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NOVEL SALICIN PHYTOSOMAL COMPLEX: DEVELOPMENT AND OPTIMIZATION USING CENTRAL COMPOSITE DESIGN

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ABSTRACT

The present study encompasses the formulation of a novel Salicin phospholipid complex (Phytosome) using Central composite design as a tool for optimization and its characterization using various techniques. Salicin is an alcoholic β-glucoside with anti-inflammatory, analgesic and antipyretic activity obtained from various natural sources like willow bark, castoreum, populous species etc. But as a phytochemical it has been reported to show low aqueous solubility, low absorption, low bioavailability and hence lower activity. Phytosomes are complexes of phytochemical and phospholipid in a stoichiometric ratio in a non-polar solvent and are thus, supposed to enhance the overall properties of the phytochemical. These complexes have the bioactive phytoconstituent of herb extracts chemically bound by a lipid. Structurally Salicin possesses five hydroxyl polar groups which have the ability to bind with the polar group of phospholipid so as to form a phytosomal complex. Salicin phytosomes were formulated by solvent evaporation method and optimized using experimental design. The optimized batch showed 4.89 times enhanced solubility in water (135.73µg/ml) than the drug alone (27.73µg/ml) with complexation rate of 99.64%. The phytosomes were evaluated using FTIR, DSC, SEM and XRD. SEM showed the surface morphology of complex depicting the conversion of crystalline drug into amorphous complex. The *in vitro* permeation in the form of suspension was significantly higher for the complex (93.43%) than the drug (19.65%) after 7 hours. Hence, Salicin showed better solubility and permeation in the form of phytosomes which may eventually increase its absorption, bioavailability and pharmacological activity.

KEYWORDS: Salicin, Phytosome, Central composite design, Partition coefficient, Solubility, Permeation.

INTRODUCTION

Plants consist of a number of secondary metabolites which are predominantly endowed with health boosting substances. A single plant cell is a huge chemical factory. But the plant constituents in isolated forms have disadvantages of limited effectiveness. This may be attributed to the lower solubility, lower absorption and lower bioavailability of these phytoconstituents which ultimately result in its lower pharmacological activity.[1] These disadvantages of phyconstituents led to the need for development of novel drug delivery systems taking into account different carriers which can bind to the plant extracts so as to improve its overall properties. The technique of phytosome or phytolipid complex is one such novel drug delivery system which involves the binding of a phytoconstituent and phospholipid in a stoichiometric ratio.[2] Phytosome chemically is a supramolecular adduct which is formed by hydrogen bonding between the polar group of the phytoconstituent and the polar group of the phospholipid.[3] These phytosomes lead to the increase in overall physicochemical properties and activity of the active phytoconstituent.

The phytoconstituent selected for the present study is Salicin, which is herbal anti-inflammatory agent. Chemically it is an alcoholic β-glucoside and is found in Willow bark (Salix) Populus bark and leaves. It is also found in castoreum which has been credited to the accumulation of salicin from willow trees in the beaver's diet and is transformed to salicylic acid and has an action very similar to aspirin. Salicin is closely related in chemical make-up to aspirin. In combination with the herb's powerful anti-inflammatory plant compounds (called flavonoids), salicin is thought to be responsible for the pain-relieving and anti-inflammatory effects of the herb.[4,5] But it has the disadvantage that it has low solubility and bioavailability in its isolated form.

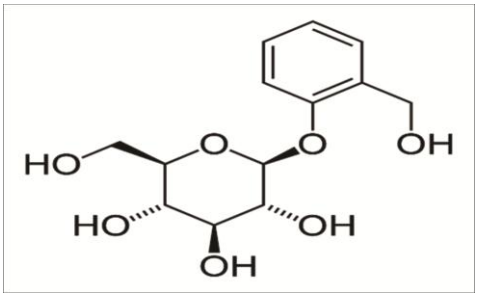

Fig. 1: Structure of salicin showing polar hydroxyl groups.

Salicin has five polar hydroxyl groups (Fig. 1) which have the affinity to bind with the phospholipid. Salicin–PHOSPHOLIPON 90H complex may be formed due to the interaction between the polar hydroxyl groups of salicin and polar choline group of phosphatidylcholine. This may lead to the enhanced solubility and permeability of Salicin which in turn will enhance its pharmacological activity.

Hence, as Salicin has not been explored as a phytosome and as it possessed the desired properties, this study aimed to prepare the phospholipid complex of salicin by solvent evaporation technique, to optimize using quality by design (Central composite design), to characterize the prepared complexes for their physicochemical properties and to compare the activity of complex with salicin.

MATERIALS AND METHODS

Materials

SALICIN was purchased from Sigma-Aldrich Inc. (USA). PHOSPHOLIPON 90 H was obtained as a gratis sample from the company Lipoid AG, Steinhausen, Switzerland. All the other reagents and solvents used were of analytical grade.

Methods

Preformulation studies

The identification of the drug was carried out by melting point determination, infrared spectrometry and differential scanning calorimetry. Melting point determination was carried using Thiele tube method.[6] DSC was carried out to study the thermal behavior of the drug.[7]

The analytical method development was carried out using ultraviolet spectroscopic analysis. Calibration of salicin was performed in solvents namely ethanol, water and pH 7.2 phosphate buffer [8]. A standard stock solution of 1000 ppm was prepared and subsequently a solution of 10 ppm was prepared from it to determine the λ_{max}. After that calibration curves were obtained using a range of solution concentrations. Then determination of validation parameters- linearity, intraday precision study, interday precision study, robustness, ruggedness was carried out in accordance with the ICH guidelines.[9]

Formulation of Salicin phytosome

The salicin phospholipid complex was prepared by solvent evaporation method. Briefly salicin and PHOSPHOLIPON 90H in a molar ratio of 1:0.5, 1:1.75, 1:3 respectively were

taken in a 100 ml round bottom flask and 30 ml of ethanol was added. The reaction temperature was controlled between 40-60 ^{0}C for a reaction time of 1-3 hours. The resultant clear solution was evaporated to 2-3 ml and excess of the non-solvent, n-hexane was added to it with continuous stirring. The complex was precipitated, filtered and dried under vacuum to remove traces of solvents. Resultant complex was stored in an amber colored glass bottle flushed with nitrogen at room temperature.[10]

Optimization using QbD concept by Central Composite Design-

Experimental design is a concept of the careful balance between several variables affecting any experiment. To reduce the number of trials and attain the highest amount of information, the screening was planned applying a central composite design to systematically study the joint influence of the effect of independent variables: Salicin – PHOSPHOLIPON 90H ratio (X_1), reaction temperature (X_2, $^{\circ}$C) and the reaction time (X_3, h) on the dependent variables complexation rate (Y_1) and partition coefficient (Y_2). In this design, 3 factors were evaluated and experimental trials were performed at all 9 possible combinations. A statistical model incorporating interactive and polynomial terms was used to evaluate the response employing the formula equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

Where Y was the dependent variable, b_0 was the arithmetic mean response of the 9 runs, and b_i was the estimated coefficient for the factor X_i.

The main effects (X_1 and X_2) represented the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2, X_2X_3, and X_1X_3) showed how the response changed when 3 factors were simultaneously changed. The polynomial terms (X_1^2, X_2^2 and X_3^2) were included to investigate non-linearity.[10]

The level values of three factors and composition of central composite design batches 1-9 were as shown in table 1 and 2.

Table 1: General batches according to central composite design.

BATCH	X_1	X_2	X_3
F1	0	0	0
F2	-1	1	-1
F3	-1	-1	-1
F4	1	1	-1
F5	-1	1	1
F6	1	-1	-1
F7	-1	-1	1
F8	1	1	1
F9	1	-1	1

Factors: X_1-Salicin-PHOSPHOLIPON 90H ratio; X_2-Reaction temperature; X_3-Reaction time

Responses: Y_1-Complexation rate; Y_2-Partition coefficient

Table 2: Dependent and independent variables in central composite design for optimization.

Types of variable	Variable	Optimization levels used		
		Low	Medium	High
Independent	X_1 (Salicin : PHOSPHOLIPON 90H)	1:0.5	1:1.75	1:3
	X_2(Temperature ^{0}C)	40	50	60
	X_3(Time hours)	1	2	3
Dependent	Y_1(Complexation rate) %	Maximize		
	Y_2(Partition coefficient)	Minimize		

Characterization of Salicin phytosome

Determination of complexation rate

Amount of complex equivalent to 10 mg of salicin was dispersed in 5 ml of chloroform. Both the complex and PHOSPHOLIPON 90H were easily dissolved in chloroform, but free salicin remained practically insoluble in chloroform. The non-complexed salicin was separated, dissolved in ethanol and volume was made to 10 ml. It was assayed using UV-spectrophotometer (V- 630 Jasco 2000 series, Japan) at 268nm. The free drug concentration was calculated from standard curves. This was performed for all the 9 batches of complex. The complexation rate of salicin was determined by the following formula:

$$\text{Complexation rate (\%)} = (m_2/m_1) \times 100 = [(m_1-m_3)/m_1] \times 100.$$

Where m_1 is the total content of salicin added, m_2 is the content of salicin present as a complex and m_3 is the free or non-complexed salicin.[10]

Determination of solubility by partition coefficient method

Complex equivalent to 10 mg of salicin was weighed and taken in a beaker. To this 10 ml of distilled water was added and it was stirred well for 20 minutes on a magnetic stirrer. In a 60 ml separating funnel, 10 ml of n-octanol was added and the dispersion of complex in water was added to the funnel. The funnel was uniformly agitated for 2 hours. It was then kept aside for 30 minutes. When two distinct layers were formed, they were separated and the solution was diluted and assayed using UV-Spectroscopy by recording absorbance in triplicate. Similar procedure was followed for the drug alone. Concentration in both the phases was calculated using calibration equation and the partition coefficient was calculated for the plain drug and for all the 9 batches by following formula:[11]

Partition coefficient =Co/Cw

Where Co – concentration in oil phase, Cw – concentration in water phase.

Determination of drug content in the complex

Complex equivalent to 10 mg of salicin of the optimized batch was dissolved in 100 ml of ethanol to form a solution of concentration 100 µg/ml approximately. Then again 2 ml solution was taken using a micropipette in a 10 ml volumetric flask and volume was made up to 10 ml with ethanol to obtain a solution of concentration 20 µg/ml and it was evaluated using UV Spectrophotometer at 268 nm. The blank was prepared by dissolving 10 mg of phospholipid in 100 ml of ethanol to form 100µg/ml and then subsequently 20µg/ml solution was prepared by dilution. The drug content was calculated for the optimized batch.[11]

Fourier Transform Infra-Red [FT-IR] Spectroscopy

The interaction between salicin and PHOSPHOLIPON 90H was studied by obtaining the FT-IR spectra using the Shimadzu- IRAFFINITY-1 Fourier transform infrared spectrophotometer. The FT-IR spectra of salicin, PHOSPHOLIPON 90H, complex and physical mixture were obtained. The sample was triturated and mixed with potassium bromide in the ratio 1:100. The mixture was then introduced in the sample holder of the FT-IR instrument and scanned to obtain the graph in the range of 4500-500cm^{-1} .Then the spectrum of the complex was compared to that of plain drug, phospholipid and their physical mixture.[12]

Differential Scanning Calorimetry

It was done with the help of a differential scanning calorimeter (R.C Patel Institute of pharmaceutical Education and Research, Shirpur). The samples sealed in the aluminum crimp

cells were heated at the speed of 5 °C/min from 0 to 300 °C under a nitrogen atmosphere. The phase transition onset temperatures of salicin, PHOSPHOLIPON 90H, complex and the physical mixture were determined and compared.[12]

Scanning Electron Microscopy

SEM imaging of complex and drug was performed by a scanning electron microscope (JEOL model JSM 6390LV- Sophisticated analytical instruments facility, STIC, Cochin) using electron beam for surface imaging.[12]

X-ray Diffraction study

The crystalline behavior of salicin, phospholipid and complex was evaluated with X-ray diffractometry. Diffraction patterns were obtained on Brisker AXS D8 Advance (Sophisticated Analytical Instruments Facility, STIC. Cochin, India.) The X-ray generator was operated at 40 kV tube voltages and 35mA tube current. The scanning angle ranged from 3 to 60 ^{0} in the step scan mode with step time of 39.8 seconds.[12]

***In vitro* permeation study of complex and salicin in suspension form**

The *in vitro* permeation study was performed using dialysis membrane 60 having diameter 15.9 mm. It was treated 24 hours prior by heating it with sodium bicarbonate solution in water for 10 minutes and then again heating it in distilled water for 10 minutes. After 24 hours the membrane was mounted on the Franz diffusion cell. The receptor compartment was filled with pH 7.2 phosphate buffer. The diffusion cell was maintained at 37±0.5 ^{0}C with constant stirring on a magnetic stirrer. Then suspension of salicin complex and plain salicin was prepared. It was evenly spread on the membrane in donor compartment. Then 1 ml sample of receptor medium was withdrawn at pre-determined time intervals and an equivalent volume of fresh buffer was added in. All samples were filtered, suitably diluted and analyzed by UV spectrophotometer. The permeation of the complex was compared to that of the plain drug.[13,14]

Stability study

A short term chemical stability of the salicin complex was examined for three months at 30±2 ^{0}C at 65% ± 5% relative humidity. The complex samples were analyzed at an interval of 30 days for three months and the *in vitro* permeation was studied and compared.[15] The data was statistically analyzed and validated using ANNOVA.

RESULTS AND DISCUSSION

Preformulation studies

The melting point range by Thiele tube method was found to be 205-209°C. The FT-IR spectrum of salicin showed similar peaks as that of standard spectrum values as shown in Fig. 4. The DSC results showed an endothermic peak at 200.97°C as shown in Fig. 5, which complied with the reported range.

The λ_{max} of salicin was found to be 268nm. The calibration curve of salicin in ethanol, water and pH 7.2 phosphate buffer along with its regression equation and correlation coefficient is shown in Fig. 5. The validation parameters evaluated were as per the ICH guidelines and the linearity range was found to be 20-100µg/ml.

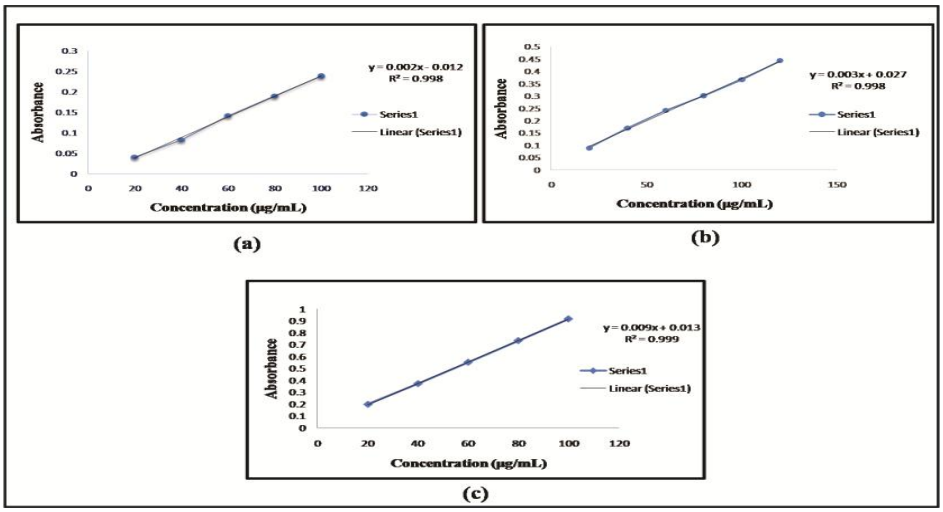

Fig. 2: Calibration curves of salicin in ethanol (a), water (b) and 7.2 pH phosphate buffer (c).

Formulation & optimization of Salicin- phytolipid complex by QbD concept using central composite design

According to central composite design 9 batches were formulated and batch F8 was found to be the optimized batch on the basis of the responses- complexation rate and partition coefficient. The optimized batch showed better solubility in water than the drug alone. The results are summarized in tables 3, 4 and 5. Only the statistically significant values were included. The contour plots and 3D surface response curves were obtained as shown in Fig. 3. They suggest that Salicin: PHOSPHOLIPON 90H ratio and temperature had positive relationship and time had an inverse relationship on complexation rate. On the other hand, temperature and time had a positive relationship and Salicin: PHOSPHOLIPON 90H ratio had an inverse relationship on partition coefficient.

Table 3: Optimization using central composite design.

Batch	X_1	X_2	X_3	Complexation rate(%)	Concentration in water	Concentration in oil	Partition coefficients $K=C_o/C_w$
F1	1:1.75	50	2	99.6147±0.23	53.19±2.0.4	832.95±1.44	15.65
F2	1:0.5	60	1	99.5804±0.29	45.17±1.43	1214.96±1.83	26.89
F3	1:0.5	40	1	99.5772±0.18	49.58±1.87	700.16±1.48	14.12
F4	1:3	60	1	99.6331±0.27	124.29±1.44	1132.96±1.77	9.1
F5	1:0.5	60	3	99.5926±0.28	67.52±1.55	976.96±1.68	14.46
F6	1:3	40	1	99.6265±0.32	115.35±1.68	1190.96±1.98	10.32
F7	1:0.5	40	3	99.5919±0.21	34.72±1.98	732.27±1.65	21.04
F8	**1:3**	**60**	**3**	**99.6485±0.28**	**135.73±1.61**	**814.73±1.58**	**6.00**
F9	1:3	40	3	99.6401±0.36	117.24±1.29	918.96±1.23	7.39

Data is presented as mean value ± SD (n=3)

Table 4: Experimental levels and evaluation of optimized batch.

Salicin: Phospholipon 90H	Temprature in ^{0}C	Time in hours	Complexation rate (Y_1)	Partition coefficient (Y_2)
1:3	60	3	99.6485±0.28	6.00

Table 5: Comparison of responses of optimized batch and plain drug.

	Complexation rate	Concentration in water	Concentration in oil	Partition coefficient
Optimized batch	99.6485	135.73	814.73	6.00
Salicin	--	27.73±1.05	843.71±1.84	30.42

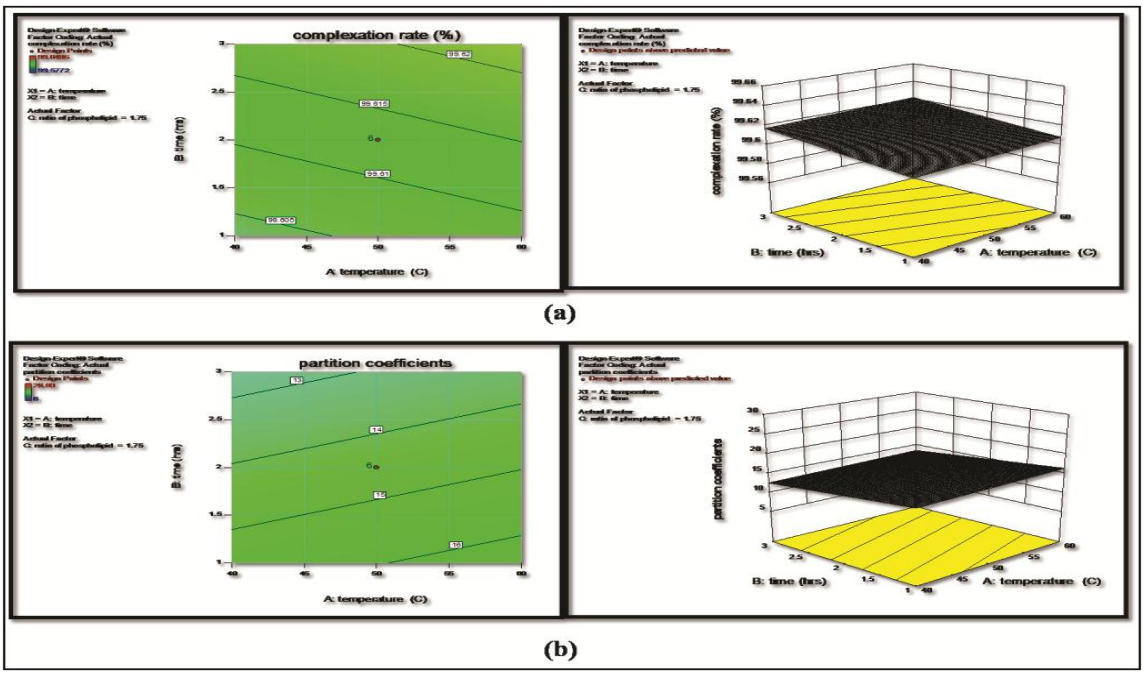

Fig. 3: Contour plots and surface response plots for the responses, complexation rate (a) & partition coefficient (b).

Characterization of Salicin phytosome

Determination of complexation rate

After evaluation of the highest complexation rate of 99.6485%, batch F8 was found to be the optimized batch. This may be attributed to the fact that phosphatidylcholine had a good affinity towards the drug salicin.

Determination of solubility by partition coefficient method

The solubility of all the 9 batches was summarized in table. The solubility of salicin and that of the optimized batch F8 in water phase were 27.73µg/ml and 135.73µg/ml respectively and the partition coefficient values were 30.42 and 6.00 respectively. This suggested that the solubility of salicin was increased almost 4.89 times in the form of phytosomal complex.

Determination of drug content in the complex

The drug content of Salicin in the form of phytolipid complex for the optimized batch F8 was found to be 94.24%. The high value of drug content in the complex indicated the formulation of a chemically stable complex.

Fourier Transform Infra-Red [FT-IR] Spectroscopy

A sharp peak at 3328.75cm^{-1} was obtained in the FT-IR spectrum of salicin for OH group. The spectrum showed aromatic C – H stretch at 2953.04 cm^{-1}, aromatic C=C stretch at 1491.61 cm^{-1} and C-O-C stretch at 1117.78 cm^{-1}. The FT-IR spectrum of PHOSPHOLIPON 90H showed the characteristic C – H stretching band of long fatty acid chain at 2918.3 and 2854.96cm^{-1},carbonyl stretching band at 1728.22cm^{-1} in the fatty acid ester, P=O stretching band at 1236.37 cm^{-1}, P–O–C stretching band at 1093.65 cm^{-1}, and $N+(CH_3)_3$ stretching at 966.34 cm^{-1}. The FT-IR spectrum of the salicin phytosomal complex showed significant changes in aromatic C – H stretching from 2953.04 cm^{-1} to 2916.05 cm^{-1}. Also the P=O absorption band of PHOSPHOLIPON 90H was found to shift to a lower wave number [16]. This depicted the successful formulation of the phytosomal complex as shown in Fig. 4.

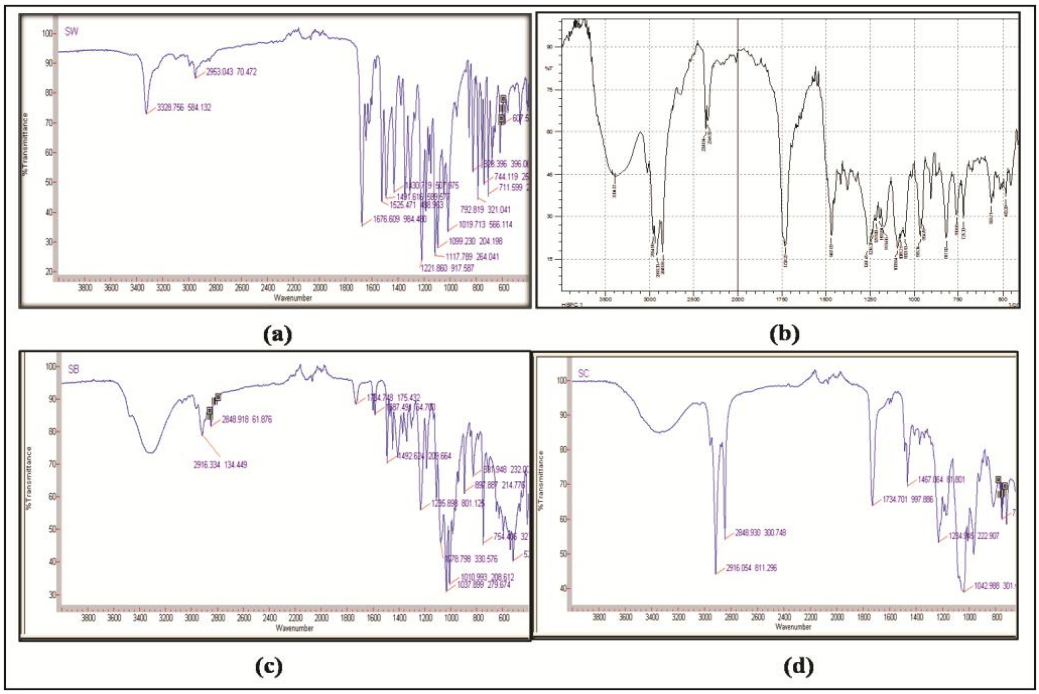

Fig. 4: FTIR spectra of Salicin (a), PHOSPHOLIPON 90H (b), physical mixture (c) & Salicin phytosomal complex (d).

Differential Scanning Calorimetry

Salicin showed endothermic peak ranging from 200.11 to 204.10 ^{0}C. PHOSPHOLIPON 90H showed two major peaks at 84.83 ^{0}C and peak at 67.16 ^{0}C. The first peak was sharp and it appeared due to the phase transition. The second peak was mild, which may be due to the movement of phospholipid polar head group. The physical mixture of salicin and PHOSPHOLIPON 90H showed two sharp peaks, one peak ranging from 62.03 ^{0}C to 73.02 ^{0}C and second peak ranging from 81.40 ^{0}C to 88.39 ^{0}C. The thermogram of the complex showed two peaks. The first sharp peak ranged from 54.94 ^{0}C to 59.55 ^{0}C and second peak from 62.41 ^{0}C to 82.72 ^{0}C which differed from the peak of salicin and PHOSPHOLIPON 90 H. It was evident that the original peak of salicin and PHOSPHOLIPON 90H disappeared from the thermogram of the complex due to shifting in the peak towards the lower temperature, thus confirming the formation of the complex as shown in Fig. 5.

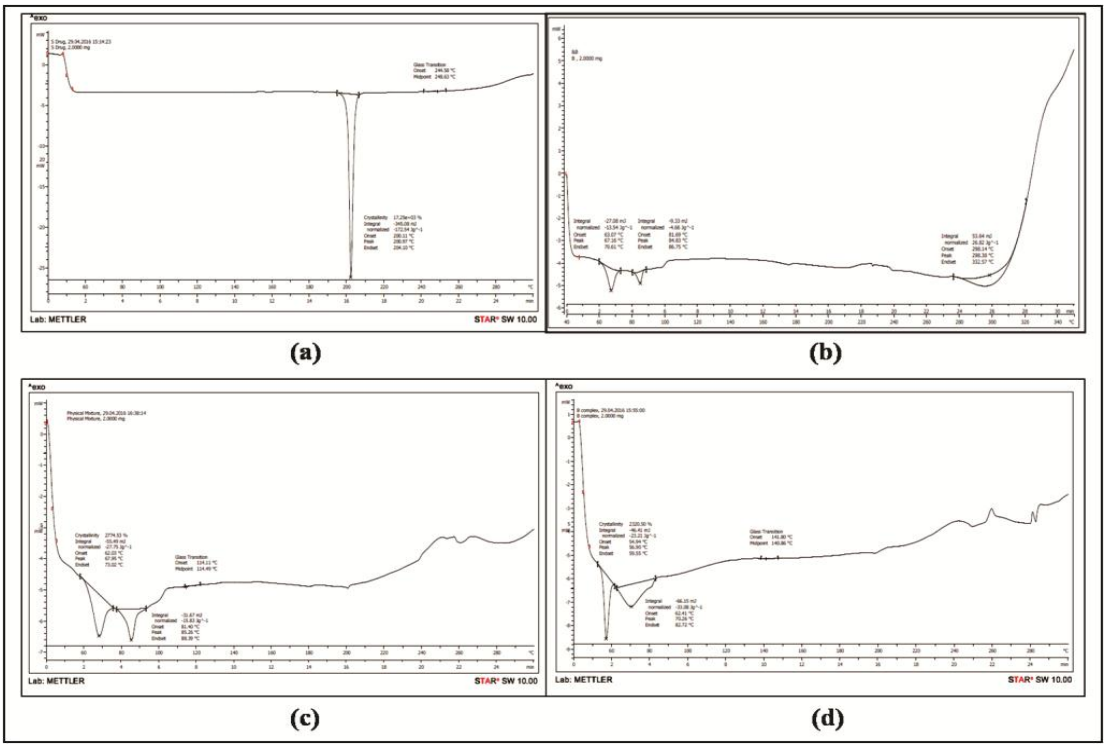

Fig. 5: DSC graphs of Salicin (a), PHOSPHOLIPON 90H (b), physical mixture (c) & Salicin phytolipid complex (d).

Scanning Electron Microscopy

The scanning electron micrographs of salicin and complex depicted in the Fig. 6 showed their surface morphology. The change from crystalline nature of salicin to amorphous complex form was evident which proved the formation of complex. This also gave the evidence for enhancement of solubility of salicin in the form of phytosome.

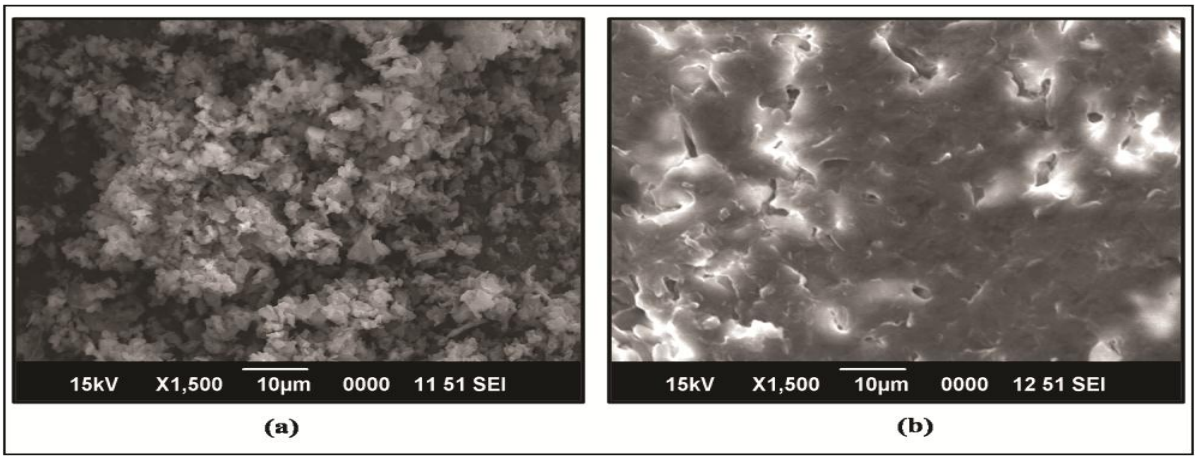

Fig. 6: Scanning electron micrographs of Salicin (a) and complex (b).

X-ray Diffraction study

Salicin exhibited many intense sharp diffraction peaks of crystallinity at a diffraction angle of 24.058 ^{0}, 21.481^0, 25.475^0, and 19.645^0 and indicated that the drug was present as a crystalline material. The phospholipid showed a single intense peak at 2θ value of 21.242. The disappearance of salicin peaks confirmed the formation of complex of salicin as shown in Fig. 7. It can be concluded that salicin phytolipid complex had an amorphous form which led to the enhancement of solubility.

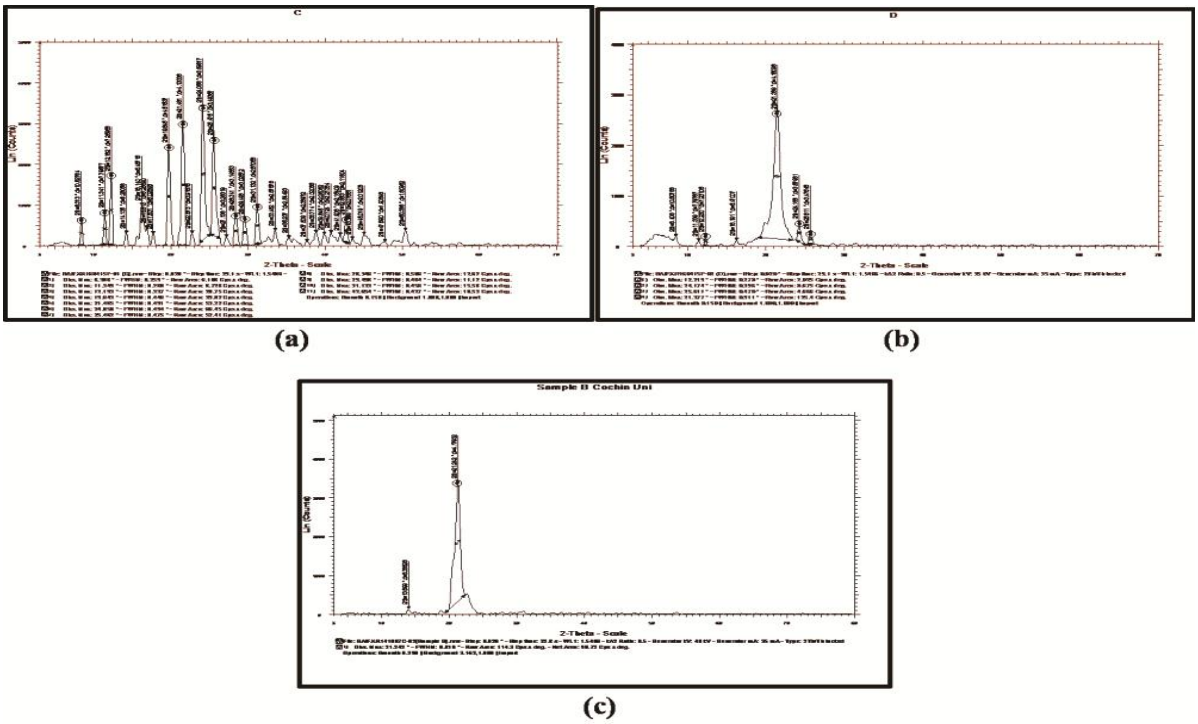

Fig. 7: X-ray diffractograms of Salicin (a), PHOSPHOLIPON 90H (b) & Salicin phytosomal complex (c).

***In vitro* permeation study of complex and salicin in suspension form**

The *in vitro* permeation of the complex and salicin in suspension was studied over a period of 7 hours as shown in table 6. The % cumulative release was calculated and the permeation was found to be higher for complex than salicin alone in the form of suspension as shown in the Fig. 8. Model fitting for *in vitro* permeation profile was performed using Zero order, First order, Higuchi and Korsmeyer-Peppas model and on the basis of the highest correlation coefficient it was found that the best fit was shown by Zero-order model. The value of the permeation coefficient (n) for complex and salicin alone was found to be $0.5<n<1$ respectively indicating non-Fickian pattern of drug release.[17]

Table 6: Comparative % cumulative release of salicin and complex from suspension.

Time in minutes	% Cumulative release of salicin from suspension	% Cumulative release of complex from suspension
30	1.589 ± 0.366	8.278 ± 0.681
60	2.012 ± 0.558	16.480 ± 0.355
90	2.513 ± 0.862	25.245 ± 0.452
120	3.013 ± 0.257	33.726 ± 0.272
150	3.751 ± 0.355	41.707 ± 0.441
180	5.432 ± 0.355	49.335 ± 0.778
210	7.869 ± 0.458	56.245 ± 0.512
240	9.412 ± 0.441	64.321 ± 0.411
270	11.369 ± 0.373	69.456 ± 0.971
300	13.867 ± 0.366	77.702 ± 0.595
330	15.307 ± 0.409	82.224 ± 0.579
360	16.201 ± 0.378	88.642 ± 0.755
390	17.729 ± 0.376	91.560 ± 0.599
420	19.658 ± 0.333	93.432 ± 0.771

Data is presented as mean value ± SD (n=3)

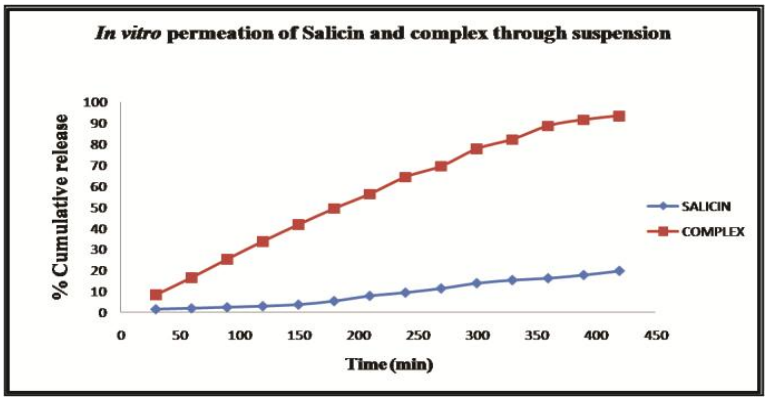

Fig. 8: *In vitro* permeation through suspension.

Stability study

The stability study at 30°C ± 2°C at 65% ± 5% RH indicated that the optimized batch did not show any physical changes during the study period. The results were as shown in table 7 for a period of 3 months.

Table 7: Stability study of salicin phytolipid complex for 3 months.

Formulation	Storage conditions (Months)	Time interval	*In vitro* release after 12 h (%)
Salicin-Phospholipon 90H complex (Batch F8)	30 ± 2 °C and 65 ± 5 % RH	0	86.68±0.24
		1	87.12±0.39
		2	89.87±0.97
		3	89.37±0.54

Data is presented as mean value ± SD (n=3)

CONCLUSION

Salicin is an effective herbal drug possessing anti-inflammatory, analgesic and antipyretic activity. During the literature survey it was found that salicin was an effective drug for the formulation of phospholipid delivery system because of its structural suitability. It possesses five polar hydroxyl groups which are suitable for complexation to the polar group of PHOSPHOLIPON 90H. Salicin has not been reported for the formulation of phytosomal complex and hence an attempt was made to formulate this novel Salicin phytosomal complex. Salicin was successfully complexed with PHOSPHOLIPON 90H to form a novel phytosomal delivery system. The preformulation study confirmed the drug salicin by FTIR, DSC and melting point determination. Analytical method development and determination of various validation parameters confirmed stability and method reliability of the drug. The complexation was carried out by solvent evaporation method using central composite design applying three factors, salicin: PHOSPHOLIPON 90H ratio, time and temperature and the batch was optimized by evaluating two responses, complexation rate and partition coefficient. The optimized batch showed increased solubility in water (135.73µg/ml) which was approximately 4.89 times that of the drug alone (27.73µg/ml) with complexation rate of 99.64%. The optimized batch (F8) was then evaluated for the drug content (94.24%). The phytosomes were characterized using FTIR, DSC, SEM and XRD. SEM showed the surface morphology of complex depicting the conversion of crystalline drug into amorphous complex which may also be attributed to the increased solubility of the complex. The *in vitro* permeation in the form of suspension was significantly higher for the complex (93.43%) than the drug (19.65%) after 7 hours of study. The stability study depicted no significant changes over the period of study. Hence, it can be concluded that the anti-inflammatory phytoconstituent, Salicin showed a significant enhancement of its overall physicochemical properties in the form of phytosomal complex which led to its improved solubility and permeation. This in turn may lead to enhancement of its bioavailability and ultimately its pharmacological activity.

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