

PREDNISOLONE ENCAPSULATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES FOR TARGET DRUG DELIVERY – DESIGN AND QUANTIFICATION

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ABSTRACT

Objective: The present study aimed to develop a novel type of superparamagnetic iron oxide nanoparticles (SPIONs) to deliver prednisolone at colon as a target site for the treatment of inflammatory bowel disease (IBD) such as ulcerative colitis and Crohn's disease which may further progress to cancer.

Methods: SPIONs were synthesized using a coprecipitation method. Further, it was encapsulated with prednisolone-polyethylene glycol by double emulsion method ($W_1/O/W_2$). The prepared formulations were characterized for its physicochemical characterization such as scanning electron microscopy, X-ray diffraction, particle size and zeta potential, encapsulation efficiency, and *in vitro* drug release.

Results: The results reveal that the physicochemical property of the formulations complies with the standard values and *in vitro* release of prednisolone in the first 18 h, attains 57 and 58% and it reaches 71 and 75% at 24 h, and this is statistically significant ($p < 0.0177$). This release result implies that the drug release from the formulations is controllable and sustains manner.

Conclusion: Our findings could be a promising approach for the delivery of prednisolone with enhanced half-life for the treatment of IBD through colon targeting.

Keywords: Superparamagnetic iron oxide nanoparticles, Prednisolone, Colon target drug delivery system, Polyethylene glycol, Coprecipitation method, Double emulsion method.

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INTRODUCTION

Targeted or site-specific drug delivery system is a kind of smart drug delivery system that delivers the medication to the target site, thus enhances the concentration of drug in particular target site when compared to non-target site. This improves the efficacy of the drug and reduces the side effects [1]. The colon is a site where both local and systemic delivery of drugs can take place. The colon drug delivery system permits the drug to absorb in particular site and overcome the drug release in the stomach and small intestine [2]. Targeting to the colon is advisable for local treatment of inflammatory bowel disease such as ulcerative colitis (UC) and Crohn's disease which may further progress to cancer, amebiasis, and systemic delivery of protein and peptide drugs [3]. To achieve the outcome of better therapeutic effect of drugs, it is essential that the designed drug delivery system especially targets the drugs into the colon.

Over the past few decades, nanotechnology is used for numerous biomedical applications where they facilitate laboratory diagnostics and therapeutics [4]. Among these, biocompatible superparamagnetic iron oxide nanoparticles (SPIONs) or magnetic nanoparticles with proper surface architecture and conjugated targeting ligands/proteins have created their attention in numerous drug delivery applications due to its biocompatibility and therapeutic efficacy [4,5].

Prednisolone is a synthetic corticosteroid with predominant glucocorticoid activity. Systemic glucocorticoids are potent immunosuppressants, potentially facilitating carcinogenesis [6]. It is the second-line drug in the therapy of UC. It can inhibit leukocyte infiltration at the site of inflammation, interfere with mediators of inflammatory response, and suppress humoral immune responses. The

combination may result in synergistic action for the treatment of the UC and may cause significant lowering of treatment failure rate along with slower development of resistance. The anti-inflammatory effect of prednisolone is primarily due to its potential to inhibit the production of prostaglandins and leukotrienes [7].

Oral route of administration is considered as the preferred route of administration of all kinds of therapeutic agents with higher patient compliance [8]. At present, oral therapy is considered as the preferred route of administration of prednisolone, possibly on account of a higher patient compliance. However, research suggests that this drug exhibits variation in bioavailability with change in dose and also possesses short biological half-life (~2–4 h) following oral administration. Hence, in our study, we selected prednisolone for colon target [7].

Numerous approaches have been attempted to enhance the therapeutic efficiency of prednisolone in treating inflammatory diseases such as the potential of liposomes in enhancing the therapeutic efficiency of prednisolone in animal models of rheumatic arthritis and long-circulating multifunctional, multimodal liposomes of prednisolone for the treatment of atherosclerotic plaque inflammation, which, in turn, enhanced the efficacy and reduce the dose. Alternatively, controlled, delayed, and extended-release dosage forms of prednisolone were also developed to enhance the therapeutic efficiency for the successful treatment of various inflammatory diseases [9]. Despite all these efforts, successful therapy of prednisolone in treating inflammatory diseases remains elusive. Thus, the present research work hypothesizes that the site-specific drug delivery to colon using SPIONs could be a promising approach for the delivery of prednisolone to enhance its therapeutic efficacy by local action.

MATERIALS AND METHODS

Materials

Prednisolone was gifted by Microlab, Bengaluru; ferrous sulfate, ferric chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, and ethanol were procured from HiMedia Laboratories Pvt. Ltd., Nasik. Ammonium hydroxide, sodium hydroxide, hydrochloric acid, polyethylene glycol (PEG), chloroform, glutaraldehyde, and distilled water were procured from Loba Chemie Pvt. Ltd., Mumbai. All the chemicals used were of analytical grade. All solutions were prepared using double distilled water.

Methods

Preparation of SPIONs

SPIONs were synthesized using a coprecipitation method. Briefly, 450 ml of deionized water was stirred mechanically for 15 min under a nitrogen gas at room temperature to remove O₂ from solution. Then, 0.19 mg FeCl₂ (4H₂O) and 0.486 mg FeCl₃ (6H₂O) were added to the vigorously stirred water and 250 mg of oleic acid was quickly added to the previous reaction mixture and the product container was placed in a water bath (75–80°C). After 15 min, 1.35 ml of NH₄OH was added during 1 min and argon gas flow was discontinued. After about 30 min, SPIONs were deposited. The product was washed 3 times with deionized water and the black precipitate was separated using a permanent magnet and lyophilized [10].

Encapsulation of prednisolone-PEG in SPIONs

Double emulsion method (W₁/O/W₂) was used for the preparation of SPION encapsulated with prednisolone using PEG. Briefly, 1 ml of SPION suspension in chloroform was mixed with 1 ml of the organic solution of the polymer (PEG) in dichloromethane. Then, 0.2 ml solution of prednisolone in deionized water was added to the organic phase, and the mixture was emulsified by probe sonication (3.5 L 100 Analytical Lab Services, Mumbai) for 1 min (0.6 Hz frequency, 90 amplitude) (W₁/O). The primary water-in-oil emulsion was added dropwise to 8 ml of ice-cold aqueous polyvinyl alcohol (PVA) solution (5%, w/v) and emulsified for 10 min using a probe sonicator (W₁/O/W₂). To evaporate the organic solvent, the resulted solution was diluted in 10 ml aqueous PVA solution (0.1%, w/v) under stirring at room temperature overnight. Then, the nanoparticles were collected by centrifugation at 14,000 rpm for 15 min and washed 3 times with deionized water. Finally, the products were freeze-dried and the dry samples were filled with N₂ gas and stored in a freezer for further use [10].

Physicochemical properties and release characteristics of SPIONs

Morphology by scanning electron microscopy (SEM)

The morphology of SPIONs was analyzed by SEM (JEOL MODEL JSM 6400). The SPIONs were mounted directly on the SEM stub, using double-sided, sticking tape and coated with platinum and scanned in a high vacuum chamber with a focused electron beam. Secondary electrons emitted from the samples were detected and the image formed [10].

Surface characteristics by zetasizer

The particle size and particle size distribution of SPIONs were measured with a Malvern instrument (Zetasizer 3000 HS, U.K). The particle size distribution is reported as polydispersity index. The samples were placed in the analyzer chamber and readings were performed at 25°C with a detected angle of 90°. The zeta potential of SPIONs was measured with a Malvern instrument (Zetasizer 3000 HS, U.K). The samples were diluted with pH 7.4 buffer and placed in electrophoretic cell and measured in the automatic mode [10].

X-ray diffraction (XRD)

To quantify the internal behavior and structure of the particles, XRD was applied [11]. XRD (fiber XRD) spectra were taken in SmartLab XRD model of Rigaku, and copper K α was used as the X-ray source and the power used was 1.2 kW [12].

Table 1: X-ray diffraction pattern peak values of prednisolone entrapped SPIONs

Sample name	Left angle	Right angle	Left int.	Right int.	Obs. max.	d (Obs. max.)	Max int.	Net height	FWHM	Chord mid.	I. breadth	Gravity C.	d (Gravity C.)	Raw area	Net area
SPIONs	28.600	29.240	72.2	72.9	28.922	3.08464	426	354	0.330	28.923	0.328	28.921	3.08472	162.6	116.1
	2-Theta	2-Theta	Cps	Cps	2-Theta	Angstrom	Cps	Cps	2-Theta	2-Theta	2-Theta	2-Theta	Angstrom	Cps*2-Theta	Cps*2-Theta

SPIONs: Superparamagnetic iron oxide nanoparticles

Table 2: Data of drug encapsulation efficiency

S. No.	Name of the formulation	% drug entrapment efficiency							
		6.8 phosphate buffer				7.4 phosphate buffer			
		Trial I	Trial II	Trial III	Mean±SD	Trial I	Trial II	Trial III	Mean±SD
1.	Prednisolone-polyethylene glycol superparamagnetic iron oxide nanoparticles	92	94	92	93±1.53	85	83	84	84±1.0

SD: Standard deviation

Table 3: Comparative prednisolone release in 6.8 and 7.4 phosphate buffer at 246 nm

S. No.	Time (h)	% drug release in phosphate buffer	
		6.8	7.4
1.	0	0	0
2.	0.5	10.73	7.723
3.	1	13.71	9.778
4.	2	20.96	14.326
5.	4	24.40	21.419
6.	6	27.28	24.267
7.	8	31.07	31.040
8.	12	37.76	32.586
9.	18	57.62	58.171
10.	24	75.03	71.193

Prednisolone encapsulation efficiency (EE) in SPIONs

The EE and loading capacity of SPIONs were determined by the separation of SPIONs from the supernatant liquid containing non-associated prednisolone obtained after cold centrifugation at 12,000 g for 30 min. The amount of free prednisolone in the supernatant liquid was measured by ultraviolet (UV)-visible spectrophotometer at 264 nm. The experiment was run in triplicate using 6.8 and 7.4 phosphate buffer and the mean values were recorded. The prednisolone EE of the SPIONs was calculated from the following equations [10].

$$\text{Encapsulation efficiency} = \frac{\text{Total amount of prednisolone} - \text{Free prednisolone}}{\text{Total amount of prednisolone}} \times 100$$

In vitro release characteristics

Dialysis bag method

The studies were performed on prednisolone and prednisolone-PEG – SPIONs and in 6.8 and 7.4 phosphate buffer. Sample equivalent to 100 mg of prednisolone was redispersed in 10 ml solution of 6.8 and 7.4 phosphate buffer and placed in a dialysis membrane bag with a molecular cutoff of (MWCO 12,000–15,000 Da, HiMedia, India) which acts as a donor compartment, tied and placed into 10 ml 6.8 and 7.4 phosphate buffer solution in a beaker which acts as a receptor compartment. The entire system was kept at 37°C±0.1°C with continuous magnetic stirring at a rotation speed of 50 rpm. At appropriate time intervals (0, 15, 30, 45, and 60 min; 2, 4, 6, 8, 12, and 24 h), 1 ml of the release medium was removed through 0.1 µm membrane filtered immediately and 1 ml fresh 6.8 and 7.4 phosphate buffer solution was added into the system. The amount of prednisolone in the release medium was determined by UV-visible spectrophotometer at 246 nm and the percentage release of prednisolone was recorded. The experiment was run in triplicate and the mean values were recorded as percentage release of prednisolone [13]. The results are expressed as a cumulative percentage of the released drug, which calculated using the equation.

$$\text{Cumulative drug release (\%)} = \frac{\text{Amount of drug released at time t (mg)}}{\text{Total amount of in nanoparticles (mg)}} \times 100$$

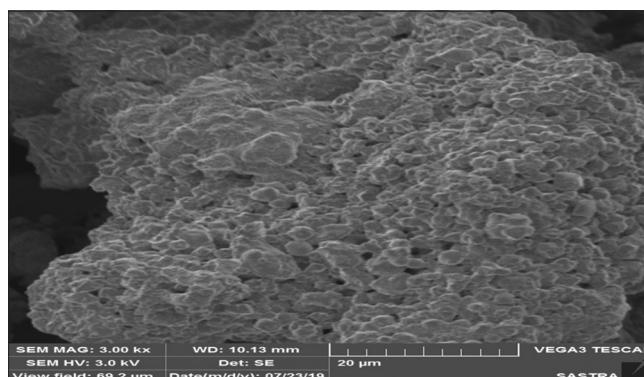


Fig. 1: Scanning electron microscopy image of superparamagnetic iron oxide nanoparticles

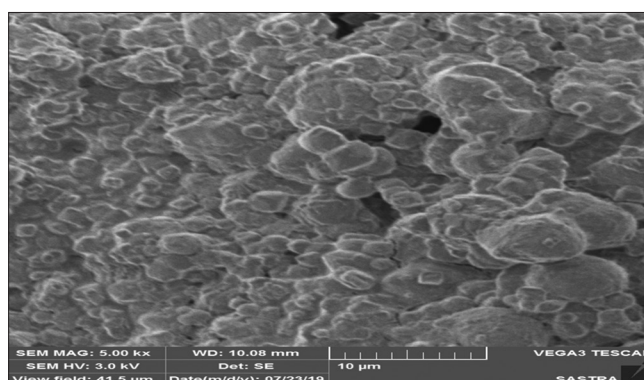


Fig. 2: Scanning electron microscopy image of prednisolone entrapped superparamagnetic iron oxide nanoparticles

Statistical analysis

The data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests with the help of GraphPad Instat software, version 3.01. All the data were presented as a mean value with its standard deviation (mean±standard deviation). p<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Recently, there is much more attention in the development and characterization of active pharmaceutical ingredients at nanotechnology level for numerous drug delivery applications. In our study, we prepared SPIONs by coprecipitation method and simultaneously encapsulated prednisolone-PEG by double emulsion method for target drug delivery system to colon.

Physicochemical properties of SPIONs

Morphology and size distribution

Morphology of prepared SPIONs and prednisolone entrapped SPIONs was characterized by SEM analysis and it is shown in Figs. 1 and 2. It illustrates that prepared SPIONs by coprecipitation method and double emulsion method are spherical in shape and uniformly distributed.

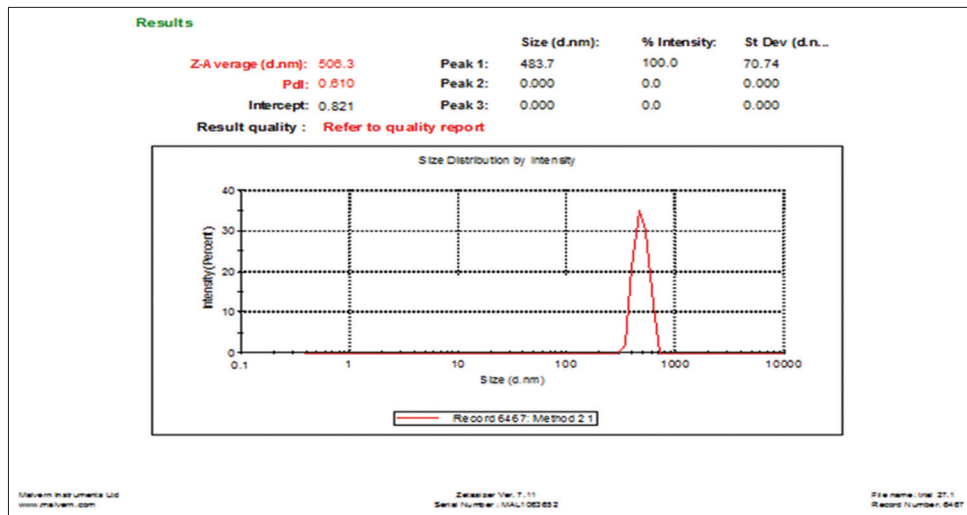


Fig. 3: Zeta size of prednisolone entrapped superparamagnetic iron oxide nanoparticles

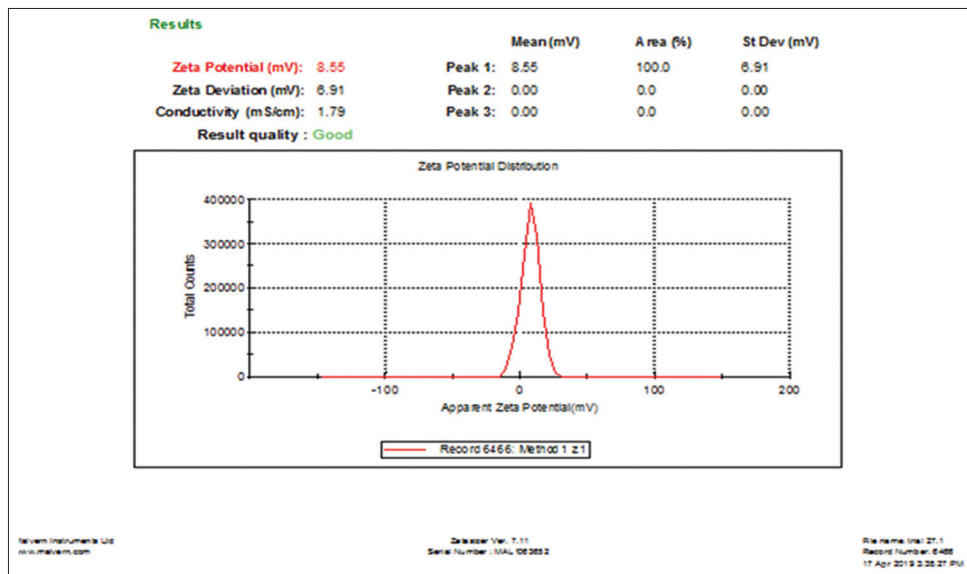


Fig. 4: Zeta Potential of prednisolone entrapped superparamagnetic iron oxide nanoparticles

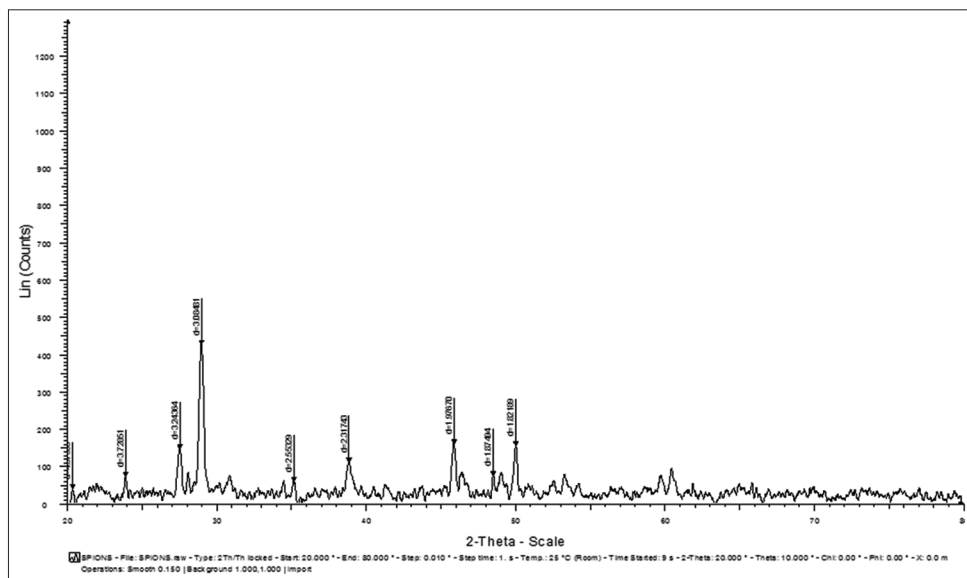


Fig. 5: X-ray diffraction pattern of prednisolone entrapped superparamagnetic iron oxide nanoparticles

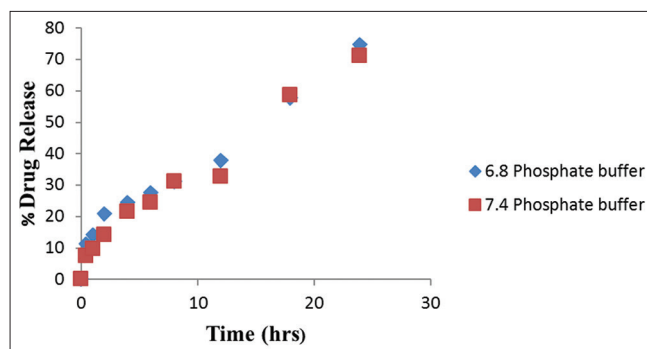


Fig. 6: Comparative prednisolone release in 6.8 and 7.4 phosphate buffer at 246 nm

Zeta size and zeta potential

In general, zeta potential analysis is a technique to determine the surface charge of nanoparticles in solution (colloids). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticles surface. This double layer of ions travels with the nanoparticles as it diffuses throughout the solution. The electric potential at the boundary of the double layer is known as the zeta potential of the particles. Zeta potential is taken as a measure for stability of nanosuspension [14]. Under most conditions, the higher the absolute value of the zeta potential of the nanoparticles, the larger the charge on their surface, leading to stronger repulsive interaction between the dispersed nanoparticles and higher stability and more uniform size [15].

The zeta size and zeta potential of prednisolone entrapped SPIONs were 506 nm and +8.55 mV and given in Figs. 3 and 4.

XRD

Powder XRD patterns recorded for the powders made of prednisolone-PEG entrapped SPIONs (Fig. 5 and Table 1) have curved baselines and diffraction peak at $2\theta=28.922$. The sharpness of the peaks represents the pure as well as crystalline sample in case of XRD analysis.

Prednisolone EE in SPIONs

The results of drug EE in 6.8 and 7.4 are given in Table 2. From the observed result reveals that EE is high in case of 6.8 phosphate buffer indicating that the drug has higher solubility in 6.8 phosphate buffer (colon pH).

In vitro release characteristics

Dialysis bag method

The primary factor for the successful development of a promising drug delivery system and assessments of the drug release profile from the delivery system is the proper design and selection of an *in vitro* drug release system, which permits the accurate evaluation and mechanistic analysis of the drug release profiles. Physiological availability of the drug depends on the rate of release from nanoparticles and permeability through our body membrane [16]. The *in vitro* method is valuable and important in screening procedure for understanding of physicochemical parameters such as flux (movement of drug between our body compartments) as well as partition diffusion coefficient. *In vitro* method may be limited predictive value, but they are the means of assessing the ability of vehicle to release the drug under experimental conditions. The constraints of such technique are that the method does not exactly simulate the *in situ* behavior, especially with respect to the unpredictable blood supply and metabolism. However, since performing biostudies on every manufactured batch are impractical and expensive, formulators must rely on *in vitro* testing to ensure batch-to-batch uniformity and consistency in bioavailability among the developed formulations [17].

Prednisolone exhibits variation in bioavailability with change in dose and also possesses short biological half-life (~2-4 h) following oral administration [7]. To overcome this lacuna, in the past few decades, nanotechnology has an improvement and has emerged as a basis for the treatment of a wide range of different diseases. Nanotechnology leads to a prolongation of the drug release and increasing the entrance of drug into the cell [10]. Nanoparticles increase the effects of drug with negligible toxicity and cause controlled transfer and accumulation in the affected site and protection of drug molecules against biodegradability and plasma clearance.

In our study, we used PEG as polymer. It has numerous advantages such as biodegradability, biocompatibility, decreasing systemic side effects, rapid clearance from the biological system, and high efficiency of drug transmission and transportation. Thus, it has been used in micro- and nano-formulations.

Following the physicochemical characterization of prepared SPIONs, *in vitro* prednisolone release from prednisolone entrapped SPIONs was performed in 6.8 and 7.4 phosphate buffer. The results are given in Table 3 and Fig. 6. As illustrated in Fig. 6, it can be seen that in the first 18 h, the drug release attains 57 and 58% and it reaches 71 and 75% at 24 h. It implies that the drug release from the formulations is controllable and sustains. The obtained results were statistically significant ($p=0.0177$). Hence, the result shows that the nanodrug delivery system (SPIONs) is suitable for controlled drug delivery at target site and also protects the drug from biodegradability and also it extends its half-life.

CONCLUSION

The present study reveals that the site-specific drug delivery of prednisolone to colon using SPIONs could be a promising approach for the delivery of prednisolone to enhance its therapeutic efficacy by local action. *In vitro* method may be limited predictive value, but they are the means of assessing the ability of vehicle to release the drug under experimental conditions. The constraints of such technique are that the method does not exactly simulate the *in situ* behavior, especially with respect to the unpredictable blood supply and metabolism. Hence, further work on biostudies to be needed to predict the bioavailability.

AUTHORS' CONTRIBUTIONS

Subashini Rajaram, Senthil Rajan Dharmalingam, Santhosh Rani A, Sathasri R, Varsha D, and Vinothini V have equally contributed to the preparation and editing of the manuscript.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest.

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