thermograms of complex α-CD/ FA

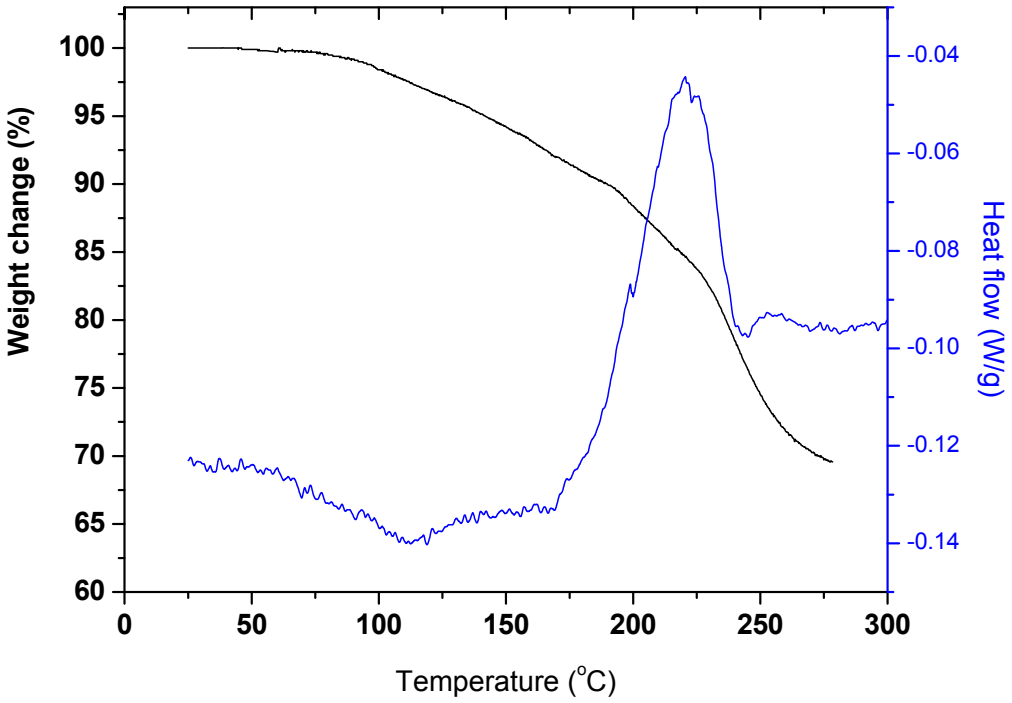

Fig. 10. TG-DSC thermograms of complex βCD/ FA

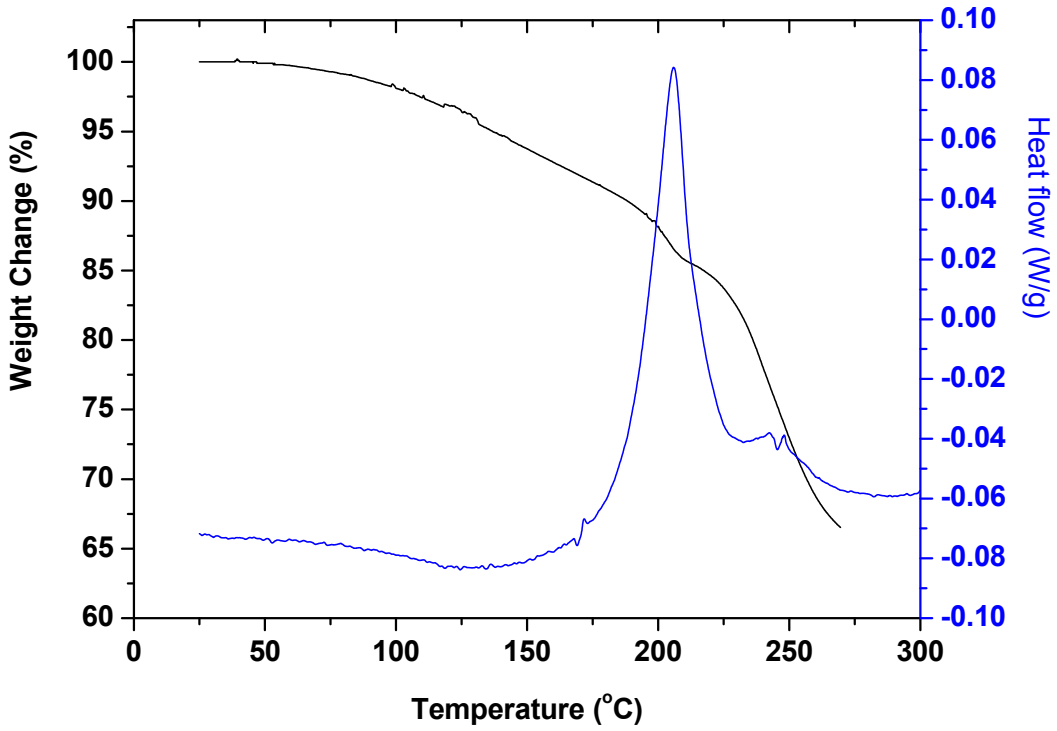

Fig 11. TG-DSC thermograms of complex γCD/ FA