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Research Article

In Vitro Investigation of Phytosomal Nanocarriers of Rutin

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ABSTRACT

The drug-lipid complex formulation is a potential drug delivery approach for the polyphenolic phytoconstituents which works by improving the drug solubility, absorption and bioavailability. Rutin is a polyphenolic flavonoid known for several biological effects such as anti-oxidant, hepatoprotective, anti-inflammatory, cardio-protective etc. but due to its low solubility, absorption and bioavailability problems the use is limited. We formulated the phytosomal nanocarriers of rutin to overcome these limitations. The rutin phytosomal nanocarriers were prepared by solvent evaporation method using different ratios of rutin and soybean phosphatidylcholine (1:1, 1:2 and 1:3) and characterised for compatibility, particle size, poly dispersity index, zeta potential, surface morphology, solubility, drug content, *in-vitro* drug release and kinetics. The formulated rutin phytosomal nano carriers were in nanometric size, negative surface charge and exhibited porous, fluffy and rough surface. The aqueous solubility of pure rutin was found to be 1.59 µg/ml which was improved in F2 formulation which showed 42.71 µg/ml aqueous solubility. The *in-vitro* drug release studies demonstrated no significant drug release from pure drug and rutin phytosomal nano carriers up to 120 min in acidic buffer pH 1.2 whereas in the phosphate buffer pH 7.4 formulation F2 showed highest drug release of 76.9 % at the end of 24 h which followed diffusion controlled drug release mechanism. The phytosomal nanocarriers of rutin with improved solubility, dissolution characteristics found promising for better drug delivery.

INTRODUCTION

Phytoconstituents are emerging as promising candidates for health maintenance. Now people are keen to use phytomedicines in the treatment of various diseases due to the minimum side effects and also cost effectiveness which highlights the era of 'back to nature'. The polyphenolic plant secondary metabolites such as terpenoids, flavonoids, alkaloids, glycosides etc. are the major plant components possess a variety of biological activities such as antioxidant, hepatoprotective, anti-inflammatory, cardioprotective, anti-cancer etc. Despite the wide therapeutic potential, these components suffer from bioavailability problem which limits their use in the pharmaceutical field [1-3].

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Rutin, also called as Quercetin-3-O-rutinoside (Fig. 1) belongs to the flavonolglycosides class, is a poly phenolic compound with excellent therapeutic potential majorly used for its antioxidant and hepatoprotective actions and also it possesses good safety profile [4, 5].

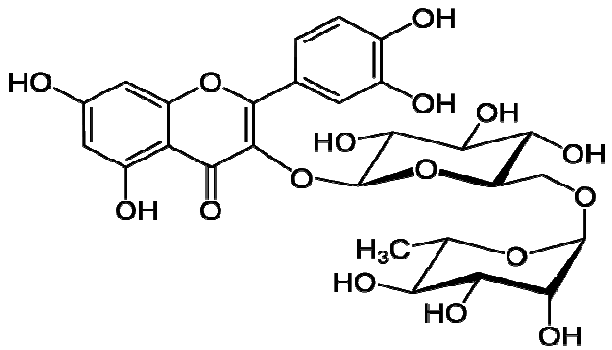

Figure 1: Chemical Structure of Rutin

The phenolic nature of the rutin leads to solubility and permeability problems which ultimately affect its bioavailability. Therefore,

usually, a large dose of 500 mg twice daily is recommended for oral dosage regimens [6]. The poor absorption of rutin is likely due to two main factors, the large molecular size which is difficult to absorb by the simple diffusion and the poor miscibility with oils and lipids which limit its ability to pass across the lipid-rich outer membranes of the enterocytes of the small intestine [7]. In order to improve rutin delivery to the systemic circulation, it is essential to formulate an appropriate drug delivery system, which can promote drug absorption and permeation across the bio membrane.

The lipid based carriers are considered as efficient and safe for the formulation of pharmaceuticals, vaccines and nutraceuticals [8]. Phytosomes are molecular complexes between phytoconstituents and phospholipids due to the formation of hydrogen bonds between the polar head of the phospholipid and the polar functionalities of the phytoconstituent. The physical stability of phytosomes is mainly due to the formation of these hydrogen bonds [9,10]. Phytosomes found effective in the delivery of different flavonoids. In this context, Maiti *et al.,* formulated the curcumin phytosomes and investigated its effectiveness on carbon tetrachloride-induced acute liver damage in rats. The complex showed enhanced aqueous and n-octanol solubility. The antioxidant and hepatoprotective activity of the complex was found to be higher than that of pure curcumin at all dose levels tested. The pharmacokinetic study indicated the better oral bioavailability of the complex compared to the pure curcumin [11]. Ying *et al.,* experimented to study the pharmacokinetics of puerarin phytosomes (puerarin-phospholipid complex) in comparison with pure puerarin in Beagle dogs *in vivo.* AUC, C_{max} values of puerarin phytosome were found to be higher compared to pure puerarin, and t_{max} value was found to be less than that of pure puerarin. This result showed that the phospholipid complex formation could effectively enhance the puerarin absorption [12].

The success of any herbal formulation depends on the delivery of active component at its effective level. Hence this study aimed to formulate rutin phytosomal nanocarriers, a self nanovesicular pre-concentrate which will convert into a liquid vesicular form on contact with GIT aqueous media. The developed rutin phytosomal nanocarriers were characterised for compatibility by FTIR, particle size, poly dispersity index and zeta potential by Zeta nano sizer, surface morphology by SEM, solubility, drug content, *in vitro* drug release and kinetics.

MATERIAL AND METHODS

Materials

The soybean phosphatidylcholine (SPC) - 'Phospholipon®' was a kind gift sample from Lipoid, Germany. Rutin was procured from Yarrow Chem, Mumbai, India. Dichloromethane and n-Hexane were purchased from Hi Media Laboratory Pvt. Ltd, Mumbai, India. All the chemicals/reagents used were of analytical grade.

Formulation of Rutin Phytosomal Nanocarriers

The rutin phytosomal nanocarriers were prepared by solvent evaporation technique as described by Maiti *et al.,* [13]. The drug (rutin) and SPC (Phospholipon®) were accurately weighed in 1:1, 1:2 and 1:3 molar ratios (Formulation F1, F2 and F3 respectively) and placed in a 100 ml round bottom flask (RBF) containing 20 ml of dichloromethane. RBF was then attached to a rotary evaporator (SuperfitRotavap – PBU-6D, Superfit continental Pvt Ltd. Mumbai, India) and refluxed at 50 rpm and 60 °C temperature. The resulting solution was concentrated to 2-3 ml to obtain a thin lipid film. Later, a sufficient amount of n-hexane was added to form the amorphous product and placed in a lyophilizer (EBT- 10N, Esquire Biotech, Chennai, India). Further, the phytosomal nanocarriers of rutin obtained were stored in desiccators over fused calcium chloride at room temperature until further use.

Evaluation of Rutin Phytosomal Nanocarriers

Compatibility by Fourier Transform Infrared Spectroscopy (FTIR)

IR spectra matching approach was used to detect possible chemical interactions between the drug and polymer. Pure rutin, SPC, physical mixture of rutin and SPC (1:2) and formulation (F2) were scanned from 4000 to 400 cm^{-1} in FTIR spectrometer (Alpha Bruker, Germany) [14].

Average Particle Size and Size Distribution

Average particle size and particle size distribution as the polydispersity index (PDI) of the rutin phytosomal nanocarriers were measured by dynamic light scattering (DLS) method using Malvern zeta sizer (Malvern Instruments, Malvern, UK). The samples were diluted with distilled water (1:10) before the measurement [15].

Zeta Potential

Measurement of the zeta potential of rutin phytosomal nanocarriers was done by electrophoretic light scattering (ELS) technique using Malvern zeta sizer (Malvern Instruments, Malvern, UK). The samples were diluted with distilled water (1:10) before measurement [15].

Drug Content

Accurately weighed 2.5 mg of phytosomal nanocarriers were dispersed in 5 ml of chloroform. The free, non-complexed rutin will be insoluble in chloroform and precipitates out. By using a filter paper, it was filtered, dried and solubilised in methanol. After appropriate dilutions, the samples were analysed by UV spectrophotometer (V-630, JASCO, Japan) at λ_{max} 257 nm. Corresponding drug concentration in the sample was calculated from the standard calibration curve [16].

Surface Morphology

Scanning electron microscopy (SEM) has been used to determine the surface morphology of the formulated phytosomal rutin nanocarriers. The samples for 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 using an analytical scanning electron microscope (JEOL-JSM 6380LA, Tokyo, Japan) [17].

Solubility

It is determined by dissolving the excess amount of rutin phytosomal nanocarriers in 5 ml of distilled water and phosphate buffer pH 7.4 into sealed glass containers at room temperature. The solution was then agitated for 24 h and further centrifuged (R-8C, Remi Elektrotechnik Ltd. Vasai, India) for 30 min at 5,000 rpm. The supernatant was filtered through a 0.2 µm membrane filter. Further 1 ml filtrate was made up to 10 ml with respective solvents in a 10 ml volumetric flask. After appropriate dilutions, the samples were analysed in a UV spectrophotometer (V-630, JASCO, Japan) at 257 nm [18].

***In-Vitro* Drug Release**

Dialysis method was used to determine the *in vitro* drug release from the rutin phytosomal nanocarriers. After proper pre-treatment (wash), one end of the dialysis sack was tied, and 100 mg of pure rutin and in one more sac rutin phytosomal nanocarriers (~100 mg of rutin) 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 (up to 2 h) and then pH 7.4. The contents of the beakers were stirred at 50 rpm using a magnetic stirrer at 37±1 °C. The samples were withdrawn (5ml) from the dissolution medium at various time intervals, and the apparatus was immediately replenished with the same quantity of fresh buffer medium in order to maintain the sink conditions. The samples were filtered and diluted accordingly and analysed using UV spectrophotometer (V-630, JASCO, Japan) at 257 nm [19].

***In Vitro* Drug Release Kinetics**

The data obtained from the *in vitro* drug release study of the selected formulation was fitted to kinetic release models (zero order, first order, Higuchi and Korsmeyer-Peppas model) to understand the mechanism of drug release from rutin pytosomal nanocarriers [19].

RESULTS AND DISCUSSION

Formulation of Rutin Phytosomal Nanocarriers

Maiti *et al.,* reported the solvent evaporation method for the formulation of phytosomal nanocarriers [13]. Therefore, the same method was considered for the preparation of rutin phytosomal nanocarriers using dichloromethane as solvent. However, out of three formulations formulated (F1, F2 and F3), the F2 formulation containing 1:2 rutin: SPC ratio was observed as clear rutin: SPC solution compared to other two formulations which may facilitate the formation of complex between rutin and SPC. However, all three formulations were prepared and evaluated further.

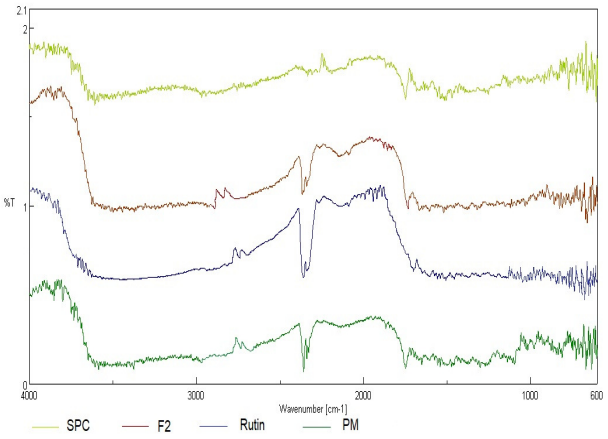

Figure 2: FTIR Spectrum of Rutin, SPC, Physical Mixture of Rutin and SPC (1:2) and Formulation (F2)

Evaluation of Rutin Phytosomal Nanocarriers Compatibility by Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of pure drug Rutin, SPC, physical mixture of rutin and SPC (1:2) and F2 formulation were compared (Fig. 2). The changes were observed between the physical mixture and formulation in the wave number ranges from 1231 cm^{-1} to 942 cm^{-1} corresponding to the region of the SPC phosphate group. Broadening of the phenolic (–OH) band of rutin at 3637 cm^{-1} was also observed, which is the sign of H-bonding [20].The spectra of formulation showed an additive effect of rutin and SPC reflecting the complex formation.

Average Particle Size and Size Distribution

The results obtained by the particle size and PDI analysis indicated that the size of the rutin phytosomal nanocarriers of F2 formulation was lesser compared to F1 and F3 formulations (Table 1). F1 formulation exhibited the average particle size and PDI of ~455.6 nm and ~0.471 respectively. F2 formulation exhibited ~301.4 nm particle size and ~0.520 PDI. The decrease in the particle size may be due to the complete physical complexation between rutin and SPC molecule where as in F1 formulation the higher particle size indicates the presence of free non complexed rutin. Further F3 formulation exhibited particle size ~342.5 nm, and PDI ~0.495, this increased particle size than the F2 formulation may be due to aggregation of extra, non-complexed SPC.

Zeta Potential

It was expected that after dispersing the rutin phytosomal nanocarriers in water, it would possess negative surface charge due to the presence of phosphate group of SPC [21]. Zeta potential of the rutin phytosomal nanocarrier formulations F1, F2 and F3 was found to be ~-36.2, ~-33.7 and ~-15.4 respectively (Table 1). F2 formulation had adequate zeta potential due to the complete complexation between rutin and SPC molecules.

Drug Content

Drug content value obtained for the rutin phytosomal nanocarrier formulations F1, F2 and F3 are shown in Table 1. F2 formulation exhibited the highest drug content value of ~57.48 % indicating the maximum complexation between the rutin and SPC molecules.

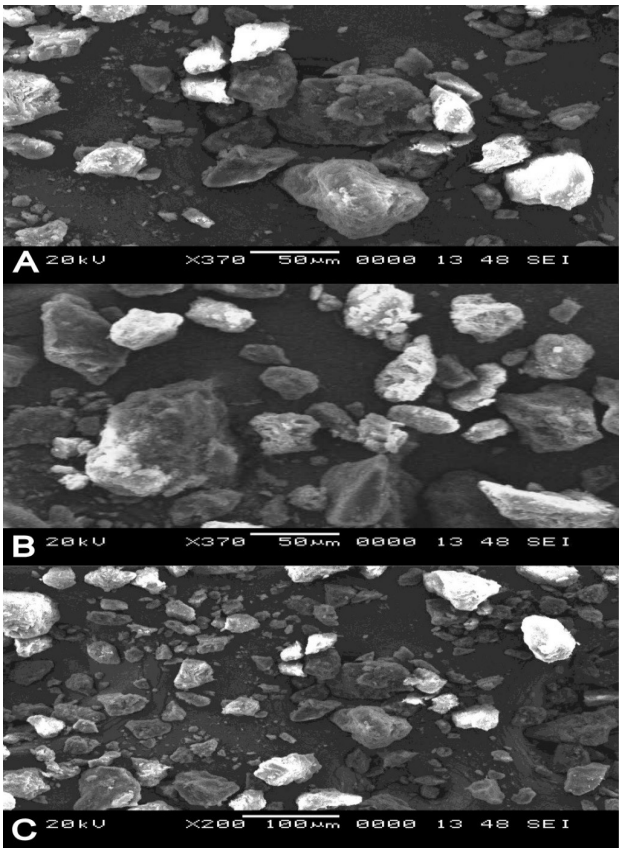

Figure 3: SEM Photographs of Rutin Phytosomal Nanocarriers F1 (A), F2 (B) and F3 (C)

Surface Morphology

SEM photographs of rutin phytosomal formulations F1, F2 and F3 are given in Fig. 3. The formulations possessed fluffy, porous and rough surface unlike the crystalline nature of the pure rutin [22]. This could be one of the reasons for increasing the solubility, dissolution of the rutin phytosomal nano complex than the pure rutin.

Solubility

Rutin is a hydrophobic drug and has poor aqueous solubility [23]. The poor absorption and bioavailability problems of the rutin are mainly due to this reason. In the solubility study we conducted, we observed the increased solubility of the rutin phytosomal nanocarriers in comparison with pure rutin in water and phosphate buffer pH 7.4. Further, the F2 formulation showed the highest solubility ~42.71 µg/ml in water and ~73.99 µg/ml in phosphate buffer pH 7.4. The increased solubility of the rutin phytosomal nanocarriers compared to pure rutin may be due to its amorphous, porous nature which is evidenced by our SEM analysis.

Table 1: Composition and characteristics of rutin phytosomal nanocarriers (F1, F2 and F3)

Formulation	Rutin: SPC ratio	Particle size (nm)	Poly dispersity index (PDI)	Zeta potential (mV)	Drug content (% w/w)
F1	1:1	455.6±2.41	0.471±0.04	-36.2±1.10	49.33±2.36
F2	1:2	301.4±1.85	0.520±0.02	-33.7±0.55	57.48±1.57
F3	1:3	342.5±2.12	0.495±0.05	-15.4±1.22	42.66±1.71

Values are mean ± SEM (n=3)

Table 2: Solubility of pure rutin and rutin phytosomal nanocarriers (F1, F2 and F3) in water and phosphate buffer pH 7.4

Medium	Solubility (µg/ml)			
	Pure rutin	F1	F2	F3
Water	1.59±0.11	38.05±2.14	42.71±1.16	32.15±1.89
Phosphate buffer pH7.4	12.41±0.56	50.72±1.77	73.99±2.39	61.29±2.59

Values are mean ± SEM (n=3)

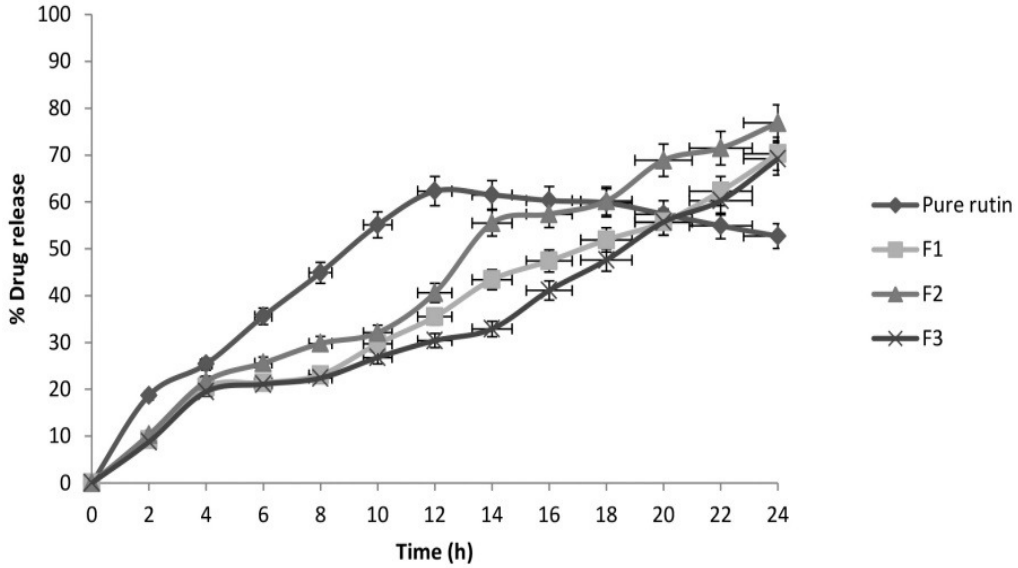

Figure 4: *In Vitro* Drug Release Profile of Pure Rutin and Rutin Phytosomal Nanocarriers (F1, F2 and F3) In Acidic Buffer pH 1.2 (up to 2 h) and Phosphate Buffer pH 7.4. Values are Mean ± SEM (n=3).

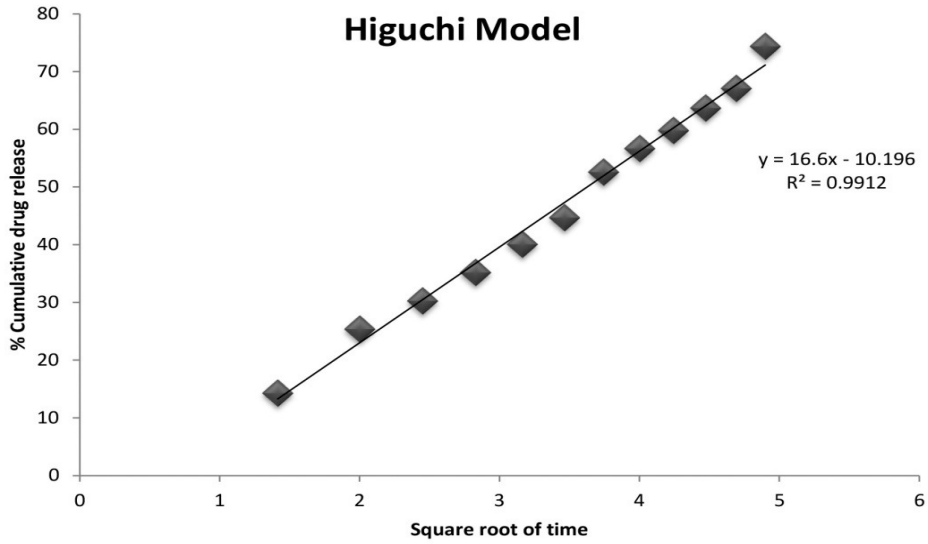

Figure 5: Higuchi's Plot for Rutin Phytosomal Nanocarriers (F2)

In Vitro Drug Release

The *in vitro* drug release study was carried out to determine the amount of drug released from the rutin phytosomal nanocarriers in various time intervals. The *in vitro* drug release profile obtained for the pure rutin, rutin phytosomal nanocarriers (F1, F2 and F3) is shown in Fig. 4. The pure rutin showed 18.7 % drug release at the end of 2 h in acidic pH 1.2 and 52.7 % drug release at the end of 24 h in phosphate buffer pH 7.4. The F2 formulation showed highest 76.9 % drug release compared to F1 and F3. These results indicated the sustained release of the drug from the formulation compared to pure drug. Here the improved drug delivery may be due to the fluffy, porous and rough surface of the formulations supported by SEM analysis, compared to crystalline nature of the pure rutin which may be facilitated the dissolution of rutin phytosomal nanocarriers.

In Vitro Drug Release Kinetics

The drug release kinetics from the rutin phytosomal nanocarriers (F2) was evaluated considering four drug release kinetic models including zero order, first order, Higuchi model and Korsmeyer-Peppas model. The correlation coefficient (r) value was used as an indicator for the selection of the best fitting model. The Higuchi's plot of the rutin phytosomal nanocarriers (F2) was found to be linear with the R^2 value 0.9912 (Fig. 5) and confirmed diffusion controlled drug release.

CONCLUSION

To achieve better drug delivery, the phytosomalnano carriers of rutin was formulated via solvent evaporation method using polymer SPC. After complexing the rutin with the SPC, the physicochemical properties of pure rutin changed significantly such as the molecular crystallinity of the pure rutin is changed to amorphous in the formulation which improved the drug solubility and dissolution which may facilitate the intestinal drug absorption and bioavailability. From the reproducible results of the executed experiments, it can be concluded that rutin phytosomal nano carriers can be considered as a promising drug delivery system to improve the systemic availability of the drug. However, further investigation in the light of *in vivo* bioavailability will further justify the better drug delivery potential of rutin phytosomal nanocarriers.

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DISCLOSURE

The authors report no conflicts of interest in this work.

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