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RESEARCH ARTICLE

FORMULATION, CHARACTERIZATION AND EVALUATION OF CURCUMIN-CHOLESTEROL PHYTOSOMAL COMPLEX

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ABSTRACT

Turmeric as been long known for its medicines properties and it is the major source for curcumin and it helps in management of various health conditions such as antioxidant, inflammatory, hyperlipidemia, anxiety and metabolic syndrome. Despite these advantages when administered alone curcumin does not attain its full therapeutic effect because of its poor absorption, fast metabolism and rapid elimination. To overcome this barrier for its efficient delivery, curcumin was incorporated into phytosome technology by reacting it with cholesterol. The term phytosome can be divided into two parts, "phyto" means plant while "some" means cell-like. Phytosome is a evolving technology in which the phytochemical agent is bounded with lipid agent. Thus, it results in increased absorption, stability which leads to better bioavailability and therapeutic effect in the treatment of various health conditions as compared to delivery of the herbal compound alone.

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INTRODUCTION

Herbal medicines and its phytoconstituents have proven their efficiency in the treatment of various health conditions, thus, this reflects the increase in demand of these products. Based on World Health Organization (WHO) statistics, about 75% of the world population relies on herbal compounds for treatment of various diseases (World Health Organization Fifty-Sixth World Health Assembly, 2003). Despite their advantages, most herbal compounds are vastly water-soluble which affects their absorption due to poor lipid solubility (Manach, 2004). Curcumin is obtained from dried rhizome of *curcuma longa* which is yellowish in color and it has been used as Ayurvedic medicine for treatment of various conditions such as anti-inflammatory, wound-healing, antiamoebic, anticancer and different pathogenic invasions (Grzegorz Grynkiewicz, 2012; Alok Abhijeet, 2015). Curcumin was discovered in 1815 and up to date many studies have been performed on curcumin for improvement of its various pharmacological activities (Vogel, 1815; Sharma, 1976). The Indian turmeric is regarded as the best in the world due to high presence of curcumin content (Priyanka Dagar, 2014). Curcumin a polyphenolic natural product exhibits therapeutic activity against a number of diseases, attributed mainly to its chemical structure and unique physical, chemical and biological properties (Kavirayani Indira Priyadarsini, 2014). The IUPAC name of curcumin is (1*E*, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, with chemical formula C21H20O6, and molecular weight of 368.38 (Priyadarsini, 2013). Structurally curcumin consist of [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione)] containing two ferulic acid residues joined by a methylene bridge. It has important functionalities such as aromatic o-methoxy phenolic group, alpha, beta-unsaturated beta-diketo moiety and a seven carbon linker. These different functional groups are crucial for its biological activity and the o-methoxypherol group and methylenic hydrogen are responsible for the antioxidant activity of curcumin, and curcumin donates an electron/hydrogen atom to reactivate oxygen species (Lobo *et al.,* 2010). Cholesterol contain glycerol and fatty acid but it has three fatty acid attached to the glycerol. Cholesterol is known as "sterol" because it is made up of an alcohol and sterol and 100% of it is made up in liver. Cholesterol is very important in the fluidity of the cell, learning capacity and memory (Antonio M. Rabasco Alvarez, 2000). Cholesterol act as source of energy, maintenance of body temperature and cholesterol takes parts in the synthesis of various hormones like steroids. Phytosome or herbosome is an improved technology for delivery of herbal products which are better absorbed and as a result can improve biological activities of the phytoconstituents. This technology was first discovered by Indena S.P.A, Italy (Goyal, 2011). The

term phytosome can be divided into two distinct parts in which "phyto" means plants and "somes" means cell-like, and it is basically a complex of herbal molecules with lipid part (Jain, 2013). The process is to incorporate standardized plant extracts or water soluble phytoconstituent into lipid to produce lipid compatible molecular complexes, called as Phytosome and so vastly improve their absorption and bioavailability. The mechanism through which phytosome reaches the systemic circulation is based on the fact that it can transition from a hydrophilic medium into lipid environment of the enterocyte cell membrane, further into the cell and finally to the blood circulation (Parris Kidd, 2005). In the Phytosome technology process, the lipid and the plant components actually form a 1:1 or a 2:1 molecular complex ratio, involving chemical bonds (Thurapati Pandu Raju, 2011). A Phytosome is an amphiphilic substance with a definite melting point, generally soluble in non-polar solvents, and moderately soluble in fats (Ajay Semalty, 2010). The main difference between phytosome and liposome is that in the late one there are hundreds or thousands of lipid molecules surrounding the plant components, while in contrast, in the phytosome technology the lipid and plant material form molecular complex of 1:1, 1:2 in which chemical bond was involved which provides better bioavailability of phytosome and provide better effect on topical and skin products as compared to liposome (Nilesh Jain, 2010).

MATERIALS AND METHODS

Materials: Curcumin was obtained from (Sigma Aldrich-Merk), Cholesterol, Dichloromethane (DCM) and n-hexane was obtained from Central Drug House (CDH) (P) Ltd. 7/28 Vardaan House, Daryaganj, New Delhi110002 (INDIA). All other chemicals were of analytical grade.

Methods: The preparation of curcumin-lipid phytosome was done as per Maiti *et al.,* 2006 in which curcumin and cholesterol was taken at 1:1molar ratio after the optimization as shown in Table . Curcumin and cholesterol was accurately weighed and taken in a 250 ml flat bottom flask with 22.07 ml of DCM (dichloromethane). The mixture was stirred and refluxed until the total volume was reduced up to 3-5 ml at a temperature not more than 60^0C. At this point, n-hexane is added at a volume of 10 ml drop wise. The complex of curcumin and cholesterol was precipitated which was then filtered (using Whatman filter paper) and dried under vacuum to remove traces of solvent. The formulated curcumin-lipid complex was stored in an air tight container and kept in freezer (2-8 ^{0}C) until used (Kuntal Maiti, 2007).

Table 1. Different batches of Phytosome formulation.

Batch	Cholesterol conc. (mg)	Dichloromethane (ml)
F1	500	10
F2	750	15
F3	1000	10
F4	1103.55	15
F5	750	7.92893
F6	750	15
F7	750	22.0711
F8	396.447	15
F9	500	20
F10	750	15
F11	750	15
F12	1000	20
F13	750	15

Formulation optimization: For the development of pharmaceutical formulations, traditionally each variable is change at the time until the optimum formulation is obtained. This approach is by its nature time consuming and the effects that independent variables can have in the formulation are not taken into consideration. Therefore, it is necessary to use statistical tools such as factorial designs in order to solve the formulation complexity of pharmaceutical products. Full-factorial experimental design was implemented to study the effects of independent variables on the dependent variables. For the optimization, cholesterol concentration (X_1) and dichloromethane (X_2) were selected as independent variables. Each independent variable was set at a high, medium and low level as shown in Figure 3 and Figure 4. The % Drug content was taken as the response parameter. Design Expert 11.1.2.0 (Trial Version Stat-Ease, Inc, USA) was implemented for the study of the effect of each variable on the designed response. ANOVA test was used to study the statistical significance of the difference in % Drug content using following equation: $Y= Y_1 + 1.52\ X_1 + 0.9166\ X_2 - 0.1350\ X_1X_2 - 3.03\ X_1^2 - 0.3157\ X_2^2$

Evaluation of curcumin-cholesterol phytosome complex: *Organoleptic properties and wavelength maxima (λ_{max})* Curcumin-cholesterol complex was observed for its organoleptic properties like color, solubility. A concentration of about 1 mg of the complex was dissolved in methanol and scanned over a wavelength range between 200-800 nm respectively in UV-Visible spectrophotometer (Shimadzu 1800) to get the λ_{max} of the formulated complex[20].

Drug content: The amount of curcumin in the complex was determined by the method described by Sauvik Bhattacharyya *et al.,* 2013 with some modification. 5 mg of the complex was dissolved in 10 ml of methanol. The mixture was sonicated for 5-10 minutes then filtered. The volume (1 ml) was taken from the stock solution in a 10 ml volumetric flask and made up with methanol. The solution was analyzed by UV spectrophotometer (Shimadzu UV, 1800) at 422 nm wavelength (Mistuni Ghosh, 2011).

Drug entrapment efficiency: It was determined after drug content analysis and the total amount of the complex yielded. Some amount of drug in complex was taken and divided into amount of initial drug that was used (Mistuni Ghosh *et al.,* 2011; Sauvik Bhattacharyya, 2013).

Percentage yield: The percentage yield was calculated to find out the total percentage of the formulated phytosomal complex. The following formula was used to calculate % yield: % yield = practical yield/ theoretical yield x 100.

Characterization of curcumin-cholesterol phytosomal complex

Scanning electron microscopy (SEM): About 5 µL of the curcumin-cholesterol phytosome suspension was transformed to a cover slip, which in turn was mounted on a specimen tab and the samples were allowed to dry at room temperature. The particle size of the formulation was analyzed and photographed using Scanning Electron Microscope (Sigma, Carl Zeiss). The particles were coated with platinum by using vacuum evaporator and thus, the coated samples were viewed and photographed in JEOL JSM-6701F Field Emission SEM.

TLC study: The TLC studies were performed as per S. Revathy *et al.,* 2011 with modifications. The sample of curcumin, cholesterol, curcumin + cholesterol (physical

mixture) and phytosome were collected in four different eppendorf containers and acetone was added to the samples. The mobile phase (dichloromethane and methanol) at a ratio of 99:1 was used for TLC. The precoated TLC plate was cut at a specific size and the samples were placed on it with the help of fine capillary tubes. Now, the TLC plate was placed in the mobile phase chamber and the solvent front was allowed to run up to 80% of the TLC plate. The TLC plate was analyzed under UV chamber and the R_f value was calculated (Amudha, 2018).

UV analysis

The UV of the formulated curcumin-cholesterol complex was analyzed by (Shimadzu, 1800) at the wavelength of 422nm.

Partition coefficient: In the first study (n-octanol + water), 2 ml of each n-octanol and distilled water were taken in an air tight container and excess of the curcumin-cholesterol phytosomal complex were added with continuous shaking for 24 hours until sample is no longer dissolving (saturation point). The sample was centrifuged at 5000 rpm for 10 minutes. The organic layer was separated with the help of separating funnel. Collected 1 ml from the organic sample and added 9 ml of methanol. The concentration was calculated with help of UV spectrometer at wavelength of 422 nm. For the aqueous phase study, Collected 1 ml from the aqueous phase sample and added 9 ml of methanol. The concentration was calculated with help of UV spectrometer at wavelength of 422 nm. In the second study (n-octanol + PBS), n-octanol and PBS 7.4 (phosphate buffer saline) were taken in an air tight container and excess of the curcumin-cholesterol phytosomal complex was also added with continuous shaking for 24 hours until sample reaches saturation point. The sample was centrifuged at 5000 rpm for 10 minutes. The two layers were separated with the help of separating funnel. Collected 1 ml from the organic sample and added 9 ml of methanol. The concentration was calculated with help of UV spectrometer at wavelength of 422 nm. For the PBS layer, 1 ml was collected and 9 ml of methanol was added. The concentration was also calculated with help of UV spectrometer at wavelength of 422nm (Kuntal Maiti, 2007).

In-vitro drug release: The determination of in-vitro release of curcumin from phytosomal suspension was performed through cellophane membrane (previously soaked overnight in diffusion medium) using Franz diffusion cell by taking phosphate buffer solution pH 7.4 as diffusion medium. The cellophane membrane was mounted on the Franz diffusion cell and the upper side was used as donor compartment and lower side as receptor compartment. The suspension (10 ml) of curcumin-phytosome complex containing 10 mg of complex was placed on the donor compartment (upper side) then closed firmly closed to avoid leakage. About 20 ml of PBS pH 7.4 was kept in the receptor compartment so that it can slightly touch the receptor membrane surface and the temperature was kept at 37±0.5 ºC. The magnetic bead was kept at the bottom of the diffusion medium at 100 rpm. The samples of 3 ml were withdrawn between 1 hour intervals for 24 hours consecutive and fresh amount of sample (3 ml) of PBS pH 7.4 was replaced to maintain the sink condition. UV spectrophotometer analysis of the samples was recorded at λmax 422 nm (Revathy, 2011).

Microscopic evaluation: Projection microscope (Impact Icon Instruments Company, IIC-604) was used for microscopic characterization of the complex. A quantity of 1 mg each of the curcumin-lipid complex, cholesterol, and curcumin was taken in three different 5 ml eppendorf tube and distilled water was added and the samples were shaken with Vortex shaker to make a uniform suspension of the curcumin-lipid complex, curcumin and cholesterol. Drop was collected from each suspended solution with the help of micropipette (10 micro-liters) and placed on three different slides (make a smear on the glass slide) and the microscopic observation was performed at a magnification of 400x.

Fourier Transformed-Infrared Spectroscopy (FT-IR): FTIR spectra of the formulated curcumin-cholesterol complex were done by SAIF PU, Chandigarh.

Nuclear magnetic resonance (NMR): Proton NMR (^{1}H and ^{13}C NMR) of curcumin-cholesterol complex was performed by Bruker Avance II 400 NMR spectrometer SAIF Panjab University, Chandigarh.

RESULTS AND DISCUSSION

Optimization study: The curcumin-cholesterol phytosomal complex was formulated after optimization by Expert Design Software to study the effect of independent variables on % drug content in phytosome. In the current study, the factors selected were X_1= concentration of cholesterol (% w/v); X_2= volume of dichloromethane (ml). The thirteen batches (F1-F13) of curcumin-cholesterol phytosome were prepared as per Design Expert Software (Table 1). With the drop wise addition of n-hexane to DCM, curcumin and cholesterol solution, the precipitates were formed which indicated the formation of phytosomal complex. A total of thirteen formulations of curcumin-cholesterol phytosome were formulated taking concentration of cholesterol (X_1) and dichloromethane (X_2) as independent variables to study their effect on drug content it has been observed that the % drug content of batch 7 (90.94%) was highest, particle size was between 8-10 µm, % yield was 80.1 % w/w. The drug content value was higher when the concentration of cholesterol and dichloromethane are high. Thus, the data revealed that concentration of cholesterol and volume of n-hexane at higher values increases the drug content. ANOVA was applied on the study of the drug content response (Table 3). F-test was carried out to compare the regression mean square the ratio F= 6.84 shows regression to be significant. The 3-dimensional response surface plots and the corresponding contour plots for drug content efficiency are shown in Figure and Figure and its constraint (Table 2).

The Model F-value of 8.39 implies the model is significant. There is only a 0.72% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 6.84 implies the Lack of Fit is significant. There is only a 4.71% chance that a Lack of Fit F-value this large could occur due to noise.

Curcumin-cholesterol organoleptic properties: Curcumin-cholesterol phytosomal complex was analyzed for the organoleptic property; the complex was found to be orange-yellowish in nature, completely soluble in ethanol, methanol, and acetone and insoluble in water.

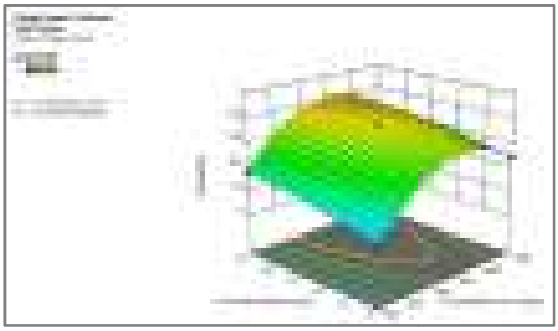


Figure 1. Three D surface of drug content as a function of formulation variables

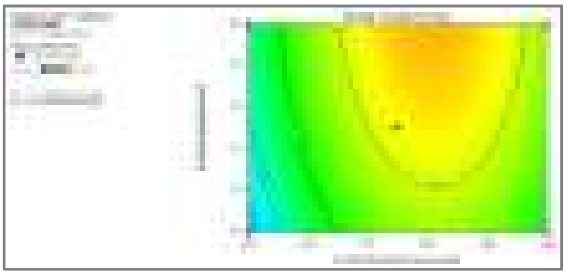


Figure 2. Contour plot of drug content as a function of formulation variables

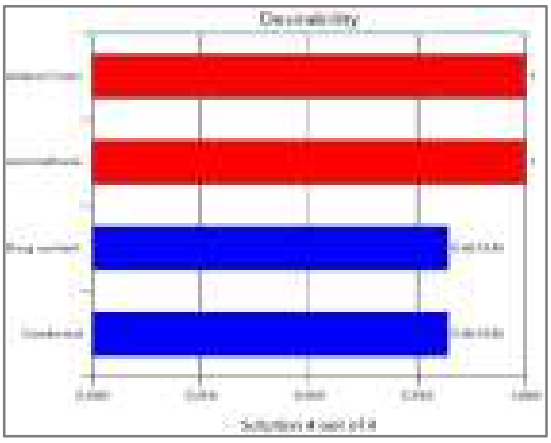


Figure 3. Independent variables set at high, medium and low levels

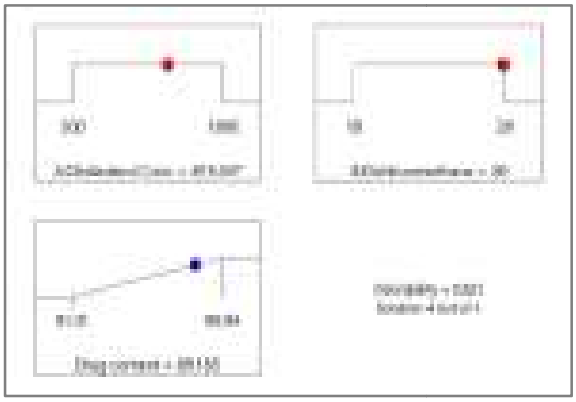


Figure 4. Result from independent variables set at high, medium and low levels

Determination of wavelength maxima: The wavelength of curcumin-cholesterol complex (1 mg concentration) in methanol was found to be 415 nm in UV-Visible spectrophotometer (Fig. 16) and is the typical spectral peak of curcumin which is present abundantly in *turmeric rhizome* (Kavirayani Indira Priyadarsini, 2014).

Standard calibration curve of curcumin-cholesterol complex in UV spectrophotometer: The UV absorbance of curcumin-cholesterol complex solution in methanol showed linearity at λ_{max} 422nm and the linearity were plotted for absorbance versus concentration and Fig 17.

Percentage yield: The total %age yield of the formulated curcumin-cholesterol complex was found to be 80.1%.

% yield = practical yield/ theoretical yield x 100
=605 mg/ 755 mg **x** 100
= 80.1 % w/w

Drug content and **entrapment efficiency:** Curcumin-cholesterol complex was prepared by solvent displacement method and the drug content (percent loading) of the optimized batch (F7) was estimated by UV spectrophotometer at 422 nm in methanol and it was found to be 90.7506% (w/w) as shown in the table below (Table 4). The entrapment efficiency was found to be 34.903% as shown bellow. The good drug loading and entrapment of curcumin makes the delivery of the drug clinically feasible.

Drug content (%) = (Amount of drug in complex/Amount of complex) x 100
Drug content (%) = 4.545/5 **x** 100
= 90.94%

Entrapment efficiency (%) = (Amount of drug in complex/Amount of initial drug used) x 100
Entrapment efficiency (%) = 4.5375/13 **x** 100
= 34.903 %

TLC of curcumin-cholesterol complex: The R_F value of curcumin-cholesterol complex, curcumin, cholesterol and physical mixture were calculated individually and the results obtained were compared. The R_F value of curcumin-cholesterol complex was 0.55 which is higher than R_F values of curcumin, cholesterol and physical mixture. These results have been represented in the and
Figure and Table .

Microscopic evaluation*:* The optimized and prepared curcumin-cholesterol phytosomal complex under projection microscopy has shown characteristic features of presence of round/spherical shape vesicles

Figure 11 and Figure 12 at the magnification of 400X which is significantly different from the structure of curcumin Figure 9 and Figure 10, and cholesterol alone Figure 7 and Figure 8.

UV: The UV spectrum analysis (qualitative study, was performed and methanol was used as blank. (THE UV RESULT SHOULD CONTAIN THE FOLLOWING LINES: The UV spectrum analysis (qualitative study, Table 1) was performed and methanol was used as blank. The samples of phytosome complex, curcumin, cholesterol, physical mixture (curcumin + cholesterol) were analyzed and the data obtained was recorded has shown in the Figure, Figure 1, Figure 15, Figure. For UV photometric analysis (quantitative study) the quantity of 1 mg of curcumin was dissolved in 1 ml methanol (1000 µg / µl) further dilution of 2, 4, 6, 8, 10 µg/ml were prepared and absorbance were determined. The calibration graph is given in Figure. The samples of phytosome complex, curcumin, cholesterol, physical mixture (curcumin + cholesterol) were analyzed and the data obtained was recorded has shown in the Figure, Figure 1, Figure 15, Figure.

Table 2. Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Cholesterol Conc.	is in range	500	1000	1	1	3
B:Dichloromethane	is in range	10	20	1	1	3
Drug content	maximize	81.01	90.94	1	1	3

Table 3. ANOVA of regression (% drug content response)

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value
Model	88.90	5	17.78	8.39	0.0072 significant
A-cholesterol	18.37	1	18.37	8.67	0.0216
B-dichloromethane	6.72	1	6.72	3.17	0.1182
AB	0.0729	1	0.0729	0.0344	0.8581
A^2	63.69	1	63.69	30.05	0.0009
B^2	0.6936	1	0.6936	0.3272	0.5852
Residual	14.84	7	2.12		
Lack of Fit	12.42	3	4.14	6.84	0.0471 significant
Pure Error	2.42	4	0.6055		
Cor Total	103.74	12			

Table 4. Drug content study (%)

Batch No	Drug Content (%)
F1	82.16
F2	88.32
F3	85.02
F4	85.92
F5	86.83
F6	88.92
F7	90.94
F8	81.01
F9	83.19
F10	89.13
F11	87.14
F12	85.51
F13	88.56

Table 5. Rf value calculation of curcumin-phytosome complex 1:1 ratio

Samples	Distance travelled by solute	Distance travelled by solvent	R_f value
Curcumin	2.80	5.4	0.51
Cholesterol	2.5	5.4	0.40
Physical mixture	2.6	5.4	0.48
Phytosome	2.97	5.4	0.55

R_f value = Distance travelled by solute/Distance travelled by solvent

Figure 5. TLC of curcumin (lane 1), cholesterol (lane 2), physical mixture (lane 3), phytosome 1:1 ratio (lane 4). Solvent system consists of 9.9:0.1 ratio DCM and methanol respectively

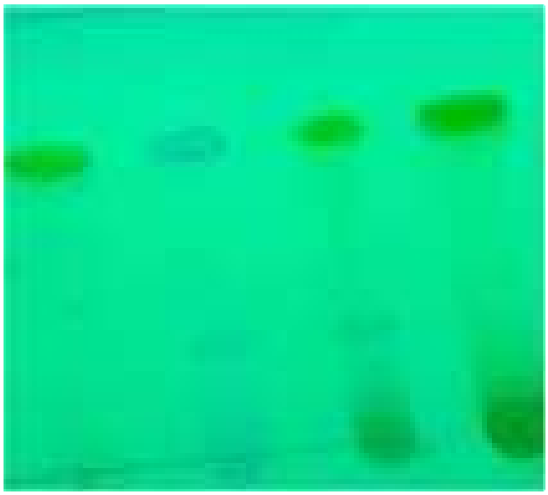


Figure 6. TLC of curcumin (lane 1), cholesterol (lane 2), physical mixture (lane 3), and phytosome 1:1 ratio (lane 4). Solvent system consists of 9.9:0.1 ratio DCM and methanol respectively under UV visible light at 366 nm wavelength.

Figure 7. Microscopic of cholesterol (in normal mode), 400x

Figure 8. Microscopic of cholesterol (in negative mode) 400x

Figure 9. Microscopic of curcumin (normal mode), 400x

Figure 10. Microscopic of curcumin (in negative mode) 400x

Figure 11. Microscopic view of curcumin-cholesterol complex (phytosome) 400x

Figure12. Microscopic view of curcumin-cholesterol complex (in negative mode), 400x

Figure 13. UV chromatogram of blank methanol (blank MeOH) UV spectrum analysis (qualitative study)

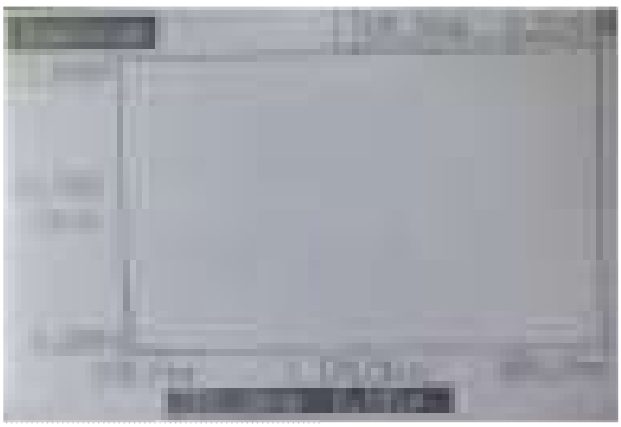


Figure 14. UV chromatogram of cholesterol (λmax 201 nm) UV spectrum analysis (qualitative study)

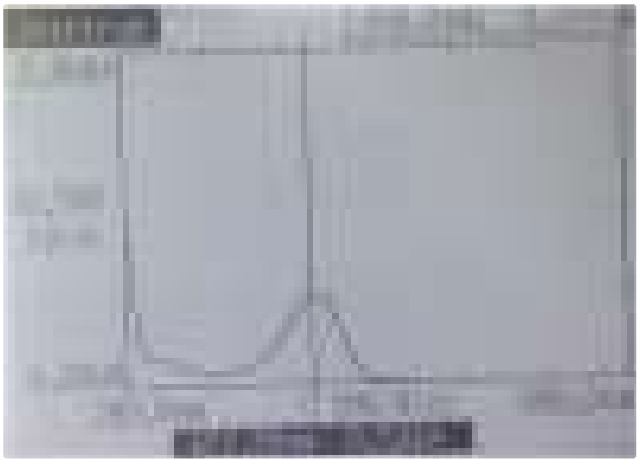


Figure 15. chromatogram of curcumin (λmax 422nm) UV spectrum analysis (qualitative study).

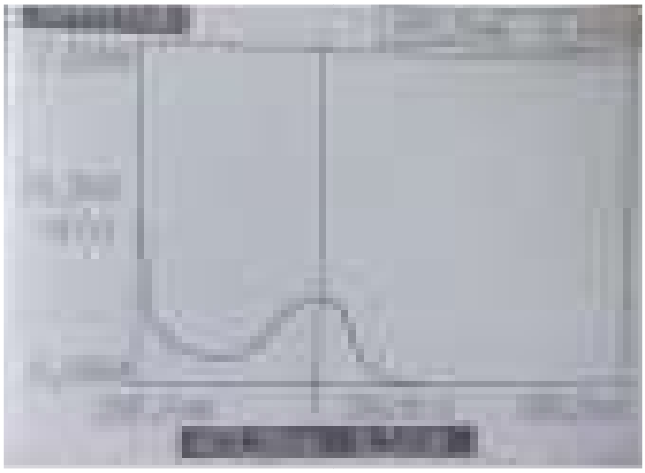


Figure 16. UV chromatogram of curcumin-cholesterol complex (phytosome formulation) λmax 415 nm UV spectrum analysis (qualitative study)

Table 1. UV photometric analysis

Sample	Concentration (µg / µl)	Absorbance	K* Absorbance
1	0	0.000 ± 0.4	0.0422 ± 0.3
2	2	0.039 ± 0.3	16.331 ± 0.3
3	4	0.051 ± 0.4	21.395 ± 0.4
4	6	0.082 ± 0.2	34.562 ± 0.3
5	8	0.108 ± 0.2	45.787 ± 0.2
6	10	0.154 ± 0.4	64.861 ± 0.2

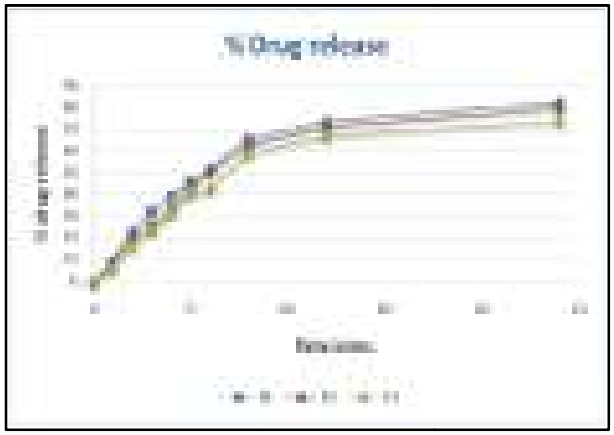


Figure 17. Calibration curve

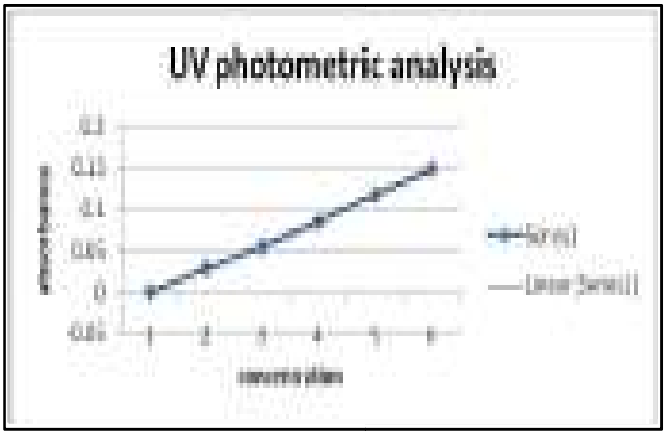


Figure 18. In-vitro drug release (%)

Figure 2. SEM image of Cholesterol

Figure 3. SEM image of Curcumin

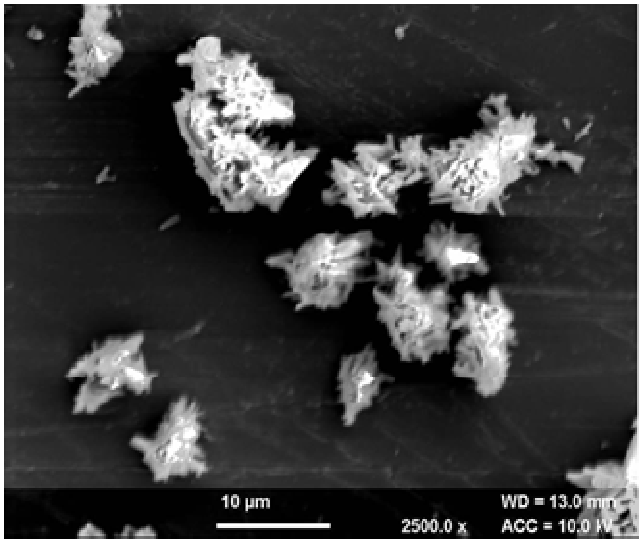


Figure 21. SEM image of Curcumin-cholesterol complex

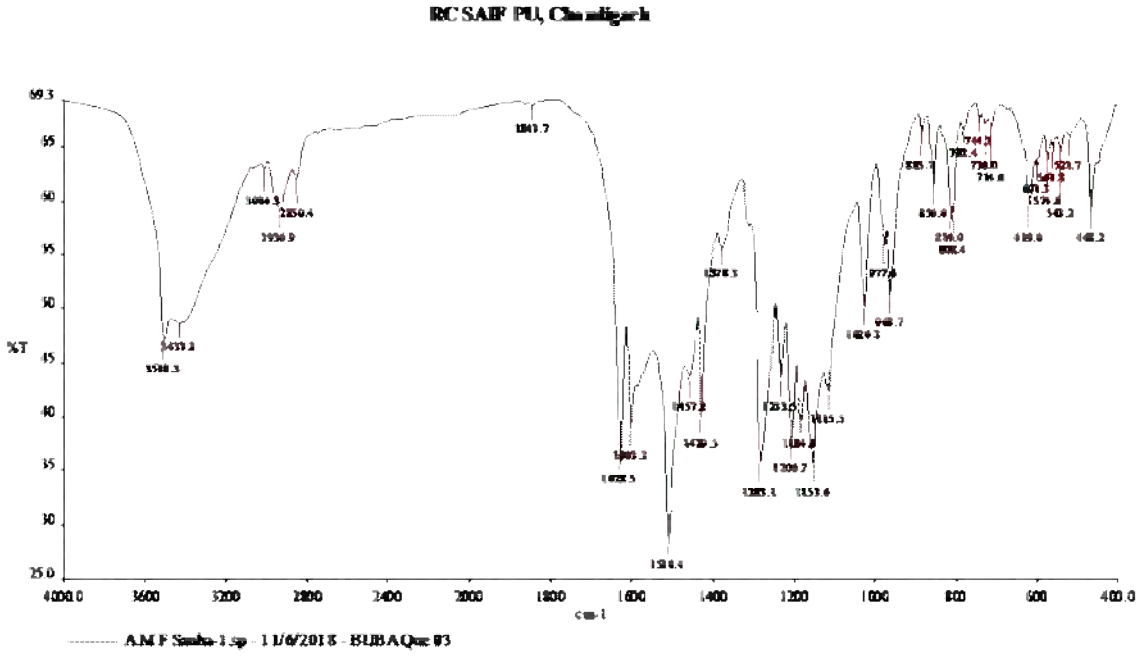


Figure 22. FTIR of curcumin-cholesterol complex

Figure 23. FTIR of pure cholesterol

Figure 24. FTIR of curcumin

Table 8. 1H-NMR of pure curcumin and curcumin-cholesterol complex

^{1}H-NMR of curcumin	Functional groups	^{1}H-NMR of curcumin-cholesterol complex	Functional groups
3.716	(s) $6H_{11}$	2.5126	More deshielded due to CH_3
		2.5083	
		2.5040	
5.812	(s) $2H_1$	3.4090	
6.463-6.515	(d) $2H_3$	3.8437	(s) $6H_{11}$
		3.8248	
7.515-7.627	(d) $2H_4$	5.7578	(s) $2H_1$
6.931-6.958	(d) $2H_9$	6.0495	(d) $2H_3$
		6.0642	
7.06	(s) $2H_6$	6.7449	(d) $2H_9$
		6.7842	
7.119-7.141	(d) $2H_{10}$	6.8218	(d) $2H_{10}$
		6.8418	
		7.1460	
		7.1500	
		7.1663	
		7.1706	
		7.3275	
		7.3311	
		7.5361	
		7.5635	(d) H_4
		7.5757	
		9.6969	Deshielded due to electronic effect
		10.1390	

For UV photometric analysis (quantitative study) the quantity of 1 mg of curcumin was dissolved in 1 ml methanol (1000 µg / µl) further dilution of 2, 4, 6, 8, 10 µg/ml were prepared and absorbance were determined. The calibration graph is given in Figure 17.

Partition coefficient: The Log P value of curcumin-cholesterol complex was found to be 3.932 in n-octanol + water and 3.812 in n-octanol + PBS 7.4 which is higher than the Log P value of curcumin alone (3.2).

As per the equation C=A/ab, where C is concentration, A is absorbance, a is specific absorbance, and b is path length (1 cm). The results are given bellow (Table 2).

Table 2 n-octanol + PBS (7.4), n-octanol + PBS (7.4), and curcumin Log P

S. No.	Log P
n-octanol + Distilled water	3.932
n-octanol + PBS (7.4)	3.8122
Curcumin	3.2

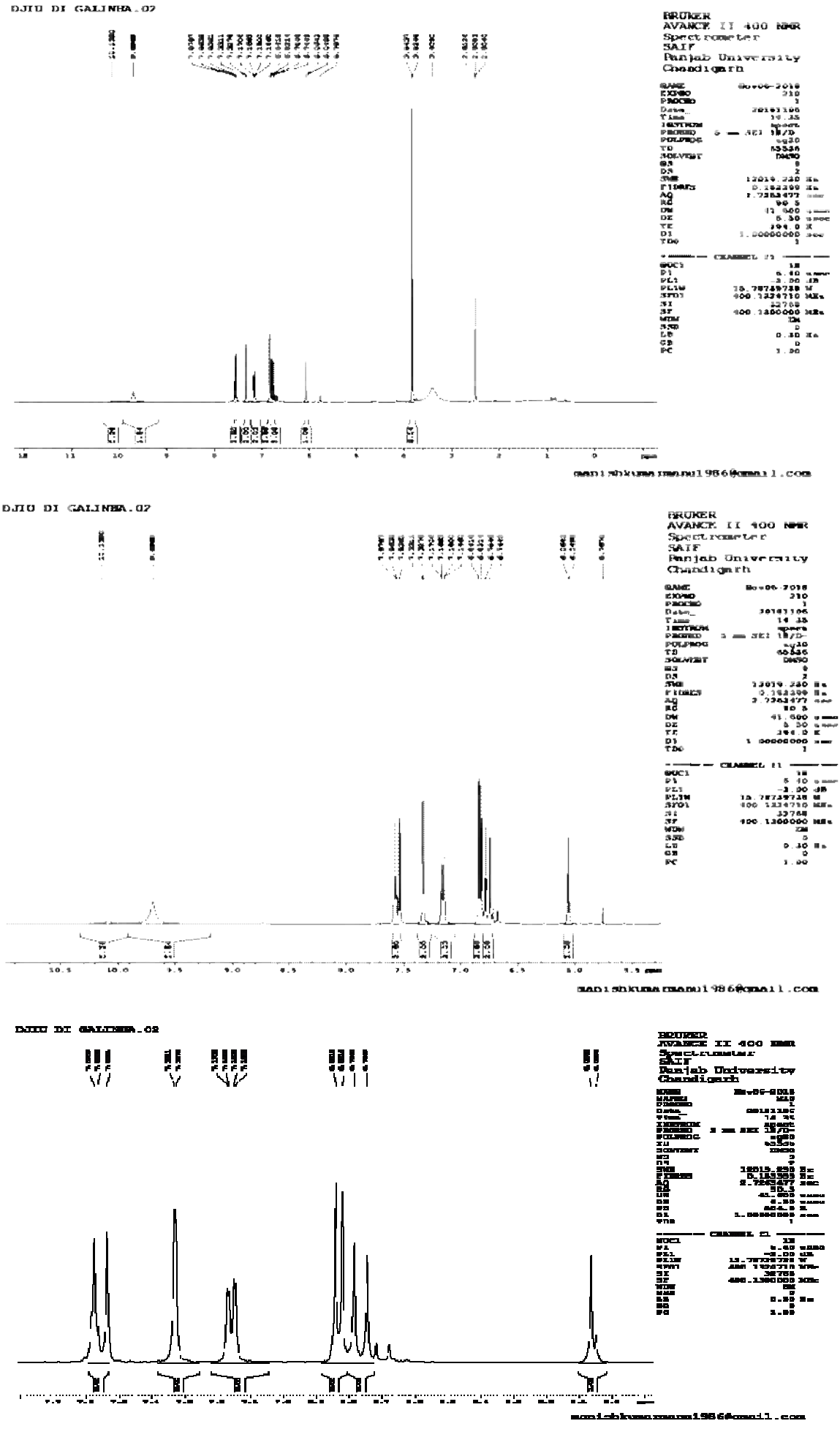
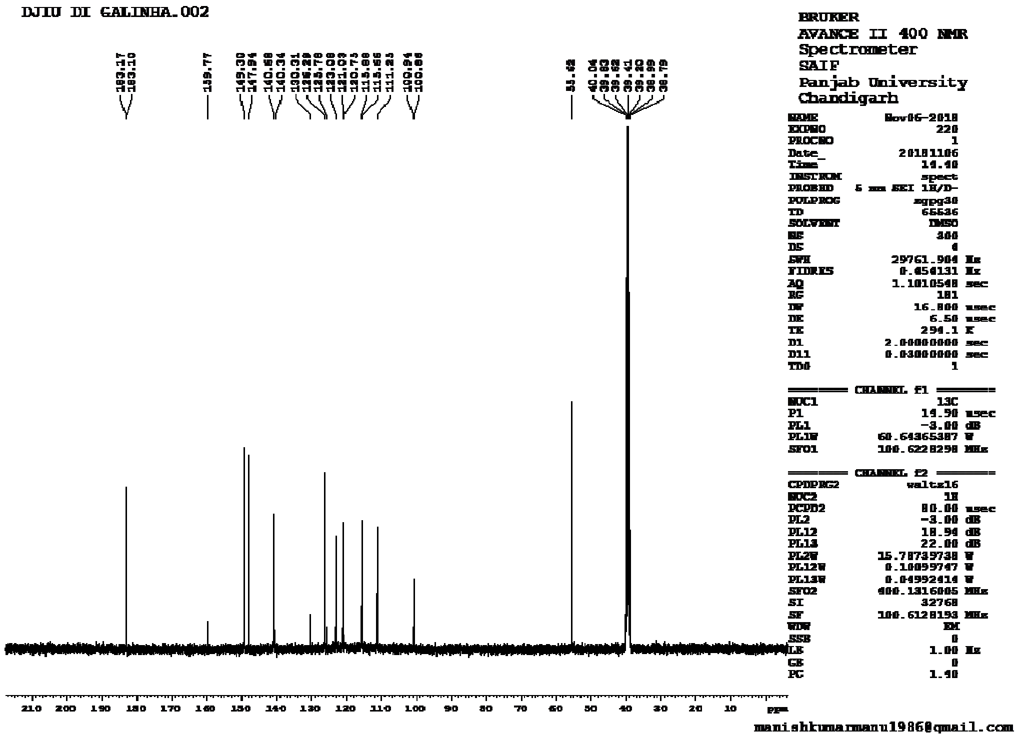


Figure 25. 1H-NMR of curcumin-cholesterol complex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BRUKER
AVANCE II 400 NMR
Spectrometer
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Panjab University
Chandigarh
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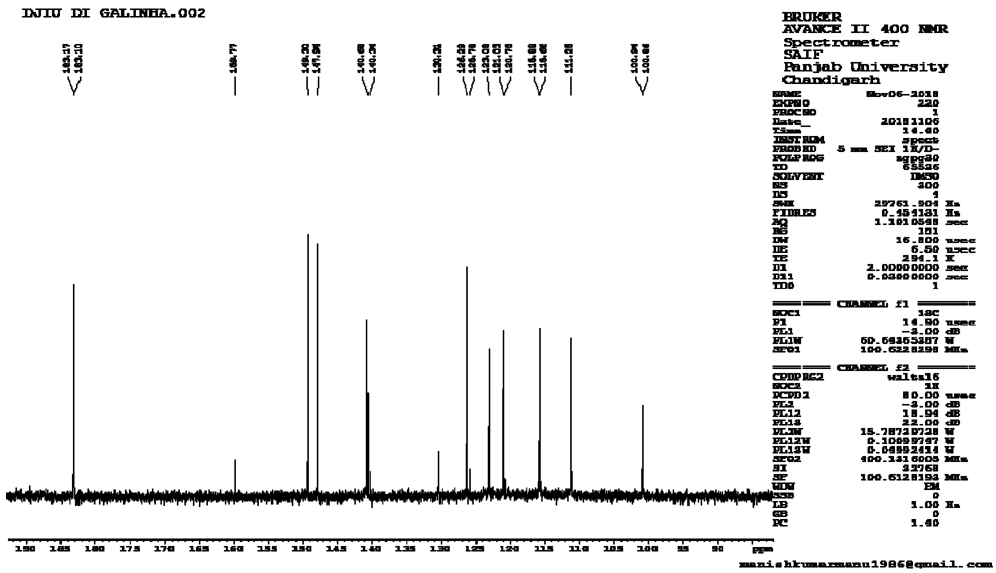
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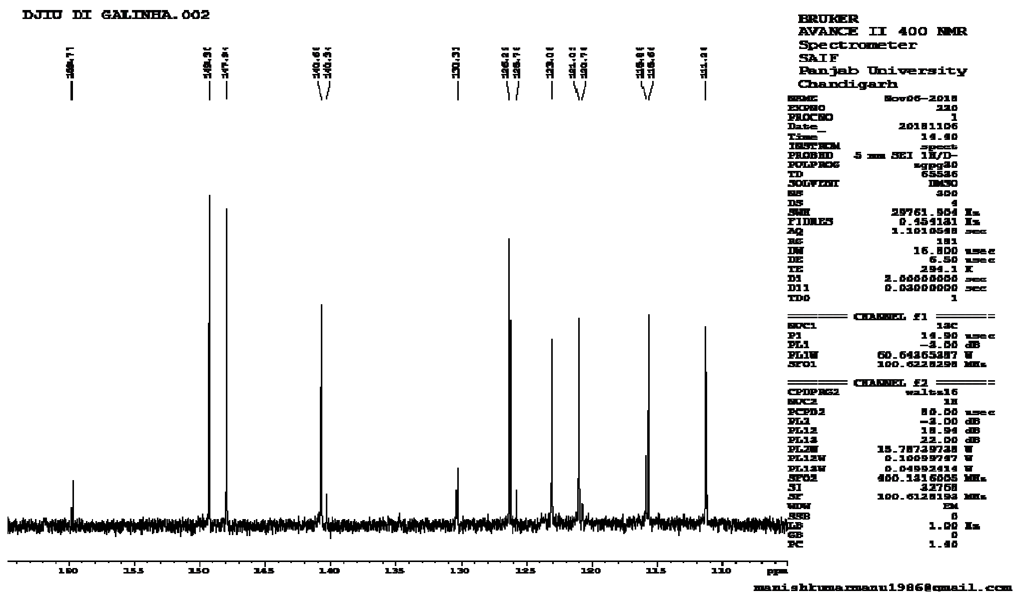
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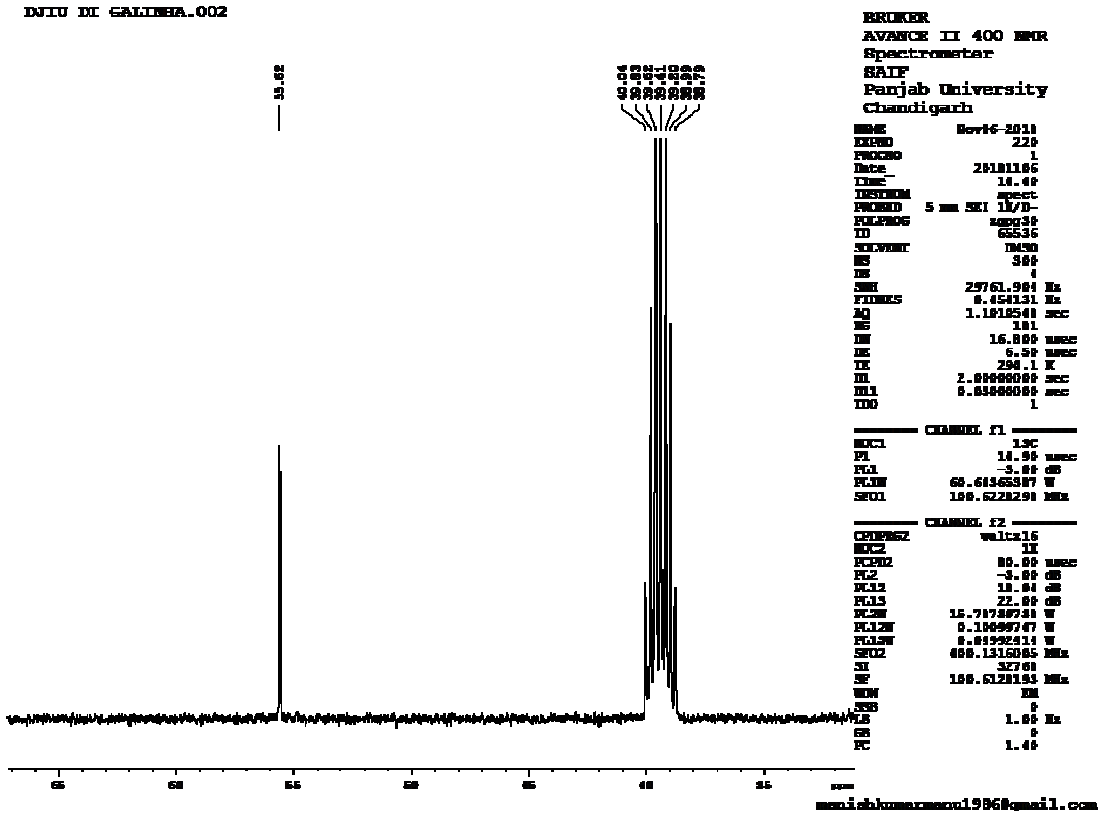

Figure 4. 13C-NMR of curcumin-cholesterol complex

Table 3. 13C-NMR of pure curcumin and curcumin-cholesterol complex (CAPTION TO BE MENTIONED)

^{13}C-NMR of curcumin	Functional groups	^{13}C-NMR of curcumin-cholesterol complex	Functional groups
56	C_{11}	38.79	
101	C_1	38.99	
111	C_6	39.20	CH_3
		39.41	CH_3
		39.62	CH_3
115	C_9	39.83	
121	C_3	40.04	
123	C_{10}	55.62	C_{11}
126	C_5	100.86	C_1
141	C_4	100.94	C_5
148	C_1	111.25	C_6
149	C_8	115.66	C_9
183	C_2	115.88	
		120.75	C_6
		121.03	C_3
		123.08	C_{10}
		125.78	
		126.29	C_5
		130.31	
		140.34	C_4
		140.68	C_5
		147.94	C_7
		149.30	C_8
		159.77	
		183.10	C_2
		183.17	C_2^1

In-vitro drug release (%): The in-vitro drug release profile of optimized curcumin-cholesterol phytosome suspension is given in Figure 18. Among the three formulations, the F1 have shown better drug release which is about 80%.

SEM: The visualization of curcumin-cholesterol complex by Scanning Electron Microscope (SEM) have shown a spherical shaped particle with a diameter ranging between 8-10 μm has shown in the Figure 19, Figure 20, Figure 21. The shape changes of both curcumin and cholesterol which was previously long fibrous shape) confirms the complex formation between curcumin and cholesterol.

FTIR: FT-IR spectroscopy helps in confirming the formation of the complex between curcumin and cholesterol by comparing the individual spectrum of curcumin, cholesterol with the spectrum of curcumin-cholesterol complex. The FTIR of phytosome (Figure 23) have shown that the OH is in bounded form and no sharp peak was observed for ketone group which suggested that the ketone group is in bounded form. The CH_2 groups and 6H in cholesterol cause the stretching to be more in curcumin-cholesterol phytosome as compared to curcumin alone (Figure 24). The changes in the peaks and positions in curcumin-cholesterol phytosomal complex differ from that of cholesterol Figure 25.

NMR study

^{1}H-NMR: Under the ^{1}H-NMR curcumin-cholesterol phytosomal complex and pure curcumin have shown various functional groups as described in the table below (Table and Figure 26). At 9.6969 no peak was observed in curcumin and cholesterol which is due to deshielded proton.

^{13}C-NMR: Under the ^{13}C-NMR curcumin-cholesterol phytosomal complex and pure curcumin have shown various functional groups as described in the Table 9.

Conclusion

In the current study curcumin-cholesterol complex was prepared by solvent displacement method and evaluated for different parameters. All the physicochemical properties evaluated have shown that curcumin and cholesterol had formed a stoichiometric complex having a increased solubility. The ^{1}H-NMR, ^{13}C-NMR, FTIR, UV, SEM studies confirmed the formation of the curcumin-cholesterol complex. The particle size of the complex was found to be 8-10 μm. Entrapment efficiency was found to be about 34.903 %. Thus, with these findings we concluded that curcumin and cholesterol complex may be of potential use for improving the bioavailability of curcumin.

Conflict of interest statement: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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