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Design and Characterization of Phytosomal Nano Carriers for Enhanced Rutin Delivery

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ABSTRACT

Development of amphiphilic drug-lipid complexes is a potential approach for improving delivery of the drugs by increasing solubility, release profile and oral bioavailability. Rutin, a polyphenolic flavonoid, shows several biological effects like capillary protectant, anti-oxidant, anti-inflammatory, anti-aging, cardio-protective, anti-thrombotic and neuroprotective, but its use is limited due to its low aqueous solubility. To overcome this limitation, phospholipid complex of Rutin was developed to improve its aqueous solubility for better absorption through the gastrointestinal tract and this might result in improved bioavailability. The Rutin phytosomes prepared by solvent evaporation method using different ratios of Rutin and Soybean phosphatidylcholine (1:1, 1:2 and 1:3) was evaluated for percentage yield, compatibility studies by infra-red spectroscopy, particle size, poly dispersity index, zeta potential, drug content and were found to be within the acceptable range. Surface morphology by scanning electron microscopy, solubility studies, *in-vitro* drug release and stability studies also were carried out. The phospholipid complex of Rutin was found to be fluffy and porous with rough surface. The water solubility of Rutin was improved from 0.058mg/ml to 0.475 mg/ml in the prepared Phytosomes. The *in-vitro* drug release studies showed that there is no drug release from pure drug and F1 formulation up to 120min in acidic buffer pH 1.2; while in phosphate buffer pH 7.4 showed releases about 49.3% and 92.85% respectively, which indicates the significant enhancement of dissolution of Rutin phytosomes compared to pure drug. Stability studies suggested that the formulations were stable. In this study, Phytosomes could be successfully tailored for Rutin with improved dissolution characteristics which is promising for lowering the influence of exogenous factors and increasing drug delivery.

Keywords: Rutin, Soybean phosphatidylcholine, Phytosomes, Solubility.

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INTRODUCTION

Herbal drugs stand out as recent promising candidates for the treatment of various diseases. Fewer side effects and lower phytochemical costs from natural resources open new avenues for health maintenance by various means and highlight the era of 'back to nature. Most of the bioactive constituents of phytomedicines are secondary metabolites like flavonoids, glycosides etc. Flavonoids are polyphenolic compounds that are ubiquitous in nature. Flavonoids like quercetin, hesperetin, curcumin, luteolin, diosmin and Rutin are phenolic compounds possess strong antioxidant activity[1] as well as other interesting potential effects including anti-inflammatory[2], anti-cancer[3]anti-ulcer[4] and anti-microbial (antibacterial[5], antifungal[6] and antiviral[7]) activities. Rutin, also called as Quercetin-3-O-rutinoside (Figure 1) belongs to the flavonolglycosides class, which contains a carbohydrate moiety (Rutinose) which is o-glycosidically linked to one of the flavonoid backbones (2-phenylchromen-4-one, 3-phenylchromen-4-one or 4-phenylcoumarin) is a poly phenolic compound with excellent therapeutic potential and good safety profile.

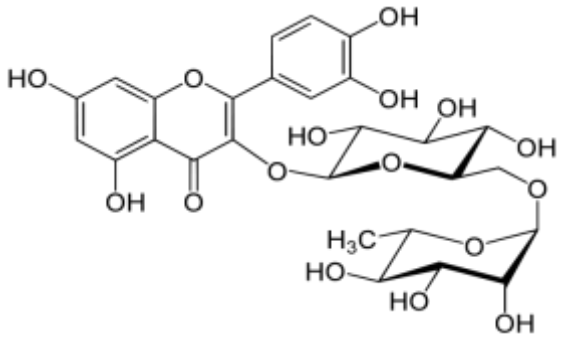

Figure 1: Chemical structure of Rutin

Rutin is one of the ancient drug has been used for its phlebotonic properties and capillary protector used for the treatment of haemorrhoids and as anti-oxidant, anti-inflammatory, cardio-protective, neuroprotective, anti-thrombotic, anti-aging agent and used in multivitamin preparations. Unfortunately, despite the wide therapeutic potential of Rutin, its phenolic nature renders it polar but has poor solubility in water and most of the organic solvents as well. Poor drug dissolution is responsible for scarce absorption, poor bioavailability and high inter-subject variation following oral administration, which is reported consequence of these features[8]. Low drug solubility results in reduced amounts of drug absorbed, paving the way for a high influence of exogenous factors, such as diet and dosage regimen. Therefore, a large standard dose of Rutin (500mg twice daily) is usually required for oral dosage regimens[9]. These aspects constitute a handicap against the widespread use of this flavonoid in the pharmaceutical field. The poor absorption of Rutin is likely due to two main factors. First, it is having multiple-ring molecules that are too large to be absorbed

by simple diffusion. Secondly, Rutin typically have poor miscibility with oils and other lipids, which limit its ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine. The effectiveness of any herbal product is dependent upon delivering an effective level of active compounds. The phytosome technology meets this challenge by markedly enhancing the dissolution of phytomedicines[10]. Rutin delivery can be improved by formulating an appropriate drug delivery system, which can enhance the rate and the extent of drug dissolution, absorption and permeation across the lipoid biomembrane. Natural soyabean phosphatidylcholines (SPC) have always presented as outstanding candidates for clinical applications including biocompatibility, biodegradability, metabolic activity, and low toxicity and cytotoxicity compared to their synthetic alternatives. In this context, SPC based drug delivery systems are promising. Phytosomes (herbosomes) constitute complexes between a natural product and natural phospholipids, such as soy phospholipids. Such a complex is obtained by a reaction using stoichiometric amounts of SPC and the substrate in an appropriate solvent. The main phospholipid-substrate interaction is the formation of hydrogen bonds between the SPC polar head and the polar functionalities of the substrate. Such bonding is the main reported cause for the better physical stability profile of phytosomes compared to liposomes. Phytosomes have successfully improved the oral bioavailability of different flavonoids. In this context, Maiti *et al.*,[11,12] developed phytosomes of curcumin and naringenin in two different studies. In the first study, phytosomes of curcumin were developed to overcome the limitation of absorption and to investigate the protective effect of curcumin–phospholipid complex on carbon tetrachloride-induced acute liver damage in rats. The complex showed enhanced aqueous and n-octanol solubility. The antioxidant activity of the complex was significantly higher than that of pure curcumin at all dose levels tested. In the other study, the developed phytosomes of naringenin exhibited better antioxidant activity than the free compound with a prolonged duration of action, which may be helpful in reducing the rapid elimination of the molecule from the body. It is anticipated that in the tailoring of such nanocarriers, obstacles, such as poor solubilizing capacity of different aqueous and oily vehicles to the drug, would render complex formation and its characterization more challenging. Accordingly, this article has considered the genesis and appraisal of Rutin loaded SPC phytosomes for enhanced drug delivery. In the present study, an attempt was made to prepare and evaluate Rutinphytosomes to improve the solubility and GIT absorption of the drug by complexing with the polymer SPC.

MATERIALS AND METHOD

The SPC phospholipid Lipoid® S 75 was kind gifts from Lipoid Co,GmBH, Ludwigshafen,

Germany. Rutin was purchased from Yarrow chemical, Mumbai, India. Dichloromethane and n-Hexane was purchased from Hi Media Laboratory Pvt. Ltd, Mumbai, India. All the reagents used were of analytical grade.

Preparation of Rutin Phytosomes

The Rutin phytosomes were prepared by solvent evaporation technique as per the method described by Maiti*et al.,*[12]. The molar ratios (1:1, 1:2 and 1:3) of drug (Rutin) and phospholipid (Lipoid® S 75) were accurately weighed and refluxed by placing into 250ml round bottom flask containing 20ml of dichloromethane and then round bottom flask was attached to a rotary evaporator and rotated at 60 rpm. Temperature is maintained above the phase transition temperature (Tm) of the phospholipid (45°C), resulting solution is then concentrated to 2-3ml to obtain a thin lipid film. Sufficient amount of n-hexane was added after reducing the solution to 2-3ml to form amorphous product. Then it is subjected to sonication for further size reduction. All formulations with drug: phospholipid ratio shown in Table 1.

Table 1: Preparation of Rutinphytosomes

Formulation code	Drug: polymer ratio	Temperature	Speed of rotation
F1	1:1	45°C	60 rpm
F2	1:2		
F3	1:3		

Evaluation of Rutin Phytosomes[15]

Compatibility studies by Fourier transform infrared spectroscopy (FTIR)

FTIR can be used to investigate and predict any physicochemical interaction between different excipients. IR spectra matching approach was used for detection of any possible chemical interaction between the drug and polymer. A physical mixture of Rutin and polymer SCP were prepared and mixed with suitable quantity of potassium bromide. It was scanned from 4000 to 400 cm^{-1} in a FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with those of pure Rutin and polymer and peak matching was done to detect any appearance or disappearance of peaks.

Percentage Yield

The percentage yield of the prepared phytosomes was determined by using the formula:

Percentage yield = Practical yield/ Theoretical yield X 100

Average particle size and size distribution

Average particle size (in nanometers) and size distribution (as the polydispersity index) of the

phytosomes was measured using a Malvern nano zeta sizer instrument. The samples were diluted with distilled water (1:10) before measurement.

Zeta Potential

Measurement of zeta potential of the Rutin phytosomes was done by using a Malvern nano zeta sizer instrument. The samples were diluted with distilled water (1:10) before measurement.

Surface Morphology

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM studies were carried out by using JEOL-JSM 6380LA analytical scanning electron microscope. The samples of SEM were prepared by lightly sprinkling the phytosomal powder on a double adhesive tape, which was stuck on an aluminium stub. The photomicrographs were taken with the help of SEM analyzer.

Drug Content

Phytosomes were accurately weighed (equivalent to 100 mg of Rutin), triturated and digested in 10 ml buffer solutions (pH 1.2 and pH 7.4) separately and kept overnight for extraction of drug. The digested homogenate was centrifuged and supernatant was collected. After appropriate dilution of supernatant with same buffer solutions, aliquots were assayed by UV spectrophotometer at λmax 256 nm for acidic buffer pH1.2 and at λmax 257 nm for phosphate buffer pH 7.4. Corresponding drug concentrations in the sample were calculated from the standard calibration curve.

Solubility Studies

It is determined by dissolving Rutin phytosomes in water, acidic buffer pH 1.2 and phosphate buffer pH 7.4. The solubility study was conducted by taking excess amount of the complex in 10 ml of solution. Then the samples were kept in the water bath shaker and agitated for 24 hrs at 37±0.5°C. The samples were filtered and diluted suitably with buffer solution. The samples were analyzed spectrophotometrically at λ max 256 nm for acidic buffer pH 1.2 and at λ max 257 nm for phosphate buffer pH 7.4 respectively. The concentration of Rutin was determined using respective standard graphs.

***In-vitro* Drug Release[16]**

In vitro drug release from the complex was determined using dialysis sacks. The sacks were washed as per the instruction given by the manufacturer. After proper pre-treatment, one end of the sack was tied and known amount of (equivalent to 100mg of Rutin) pure Rutin/Rutin phytosome was placed inside the sack. The other end of the sack was tied and then suspended vertically into a beaker containing 200 ml of buffer solution (pH 1.2 and pH 7.4).The content of the beakers were

stirred at 50 rpm using a magnetic stirrer at 37±1°C.The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 hrs and then replace with pH 7.4. The samples were withdrawn (5ml) from the dissolution medium at various time intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium. The rate of drug release was analyzed using UV spectrophotometer.

Stability studies[17]

Selected formulation was subjected to stability studies for a period of 2 months at an accelerated condition at 40±2 ºC/ 75±5% RH due to lack of time to carry out for 6 months as per new ICH guidelines. Phytosomes were evaluated for drug content and *in-vitro* drug release studies.

RESULTS AND DISCUSSION

Compatibility Studies By FTIR

The IR spectra of the Rutin-SPC and formulations (F1 and F2) were compared with the standard spectrum of pure drug Rutin and the characteristic peaks associated with specific functional groups and bonds of the molecule and their presence/ absence were noted in Table 2and the overlay of pure drug Rutin, Rutin-SPC and formulations (F1 and F2) is shown in the Figure 2.The prominent peaks associated with O-H bonded (3650-3600), C-H stress (3000-2850), C=O (Ketone) (1752-1705), C=C (Alkene) (1680-1600), C=C (Aromatic) (1600-1475) and C-O-C (Ether) (1300-1000) were analysed. The range of peak values were found to be the same indicating that there were no interaction of Rutin with polymer confirming the stability of drug in the formulations.

Table 2: Comparison of FT-IR spectra of Rutin and polymer

Sr. No.	Functional Groups	Reported Frequency (cm-1)	Observed Frequency (cm-1)			
			Drug	Drug + SPC	F1	F2
1	O-H (Bonded)	3650-3600	3642.71	3640.95	3640.7	3643.84
2	C-H (Stretch)	3000-2850	2920.66	2918.33	2918.4	2913.91
3	C=O (Ketone)	1725-1705	1708.62	1710.55	1706.6	1705.01
4	C=C (Alkene)	1680-1600	1605.9	1609.27	1606.6	1607.13
5	C=C (Aromatic)	1600-1475	1585.2	1523.27	1581.0	1587.59
6	C-O-C (Ether)	1300-1000	1057.76	1058.32	1055.2	1050.05

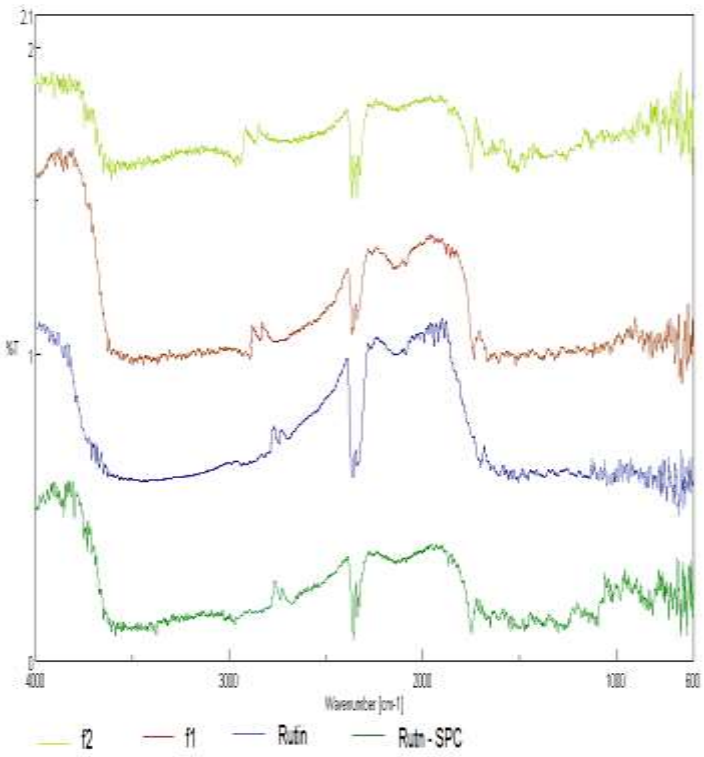

Figure 2: FT-IR Spectrum of Rutin, Rutin-SPC, F1 and F2

Percentage Yield

The percentage yield of formulations, F1, F2 and F3 was found to be 77.44, 67.15, and 67.38% respectively. The results indicated that as the amount of polymer increases, the number of phytosomes formed decreases and hence the percentage yield decreases. The results obtained were showed in Table 3.

Average Particle Size and Size Distribution

Particle size analysis of the phytosomes was determined using a Malvern zeta sizer instrument. The size distribution (as the poly dispersity index) of Rutin phytosomes (F1 and F2) was measured by Dynamic Light Scattering phenomenon using Malven Nano Zeta Sizer. The results obtained by the analysis indicated that the size of the phytosomes of F1 formulation was lesser compared to the size of phytosomes of F2 formulation. It was found that the average particle size of the F1 formulation was approximately 293nm, whereas the average particle size of F2 formulation was found to be 305nm. Poly dispersity index of F1 formulation was found to be 0.46 and increased to 0.48 in F2 formulation. All the results were shown in Table 3.

Zeta Potential

Zeta potential of the Rutin phytosomes (F1 and F2) was determined by Malvern nano zeta sizer instrument. It was found that zeta potential of the F1 formulation was negative i.e., -36.2mV and

zeta potential of the F2 formulation was also negative, i.e., -33.3mV. The negative potential indicates that the particles have no charge and as a whole system is stable. The results obtained are shown in Table 3.

Surface Morphology

The SEM studies of Rutin phytosomes showed that formulations (F1 and F2) exhibited fluffy, porous and rough surface. SEM photographs of formulation F1 and F2 were shown in Figure 3 and Figure 4 respectively.

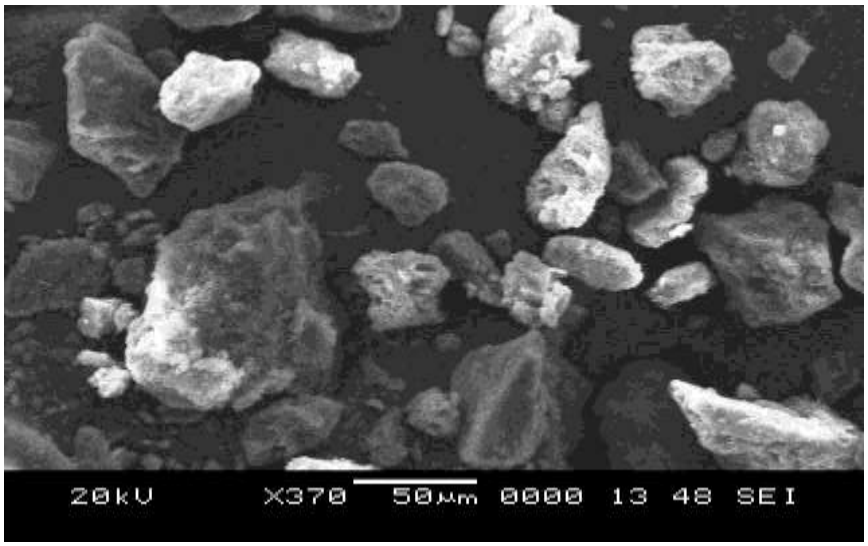

Figure 3: SEM of F1 formulation of Rutin phytosomes

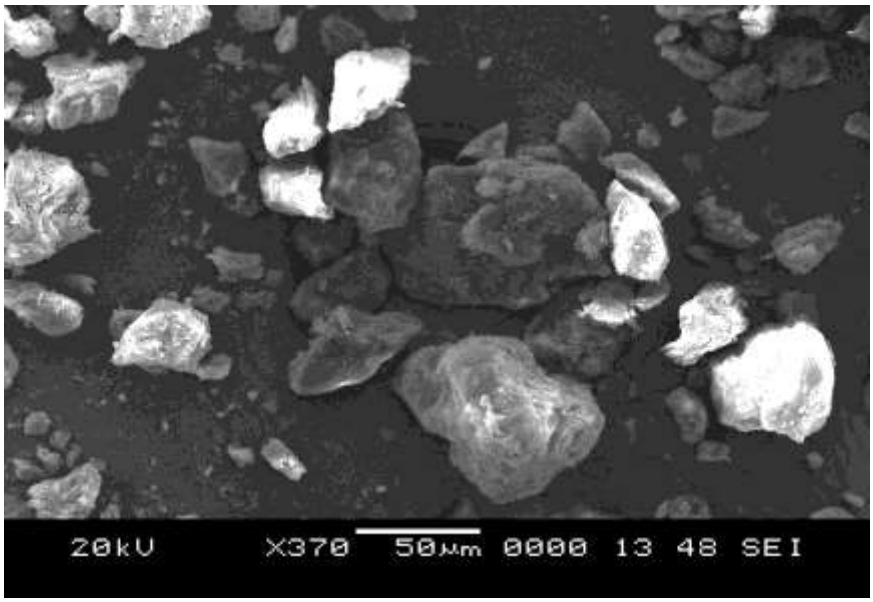

Figure 4: SEM of F2 formulation of Rutin phytosomes

Drug Content

As the polymer ratio was increased, the drug content decreased with 64.25mg, 56.78mg and

47.93mg for formulations F1, F2 and F3 respectively. High drug content was observed for F1 formulation, thereby indicating that the polymer concentration plays a major role in drug content. The results obtained were depicted in Table 3.

Table 3: Evaluation of Rutin phytosomes

Formula	Rutin/SPC ratio	%Yield (%)	Particle size (nm)	Poly dispersity index	Zeta potential	Drug content (mg)
F1	1:1	74.44	293	0.46	-36.2	64.25
F2	1:2	67.15	305	0.48	-33.3	56.78
F3	1:3	67.38	-	-	-	47.93

Solubility Studies

Rutin is extremely hydrophobic in nature and slightly soluble in aqueous media. Its poor solubility leads to its poor absorption/ permeation across the intestinal epithelial cells of the gastrointestinal (GI) tract leading to low bioavailability of the drug. Aqueous solubility of Rutin improved from 0.058mg/ml to 0.475mg/ml, 0.4353mg/ml and 0.427mg/ml in the prepared complex F1, F2 and F3 respectively. Solubility of the Rutin in Phosphate buffer pH 7.4 was improved from 0.50mg/ml to 4.5902mg/ml, 4.537mg/ml and 4.4651mg/ml in F1, F2 and F3 respectively. Same as pure drug all formulations are also remained insoluble in acidic buffer pH 1.2. The results obtained were depicted in Table 4.

Table 4: Solubility of pure drug and Rutin phytosomes in Water, Acidic buffer pH 1.2 and Phosphate buffer pH 7.4

Medium	Solubility (mg/ml)			
	Pure drug	F1	F2	F3
Water	0.058	0.475	0.4353	0.427
Acidic buffer pH 1.2	0.0	0.0064	0.0068	0.0065
Phosphate buffer pH 7.4	0.50	4.5902	4.537	4.4651

***In-Vitro* Drug Release**

The *in-vitro* release profile obtained for the pure drug Rutin and for all the formulations (F1, F2 and F3) were shown in Figure 5. The cumulative percent drug release after 180min were found to be 49.3, 92.85, 83.27 and 70.99 for pure drug, formulation F1, F2 and F3 respectively. As expected, there was no measurable drug release observed at acidic pH 1.2 till 120min. Results revealed significant enhancement of Rutin release in Phosphate buffer pH 7.4 from all phytosomal formulations compared to pure drug. The dissolution of solid dosage forms is a complex operation influenced by a number of factors. Differences in surface area, surface energies, particle size, and wetting properties may all play a role in affecting the dissolution rate of powder. Such significant

enhancement in dissolution of phytosomes compared to pure drug could be ascribed mainly to the amphiphilic nature of the complex. Soybean phospholipids, being an amphiphilic surfactant, are anticipated to increase Rutin solubility through the action of wetting and dispersion. Furthermore, the nano size of the phytovesicles could be another factor for dissolution enhancement of Rutin. This explanation supported by particle size results demonstrating a nano-metric range for all complex ratios (F1 and F2) reflecting high specific surface area compared to plain drug (Table 3). Here as F1 formulation exhibited the lowest particle size and poly dispersity index, adequate zeta potential, high drug content parallel to significant dissolution enhancement. Therefore, phytosomal formula F1 with Rutin: SPC ratio of 1:1 was selected as the best formulation for stability studies.

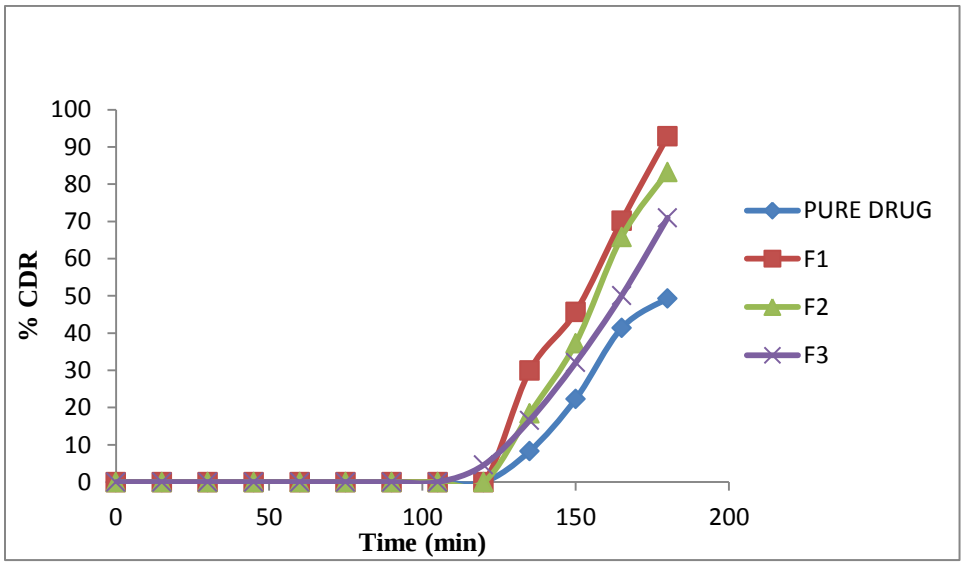

Figure 5: Percentage CDR of pure drug Rutin and formulations (F1, F2 and F3)

Stability Studies

The results of the stability studies indicated that the phytosomes did not show any changes in the drug content during the stability study period. The percentage cumulative drug release after 60 days showed 92.84% after 3 hrs indicating no significant changes. The results obtained were depicted in Figure 6.

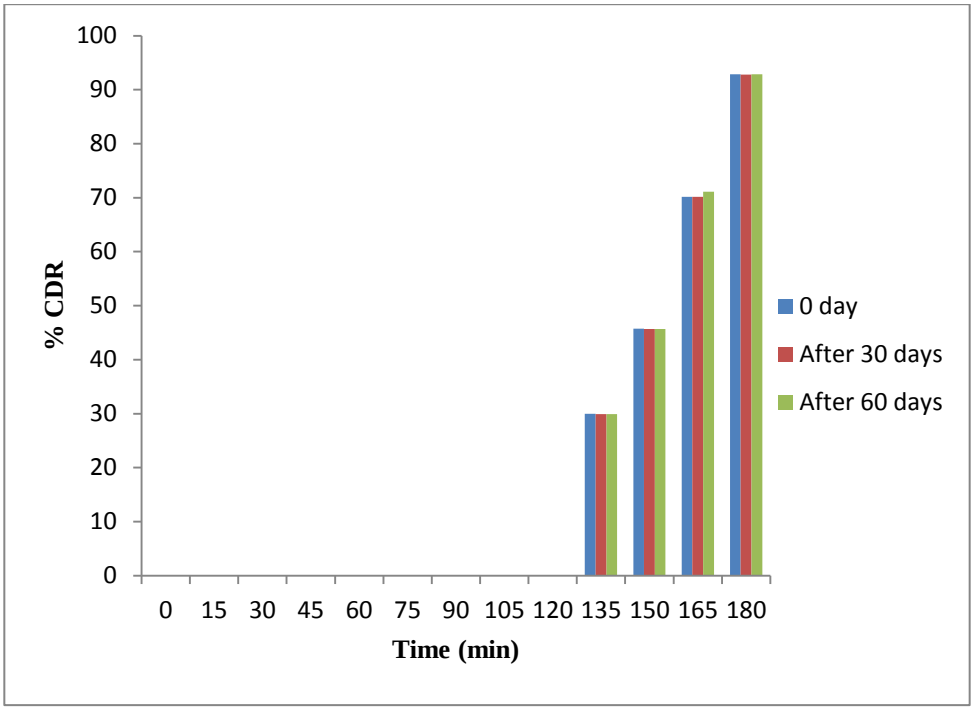

Figure 6: *In-vitro* release data of F1 during stability study

CONCLUSION

The present study has been a satisfactory attempt to formulate phytosomal nano carriers for enhanced delivery of Rutin using polymer Soybean phosphatidyl choline. From the reproducible results of the executed experiments, it can be concluded that: The IR spectra revealed that, there was no interaction between Rutin and polymer, thus indicating the compatibility of Rutin with the polymer used. The percentage yield and drug content of the prepared Rutin phytosomes decreased as the concentration of polymer increased and from the study it can be seen that formulation F1 with Rutin: SPC ratio of 1:1 showed higher percentage yield and drug content. Solubility studies revealed that the phytosomal nano carriers enhanced the solubility of the drug Rutin compared to its crude form in aqueous medium and Phosphate buffer pH 7.4. Both crude form of the drug and formulations remained insoluble in Acidic buffer pH 1.2. *In-vitro* release studies showed that as the amount of polymer increases the extent of drug release decreases. Results of dissolution studies contended the ability of phytosomalnano carriers to enhance dissolution rate of the drug compared to its crude form. The cumulative drug release from formulation F1 with Rutin: SPC ratio of 1:1 showed desired release rate, compared to other formulations, as there was no drug release observed up to 120min and showed desired drug release of about 92.85% after 180min.Stability studies were carried out for the best formulation F1. The results of drug content and *in-vitro* drug release studies showed no significant changes indicating the formulation is stable.

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