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SPECTRAL CHARACTERISTICS AND MOLECULAR DOCKING STUDIES ON THE α-CD INCLUSION COMPLEXES OF 2-Methyl-4-nitrophenol And 4-Methyl-2-nitrophenol

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Abstract - *Nitrophenols are generally used as intermediates in the synthesis of dyestuffs, pharmaceutical products, pesticides and herbicides. They are highly toxic to animals and human community and of environmental concern. Inclusion of 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol with α-Cyclodextrin may be considered as a safer means to separate the traces of these compounds present in the environment. The inclusion complexes prepared are characterized using different techniques. The inclusion complexes in solution state are characterized by UV-Visible spectrophotometer(UV-Vis) and fluorimetry. Inclusion complexes in solid state are characterised by Fourier Transform Infra Red Spectroscopy(FTIR)and Scanning Electron Microscopic(SEM) techniques and in virtual state by Molecular docking studies. The results obtained from molecular docking studies are found to be in good correlation with the results obtained through experimental methods. Therefore complexation of 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol with α-CD can be considered as a better option and suitable measure for separation of contaminants from the environment.*

Key Words: 2-Methyl-4-nitrophenol, 4-Methyl-2-nitrophenol, inclusion complexes, α-Cyclodextrin, UV-Visible spectrophotometer, fluorimetry, Molecular docking.

1. INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of 6, 7, and 8 units of 1, 4-linked glucose units, and are named as alpha (α), beta (β) and gamma (γ)-cyclodextrins respectively [1].They have internal cavities capable of forming complexes with hydrophobic organic molecules in aqueous solutions [2]. The inner diameters of the cavities are approximately 4.5A in α-CD, 7.0A in β-CD and 8.5A in γ-CD [3]. The CDs are capable of incorporating a high range of guest molecules based on hydrophobic and geometrical cavities. They have a toroidal shape with an internal hydrophobic surface and an external hydrophilic surface and they are acting as a host molecule. These cyclodextrins are well known as they form stable host-guest inclusion complexes which have the interesting property of including organic, inorganic and biological molecules in their cavities[4,5] Once the inclusion compound is formed, the stability of the guest molecules increases due to the binding forces (van der Waals attractions, hydrogen bonding, hydrophobic interactions, etc.) between the host (CDs) and guest molecules[6] The chemical properties of cyclodextrins combined with their non-toxic character to humans have led to their use in pharmaceuticals, as food additives as well as in the environmental de-contamination procedures of wastewater, aquifer, air, and soil [7,8]. Particularly cyclodextrins and their derivatives have been use to remove contaminations by the formation of inclusion complexes or to enhance the solubility of several compounds[9-12].

Nitrophenols belong to major organic pollutants that have been analysed in the environment [13]. Nitrophenols, coming from pesticide degradation products, car exhaust, and industrial waters are listed as pollutants with great potential toxicities of carcinogenesis, teratogenesis, and mutagenesis [14] because of their detriment and vast scale distribution in the ecological environment. It has been used in making dyes, wood preservatives, explosives, insect control substances, and photographic developer. It is released into the air, water and soil and environment from landfill leaks and accidental spills. Consequently, due to the harmful effects of these organic compounds, the waste waters containing them must be treated before being discharged to receiving water bodies. The secondary biological processes are commonly used for domestic and industrial waste water treatment, but their efficiency is not satisfactory at high pollutant concentration [15].

2-Methyl-4-nitrophenol is suitable as a target compound in the study on measurement of methyl nitrophenol concentrations and stable isotope ratios in the atmospheric particulate matter [16] and as an internal standard in the determination of monoaromatic nitro compounds in atmospheric aerosols using spectrometry (HPLC/MS/MS) method [17]. It may also be used as a starting material in the synthesis of 2-bromo-4-nitro-6-methylphenol [18] is used as intermediate in dyestuffs, pesticides etc. In order to assess the fate of 2-Methyl-4-

nitrophenol and 4-Methyl-2-nitrophenol in waste water and to control their mobility and reactivity during remediation processes, the sorption behavior of these toxic contaminants must be understood and revealed. Hence the host-guest inclusion complex mechanism between 2-Methyl-4-nitrophenol/ 4-Methyl-2-nitrophenol and α-CD in different techniques was studied. In this investigation, the spectral and docking behaviours of 2-methyl-4- nitrophenol and 4-methyl-2-nitrophenol with α-cyclodextrin the formation of host – guest inclusion complex of 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol with α-CD in solution phase was studied by UV-Visible spectroscopy (UV-Vis) and fluorimetry. The solid complex was prepared and characterized by FTIR and SEM techniques. The inclusion complexes were characterized using docking studies in virtual state.

2. MATERIALS AND METHODS

2.1 Reagents and Materials

Analytical grade of 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol and α-cyclodextrin were purchased from Sigma Aldrich. The solvents used were also of analytical grade. Triply distilled water was used for the preparation of stock solutions. The solutions were prepared just before taking measurements.

2.2 Preparation of liquid inclusion complexes of 2-Methyl-4-nitrophenol:α-CD/ 4-Methyl-2-nitrophenol:α-CD

About 0.0036g of 2-Methyl-4-nitrophenol / 4-Methyl-2-nitrophenol was dissolved separately in 10 ml of methanol. About 0.5836 g of α-CD was dissolved in 60 ml of distilled water in a 250 ml beaker. Inclusion complexes of 2-Methyl-4-nitrophenol:α-CD/4-Methyl-2-nitrophenol :α-CD were prepared by varying the concentration of α-CD from $2x10^{-3}$ M to $1x10^{-3}$ M with 2-Methyl-4- nitrophenol / 4-Methyl-2-nitrophenol.

2.3 Preparation of solid inclusion complex of 2-Methyl-4-nitrophenol:α-CD/ 4-Methyl-2-nitrophenol:α-CD

About 0.0459g of 2-Methyl-4-nitrophenol/4-Methyl-2-nitrophenol was dissolved separately in 60 ml of methanol. About 0.5836 g of α-CD was dissolved in 60 ml of distilled water in a 250 ml beaker. Both the solutions were mixed together in a beaker and put over electromagnetic stirrer continuously for 48 h at room temperature. The precipitate formed after evaporation was dried and used for characterization.

2.4. Molecular docking study

The most probable structure of 2-Methyl-4-nitrophenol: α-CD inclusion complex were determined by molecular docking studies using the PatchDock server. The 3D structural data of α-CD was obtained from the Chem-Spider database using the search interface. The 3D structural data of 2- Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol was obtained by translating its SMILES formula generated by CORINA server (http://www.molecular.networks.com/online–demos /corina-demo). Then the 3D structure of 2-Methyl-4-nitrophenol and α-CD was energy minimized using the Pymol software. The energy minimized structure of guest (2-Methyl-4-nitrophenol) was docked into the host (α-CD) cavity using PatchDock server by uploading the 3D coordinate data file (i) α-CD as receptor molecule and (ii) 2-methyl-4- nitrophenol as ligand molecule. Similarly the energy minimized structure of guest (4-Methyl-2-nitrophenol) was docked into the host (α-CD) cavity using PatchDock server by uploading the 3D co-ordinate data file of (i) α-CD as receptor molecule and (ii) 4-Methyl-2-nitrophenol as ligand molecule. The PatchDock server carries out thorough conformational search on a geometry-based molecular docking algorithm to identify the docking transformations with effective molecular shape complementarity. PatchDock algorithm separates the Connolly dot surface representation of the host and guest molecules into concave, convex and flat patches. These divided complimentary patches are matched to generate candidate transformations and evaluated by geometric fit and atomic desolvation energy scoring function. PatchDock algorithm uses root mean square deviation clustering method to select the non-redundant docked models and to omit the redundant docked models. The conformational search by PatchDock server yields 100 different models of 2-Methyl-4-nitrophenol:α-CD and 4-Methyl-2-nitrophenol:α-CD inclusion complex. During the conformational search process, the PatchDock server validates each conformation and ranks all the models with a score. The highly favorable model will have a high score. The first model will be the model with high score and energetically favorable model. Hence the first model was selected for the analysis and compared with the model predicted based on the experimental (UV-Vis, Fluorimetry, FTIR, SEM) investigation.

3. RESULTS AND DISCUSSION

3.1 Effect of α-cyclodextrin Absorption studies on 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol.

The absorption maxima of 2-Methyl-4-nitrophenol in varying concentrations of α-CD are shown in Fig-1 and Table 1. The absorption maxima are red shifted from λ_{max}

232.5 nm to 243.1 nm with increase in absorbance values with increase in α-CD concentration. This shift in wavelength indicates the formation of inclusion complex.

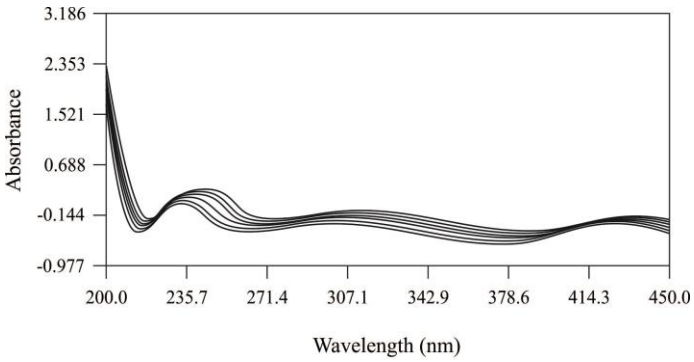


Fig-1: Absorption Spectra of 2-Methyl-4-nitrophenol:α-CD

Table-1 Absorption Spectral data of 2-Methyl-4-nitrophenol α-CD

S.No	2-Methyl -4-nitrophenol:α-CD					
	Concn.Of $\alpha-CD$	λ_{abs}	Abs	$\frac{1}{A-A_0}$	log ε	$\frac{1}{[\alpha-CD]}$
1	0	232.5	0.188		1.97	
2	0.002	234.5	0.209	47.61	2.02	500
3	0.004	237.5	0.224	27.77	2.05	250
4	0.006	239.5	0.235	21.27	2.07	166.6
5	0.008	240.1	0.242	18.51	2.08	125
6	0.010	243.1	0.253	15.38	2.10	100

Abs – Absorbance, Concn – Concentration, ε- Molar Absorptivity

$$\frac{1}{A-A_0} = \frac{1}{A'-A_0} + \frac{1}{K_B(A'-A_0)\,[\alpha-CD]} \quad \text{----- (1)}$$

Where $A - A_0$ is the difference between the absorbance of 2-Methyl-4-nitrophenol in the presence and absence of α-CD, A good linear correlation is obtained, confirming the formation of 1:1 inclusion complex. The binding constants for the formation of 2-Methyl-4-nitrophenol:α-CD complex is calculated and it is found to be 171.52M^{-1}.

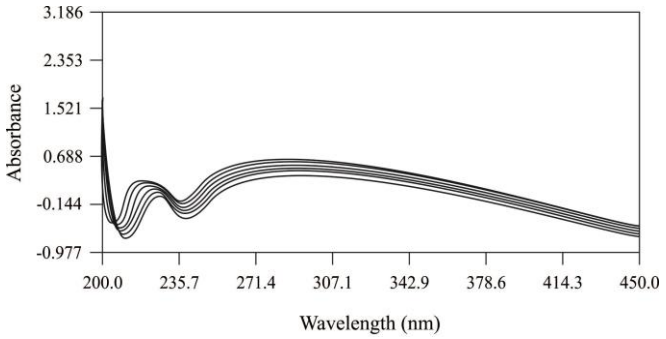


Fig-2: Absorption Spectra of 4-Methyl-2-nitrophenol:α-CD

Table-2: Absorption Spectral data of 4-Methyl-2-nitrophenol:α-CD

S.No	4-methyl-2-nitrophenol:α-CD					
	Concn .Of $\alpha-CD$	λ_{abs}	Abs	$\frac{1}{A-A_0}$	log ε	$\frac{1}{[\alpha-CD]}$
1	0	299.6	0.079		1.59	
2	0.002	288.8	0.134	18.18	1.82	500
3	0.004	286.2	0.175	10.41	1.94	250
4	0.006	284.3	0.193	8.77	1.98	166.6
5	0.008	282.1	0.217	7.24	2.03	125
6	0.010	280.7	0.242	6.134	2.08	100

The absorption spectra of 4-Methyl-2-nitrophenol are blue shifted from λ_{max} 299.6 nm to 280.7 nm with increase in absorbance upon increase in α-CD concentration. The absorption spectra is shown in Fig-2 and the data are tabulated in Tab 2. The spectral shift is attributed to the formation of inclusion complex between 4-Methyl-2-nitrophenol and α-CD.

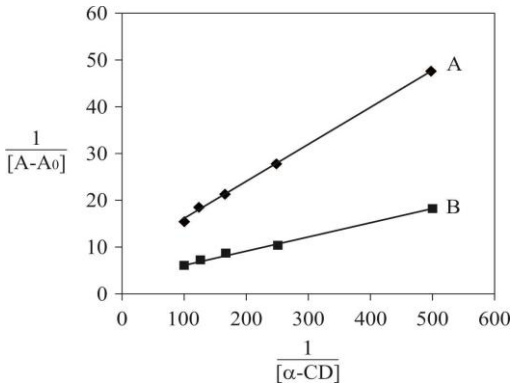


Fig-3: Benesi-Hildebrand plot of (A) 2-Methyl-4-nitrophenol:α-CD and B) 4-Methyl-2-nitrophenol:α-CD

The stoichiometry of the complex can be obtained from the Benesi-Hildebrand equation (1) and it is found to be 1:1. The binding constant value calculated is found to 181.81 M^{-1}.

Fluorescent Studies

The emission spectra of 2-Methyl-4-nitrophenol is depicted in Fig-4 and the spectral data is shown in Tab 3. There is a red shift in the emission spectra from 367 nm to 376 nm as the concentration of α-CD is increased. The shift in the spectrum indicates the formation of inclusion complex between 2-Methyl-4-nitrophenol and α-CD.

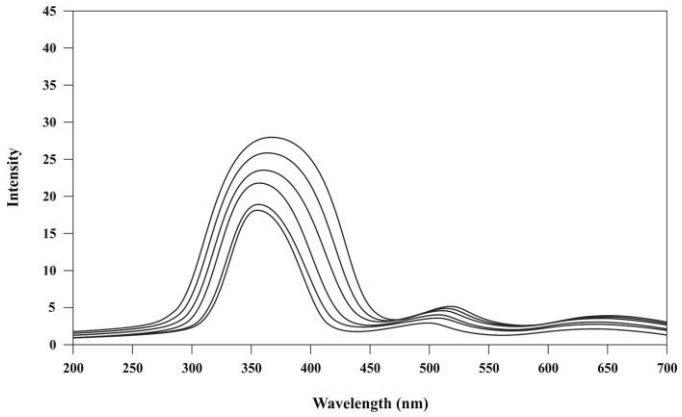


Fig-4: Emission Spectra of 2-Methyl-4-nitrophenol:α-CD

The emission spectra of 4-Methyl-2-nitrophenol shows a blue shift from 321 nm to 308 nm with increase in intensity upon increase in α-CD concentration as shown in Fig-5 The spectral shifts confirm the formation of inclusion complex of 4-Methyl-2-nitrophenol and α-CD.

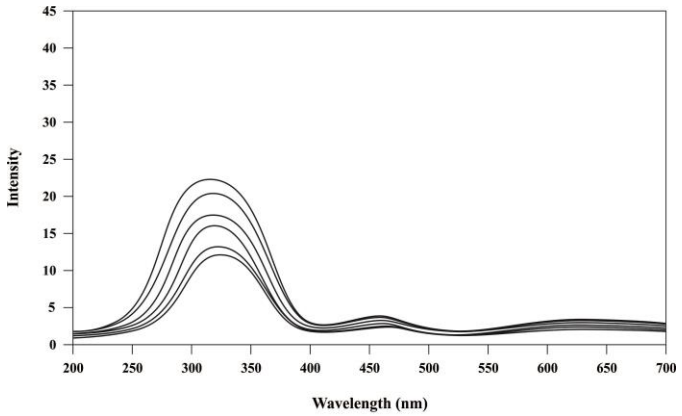


Fig-5: Emission Spectra of 4-Methyl-2-nitrophenol:α-CD

$$\frac{1}{I-I_0} = \frac{1}{I'-I_0} + \frac{1}{K_B(I'-I_0)\,[\alpha-CD]} \text{ -------- (2)}$$

Table-3: Emission Spectral Data of 2-Methyl-4-nitrophenol:α-CD

S.No	2-Methyl 4-nitrophenol:α-CD				
	Concn. Of $\alpha - CD$	λ_{flu}	Int	$\frac{1}{I - I_0}$	$\frac{1}{[\alpha - CD]}$
1	0	367	18.65		
2	0.002	368	19.23	1.72	500
3	0.004	369.5	21.45	0.357	250
4	0.006	372.0	23.50	0.206	166.6
5	0.008	374.0	25.8	0.139	125
6	0.010	376.0	27.9	0.108	100

Concn – Concentration, Int - Intensity

Table-4: Emission Spectral Data of 4-Methyl-2-nitrophenol:α-CD

S.No	4-Methyl 2-nitrophenol:α-CD				
	Concn. Of $\alpha - CD$	λ_{flu}	Int	$\frac{1}{I - I_0}$	$\frac{1}{[\alpha - CD]}$
1	0	321	11.423		
2	0.002	319	13.251	0.5470	500
3	0.004	315	15.73	0.2321	250
4	0.006	312	17.38	0.1678	166.6
5	0.008	310	20.75	0.1072	125
6	0.010	308	22.11	0.0935	100

Concn – Concentration, Int – Intensity

The stoichiometry of the inclusion complexes formed for both 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol is found to be 1:1. The binding constant value of 2-Methyl-4-nitrophenol calculated is found to be 167.56 M^{-1} and 4-Methyl-2-nitrophenol is 267 M^{-1}.

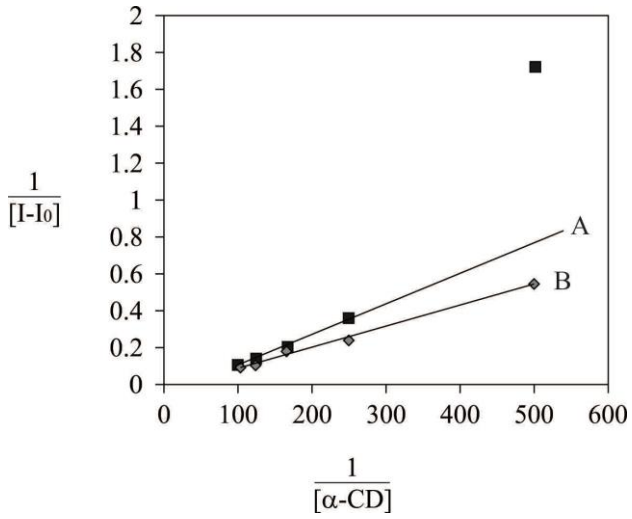


Fig-6: Benesi-Hildebrand plot of (A) 2-Methyl-4-nitrophenol:α-CD and B) 4-Methyl-2-nitrophenol:α-CD

3.3. FTIR Spectroscopic Studies

The inclusion complex formation in solid state is confirmed by FTIR studies. In 2-Methyl-4-nitrophenol, the two peaks obtained at 1537.27 cm^{-1} and 1332.81 cm^{-1} correspond to the characteristic stretching of aromatic nitro group. The peak observed at 839.03 cm^{-1} corresponds to the -C-N stretching vibration for aromatic nitro group. The characteristic peak at 1624 cm^{-1} and 1579 cm^{-1} corresponds to -C=C- stretching vibrating of aromatic nuclei. The =C-O stretching vibration is observed at 1153.4 cm^{-1}.

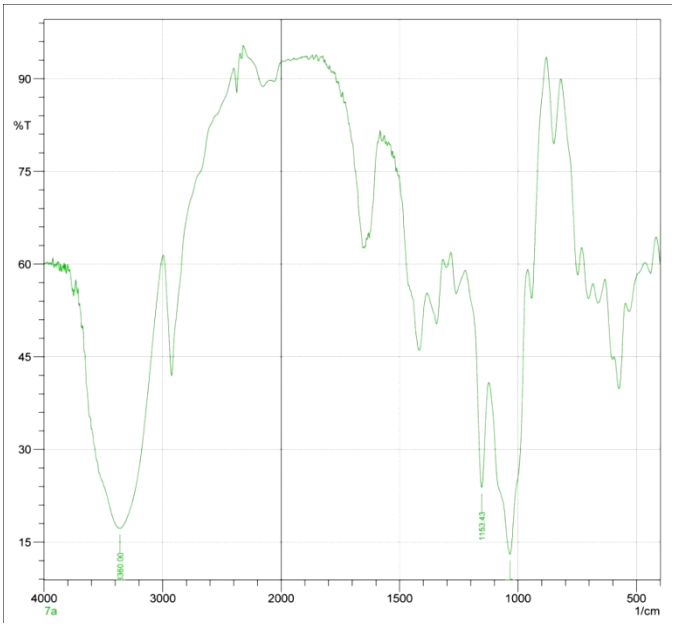


Fig-7:FTIR Spectra of α-CD

In the 2-Methyl-4-nitrophenol: α-CD complex, the characteristic stretching of aromatic group is shifted to 1338.6 cm-1 the characteristic -C-N stretching vibration for aromatic nitro group disappeared in the complex. The -C=C- stretching vibration remain as such in the complex, but the intensity is decreased. From all the above details it is inferred that the nitro group and the benzene ring is encapsulated into the α-CD cavity. The -OH group is projected above the α-CD rim. All the above data confirms the formation of solid inclusion complex and thereby confirms that 2-methyl-4- nitrophenol is included into the α-CD cavity.

In the case of 4-Methyl-2-nitrophenol, the characteristic peaks at 1537.27 cm^{-1} and 1371.39 cm^{-1} correspond to the stretching vibration of aromatic nitro group. The characteristic peak at 885.53 cm^{-1} corresponds to -C-N stretching for aromatic nitro group. For aromatic nuclei, the -C=C- stretching vibration is observed at 1178.51 cm^{-1} corresponds to =C-O stretching vibration. In the 4-Methyl-2-nitrophenol the characteristic vibration stretching for aromatic nitro group is shifted to lower wavelength with decreased intensity. The -C-N stretching vibration is also shifted to 848.68 cm^{-1}. The characteristic -C=C- stretching vibration is shifted to 1643.35 with decreased intensity. The =C-O stretching vibration is shifted to 1157.29 cm-1. The FTIR spectral data confirms the formation of solid inclusion complex between 4-methyl-2- nitrophenol and α-CD. This further serves as evidence that the nitro group and the aromatic ring is encapsulated into the α-CD cavity.

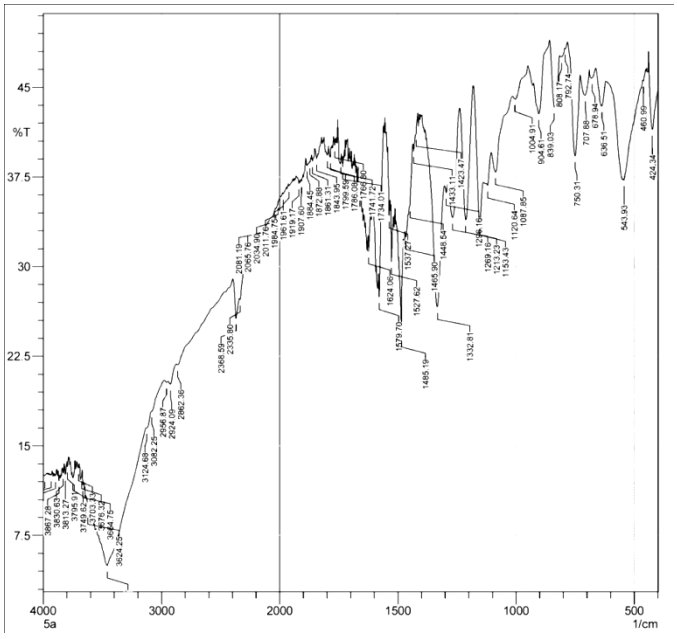


Fig-8:FTIR Spectra of 2-Methyl-4-nitrophenol

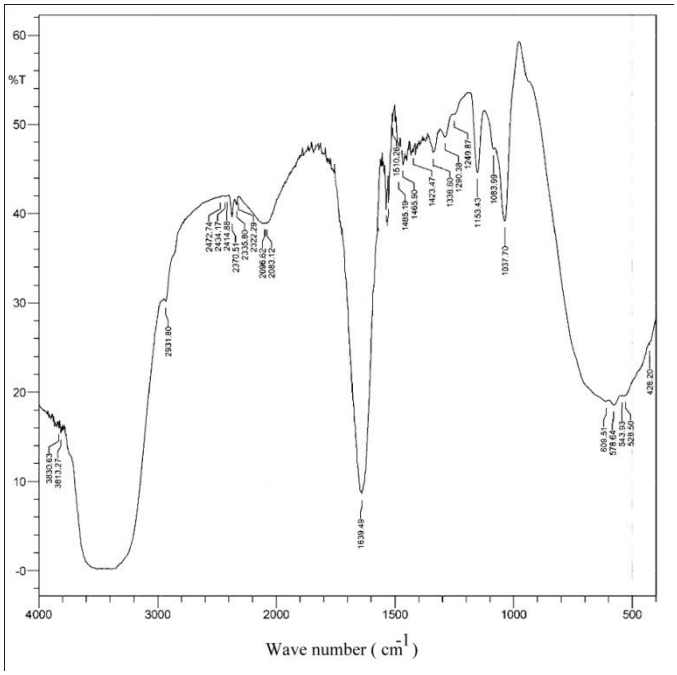


Fig-9: FTIR Spectra of 2-Methyl-4-nitrophenol:α-CD

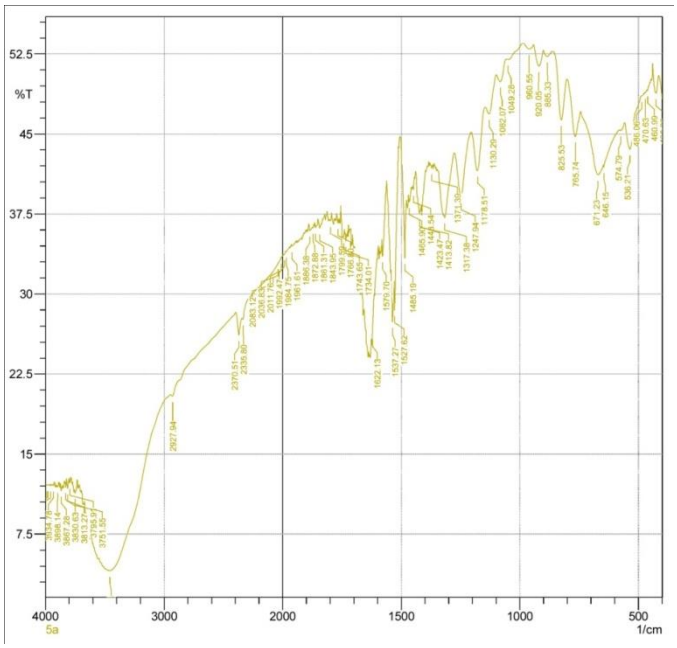


Fig-10:FTIR Spectra of 4-Methyl-3-nitrophenol

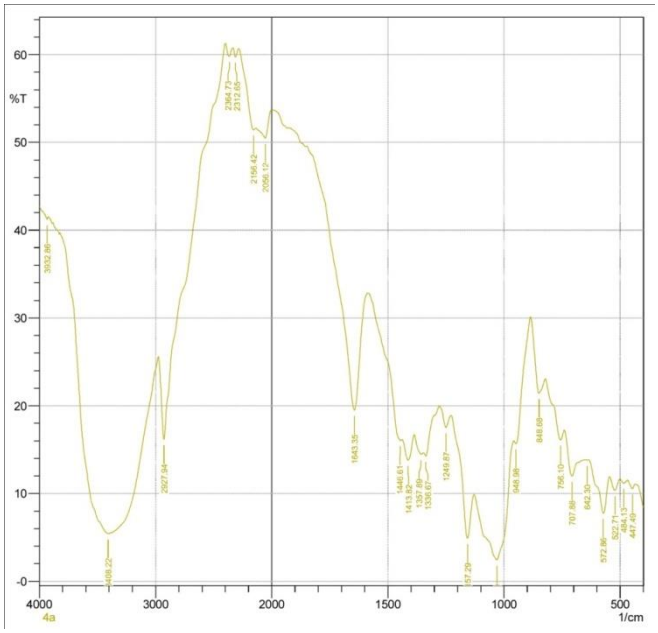
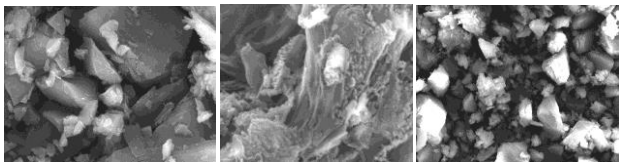


Fig-11:FTIR Spectra of 4-Methyl-3-nitrophenol:α-CD

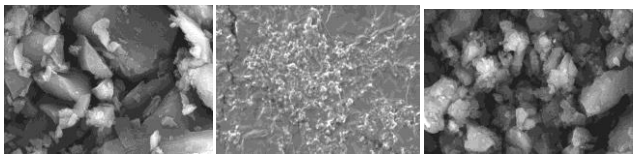
3.4 Scanning Electron Microscopic Studies

The surface morphology of pure 2-Methyl-4-nitrophenol, α-CD, 4-Methyl-2-nitrophenol solid inclusion complex of 2-methyl-4-nitrophenol with α-CD and 4-methyl-2-nitrophenol with α-CD are examined in their powder form. Pure α-CD shows plated crystalline structure. Whereas 2-Methyl-4-nitrophenol appears as rock stone crystals. The 2-Methyl-4-nitrophenol: α-CD has morphological features different from that of pure α-CD and 2-Methyl-4-nitrophenol. This serves as a proof for the formation of inclusion complex between 2-Methyl-4-nitrophenol and α-CD.



(a) (b) (c)

Fig-12: Scanning Electron Microscopic Photographs (a) α-CD (b) 2-Methyl-4-nitrophenol (c) 2-Methyl-4-nitrophenol:α-CD



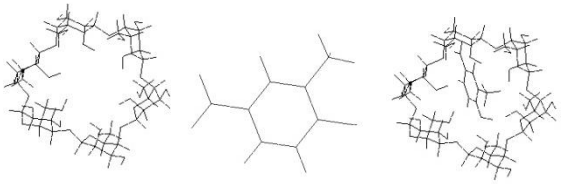
(a) (b) (c)

Fig-13: Scanning Electron Microscopic Photographs (a) α-CD (b) 4-Methyl-2-nitrophenol (c) 4-Methyl-2-nitrophenol:α-CD

Pure 4-Methyl-2-nitrophenol appears as crystalline rock structure. α-CD is observed as plated crystals. The 4-Methyl-2-nitrophenol:α-CD complex has a different morphological texture from that of pure 4-Methyl-2-nitrophenol and α-CD. This is an indication that an inclusion complex is formed between 4-Methyl-2-nitrophenol and α-CD. Thus SEM studies confirm the formation of solid inclusion complex.

3.5 Molecular docking studies of inclusion process.

The 3D structure of 2-Methyl-4-nitrophenol is obtained from crystallographic databases. Using PatchDock server version 1.0 the guest molecules of 2-Methyl-4-nitrophenol is docked into the α-CD cavity. The PatchDock server program gave several possible models for the most probable structure depending on the energetic parameters; geometric shape complementarity score, approximate interface area size and atomic contact energy of 2-Methyl-4-nitrophenol : α-CD inclusion complexes. Therefore the docked 2-Methyl-4-nitrophenol : α-CD 1:1 model with the highest geometric complementarity score 2356 for the approximate interface area size of 247.60 Å and atomic contact energy of -197.72 kJ/ mol for 2-methyl-4-nitrophenol: α-CD was calculated. This is highly probable and energetically favourable model and is in good correlation with results obtained through experimental methods.

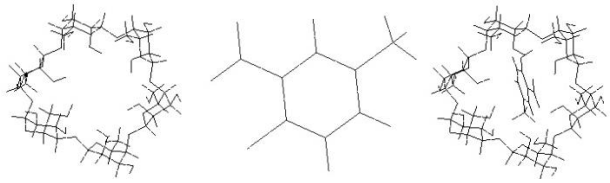


(a) (b) (c)

Fig-14: Docking Studies of (a) α-CD (b) 2-Methyl-4-nitrophenol (c) 2-Methyl-4-nitrophenol:α-CD

Similarly the 3D structure of 4-methyl-2- nitrophenol is obtained from crystallographic databases. The guest molecules of 4-methyl-2- nitrophenol is docked into the cavity of α-CD using PatchDock server version 1.0. The PatchDock server program gave several possible docked models for the most probable structure based on the energetic parameters, geometric shape complementarity score, approximate interface are a size and atomic contact energy of the 4-Methyl-2-nitrophenol:α-CD inclusion complexes.

The docked 4-Methyl-2-nitrophenol:α-CD 1:1 model with the highest geometric shape complementarity score 2340, approximate interface area size of 253.90 Å and atomic contact energy of -210.93 KJ/mol is calculated. This is the highly probable and energetically favorable model and it is found to be in good agreement with results obtained through experimental methods.



(a) (b) (c)

Fig-15: Docking Studies of (a) α-CD (b) 4-Methyl-2-nitrophenol (c) 4-Methyl-2-nitrophenol:α-CD

4. CONCLUSIONS

Both 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol forms inclusion complexes with α-CD in aqueous solution. The formation of the inclusion complexes is confirmed by UV spectroscopy and fluorimetry studies. The inclusion complex is formed as a 1:1 stoichiometry of 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol to the α-CD while FT-IR and SEM analysis showed that the $-NO_2$ group attached ring of the 2-Methyl-4-nitrophenol, 4-Methyl-2-nitrophenol, are completely encapsulated in the α-CD cavity. The energetically favorable and most probable structure proposed by molecular docking studies is in good correlation with the inclusion model predicted through experimental investigation. Therefore complexation of 2-Methyl-4-nitrophenol and 4-Methyl-2-nitrophenol with α-CD can be considered as a better option and suitable measure for separation of contaminants from the environment.

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