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ORIGINAL RESEARCH ARTICLE

Development of biocompatible nanoparticles for sustained topical delivery of Rutin

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ABSTRACT

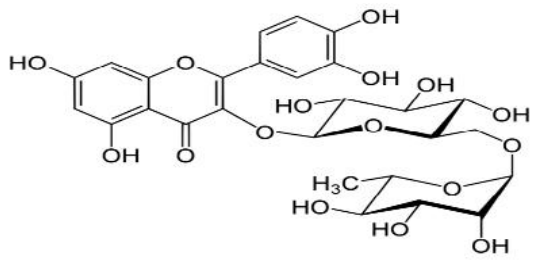
The purpose of this study was to develop sustained release nanoparticles of Rutin delivered by topical route. Rutin-loaded nanoparticles were prepared by nanoprecipitation technique using ethylcellulose as polymer. Nanoparticles made up of non-biodegradable polymer such as ethylcellulose may act as a reservoir on any of the appendages of the skin to release the drug in a sustained manner.. Nanoparticles were characterized by particle size, morphology, yield, encapsulation efficiency and *in-vitro* release. It was observed that the weight ratio of RU: EC: T80 at different ratios carried varied particle sizes along with yield and encapsulation efficiency. These nanoparticles can be used as the convenient model system for increasing the retention of the Rutin in the skin.

Keywords: Nanoparticles, Rutin, Eudragit, Ethylcellulose, Ex-vivo study

1. INTRODUCTION

Rutin is a flavonoid present in the plant kingdom as a secondary metabolite. Rutin is obtained from commonly consumed fruits and vegetables like apple, onion, tea, berries and brassica.It 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-{[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-({[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}methyl)oxan-2-yl]oxy}-4H-chromen-4-on It shows numerous effects on the body. Rutin shows anti-proliferative effects and aids to the effectiveness of chemotherapeutic agents and is effective against ultra-violet radiation induced damage Various mechanisms for cancer-preventive effects of Rutin are their anti-oxidative activity, the inhibition of enzymes that activate carcinogens, the modification of signal transduction pathways, and interactions with receptors and other proteins. Rutin shows its action on p53 and p21 gene; tyrosine kinase and estrogen receptor are other sites of action.

Structure of Rutin



Rutin is a natural plant flavonoid with reported anticancer activity but it has not been explored clinically because of low absorption when given orally. To reach the therapeutic level 10micro M, 1500mg of daily dose is required which is practically not beneficial.The poor solubility and low stability of Rutin in aqueous alkaline medium also restricts its application in oral use. Our aim is to deliver Rutin topically as nanoparticles to overcome all these drawbacks. Though yet to be explored, topical chemotherapy is a well-known agenda in this era. Transdermal system is desired to maintain a constant and prolonged drug level with reduced frequency of dosing Nanoparticle can be preferably used as a carrier for controlled release of drug Nanoparticles are selected because of their increased surface area, they have greater efficiency in enhancing the permeation of drugs into skin than many other vehicles. The nanosized particles tend to reside in the outer layers of the stratum corneum and epidermis, with negligible penetration into the dermis

2. MATERIALS AND METHODS

MATERIALS

Rutin was purchased from sigma laboratories. Ethylcellulose and potassium bromide were obtained from Hi-media laboratories Pvt. Ltd. Tween-80, IPA and octanol were obtained from Loba Chemie. Pvt. Ltd. All the other chemicals and reagents used were of analytical grade

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INSTRUMENTATION

Absorbance was recorded using UV- Visible spectrophotometer (Shimadzu, Pharmaspec-1700) and interaction study was done using FTIR (Shimadzu 8400S FTIR spectrometer). The TEM analysis was performed from sophisticated analytical instrumentation facility, New Delhi. The particle size and zeta potential was measured using Zetasizer (Malvern DTS, UK) from CIF, BITS, Ranchi.

METHODS

Preformulation study: (Gibson M)

Physiochemical properties of drug affect the skin permeability of drug. (Harada *et al*., 2011) To analyse the properties of drug (Rutin) was evaluated for its appearance, melting point, solubility studies and partition coefficient. Rutin is a yellow colored, odorless and crystalline compound. Its melting point was found to be 183.5º C, which confirms its stability at room temperature under working conditions. The Rutin was found to be soluble in most of the organic solvents and was insoluble in distilled water showing its hydrophobic nature. The partition coefficient of Rutin was found 2.78, which confirm the lipophilicity of the drug, and hence the nanoparticulate delivery of the drug offers a good alternative for the water insoluble drugs.

Selection of polymer:

The polymer was selected on the basis of the solubility studies. Ethylcellulose can be a good choice for the preparation of nanoparticles. Drug excepient compatibility can alter the physicochemical properties and bioavailability of the drugs. This incompatibility there by affects its safety and/or efficacy. In the present study, the drug excepient compatibility of the Rutin with ethylcellulose was determined by and UV spectrophotometric methods. The drug and polymer mixture of Rutin with polymer was prepared at 1:2 ratio. The drug and polymer was individually weighed in a 10 mL glass vial and mixed for 2 min. Each vial was sealed Teflon-lined screw cap and stored at 50 °C for 2 weeks. These samples were periodically examined for any change of unusual color change.

Characterization by UV spectrophotometer: The samples after 2 weeks were withdrawn from storage and analyzed by UV- Visible spectrophotometer. The drug content was determined at initial and stored samples in triplicate. An accurately weighed amount of the drug-polymer mixture was taken and suitably dissolved under sonication in iso-propyl alcohol and filtered through 0.45 µ (Millipore) filters. The sample was analyzed after making appropriate dilutions using UV- Visible spectrophotometer at 381 nm against blank.

Preparation of nanoparticles systems:

Drug-loaded Ethylcellulose nanoparticles were prepared by desolvation method. The method involves the formation of a conventional oil in water emulsion between a partially water miscible solvent containing the polymer and the drug, and an aqueous phase containing stabilizer. Among the two variance, solvent evaporation method and emulsification diffusion method the former was selected. The organic phase consists of drug and polymer in isopropyl alcohol. The concentration of the drug was kept constant and the polymer concentration was varied. The aqueous phase consist of distil water containing tween-80 as stabilizer. The organic phase was inserted into the aqueous phase dropwise by using syringe. Both the phases were desolvated by stirring at 3000 rpm at room temperature using mechanical stirrer. The resultant solution was then sonicated for 5 mins for size reduction. The resultant turbid solution was again stirred vigorously at 800 rpm for 4 h to evaporate the organic solvent using magnetic stirrer. Following above procedure, varying the drug: polymer ratio and surfactant concentration EC nanoparticles were prepared and coded as NP1, NP2, NP3 (a-f).

Optimization of Process Variable:

Surfactant ratio had a significant effect on the percent production yield.Therefore keeping the drug: polymer ratio and other parameters constant, the effect of concentration of stabilizer had been studied.

Photomicroscopy:

For the purpose of checking the stability of the nanoparticles solution digital labomed camera (40 X zoom) was used. The nanoparticle suspension was placed over the glass slide and cover slip was fixed over it and then pictures were taken.

TEM Study:

The formulations NP1, NP2 and NP3, which were found to be stable from the labomed study were futher subjected to TEM study for the morphology study and aggregation status. Formulations were visualized by transmission electron microscopy (TEM) (Morgagni 268-D) at an accelerating voltage of 100 kV. A drop of the sample was placed on a carbon-coated copper grid to form a thin film and negatively stained by adding a drop of 1% w/v phosphotungstic acid. The grid was allowed to air dry and the samples were viewed and photo As the concentration of surfactant was finalized, keeping that constant 6 formulations

were prepared by varying drug:polymer ratio which were subjected to further characterization.

Characterization of optimized nanoparticles

The characterization of nanoparticles was performed by the determination of encapsulation efficiency and the *in vitro* release study. Further particle size analysis, zeta potential determination and morphology study was performed.

Drug Entrapment efficiency and loading capacity:

For determination of drug entrapment, the suspension was centrifuged at 15,000 rpm for 30 minutes in a micro ultra-centrifuge. The supernatant was analyzed spectrophotometrically for Rutin content at 259 nm. From the concentration of the drug in the supernatant, amount of drug adsorbed on the surface of particles was determined. The amount of drug in supernatant (w) was then subtracted from the total amount of drug added (W). Effectively, this subtraction (W-w) gave the amount of drug entrapped in the nanoparticle. The percent entrapment was calculated from the formula:

$$\frac{W-w \times 100}{W}$$

The percent loading capacity was calculated from the formula:

$$\frac{W-w \times 100}{W + P}$$

Where; W = the weight of drug added to the system
W = the weight of drug in the supernatant
P = the weight of polymer added to the system

***In vitro* release study:**

In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane was prepared by treating the cellophane membrane.

Treatment of cellophane membrane:

Cellophane membrane, it was boiled for 1 hr in 1 hot bottle on water bath, for the removal of glycerol. The process was repeated by transferring the membrane in fresh distil water for the complete removal of glycerol. Then the membrane was kept in ethanol for 24 hrs followed by washing with distil water. For the removal of polysulphide from the membrane, it was treated with 0.3 % w/v solution of sodium sulphide for 1 min followed by washing with distill water. The membrane was finally acidified using 0.2% v/v H_2SO_4 and then was washed with distil water. The prepared membrane was stored in the saline phosphate buffer (Ph 4.5).

In-vitro release studies:

Phosphate buffer pH 5.6 containing 0.5% w/v of polysorbate 80 was used as release media. Nanoparticle dispersion (2 ml) was placed in the donor compartment prepared by closing the 1 side of a both open sided test-tube with the membrane and the receptor compartment was filled with 0.5% polysorbate 80 in phosphate buffer, pH 5.6 (40 ml). During the experiments, the solution in receptor side was maintained at 37°C ± 0.5°C and stirred at 800 rpm with Teflon-coated magnetic stirring bars. At fixed time intervals, 2 ml of the sample was withdrawn from receiver compartment and analyzed by UV spectroscopy.

Kinetic analysis of drug release data:

Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of Rutin release from EC and ES nanoparticles. The kinetic models used were zero order equation, first order equation, Higuchi release and Korsemeyer-Peppas .

Various kinetic models:

Zero order (Equation 1) as cumulative amount of drug released vs time, first order (Equation 2) as log cumulative percentage of drug remaining vs time, and Higuchi's model (Equation 3) as cumulative percentage of drug released vs square root of time.

$$C = K_0 t \text{ ------ (1)}$$

Where; K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours. A graph of concentration vs time would yield a straight line with a slope equal to K_0 and intercept the origin of the axes. (Hadjiioannou *et al*., 1993)

$$Log\ C = Log\ C_0 - k\ t / 2.303 \text{ -------- (2)}$$

Where; C_0 is the initial concentration of drug, k is the first order constant, and t is the time. (Bourne, 2002))

$$Q = K\ t^{1/2} \text{ -------- (3)}$$

Where; K is the constant reflecting the design variables of the system and t is the time in hours. Hence, drug release rate is proportional to the reciprocal of the square root of time. (Higuchi, 1963)

Mechanism of Drug Release:

To evaluate the mechanism of drug release from QN, data for the drug release were plotted in Korsmeyer *et al*'s equation (Equation 4) as log cumulative percentage of drug released vs log time, and the exponent n was calculated through the slope of the straight line.

$$M_t / M_\infty = K\ t^n \text{ -------- (4)}$$

Where; Mt/M_∞ is the fractional solute release, t is the release time, K is a kinetic constant

characteristic of the drug/polymer system, and *n* is an exponent that characterizes the mechanism of release of tracers. (Korsmeyer RW et al, 1983) if the exponent $n = 0.5$, then the drug release mechanism is Fickian diffusion, and if $0.5 < n < 1$, then it is non-Fickian or anomalous diffusion. An exponent value of 1 is indicative of Case-II Transport or typical zero-order release. (Siepmann *et al*., 2001)

Optimization of formulation:

Optimization of formulation was done by response surface method using stat-ease 7.1.6 software. (Li et al., 2011; Gamal *et al*., 2011) Optimum formula was developed which designates the level of independent variable that results in maximum percent drug entrapment efficiency and percent drug loading capacity with best release kinetics.

***Ex-vivo* skin penetration study**:

A system employing modified Franz diffusion cells with a diffusional area of 2.50 cm^2 was used for penetration studies. The excised goatskin was set in place with the stratum corneum facing the donor compartment and the dermis facing the receptor compartment.

***Preparation of goatskin*:**

Fresh abdominal skin of goat was used for the experiment. Skin hairs were shaved and the skin was stored in normal saline solution. The adhering fats were removed by rubbing with a cotton slab.

***Drug release study*:**

Two ml of nanoparticle dispersion of Rutin were applied to the skin surface in the donor compartment and the receptor compartment of the cell was filled with 40 ml of phosphate buffer (pH 7.4) with 0.5 % polysorbate 80. During the experiments, the solution in receptor side was maintained at 37 ± 0.5°C and stirred at 800 rpm with Teflon-coated magnetic stirring bars. After application of the test formulation on the donor side, 2 ml aliquots were collected from the receptor side at designated time intervals (1, 2, 4, 8, 10, 12 and 24 h). Thereafter, an equivalent volume of receptor fluid was supplied to the receiver compartment immediately after each sample collection. The samples were analyzed using UV-VIS spectrophotometer at 259 nm and cumulative amount diffused Q (mg/cm2) at each sampling time points was calculated. At the end of 24 h, the skin was cut, into and small pieces and extracted, with isopropyl alcohol and analyzed spectrophotometrically at 259 nm.

Surface/ shape morphology of nanoparticles:

The morphology and size of the nanoparticles were analyzed using transmission electron microscope. Samples were prepared by placing a drop of nanoparticle formulation on to a copper grid and air dried, followed by negative staining with a drop of aqueous solution of sodium phosphotungstate for contrast enhancement. Finally, the air-dried sample was examined under the transmission electron microscope.

Particle size and Zeta potential determination

The particle size and zeta potential of nanoparticles were determined by Dynamic light scattering (DLS) method using a Malvern Zetasizer 4700 (Malvern Ltd., Malvern, UK) with a 25 mW He–Ne laser and the Automeasure (version 3.2) software.

3. RESULTS AND DISCUSSION

The purpose of this study was to develop Rutin - loaded nanoparticles by a nanoprecipitation technique. The overall objectives were reduction of dose, sustained release, convenient route and avoidance of side effect to other organs. The Rutin, yellow coloured odourless and crystalline compound was obtained from the sigma laboratories. The abosorbance maxima were found to be 259 nm by the U-V Spectroscopy. The other most important study performed was the drug-interaction study of the drug and polymers. The presence of the peaks of the pure drug belonging to different functional group of the drug in the drug polymer mixtures (Rutin and ethylcellulose) According to the literature survey the tween-80 had been selected as the stabilizer for the preparation of the ethylcellulose nanoparticles. The formation of nanoparticles using ethylcellulose and tween-80 had been confirmed by the formation of dummy particles. Keeping the drug: polymer ratio and other parameters constant, the effect of concentration of stabilizer had been studied, shown in (**Table 1**)

Table 1Optimization of percentage of tween-80

Formulation code	Drug : polymer (D:P) ratio	Percent of tween-80 used	Initial Status of Nanoparticles	Status of nanoparticles after 1 week
NP1	1:1	1%	Prepared	Stable
NP2	1:1	1.5%	Prepared	Stable
NP3*	1:1	2%	Prepared	Stable

** Optimized preparation*

Using the results obtained from photomicroscope and the TEM studies as shown in (**Fig 2**) NP3 was selected for further studies. The prior optimization of the surfactant concentration was for the sake of

time and materials. For the characterization of nanoparticles sample NP3a-NP3f, encapsulation efficiency, *in vitro* release study, ex-vivo permeation study, size analysis, zeta potential determination and morphology study was performed. Results of the following parameters are shown in (**Table 2**)

Table 2: characterization of ethylcellulose nanoparticles

Formulation code	Drug: polymer ratio	Amount of drug (mg)	Amount of polymer (mg)	Percent v/v of stabilizer used	Encapsulation efficiency (Response 1)	Loading capacity (Response2)	*In-vitro* release after 24 hrs (Response 3)
NP3a	1:1	25	25	2	61.95±0.31	44.15±0.41	60
NP3b	1:2	25	50	2	62.28±0.18	21.75±0.13	36.8
NP3c	1:4	25	100	2	62.70±0.15	15.61±0.08	31.6
NP3d	1:6	25	150	2	62.65±0.10	10.56±0.03	26.4
NP3e	1:8	25	200	2	62.77±0.05	6.19±0.01	20.3
NP3f	1:10	25	250	2	63.83±0.1	5.19±0.02	14.2

The statistical analysis of the experimental data by the one-way ANOVA was performed and the differences were considered as statistically significant at p<0.001.

Release profile of the Rutin was fit into various kinetic models to find out the mechanism of drug release. Among this highest correlation coefficient was shown Higuchi plot as shown in (**Table 3**)

Table 3: *In-vitro* Release profile of Rutin

Formulation code	Higuchi model r	Zero-order R	First-order r	Krosemeyer-peppas	
				k	n
NP3a	0.9100	0.9103	0.9617	0.9858	0.471
NP3b	0.9629	0.8735	0.8823	0.9611	0.4543
NP3c	0.982	0.9751	0.9516	0.9376	0.4388
NP3d	0.9722	0.9611	0.9623	0.9498	0.2480
NP3e	0.9834	0.9833	0.9378	0.9719	0.4256
NP3f	0.9219	0.9815	0.9856	0.9324	0.4123

The data obtained was also fit in to the Krosemeyer–Peppas in order to find out the 'n' value, to describe the drug release mechanism. The 'n' value ranged between 0.248 and 0.471 and was found to be less than 0.5 indicating the mechanism of drug release is diffusion controlled (Fickian diffusion) and Korsmeyer–Peppas model showed high correlation between each other Optimization of formulation was done by response surface method variables % drug entrapment efficiency and% drug loading capacity with best release kinetics. The data generated from the software are shown in (**Table 4**).

Table 4 :tables generated from software: a.Design summary: ECN; b. Solutions for 6 combinations of categoric factor levels: ECN

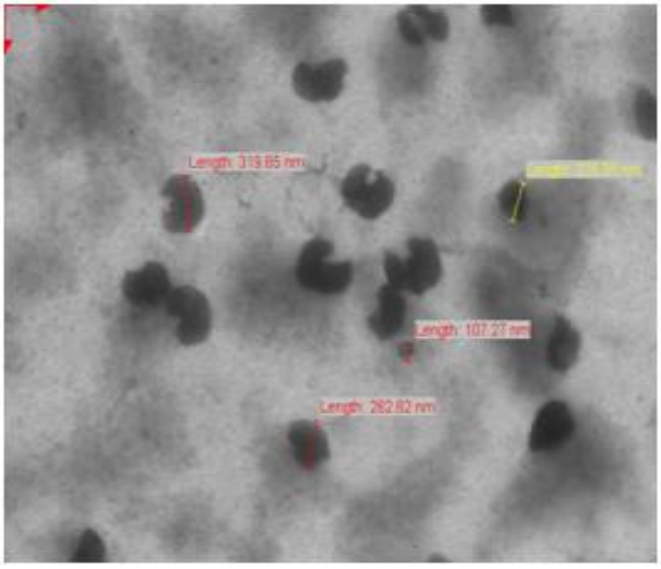
Design Summary:

Study Type: Factorial
Initial Design: Full Factorial
Design Model: Main effects
Runs: 6

Response	Name	Units	Minimum	Maximum	Mean	Std.dev	Ratio
Y1	% EE	%	60.91	64.97	62.09	0.67	1.03
Y2	% LC	%	51.23	54.78	53.05	11.25	6.67
Y3	% DR	%	12.6	50	30.91	12.75	3.96

The % EE was found to range from 60.91 to 64.97 and % LC was found to range from 51.23 to 57.78. The best formulations from among the different ECN were found to be NP3b with the goal of achievement of maximum magnitude of responses selected for the optimization The further studies of these formulations revealed that the nanoparticle suspension was stable and suitable for topical delivery. The *ex-vivo* study using goatskin showed the sustained release of the drug from the formulation. From the percent cumulative drug permeated versus time plot, the slope values were determined as the skin permeation rate. The cumulative amount of drug permeated at the end of 24 h was found to be 76.24 μg/cm^2 with skin permeation rate constants of 0.415 percent/cm^2/h. The release of drug from these formulations followed a Fickian pattern with n value 0.418-0.423. A greater amount of drug was present in the skin (26.46%) subjected to the extraction in comparison to the drug released in the receptor compartment 18.5% The slower release of drug from nanoparticle dispersions maintained the drug concentration for longer period of time in the skin. The results of drug permeation through the goatskin confirmed that the Rutin could possibly permeate through the human skin maintaining the sustained release. Further the **zeta** potential and particle size of the selected formulation were found to be –17.54 and 51.34 respectively, which confirms the stable nature and appropriate particle size of the preparation. TEM image of the optimized formulation is shown in (**Fig 2**)

Fig 2: TEM of NP3b



4. CONCLUSION

The topical use of Rutin had been suggested in many papers.This study confirms that the nanoparticles prepared from solvent evaporation technique are suitable for loading Rutin. This formulation approach can be used to improve the therapeutic efficacy of poorly soluble drugs. The change in nanoparticle size and release performance was affected by changes in polymer concentration. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin, and, because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin. The sustained release of drug from the Rutin nanoparticle suggests that the frequency of administration, dose and adverse effects of this molecule could be reduced. There is large scope for improving the use of Rutin in cancer treatments through nanoparticle as a drug delivery system. The development of aerosol vaccine is undergoing which could provide a great potential.

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