Augmentation of the Antioxidant Activity of Stigmasterol on Inclusion with Alpha-Cyclodextrin

K. Francy and *J. Premakumari
Department of Chemistry, Holy Cross College (Autonomous), Nagercoil - 629004.
Affiliated to Manonmaniam Sundaranar University, Tirunelveli - 627012.
Email: francy.k@holycrossngl.edu.in
Department of Chemistry, Scott Christian College (Autonomous), Nagercoil - 629004.
*Corresponding Author - Email: premaisaac67@gmail.com

ABSTRACT

Cyclodextrins aid in enhancing solubility, stability and bioavailability of various bioactive hydrophobic compounds by complex formation. The aim of this work is to analyse the anti-inflammatory activity of stigmasterol inclusion complex with α–cyclodextrin. The newly synthesised inclusion complex exhibit higher activity compared to pure stigmasterol. Absorption, NMR andDSC studies confirm the presence of inclusion. This is a suitable method for designing a novel drug.

Keywords: *Cyclodextrin, Stigmasterol, Inclusion complex and Anti-inflammatory*

1. Introduction

Stigmasterol is an unsaturated 6-6-6-5 tetracyclic phytosterol [1] with a polar hydroxyl group at one end and a large non polar lipophilic planar and rigid 6-6-6-5 skeleton at the other end. It has a flexible C_{10} branched-chain which makes it an interesting amphiphile.To augment the aqueous solubility of lipophilic moieties such as stigmasterol, researchers have developed traditional and novel approaches. A well recognised strategy in practice for more than a century is the formation of cyclodextrin inclusion complexes.It has been shown to be a promising technique for enhancing solubility, improving stability and bioavailability of poorly water-soluble drugs.

Cyclodextrins are cyclic organic compounds obtained by enzymatic transformation of starch. Among the class of host molecules, the α-CD is one of the most abundant natural oligomers with 6 glucose units [2]. The inner cavity is hydrophobic [3] whereas the exterior is strongly hydrophilic [4]. This unique structure allows various substrates to be included in the cavity through non covalent bonds to form inclusion complexes.

2. Materials and Methods

2.2 Reagents

Analytical grade of stigmasterol and α-CD were purchased from Sigma Aldrich.Ethanol was purchased from Himedia. The chemicals were used as purchased. The solvents used were of analytical grade.

2.3 Preparation of solid inclusion complex of stigmasterol and α-CD

About 0.1238g of stigmasterol was accurately weighed and dissolved in 30mL ethanol. About 0.2919g of α-CD was dissolved separately in 30mL double distilled water. Both the solutions were mixed together in the beaker and put over electromagnetic stirrer to stir continuously for 48hrs at room temperature. The precipitate obtained after evaporation was dried and used for characterisation.

2.4 Characterisation Techniques

2.5 UV – VIS spectroscopy

Absorbance values were recorded for the liquid inclusion complex of stigmasterol with α-CD using UV-1800, (Shimadzu) spectrophotometer.

2.6 Nuclear Magnetic Resonance (NMR) spectroscopy

^{1}H NMR spectroscopy studies of the solid inclusion complex was recorded in Bruker 400MHz FT-NMR spectrometer. For the samples $CDCl_3$ was used as solvent and Tetramethylsilane (TMS) as internal reference. The chemical shifts (δ) were reported in ppmrelative to TMS at 298K.

2.7 Differential Scanning Calorimetry (DSC)

DSC analysis of the solid inclusion complex was carried out on NETZCH DSC 204 calorimeter. A sample of approximately 1.4mg was weighed in aluminium pans. These samples were heated over a range of 25°C - 300°C at a constant rate of 10°C/mins in a nitrogen purge of 50 mL/minutes. An empty aluminium pan was used as reference.

2.8 Anti-inflammatory Assay

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi (1968) and Sakat *et al*. (2010) [4] with slight modifications. 500 µL of 1% bovine serum albumin was added to the test samples with varied concentration (100, 90, 70, 50, 30, 15, 10, 5, 1 and 0.5 µg/mL). This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. The experiment was carried out in triplicates and percentage of inhibition for protein denaturation was calculated using the following formula

$$\% \text{ Inhibition} = 100\frac{(A_1 - A_2)}{A_0} \text{x}100$$

Where A_1 is the absorbance of the sample, A_2 is the absorbance of the negative control and A_0 is the absorbance of the positive control.

3. Results and Discussion

3.1 Absorption studies on α-CD: stigmasterol inclusion complex

The absorption maxima of stigmasterol in varying the concentrations of α-CD are shown in figure 1. The λ_{max} and the absorbances are listed in table 1From the figure and table, it is clear that the absorption maxima shift towards higher wavelength and the intensity of the peaks rises on increasing the concentration of α-CD. The absorption maxima exhibit a red shift in stigmasterol from λ_{max}~262 to λ_{max}~268nm. The shifting of absorption peak position is due to the complex formation between stigmasterol and α-CD and the complex is stabilized within the cavity of α- CD, through weak intermolecular forces.

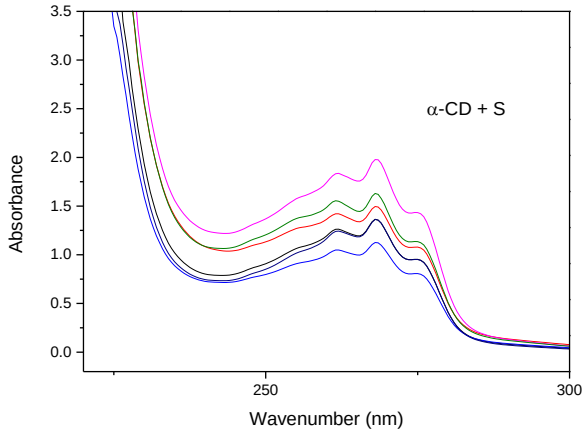

Fig. 1Absorption spectra of stigmasterol at various concentration of α-CD

Table . 1 Absorption spectral data of stigmasterol with α-CD

S.No	[α-CD]	λ_{abs}	Absorbance	$\frac{1}{A-A0}$	Log ε	$\frac{1}{[\alpha-CD]}$
1	0	262	1.0494	-	-	-
2	0.002	263.5	1.2430	5.16	4.427	500
3	0.004	264	1.2633	4.67	4.501	250
4	0.006	265	1.4208	2.69	4.508	166.6
5	0.008	266	1.5547	1.97	4.559	125
6	0.010	268	1.8363	1.27	4.670	100

3.2 Nuclear Magnetic Resonance (NMR) Spectroscopic Studies

Proton NMR is an important technique in analysing inclusion complexes of cyclodextrins in solid and liquid state [3]. The encapsulation of a guest molecule into the cavity of cyclodextrin is indicated by the changes in chemical shift of both guest and host NMR

spectrum (Panda *et al*., 2016). As shown in figure 2. H_3, H_5 and H_6 protons are located near the wider and narrow rim of the CD cavity respectively. H_1, H_2 and H_4 are located on the exterior of the cavity. Upfield shifts that are noted for H_3, H_5 and H_6 internal protons of CD are due to the substitution of water molecules by the hydrophobic guest molecule [5]. When$\delta H_3 \leq \delta H_5$complete inclusion of the guest into CD cavity occurs and $\delta H_3 > \delta H_5$ denotes partial inclusion [6].

Table 2Chemical shifts of stigmasterol, α-CD:S inclusion complex

Position of Hydrogen (Stigmasterol)	δ of stigmasterol S	δ of the complex α-CD :S	Δδ (δ S -δ α-CD : S)
C_3-OH	2.285	2.285	0
C_6-H	5.027	5.027	0
C_{18}	0.839	0.839	0
C_{19}	0.788	0.788	0
C_{21}	0.855	0.840	-0.015
C_{22}	5.127	5.027	-0.100
C_{24}	2.271	2.263	-0.008
C_{25}	2.009	2.008	-0.001
$C_{26, 27}$	1.495	1.490	-0.005
C_{28}	1.998	1.984	-0.014
C_{29}	1.823	1.731	-0.092

Table 3Chemical shifts of α-CD and the α-CD:S inclusion complex

H	δα-CD	δα-CD : S	Δδ
1	4.00	4.00	0
2	3.517	3.515	-0.002
3	3.936	3.926	-0.010
4	3.563	3.563	0
5	3.605	3.600	-0.005
6	3.914	3.914	0

3.3 DSC Analysis

DSC thermograms of stigmasterol and α-CD the inclusion complex is shown in figure 2(a-c). The DSC thermogram of α-CD shows three exothermic peaks at 69.6°C. 102.6°C, 108.6°C which correspond to the dehydration of α-CD.

DSC thermogram of stigmasterol exhibits a sharp exothermic peak at 169.6°C corresponding to its melting point, but in the case of stigmasterol:α-CD inclusion complex, a shift in the exothermic peak to the left at 167.2°C occur.

These visible shifts in the thermograms of the complex serve as evidence for the partial encapsulation of stigmasterol in the CD cavity. The significant thermal peak of stigmasterol appeared at low temperature but intensity is reduced considerably in the complex. This could be attributed to the presence of inclusion complex in amorphous state. Thus, DSC analysis measures the existence of an interaction between guest and host in the inclusion complex.

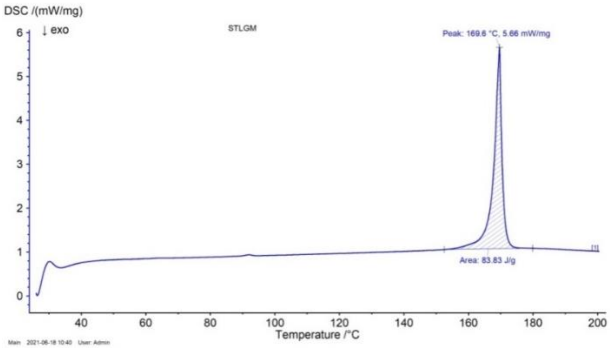

Fig. 2.a. Differential scanning calorimetric thermogram of stigmasterol

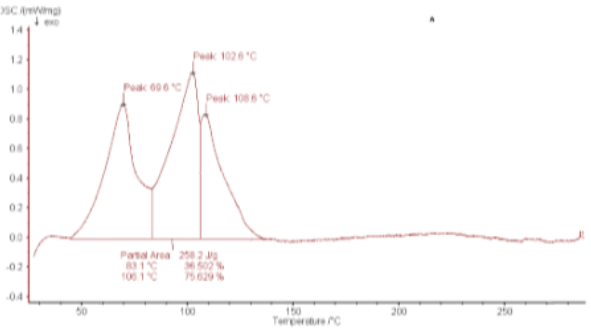

Fig. 2.b. Differential scanning calorimetric thermogram of α-CD

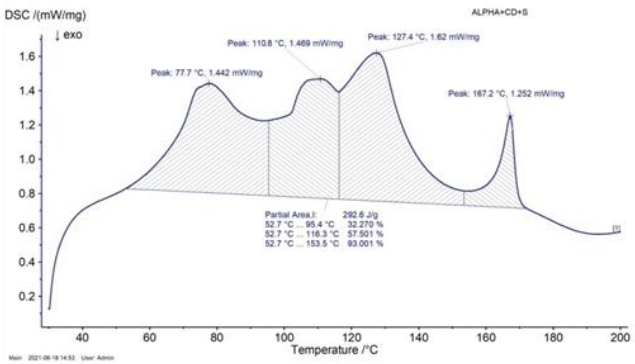

Fig. 2.c. Differential scanning calorimetric thermogram of stigmasterol:α-CD complex

3.4 Anti-inflammatory studies

Table: 4 denote the IC_{50} values of the analyzed samples. Stigmasterol is effective in inhibiting heat induced albumin denaturation with the IC_{50} value 25.60 µg/ml. Whileα-CD+S demonstrated lower activity with IC_{50} 14.73 µg/mL. The dose response for the anti-inflammatory activity of the samples is displayed in figure 3a,b From the results obtained it is visible that the complex demonstrated good anti-inflammatory potential suggesting the possibility that it can be used to control pain and inflammation.

Table .4 Anti-inflammatory property of stigmasterol and its inclusion complex

Sample	IC_{50} (µg/ml) Albumin denaturation assay
Stigmasterol	25.60
α-CD +S	14.73

Table. 5 Percentage of albumin denaturation by stigmasterol and its inclusion complex

S. No	Concentration (ug/mL)	Albumin denaturation (%)	
		S	α-CD +S
1.	Control (10)	100 ± 0	100 ± 0
2.	100	63.36 ±1.41	40.84 ±3.56
3.	90	59.05 ±1.17	27.52 ±2.41
4.	70	55.74 ±0.62	26.21 ±0.82
5.	50	53.84 ±0.51	20.89 ±2.40
6.	30	51.94 ±0.19	19.87 ±1.09
7.	15	50.33 ±1.24	15.31 ±0.78
8.	10	48.68 ±0.69	13.16 ±0.74
9.	5	45.25 ±2.09	12.14 ±2.09
10.	1	41.28 ±2.09	8.45 ±0.66
11.	0.5	38.36 ±0.59	3.28 ±3.22

Results are mean ± SD of three observation. S-stigmasterol; α-CD+S-stigmasterol: alpha cyclodextrin complex; The percentage of denaturation was calculated as a ratio of the OD of stigmasterol and α-CD+S treated cells and control cells ($P \leq 0.001$).

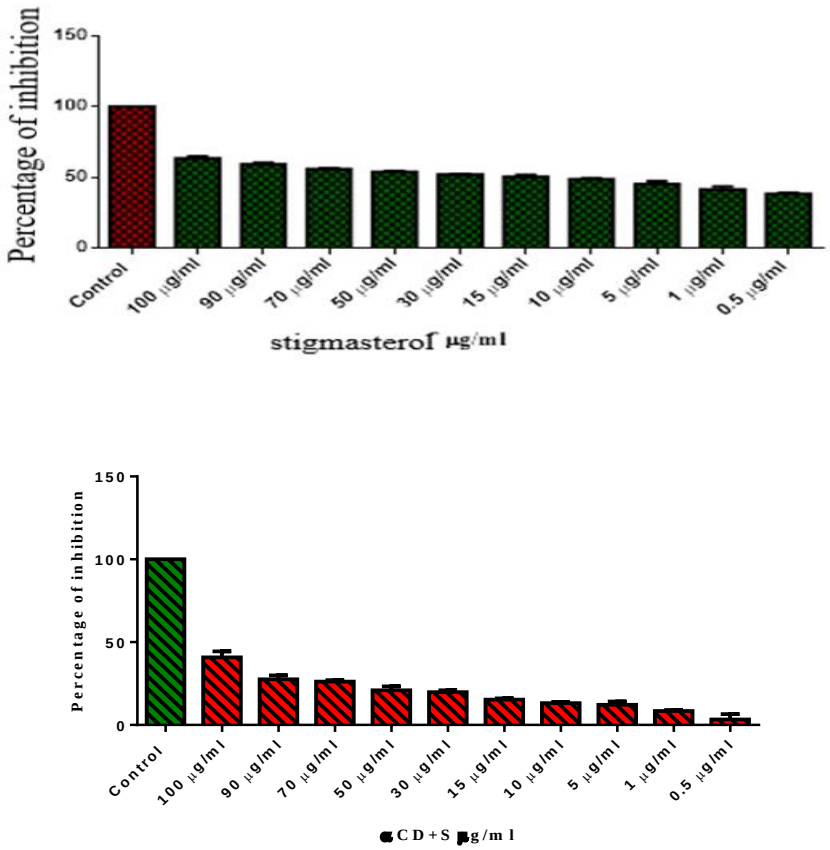

Fig. 3 (a,b) Percentage of albumin denaturation by (a) Stigmasterol (b) α-CD+S

Conclusion

The need to find alternate therapies has witnessed the revolutionary boom in the phytochemical market over the last decade as well as the increase in the amount of research done on them. Phytochemicals from the medicinal plants have found applications as pharmacologically active agent in curing diseases.Stigmasterol is effective in inhibiting heat induced albumin denaturationwith the IC_{50} value 25.60 µg/ml. whileα-CD+S demonstrated better activitywith an IC_{50}14.73 of µg/ml. Thus cyclodextrin inclusion complex can be used as effective agent in pharmaceutical industry.

References

1. Bag, BG and Barai, AC 2020, 'Self-assembly of naturally occurring stigmasterol in liquids yielding a fibrillar network and gel', Royal Society of Chemistry, 10: 4755 - 4762.
2. Jiang, H, Xu, Y, Na, L, Jin, R and Zhang, S 2008, 'UV-Vis spectral analysis of Inclusion complexes between β-Cyclodextrin and aromatic/aliphatic guest molecules', Current Drug Discovery Technologies, 5: 173 - 176.

3. Upadhuay, SK and Ali, SM 2018, 'Molecular recognition of flunarizine dihydrochloride and β–cyclodextrin inclusion complex by NMR and computational approaches', Chemistry Central Journal, 33: 1 - 9.
4. Sakat, S, Juvekar, AR and Gambhire, MN 2010, '*In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn', International Journal of Pharmacy and Pharmaceutical Science, 1: 146 - 56.
5. Francisco, BT, Pessine, Adriana, Calderini and Guilherme L, Alexandrino review, 'Cyclodextrin inclusion complexes probed by NMR technique', Magnetic resonance spectroscopy, ISBN: 978 - 953 - 51 - 0065 - 2.
6. Santos, CD, Maria, PB, and Maria, FM 2011, 'Phase solubility studies and stability of cholesterol/β-cyclodextrin inclusion complexes', Journal of Science Food and Agriculture, 91: 2551 - 2557.