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p-amino benzoic acid Schiff base and their cyclodextrins inclusion complexes

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ABSTRACT: The Schiff base of p-amino benzoic acid and ethyl vanilline was prepared and characterized by IR , ^{1}HNMR and mass spectra . The Schiff base transformed into inclusion Complexes with three types of cyclodextrins namely α-CD , β-CD and, 2HP-β-CD. The inclusion complexes were prepared following two different methods freeze drying and co-precipitation. Improvement of aqueous solubility were observed , the stability constant of inclusion complexes were calculated and the results confirmed 1:1 complexes formation.
KEYWORDS: Antranilic acid , Phase solubility , SEM , ethyl vanilline , inclusion Complexes.

I. INTRODUCTION:

Schiff bases are compounds containing azomethine group resulting from condensation of primary amines with carbonyl compounds[1]. Cyclodextrins are cyclic oligosaccharides having toroid shape and particular structure composed of a lipophilic cavity and a hydrophilic outer surface [2–4]. It is widely used as a host for many chemicals especially drugs to form a system called inclusion complexes[5,6]. Many advantages obtain from formlation the drugs as an inclusion complex with cyclodextrins such as enhancement of aqueous solubility , increased the stability of sensitive drugs to light , heat , oxidation and modification of most physical properties of the guest[7–9]. Several methods have been reported for the preparation of inclusion complexes such as freeze drying , kneading , spray drying[10–12] etc…..

The driven forces to formation the inclusion complexes are vander wales forces , relief of strain in the cyclodextrine ring , release of water molecules which naturally located in the cavity of cyclodextrine and substituted by a part of lipophilic parts of the guest and this process is preferable from a thermodynamic point of view[13–15].

II. METHODS AND MATERIALS:

The solvents used were of analytical grade and used as purchrsed. α-cyclodextrin and β-CD from ACROS organics company , ethyl vanillin and p- amino benzoic acid from BDH , 2HP-β-CD from Chem.center Lajolla BI.

UV-vis spectra were recorded on a (CE7200) using 1 cm quartz cell.IR spectra were recorded as apellets using an FT-IR spectrophotometer (SHIMADZU) within 400 – 4000 cm^{-1}. ^{1}HNMR spectra were recorded as DMSO-d6 solution on a Bruker(500MHz) at room temperature using TMS as an internal reference.

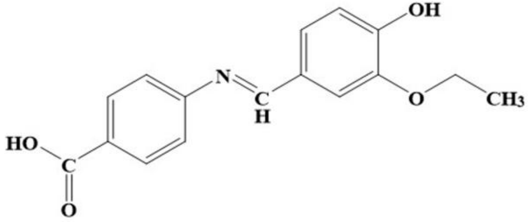
EI-Mass spectra were obtained from Agilent technologies 597SC spectrometer

SEM images were performed using ZEISS SIGMA vp from Carlzeiss Microscopy (Germany) , the images were obtain through secondary electrons with attention of 15 kV and the magnifications 10-20 KX and the samples was coated with gold to render them electrically conductive.

Preparations:

Preparation of Schiff base :

Preparation of : (E)-4-((3-ethoxy-4-hydroxybenzylidene)amino)benzoic acid (M1)



M1 : 10 mmole (1.16 g) of ethyl vanilline dissolved in 20 ml of ethanol was add drop wise to p-amino benzoic acid 10 mmole (1.37 g) dissolved in 20 ml of ethanol with continuous refluxing for 4 hrs , the reaction process was monitored by TLC ($CHCl_3$: EtOH 9:1) as eluent. The solvent was evaporated and the residual precipitate was recrystallized from hexane. The products was dried in desiccators over silica gel. The Schiff base was as following yellow crystal M.P 219-221C , yield 76%.

Preparation of Schiff base…… CDs inclusion complexes:

Freeze drying method , 1:1 molar ratio of Schiff base and α-CD , β-CD , 2HP-β-CD were mixed in deionized water and the resulting mixture was stirring for 72 hrs at room temperature. The solution freezing and then lyophilized in a freeze drying type CHRIST, modle alpha LD plus antil completely drying and the resulting fine powders kept in desiccator over silica gel.

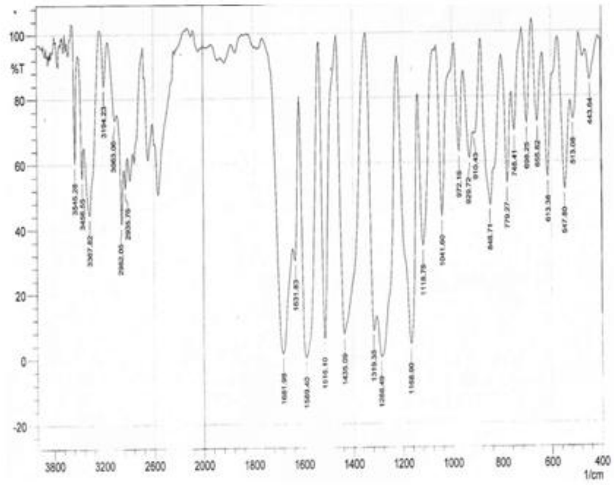


Fig (2) : EI-mass Spectrum of M1

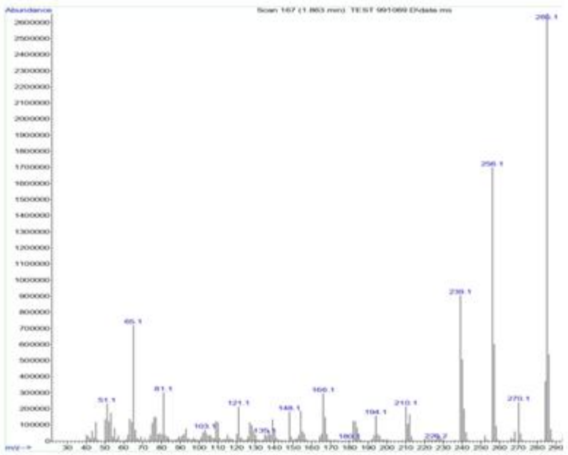


Fig (1) : spectrum of M1

The ^{1}HNMR spectrum of Schiff base (Fig 3) shows the following signals , a brood signal at δ 12.59 ppm attributed to OH proton of carboxylic group. another singlet signal at δ 9.76 ppm attributed but led to phenolic OH proton. The singlet signal at δ 8.46 ppm attributed to HC=N proton which confirm the formation of Schiff base[16]. The signals at the region 6.9 – 7.9 ppm attributed to aromatic protones, the quartet signal at δ 4.1 ppm (J = Hz) attributed to CH_2 group of ethyl moiety , also the triplet signal at δ 1.36 ppm (J = Hz) attributed to CH_3 protons.

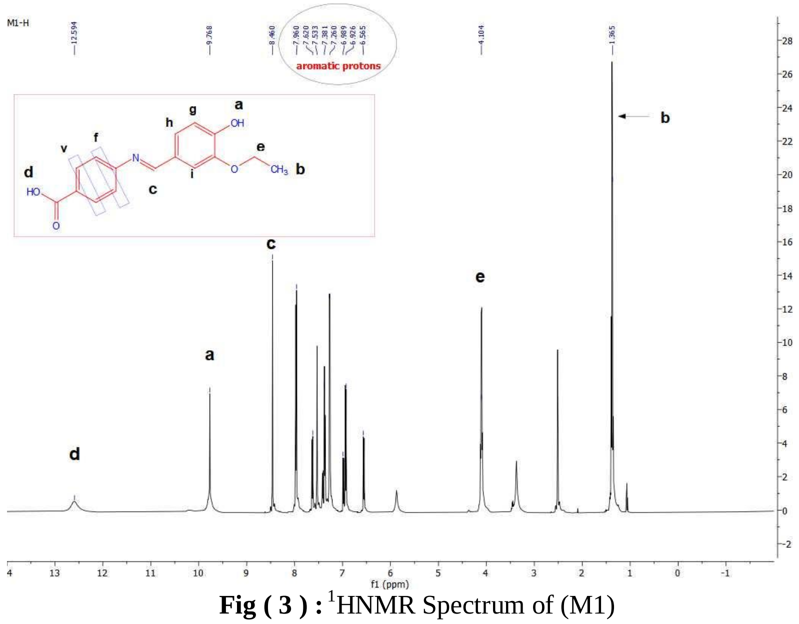


Fig (3) : ^{1}HNMR Spectrum of (M1)

Mode of Host-quest interaction :

The formation of inclusion complexes between CDs as a host and Schiff base as a guest was studies by a compared of IR and HNMR spectra of Schiff base and their inclusion complexes. Acomparison of IR spectrum of Schiff base with corresponding Schiff base - α-CD inclusion complex which prepared by both method freeze drying and co-precipitation a significant change were observed in the position and intensity of the some bands , were the stretching vibration of C = N was shifted to lower frequency (-23 cm^{-1}) , also the C – O stretching of C – OH and C – OCH$_3$ were shifted to lower frequency (-34 cm^{-1} and -38 cm^{-1} respectively).also the bonds attributed to α-CD moiety the O – H and C – H were shifted , the results was tabulated in Table (1). The IR spectrum of inclusion complex which prepared by co-precipitation show the same results which indicate the formation of inclusion complex by path two methods. The IR spectrum of Schiff base – β-CD inclusion complex show a broad band at characteristic for all β-CD inclusion complex which attributed to OH stretching these band a pear at 3378 cm^{-1} in pure β-CD and shifted to higher frequency where appear at 3408 cm^{-1} in the spectrum which prepared by freeze drying method and at 3414 cm^{-1} in the IR spectrum of the sample prepared by co-precipitation method. Asignificant charge in the position of C – H aliphatic stretching from 2924 cm^{-1} for pure β-CD to 2928 cm^{-1} , also the azomethine stretching shifted to lower frequency (- 30 cm^{-1}) , when compared these result with that in Schiff base – α – CD spectrum you can say that the wider cavity of β-CD than α-CD may be lead to strong interaction between guest groups and the hydrophobic cavity of CD. The IR spectrum of Schiff base – 2HP - β-CD show the same result with that for Schiff base - β-CD where the C = N shifted to a higher frequency about 1600.97 cm^{-1} in the sample prepared by co-precipitationand 1589.40 cm^{-1} for the sample prepared by freeze drying method. It seems that the hydroxypropyl substituent has no effect on the interaction between the quest group and the hydrophobic cavity. The other bands frequencies are listed in Table (1) and Fig(4). The mode of interaction between Schiff base as a guest with CDs as a hosts were studied by a comparison of HNMR spectra of Schiff base , α-CD , β-CD , 2HP – β –CD and their inclusion complexes Fig(5) and Table (2).

band	α-CD	M1 $_{free}$	Complex$_{(M1+\alpha\text{-CD})}$	$\Delta\upsilon$ (cm^{-1})
OH	3406.05 3382.91	3545.28 3456.55 3367.82	3363.97 3325.39 3201.94	-181.31 -131.16 -165.88
C=N $_{azomethine}$		1631.83	1608.69	-23.14
C=O	1641.31	1681.98	1693.56	+11.58
C-H$_{alph}$	2924	2982.05 2935.76	2931.90	-50.15
C=C		1516.10 1435.09 -	1516.10 1446.66 1400.37	0 +11.57
C-O		1168.90 1118.75 1041.60 972.16	1153.47 1030.02	-15.43 -88.73

Table (1) : The IR spectra of M1 and α-CD by co-precipitation method

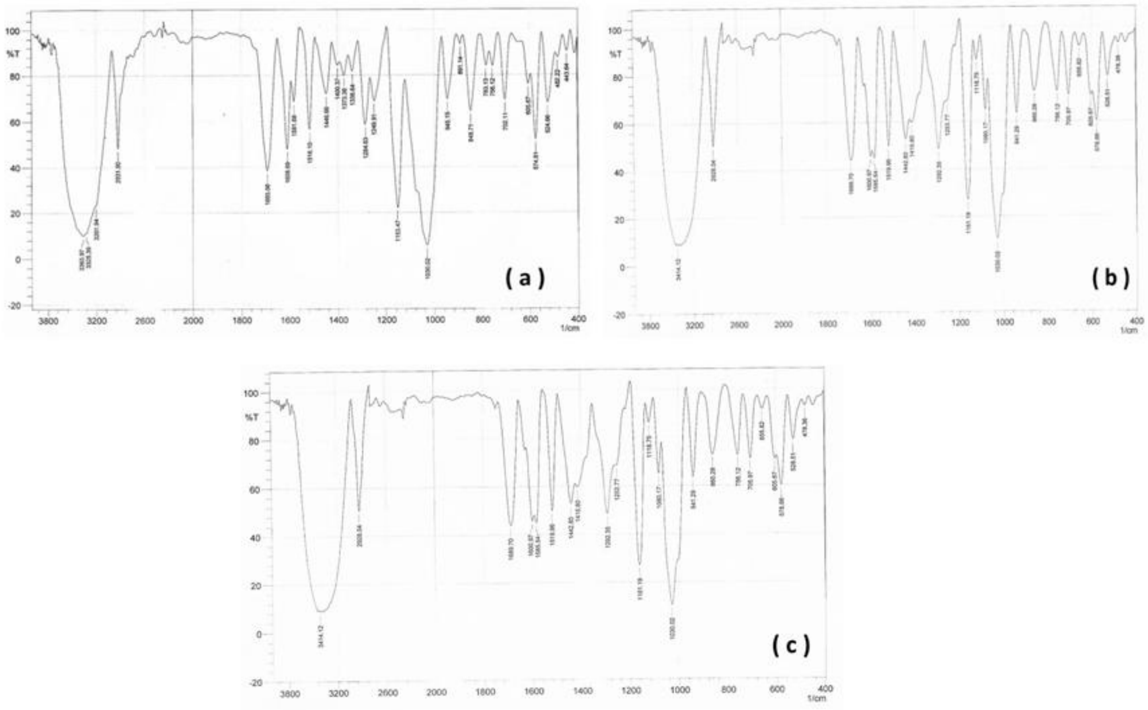


Fig (4) :IR Spectra of inclusion complexes M1: α-CD (a) , M1:β-CD (b) and M1: 2HP-β-CD (c).

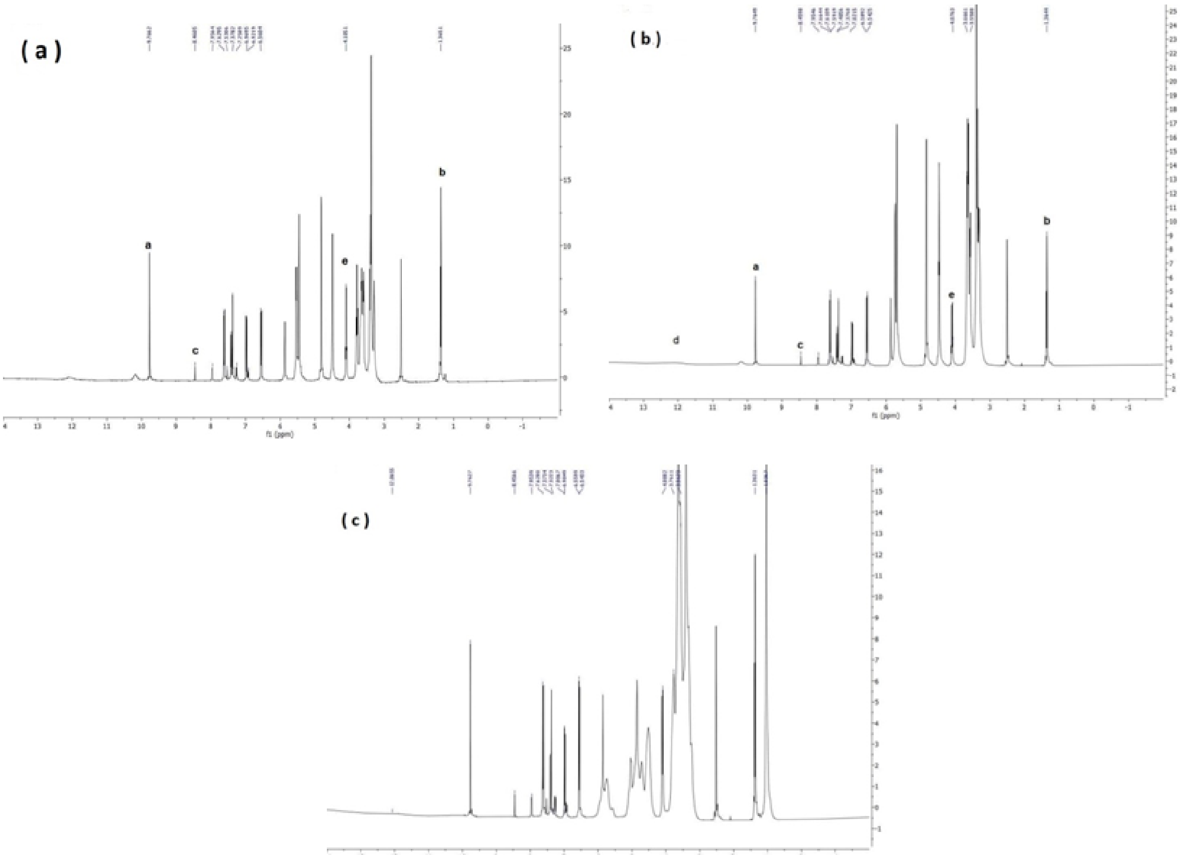


Fig (5) : ^{1}HNMR Spectra of inclusion complexes M1: α-CD (a) , M1:β-CD (b) and M1: 2HP-β-CD (c).

Substance	Proton	Free (δ)	α-CD Complex (δ)	Δδ	β-CD Complex (δ)	Δδ	2HP-β-CD Complex (δ)	Δδ
M1	a	9.7679	9.7652	-0.0027	9.7669	-0.001	9.7662	-0.0017
	b	1.3651	1.3644	-0.0007	1.3522	-0.0129	1.3522	-0.0129
	c	8.4600	8.4598	-0.0002	8.4615	+0.0015	8.4607	+0.0007
	d	12.5935	12.0401	-0.5534	12.0601	-0.5334	12.0432	-0.5503
	e	4.1038	4.1046	+0.0008	4.0787	-0.0251	4.0782	-0.0256
	Aromatic	6.5655-7.9603	6.5592-7.9551	-0.0063, -0.0052	6.5440-7.9564	-0.0215 , -0.0039		
α-CD	H3	3.768	3.786	+0.010				
	H5	3.580	3.585	+0.005				
β-CD	H3	3.882			3.6776	-0.2044		
	H5	3.754			3.560	-0.194		
2HP-β-CD	H3	3.840					3.759	-0.081
	H5	3.750					3.589	-0.161

Table (2) : Variation of the 1H NMR chemical shifts (δ /ppm) of M1, α-CD, β-CD , 2HP-β-CD and protons in free and complex by co-precipitation method

The spectrum of Schiff base – α - CDshow a shifted of $H_3$and H_5 of α-CD to down field (Δδ + 0.01 , + 0.005 respectively) , a significant change in chemical shift of hydroxyl group of carboxylic moiety to a high field region (Δδ = - 0.553 ppm) , which indicate the location of carboxylic group in the cavity. The same results were obtain from the sample which prepared by freeze drying method as show in Table (2).

The 1HNMR spectrum of Schiff base – β – CD show that the H_3 and H_5 signals shifted from δ 3.882 in pure β-CD to δ 3.677 for H_3 and H_5 signal shifted from δ 3.75 to δ 3.56 ppm in inclusion complex which prepare by co-precipitation method , while the sample which prepare by freeze drying method show a more high field where the H_3 and H_5 appear at δ 3.666 and 3.558 respectively[17–19].

Pure 2HP - β - CD ^{1}HNMR spectrum show the signal of H_3 and H_5at δ 3.84 and 3.75 ppm respectively[20]. a comparison of these result with that for their inclusion complex which prepared by co-precipitationmethod als shifted to high field region at 3.759 and 3.589 ppm respectively which gunfirethe in certain of the guest molecule in to 2HP – β – CD cavity also most of the signals attributed to Schiff base are shifted and the result listed in Table (2).

The same result was obtain for the sample prepared by freeze drying method.

SEM : The SEM analysis gave evidence of the formation of an inclusion complexes. The SEM images were recorded for Schiff base , α-CD , β-CD , 2HP - β-CD and their inclusion complexes**.** it can be noted that at α-CD , β-CD , 2HP - β-CD particles have amorphous shapes while the Schiff base particles have irregular shape in different size. A comparison of these images of raw materials with their inclusion. All complexes which prepared by co-precipitation or freeze drying methods have an amorphous shapes except the Schiff base – 2HP - β-CD (Fig6) show a special shape having almost the same size[20–23].

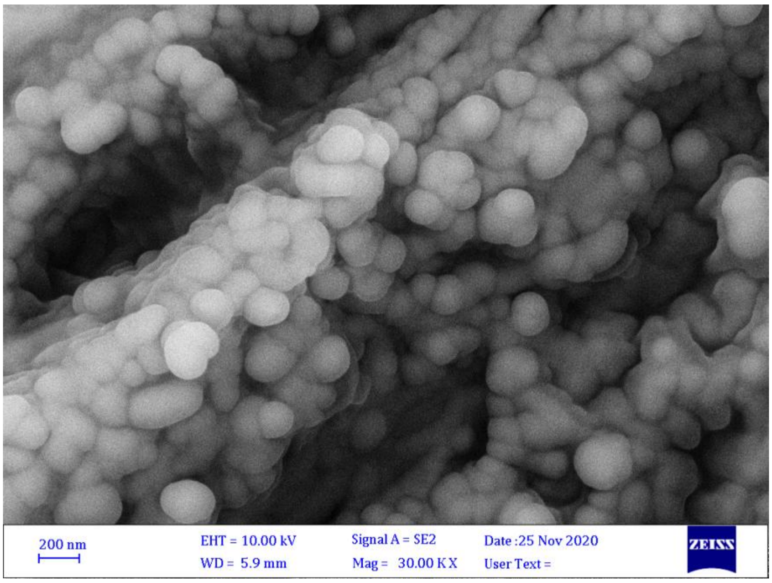


Fig (6): SEM of M1: 2HP-β-CD Inclusion Complexe

Phase solubility studies were performed according to the Higuchi & Connors method[24]. First the 10^{-4} M solution of Schiff base was prepared in distilled water and the UV spectrum was recorded to evaluate the λ max and the molar absorptivity (E) , the second step an excess amount of Schiff base was added to various concentration of aqueous solutions of cyclodextrins (0.001 – 0.012M) in a 25 ml conical flask and the mixtures were shaken for 72 hrs at room temperatures , after that the solution were filtered and 1 ml of each solution was with drawin and diluted suitably then the concentration of Schiff base in each solution was determined at the same wave length by using Beer lambert law. The phase solution diagrams for the complexes formation (Fig7) were show.

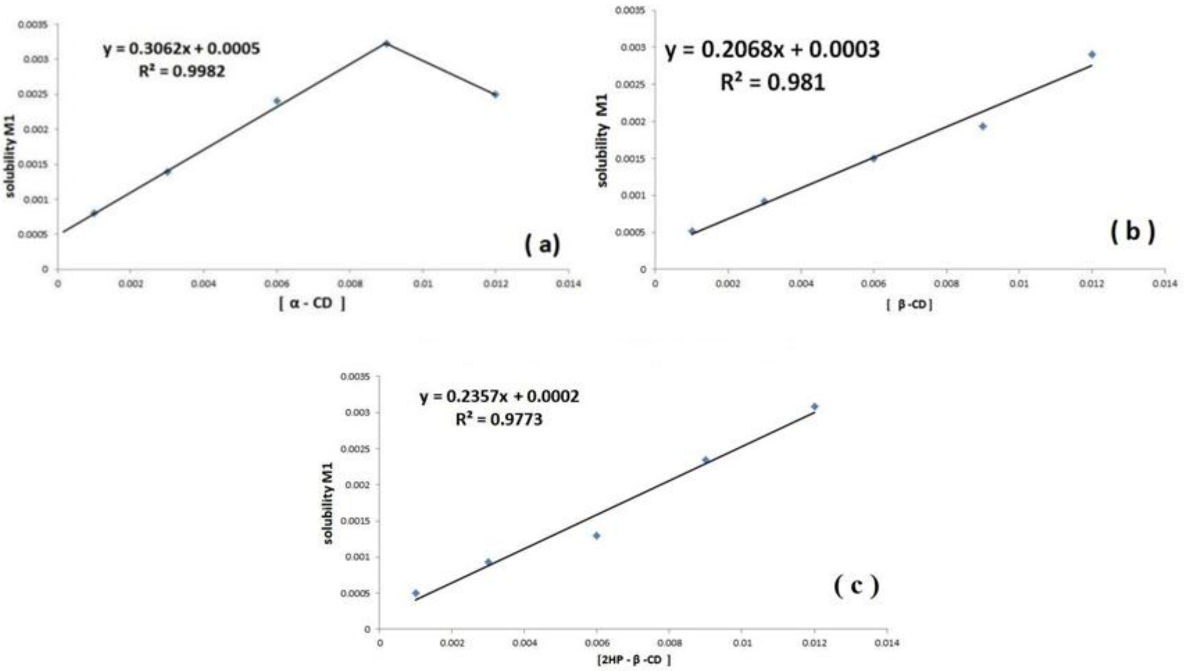


Fig (7) : The Phase solubility digrams of (a) M1-α-CD Inclusion Complexe , (b) M1-β-CD Inclusion Complexe , (c) M1- 2HP-β-CD Inclusion Complexe

The aqueous solubility of Shiff base in β-CD , 2HP-β-CD increase and the solubility studies for the types of solubility was classified as $A_L$type , while α-CD increase up to 0.009 M then the solubility decrease and classified as A_N. you can say this results indicate the self-assembly α-CD at higher concentration [24].

The stability constant for each complexes was calculated and the solubility enhancement was found to be 5 times for α-CD, 9.66 times for β-CD and 15.4 times for 2HP-β-CD. And the values of K indicate the 1:1 complexes formation [25].The stability constant was calculated from the straight line slope of the experimental diagrams using the relation

K= slope / S°(1-slope)……….(1)

Where So is the solubility of Schiff base in the absence of cyclodextrines and calculated from the intercept.

phase solubility data		**Concentration of CDs**						**So Mg/L**	**S_{max} Mg/L**	**Kc M^{-1}**
		0	**0.001**	**0.003**	**0.006**	**0.009**	**0.012**			
Solubility M1	**α-CD**	0.0005	0.0008	0.001389	0.0024	0.00322	0.0025	142.5	712.5	882.675
	β-CD	0.0003	0.008625	0.00915	0. 01	0.0107	0.0131	85.5	826.65	869
	2HP-β-CD	0.0002	0.0005	0.000932	0.0013	0.00234	0.00308	57	877.8	145.979

Table (3) : phase solubility data of M1

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