International Journal of Latest Trends in Engineering and Technology
Special Issue - International Conference on Nanotechnology: The Fruition of Science-2017, *pp.016-021*
e-ISSN:2278-621X, p-ISSN: 2319-3778

SYNTHESIS AND CHARACTERISATION OF AN INCLUSION COMPLEX WITH A-CYCLODEXTRIN AND GRANATONINE

K. Francy[1] and J. PremaKuamri[2]

Abstract-A α-cyclodextrin (α-CD) inclusion complex containing granatonine obtained from pomegranate peel, as a guest was synthesised by kneading method with aliquot addition of alcohol. The inclusion complex was characterized by Fourier Transform Infrared (FTIR),1H Nuclear Magnetic Resonance (1H NMR) and Fluorescence spectral techniques.SEM analysis confirmed that the morphology of inclusion complex was crystal in nature. Solvent effect of the dye was also studied. The interaction of α -CD and granatonine was analyzed by means of UV-Vis absorption spectral techniques. The formation constant was calculated by using a modified Benesi-Hildebrand equation at 25°C. The formation constant obtained was 5x102M-1. Besides that, the stoichiometry ratio was also determined to be 1:1 for the inclusion complex of α -CD with granatonine.
Key words: Inclusion complex,Granatonine, α-Cyclodextrin.

1. Introduction

Supramolecular studies give a broad idea of intermolecular interactions where covalent bonds are not likely to form between the interacting species. Thus, most of this interaction has been performed by host-guest interaction. Among the host molecules, cyclodextrin seems to be the most promising to form inclusion complexes, especially with various guest molecules with suitable polarity and dimensions [1]. The special characteristic of cyclodextrins is the ability to form an inclusion complex with various organic molecules through host-guest interaction with the interior cavity that provides hydrophobic environment to trap a non polar pollutant [2]. The inclusion complex of these host–guest systems occur through various interactions, such as hydrogen bonding, van der Waals interaction, hydrophobic interactions and also electrostatic attractions [3]. Thus the physical, chemical and biochemical properties of guest molecules will be modified and the application criteria of those guest molecules also can be improved.

In this present study the guest molecule is granatonine, a dye molecule obtained from pomegranate peel. The main colouring agent in the pomegranate peel is granatonine which is present in the alkaloid form N-methyl granatonine.Pomegranate peel contains medicinal value to treat different diseases and also to prevent sickness [4]. In addition to their nutritional value, pomegranate peels were used since ancient times as anti-thelmintic, anti-tracheobronchitis, for healing wounds, ulcers, bruises, stomatitis, diarrhoea, vaginitisand against excessive bleeding. It also exhibits textile property in dyeing and these medicinal and textile propertiesare enhanced when complexed with α-Cyclodextrin, for various purposes such as pharmaceutical, food additives, nutrient supplements and dyeing purposes [5].

2. Materials and Methods

Fresh and healthy pomegranates were purchased from Apta market, Nagercoil, Kanyakumari District, Tamilnadu. The peels of the fruits were carefully collected and washed well and shadow dried and powdered for further research.α-CD was procured from Merck. Double distilled water was used throughout the experiment.

2.1 Preparation of extract

2.1.1Extraction of Crude Dyestuff:

100g of sample was weighed and taken in a round bottom flask and 500ml of solvent (ethanol and water) in the ratio 40:60 was added to it. The flask was heated in a water bath at 60°C for 1 hour.The solution was then filtered to obtain crude dyestuff.

2.1.2 Purification of Crude Dyestuff:

The crude dyestuff is distilled to get 1/3rd of the solution using the Soxhlet apparatus at 70°C for 3 hours. In this process ethanol is recovered and the concentrated dye is obtained. The solution is kept overnight at room temperature for evapotation. The obtained particles are dried in the oven overnight at 60°C[6].

2.2 Inclusion Complex of α-Cd with Granatonine

[1] *Department of Chemistry, Holy Cross College (Autonomous), Nagercoil-629 004*
[2] *Department of Chemistry and Research Centre, Scott Christian College (Autonomous),Nagercoil-629 003.*

0.0459g of Granatonine was dissolved in 30ml of alcohol and 0.2918g of α-CD was dissolved in 30ml of double distilled water. These two solutions were mixed in a 100ml beaker and stirred with magnetic stirrer on a hot plate till inclusion was completed. This was confirmed by the appearance of pasty like substance. The substance was carefully removed, dried and packed for further characterization.

2.3 Characterization techniques

The absorption spectral studies were carried out using Shimadzu UV-1800 spectrophotometer. FTIR analysis of the dye and inclusion complex was done by KBr pellet in 1:100 ratio and the spectrum was recorded using Shimadzu IR Affinity-1. For SEM analysisTungsten filament is used and the images are taken with working distance between 8.5 to 12mm at 20KV.Solvent used was D_2O for NMR studies.

3. Results and Discussion

3.1 UV absorption spectral analysis

UVabsorption measurement is proved to be a very convenient method to explore the structural changes and formation of a complex. The simple superposition of the characteristic absorption peaks corresponding to the dye and the inclusion complex can be determined. This means binding has occurred between α-CD and the dye pigment.

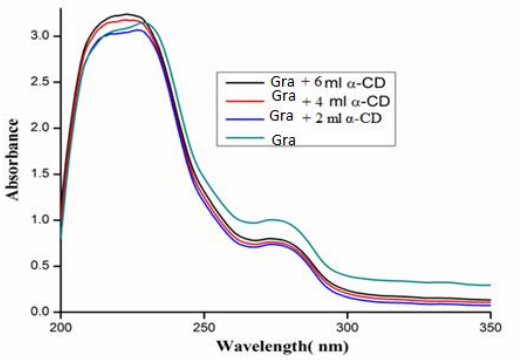


Fig. 1 UV Spectrum of Granatonine at different concentrations of α-CD

The **Fig.1** shows the UV spectrum and λ_{max} value of granatonine at different concentrations of α-CD. The λ_{max} value decreases from 285nm to 279nmas the concentration of the α-CD increases. Inorder to determine the stiochiometry of the inclusion complex, the absorbance and fluorescence dependence behavior of the dye molecule on α-CD, are analysed using the Benesi Hildebrand equation and binding constant between granatonine and α –CD is calculated. The calculated binding constant is $5x10^2M^{-1}$.The binding constant proves binding between Granatonine and α-CD. This higher binding constant is an evidence for the formation of hydrogen bonding. As there is hydrogen bonding interaction, the inclusion is higher between the cyclodextrin and the dye pigment.This hydrogen bondingmay be due to the interaction between the methyl hydrogens of granatonine with the hydroxyl groups of cyclodextrin.

3.2 Fluorescence Spectral Analysis

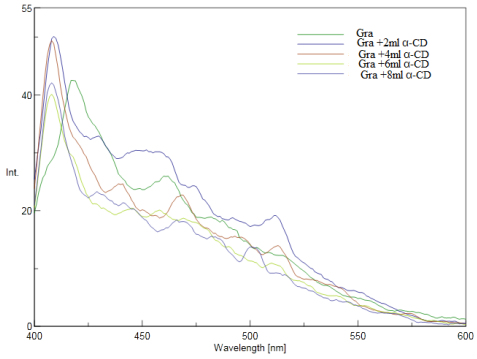


Fig. 2 Emission spectrum of Granatonine with α–CD.

In this work the concentration of dye were stabilized and concentrations of α-CD varied. From the spectra, it is vivid that the dye pigment shows emission wavelength at 420nm. As the α–CD concentration increases, the wavelength acquires a hypsocromic shift. But the intensity increases. This can reasonably attributed to a change in polarity of

the formed complex. Table 1 shows the absorption and emission spectral values of granatonine at at various concentration of α –CD

Table 1: Absorption and emission spectral values of Granatonine at different concentrations of α –CD

S.No.	Concentration of α –CD	Emission		Absorption	
		Granatonine			
		$\lambda_{flu(nm)}$	Intensity	$\lambda_{max(nm)}$	Absorbance
1	0	420	42	285	3.029
2	0.01	416	43	283	3.061
3	0.05	414	45	281	3.179
4	0.025	410	49	279	3.243
5	0.012	408	52	-	-

3.3Infrared spectral analysis

FTIR is a very useful tool to prove the existence of both guest and host molecules in their inclusion complexes [7]. Fig. 3 and Fig. 4, 4a show the FTIR spectrumof thedye the inclusion complex and α–CD.The presence of bridging nitrogen in granatonine causes the peak at 3300 $cm^{-1.}$ The peak at 3440 cm^{-1} in the guest molecule is shifted to 3465 cm^{-1} in the inclusion complex. The C=O bending vibration of guest molecule at 1750 $cm^{-1}$is shifted to 1690 cm^{-1} in the complex. The band at 1350 cm^{-1} corresponds to thepresence of C-N stretching frequency, which is shifted to 1250 cm^{-1}in the complex. Besides that, a broad band of granatonine at 3470.72 cm^{-1} wasfound to be narrowed in the FTIR spectrum of the inclusion complex which is a good indication of theformation of the inclusion complex.

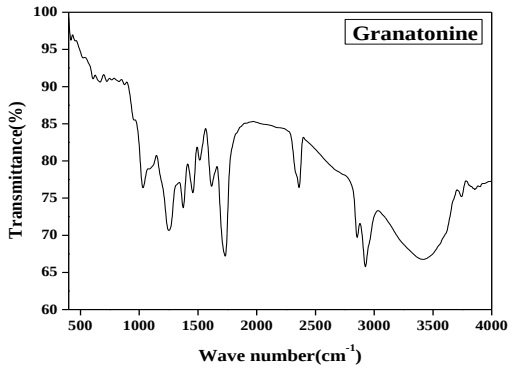


Fig. 3 FTIR spectrum of granatonine

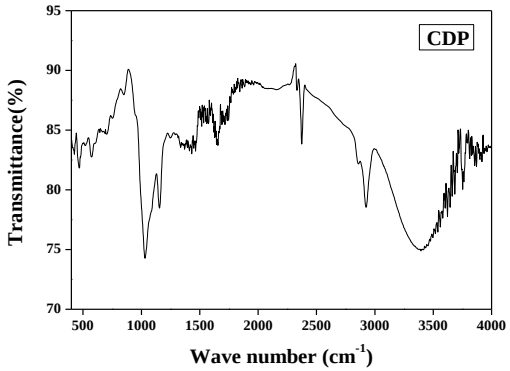


Fig. 4FTIR spectrum of inclusion complex

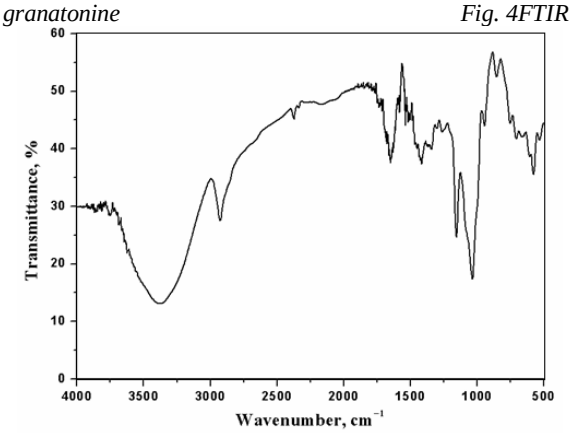


Fig. 4(a)FTIR spectrum of α-CD

3.3. ^{1}H NMR Spectral Analysis

Fig.5 is the ^{1}H NMR spectrum of pomegranate dye. The chemical shift at 4.7ppm shows the presence of α monosubstituted aliphatic proton in the dye pigment. Presence of nitrogen in the dye is confirmed by the shift at 1.2ppm. The shift at 0.8ppm may be due to the presence of axial protons of the dye.

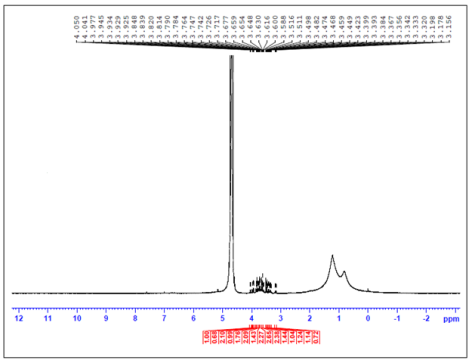


Fig. 5 ^{1}H NMR spectrum of pomegranate dye

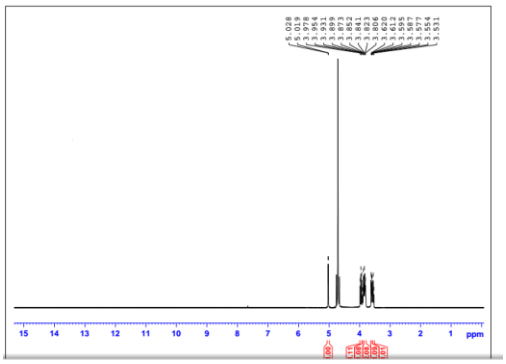
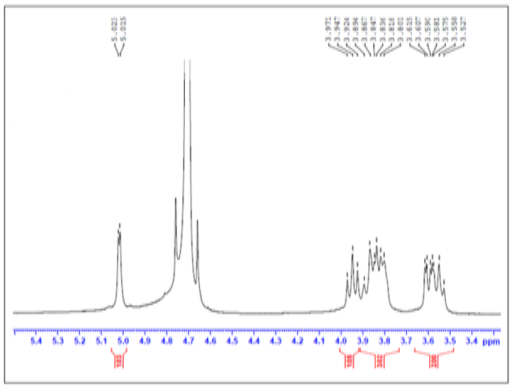


Fig. 6 ^{1}H NMR spectrum of α-CD



Fig, 7^{1}H NMR spectrum of complex

Fig.6 shows the ^{1}HNMR spectrum of α-CD. The sharp peaks at 5.028, 4.1, 3.899, 3.577 and 3.531 indicate the different protons of the cyclodextrin

Fig.7 shows the ^{1}H NMR spectrum of inclusion complex of α CD with granatonine. The chemical shift in inclusion complex is upfield when compared with inclusion complex. So the protons are shielded in the complex. This is confirmed by the low value of chemical shift (δ) in the inclusion complex spectrum.The cyclodextrin peak found at approximately 3.85ppm is shifted upfield upon complexation. In the free α-CD, peaks were superimposed at approximately 3.75ppm. Upon complexation those protons are resolved and shifted upfield. Unlike the cyclodextrin protons that are generally shifted upfield, guest molecule protons exhibits a downfield shift upon complexation.From these we can conclude that inclusion has taken place between α-CD and dye pigment, and it is well known that if a complex is formed by inserting the guest molecule into the cavity, the interior protons of the α-CD are considerably shielded leading to an upfield chemical shift, as first observed by Demarco and Thakkar [8]. Thus the spectral pattern confirms the formation of inclusion.

The above specctral studies confirm that the chemical compound present in the dye is granatonine and its structure is shown in Fig.8

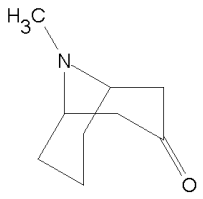


Fig. 8 Structure of granatonine

3.4.Scanning Electron Microscope (SEM) Analysis

The SEM analysis is used to determine the morphological structure of α-CD, Granatonine and its synthesized inclusion compounds (Figs. 9-11).

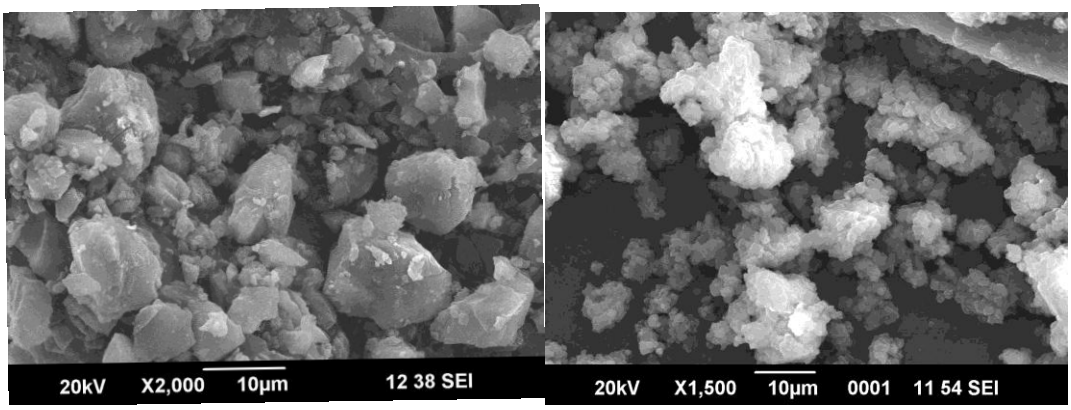


Fig.9 SEM image of α-CDFig. 10 SEM image of Granatonine

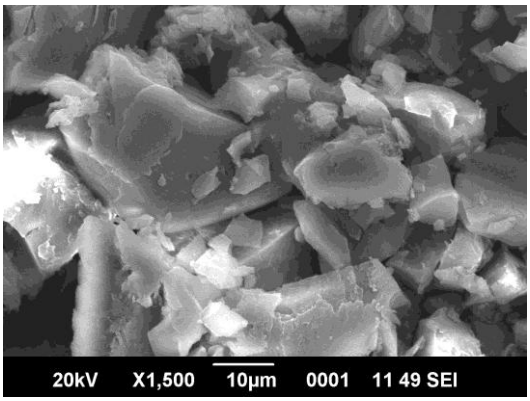


Fig.11 SEM image of inclusion complex

The SEM images show individual dye particle size, nature and the structure of the synthesized inclusion complex. SEM image of Granatonine appeares as sheated structure and the inclusion complex is different from Granatonine. From the images it is confirms that the dye extracted and synthesized inclusion complex are crystalline in nature. The clear images prove the fact that dyes containing the chemical compounds are included in the α-CD.

3.5Solvent Effect

The UV spectrum of the dye sample is taken in four solvents such as water, ethanol, ether and hexane. The spectrums are shown in Fig.12. When granatonine is dissolved in water, the absorption peak appears at 278nm. For alcohol it appears at 275nm. The absorption peak appears at 268nm for hexane and no peak for ether. These results imply that the increase in the polar nature of the solvent increases the absorption of wavelength. The absorption intensity is higher for the solvent water, followed by alcohol, hexane. This shows that for polar solvents the spectral wavelength and absorption are higher to that of the non-polar solvents.This may be due to the presence of hydrogen bond formation between the dye and the solvents.

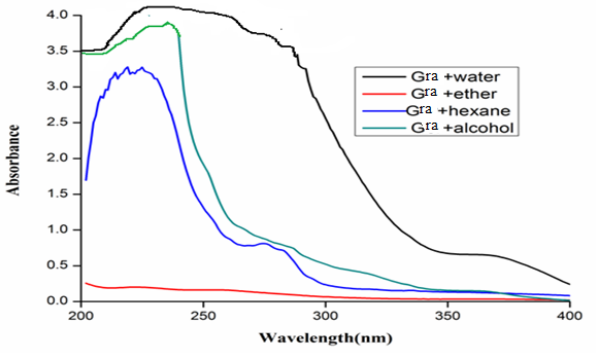


Fig. 12 UV spectrum of Granatonine with different solvents

4. Conclusion

Natural dyes cannot be used as simple alternatives to synthetic dyes and pigments. The major problem, when dealing with natural dye is the colour, not fasting on the fabric. The colour is easily removed from the textile. This research proposes a new idea and technology that can overcome this problem. The property of dyes can be improved with α-Cyclodextrinencapsulation. This encapsulation enhances the textile properties as well as medicinal properties of the natural dye.

UV spectrum at various concentarations of α – CD with granatonin reveals the formation of inclusionbetweenα - CD and the dye. This is proved by the increase in absorbance as the concentration of α - CD is increased. Binding constant is calculated using the data obtained from UV spectrum. Binding constant of Granatonine inclusion complex is $5x10^{2}M^{-1.}$ The emission studies give a detailed idea about the formation of inclusion between dye and α-CD. The steady increase in the emission peak with the addition α-CD confirms the binding between α-CD and the dye. ^{1}HNMR spectra for the dye pigment and the inclusion complex with α-CD show the formation of inclusion. The spectral pattern confirms the formation of complexation. FTIR studies also confirm the formation of inclusion complex between dye pigment and α-CD. The characteristic stretching and bending vibrational frequencies confirms the presence of chemical compound granatonine in pomegranate peels.The presence of hydrogen bonding between dye and solvent is confirmed by the increase of wavelength with the increase of absorbance. Thus the synthesised inclusion complex can be used in textile and pharmaceutical industries.

5. References

[1] Connors K.A.,1997. The stability of cyclodextrin complexes in solution.*Chem. Rev.*, *97*,1325–1358.

[2] Lee C.W., Kim S.J., Youn Y.S., Widjojokusumo E., Lee YH., Kim J., Lee Y.W., Tjandrawinata R.R.,2010. Preparation of bitter taste masked cetirizine dihydrochloride/β-cyclodextrininclusion complex by supercritical antisolvent (SAS) process. *J. Supercrit. Fluids 55*,348–357.

[3] Szejtli J., 1998. Introduction and general overview of cyclodextrin chemistry.*Chem. Rev*, *98*,1743–1753.

[4] Jennifer, H.S., 2010. Structural characterization of the Brooker'smerocyanine/Cyclodextrin complex using NMR spectroscopy and modeling,*Journal of Molecular structure*. 965, 35-38.

[5] HamadaF., IshikawaK., HiguchiY., AkagamiY., uenoA.J., 1996. .Inclusion Phenom, Mol.Recog.chem,25, 283-294.

[6] Kulkarni S.S., Gokhale A.V., Bodake U.M., Pathade G.R., 2011. Cotton Dyeing with Natural Dye Extracted from Pomegranate (Punicagranatum) Peel, Universal Journal of Environmental Research and Technology. ISSN 2249 0256,2, 135-139.

[7] Jiao, H., Goh, S.H.,Valiyaveettil, S. 2011. Inclusion complexes of poly(neopentyl glycol sebacate) withcyclodextrins. *Macromolecules 34*, 8138–8142.

[8] Demarco P.V., Thakkar A.L., 1970. Cyclodextrin and their complexes, Journal of chemical Soc., Chem Commun., 2, 317-318.