Evaluation of the Kinetics and Mechanism of Drug Release from Econazole nitrate Nanosponge Loaded Carbapol Hydrogel

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ABSTRACT

The objective of this study was to investigate the mechanism of release of econazole nitrate (EN) nanosponges loaded hydrogel and to compare it with EN hydrogel so as to develop an extended release topical drug delivery system of EN. Nanosponges of EN were prepared using ethyl cellulose and PVA by emulsion solvent evaporation method. On the basis of pharmacotechnical evaluation nanosponges with least particle size of 230.1 nm and good rheological properties were formulated as hydrogel (F1 – F7). In vitro drug release data of EN nanosponges loaded hydrogels in phosphate buffer pH 6.8 and 7.4 when analysed by GraphPad Prism software version 4.0 San Diego, USA best fitted the Makoid-Banakar model (R^2 value greater than 0.98). The Korsmeyer-Peppas release exponent (n) ranged between 0.331 – 0.418, which confirmed diffusion as the principle mechanism of drug release. The release mechanism was further confirmed by calculating the ratio of exponents A/B ratio derived from the Kopcha model.

KEYWORDS: Nanosponge, hydrogel, Makoid-Banakar, Kopcha model, Korsmeyer-Peppas model.

INTRODUCTION

The transdermal therapeutic system is a drug carrier system used for topical drug delivery, including ophthalmic and dermal treatments for eye and skin diseases, and for systemic activity, by delivering drugs across the skin to blood circulation. A significant restriction for most transdermal formulations may be the percutaneous permeation rate across the skin. Nanosponge loaded topical formulations may serve as a solubilizing matrix for poorly soluble drugs and as a local depot ("micro-reservoirs") for sustained drug release, as well as a rate-limiting membrane barrier for modulation of systemic absorption[1] thus overcoming the limitations of transdermal therapeutic formulations.

Local anesthetics, antifungal, and antibiotic agents, are among the substances whose incorporation into nanosponges satisfy all the requirements necessary for topical application and localized drug delivery.

Nanosponges have emerged as one of the most promising fields of science because of their perceived application in controlled drug delivery. Nanosponge technology offers entrapment of ingredients and is believed to contribute towards reduced side effects, improved stability, increased elegance, and enhanced formulation flexibility. In addition, nanosponge systems are non-irritating, non-mutagenic, non-allergenic, and non-toxic[2]. This system is employed for the improvement of performance of topically applied drugs[3].

These nanosponges can be effectively incorporated in to topical hydrogel drug delivery system for prolonged drug release and retention of dosage form on skin, thus reducing the fluctuations in concentration of drug, reducing drug toxicity and improving patient compliance by prolonging dosage intervals[4]. Nanosponges can be designed to release given amount of active ingredients over time in response to pressure, temperature and solubility. The release can also be activated by diffusion taking into consideration the partition coefficient of the ingredient between the nanosponges and the outside system.

Sustained release nanosponges can also be developed. Various factors that are to be considered during development of such formulations include physical and chemical properties of entrapped actives, physical properties of nanosponge and properties of vehicle in which the nanosponge are finally dispersed. Our selection of the vehicle was hydrogel, that when applied to the skin forms a thin transparent film suitable for incorporation of a topical agent intended for sustained release. Econazole nitrate, an

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imidazole antifungal is used topically to relieve the symptoms of superficial candidiasis, dermatophytosis, pityriasis versicolor and skin infections. It is commercially available as 1% cream, 1% ointment, 150 mg vaginal tablet, lotion, powder and solution. The absorption is not significant when econazole nitrate is applied to the skin or vagina. Following topical application of econazole nitrate cream to the skin, less than 1% of the applied dose is excreted in urine and feaces and most of the systemically absorbed fraction of the dose is excreted in the urine within 24 hr[6]. These delivery systems require high concentration of active agents to be incorporated for effective therapy because of their low efficacy as delivery systems. Thus the present study was aimed to evaluate the potential of econazole nitrate nanosponge loaded hydrogel as a local depot for sustained drug release and analyze the release mechanism of the drug.

MATERIALS AND METHODS

Econazole nitrate was received from FDC ltd. Mumbai, India). Other ingredients included Ethyl cellulose (SD Fine Chem. Ltd., Mumbai, India), Polyvinyl alcohol (Qualigens Fine Chemicals, New Delhi, India), Dichloromethane (Qualigens Fine Chemicals, Mumbai, India), Carbopol 934 NF (Central drug house ltd, New Delhi, India), Triethanolamine (Qualigens Fine Chemicals, Mumbai, India), Propylene glycol (Ranbaxy Fine Chemicals Ltd., New Delhi, India) and N-methyl-2-pyrrolidone (Thomas Baker Chemical Ltd., Mumbai, India). All other ingredients used were of analytical grade.

Methodology

Fabrication of Econazole Nitrate Nanosponges by Emulsion solvent diffusion method

Four batches of nanosponges coded N1- N4, utilizing different proportions of ethyl cellulose and polyvinyl alcohol were prepared by emulsion solvent diffusion method. Briefly, the disperse phase consisting of EN (100mg) and requisite quantity of ethyl cellulose (Table 1) dissolved in 20 ml dichloromethane was slowly added to a definite amount of polyvinyl alcohol in 150 ml of the aqueous continuous phase. The reaction mixture was stirred at 1000 rpm for two hours on a magnetic stirrer (HICON ®, New Delhi, India). The nanosponges formed were collected by filtration and dried in oven (HICON ®, Delhi, India) at 40^{0}C for 24 hr. The dried nanosponges were stored in vacuum desiccator to ensure the removal of residual solvent.

Particle size measurement and visualization of nanosponges

The mean particle size of nanosponges was measured by Malvern Zeta sizer (Malvern Instrument Ltd). The dispersions were diluted with Millipore-filtered water to an appropriate scattering intensity at 25^{0}C and sample was placed in disposable sizing cuvette at a count rate of 372.0 (kcps) for 20 sec. The particle measurement data is reported in Table 1.

Scanning electron microscopy of N1 was carried out at 200 X magnification, using LEO electron microscopy, U.K at an accelerating voltage of 15 kV. The sputter gold coated nanosponges were mounted on a double-faced adhesive tape and observed.

Formulation of nanosponge loaded hydrogel

Gel forming polymer was soaked in water for 2 h and then dispersed by agitation at approximately 600 rpm with the aid of magnetic stirrer to get a smooth dispersion. The stirring was stopped and dispersion was allowed to stand for 15 min to expel entrained air. To this aqueous solution, triethanolamine

Table 1: Formulation design and particle measurement data of econazole nitrate nanosponges prepared by emulsion solvent diffusion method

	Formulation code			
Component	**N1**	**N2**	**N3**	**N4**
Drug : PVA : Ethyl cellulose (%w/w)	1 : 3 : 2	1 : 3 : 3	1 : 2 : 3	1 : 2 : 2
Dichloromethane (ml)	20	20	20	20
Distilled water (ml)	150	150	150	150
Z-average (nm)	230.1	470.0	420	342
Polydispersity index	0.905	1.103	1.000	1.000
Entrapment efficiency (%)	90.43±0.09	94.45±0.08	81.8±0.16	91.55± 0.05

(2 % v/v) was added with slow agitation[7]. At this stage, nanosponges and permeation enhancers were incorporated in to the prepared base as ethanolic solution. The formulation design is summarized in Table 2.

Table 2: Formulation design of econazole nitrate nanosponge loaded carbapol hydrogels

Component	Formulation Code							
(% w/w)	F0	F1	F2	F3	F4	F5	F6	F7
Econazole nitrate nanosponges	-	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Econazole nitrate	1.0	-	-	-	-	-	-	-
Triethanolamine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Ethanol (95%v/v)	20	20	20	20	20	20	20	20
Propylene glycol	-	-	10	-	10	10	10	15
N-methyl-2-pyrrolidone	-	-	-	10	10	20	40	45
Carbapol 934 NF	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Distilled water (q.s)	100	100	100	100	100	100	100	100

Physicochemical evaluation of nanosponge loaded hydrogels

The viscosity of hydrogel was measured by using a Brookfield viscometer (DV-II + Pro, Brookfield engineering laboratories, INC. USA) attached with T-bar spindle (spindle-C, S-96, width 11mm). Hydrogel was filled in a 10 ml beaker (dia 25mm) and the spindle lowered perpendicularly taking care that the spindle did not touch the bottom of the beaker. The spindle was rotated at a speed 50 rpm and readings were recorded after 30 sec, when the hydrogel level stabilized. The pH of EN hydrogel was recorded using a calibrated pH meter (DB-111, HICON, New Delhi, India). One gram of nanosponge loaded hydrogel was dispersed homogeneously in 100 ml of distilled water and stored at room temperature for 2 hr. The pH of the dispersion was measured at 25°C.

Drug content estimation was done by accurately weighing a specified quantity (100 mg) of each hydrogel and extracting it with 5 ml of methanolic HCl using a vortex mixer. The volume was made up to 10 ml and the resultant dispersion filtered, appropriately diluted and the concentration of EN was determined spectrophotometrically at 271 nm. The estimation of percentage drug content of each formulation was done in triplicate and the average value is reported in Table 3.

Table 3: Pharmacotechnical properties of EN nanosponge loaded carbapol hydrogels

Hydrogel code	Viscosity (cp)	Drug content (%)	pH
F0	4720± 0.10	92.55± 0.80	6.54
F1	7760± 0.56	82.34± 0.45	5.47
F2	5680± 0.45	84.85± 0.95	6.44
F3	6782± 0.67	86.21± 0.42	4.82
F4	4440± 0.46	91.61± 0.80	5.51
F5	5540± 0.28	89.43± 0.92	5.60
F6	4580± 0.35	87.06± 0.47	6.25
F7	4420± 0.12	92.57± 0.36	6.49

In-vitro drug release across dialysis membrane

Econazole nitrate release from the hydrogels was measured across Himedia dialysis membrane 50 using Franz diffusion cell, with a diffusional area of 2.26 cm^2 and receptor volume of 11 ml. The membrane soaked in receptor medium for 8 hr was used as a barrier membrane and mounted between the donor and the receptor compartment. One gram hydrogel was placed on the membrane surface in the donor compartment that was sealed from the atmosphere with aluminum foil. The receptor compartment of cell was filled with 11ml of phosphate buffer pH 6.8 (pH of skin) and 7.4 (physiological pH) separately for two different experiments.

During the experiments, the solution of receptor side was kept at 37± 0.5^0 C and was stirred at 100 rpm with teflon-coated magnetic stirring bars. One ml aliquots were collected from the receptor side at designated time intervals of 0, 5, 10, 15, 30, 60, 120, 240, 480, 720 minutes and replaced by the same volume of fresh receptor solution to maintain sink condition and constant volume. The sample was analyzed by UV-spectrophotometer at 266 nm and 272 nm for pH 6.8 and 7.4

respectively. The percentage cumulative drug release was determined from the calibration curves.

Evaluation of release kinetics

To investigate the mechanism of drug release from the nanosponge loaded hydrogel, the release data was analyzed using zero order, first order, Higuchi, Peppas, and Hixson-Crowell, Kopcha, and Makoid-Banakar models. The data was analyzed using GraphPad Prism Software Version 4.0, San Diego, CA, USA. The software estimates the parameters of a nonlinear function that provides the closest fit between

Table 4: Mathematical equations for the models used to describe release characteristics of econazole nitrate from carbapol hydrogels

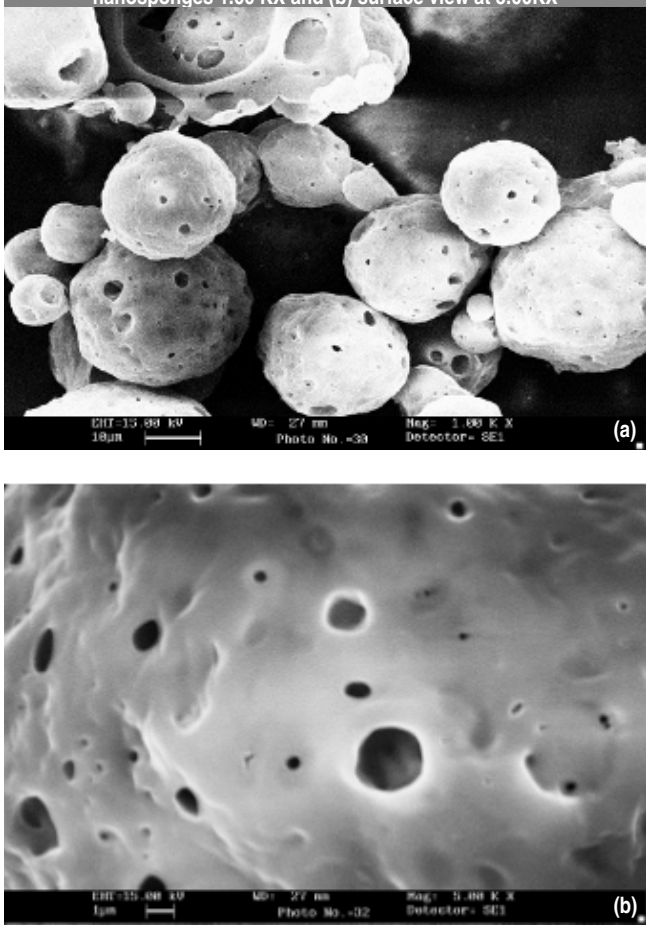
Model	Equation
Zero order	$Q_t = Q_0 + K_0 t$
Higuchi	$Q_t = Q_0 + K_H t^{1/2}$
Korsmeyer-Peppas	$Q_t = K_{KP} t^n$
Kopcha	$Q_t = At^{1/2} + Bt$
Makoid-Banakar	$Q_t = K_{MB} t^n e^{(-ct)}$

experimental observations and nonlinear function. The mathematical expressions that describe the models used to describe the dissolution curves are summarized in Table 4.

RESULTS AND DISCUSSION

The nanosponges prepared by emulsion solvent diffusion method were discrete, nearly spherical nanometeric particles in the size range of 230.1- 470 nm (Table 1). Nanosponge formulation N1 with least particle size and maximum entrapment efficiency was investigated for surface morphology by visualization under scanning electron micrograph. SEM analysis revealed nanosized, almost spherical particles with numerous pores on the surface (Fig 1a). The pores characteristically tunneled inwards (Fig 1b) that were probably the impressions of diffusion of solvent (dichloromethane) from the surface of nanosponges. Any residual crystals of EN could not be seen on the surface of the nanosphere indicative of the matrix being constructed from drug and polymer[8]. Based on these findings the nanosphere N1 with an average particle size of 230.1 nm was selected for topical formulation for the following considerations. A nanoparticulate system can prolong the release of EN, reduce fluctuations in concentration of drug and improve patient compliance by prolonging dosing intervals. Secondly when a nanosized system is formulated as topical drug delivery system**,** it will not impart gritty feeling[9] and will help in prolonged retention of drug on skin.

Fig. 1. Scanning electron micrograph of (a) econazole nitrate nanosponges 1.00 KX and (b) surface view at 5.00KX



Thus a total of seven hydrogels (F1-F7) were formulated using Carbapol 934 NF as the gelling polymer with variable permeation enhancers and evaluated for physicochemical properties (Table 3). Carbapol 934 NF (crosslinked acrylic acid polymer) is hygroscopic in nature and is good choice for thick formulations such as medium to high viscosity gels. The gelation mechanism depends on neutralization of the carboxylic acid moiety to form a soluble salt. When neutralized it produces sparkling clear gels that have good thermal stability and as a topical product, carbapol gels possess optimum rheological properties[10]. Based on these considerations carbapol was selected for formulation of hydrogels, F1-F7. These formulations differed in the type and the concentration of the permeation enhancers. Though the drug was intended for topical delivery**,** inclusion of permeation enhancers to achieve therapeutic efficacy in such formulations has been frequently reported by various authors [4,7,11].

The formulated hydrogels were transparent, pleasant and smooth in appearance. These were similar in color and smell to the original raw material and had the appearance of a solid-

like gel. The presence of nanosponges could be faintly recognized on visual observation. The physicochemical characteristics were investigated and the drug content of the formulations ranged between 82.34% - 92.57%. The viscosity values widely ranged between 4420 ± 0.12 cps to 7760 ± 0.56 cps. The concentration of gelling agent was kept constant in all the hydrogels; hence the variation in the viscosity was attributable to the total concentration of permeation enhancers. Thus when the total concentration of permeation enhancer was beyond 30% by weight**,** the viscosity values were lowest. Thus F5, F6 and F7 with total permeation enhancer more than or equal to 30% by weight had viscosity values in the lower range of 4580 ± 0.35 - 4420 ± 0.12 cps whereas other formulations with lesser permeation enhancers displayed higher viscosity values. This is quite understandable as the permeation enhancers were liquids that would definitely affect the viscosity in a predictable manner. The pH of all hydrogels was between 4.82 and 6.54 that is well within the normal pH range of skin 4.0-6.8, hence the preparations will potentially be irritation free[12] .

The *in vitro* release studies were carried out both in pH 6.8 and pH 7.4 using Himedia dialysis membrane (MWCO 50k). Artificial membrane was used for the study in order to minimize the number of experimental variables while trying to understand the release of econazole nitrate from nanosponge loaded hydrogel. All formulations exhibited slightly higher drug release in pH 6.8 that is correlatable to the basic nature of the drug, an advantageous aspect as better release of drug in inflamed conditions is desirable to achieve the therapeutic effect. Amongst the various formulations, F7 exhibited highest drug release when compared to other formulations in both the test media 58.79% ± 0.15 at pH 6.8 (Fig 2) and 54.75.25% ± 0.11 at pH 7.4 (Fig 3). This is in contrast to the release of EN from F0 (hydrogel without EN nanosponges) amounting to 86.60% ± 0.09 in pH 6.8 and

Fig. 2. *In vitro* release profiles of econazole nitrate loaded hydrogels in phosphate buffer pH 6.8

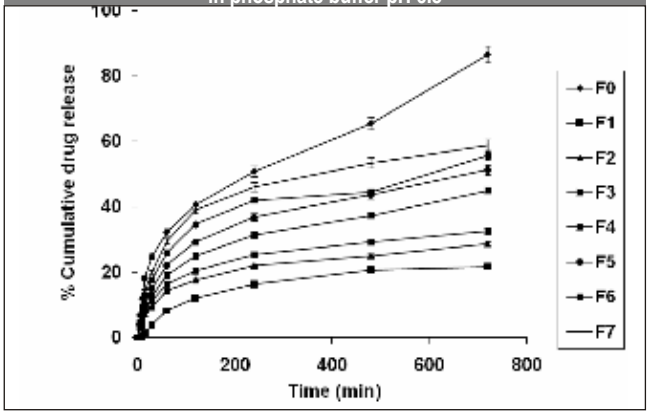
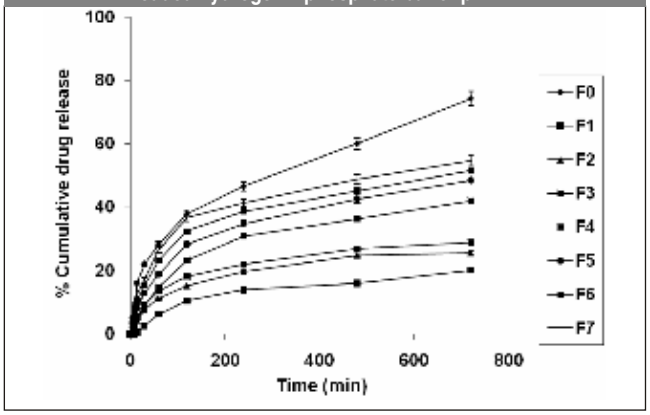


Fig. 3. *In vitro* release profiles of econazole nitrate nanosponge loaded hydrogel in phosphate buffer pH 7.4



74.29% ± 0.51 in pH in 7.4 signifying the role of nanosponges in controlling the release of EN from hydrogel.

Consequently the descending release order was F0> F7>F6>F5>F4>F3>F2>F1. Interestingly the formulations F1, F2, F3 displayed a delayed onset of release (beyond 5 min for F1 and F3 and beyond 10 min for F1) whereas the onset of release was within 5 min for rest of the formulations that is correlatable to lower viscosity values and the presence of higher content of permeation enhancers in the latter. The highest *in-vitro* release of econazole nitrate from F7 may be attributed to highest increase in solubility of drug within the gel matrix due to permeation enhancers that consequently facilitated the drug to release from 3D network of hydrogel into the test media. The total cumulative drug released at the end of 12 hr was below 100% for all dosage forms. This may be due to very slow erosion of the polymeric matrix under the test conditions that resulted in slow diffusion of entrapped drug.

Data obtained from *in-vitro* release studies was modeled to various kinetic equations to find out the mechanism of drug release from EN loaded nanosponges hydrogel. The development of advanced drug delivery systems relies on a judicious and careful selection of components, configurations, and geometries, which can be facilitated through mathematical modeling of controlled release systems. Mathematical modeling aids in predicting the drug release rates and diffusion behavior from these systems by the selection of an appropriate model, thereby reducing the number of experiments needed. It also aids in understanding the physics of a particular drug transport phenomenon, thus facilitating the development of new pharmaceutical products.

In the present study the release profiles were not linear suggesting that the drug release from the formulation was not zero order that was confirmed by low R^2 values of 0.742-

0.887 . The *in vitro* release data for both test media fitted best to the Higuchi order release pattern (Tables 5 & 6). Release data again fitted the Kopcha matrix model. However, the mathematical expression that best described drug release from nanosphere loaded hydrogel was Makoid-Banakar model, with resultant R^2 value greater than 0.98. The Korsmeyer-Peppas release exponent (n) ranged between 0.331 – 0.418, which confirmed that diffusion as the principal mechanism of drug release.

The release mechanism was further confirmed by calculating the ratio of exponents A/B ratio derived from the Kopcha model. The A/B ratio for all the nanosphere loaded hydrogels was greater than 1 and was highest for F7 suggesting a fastest diffusion of EN from the formulation. The Kopcha model can easily be used to help quantify the contribution of diffusion and polymer in drug release. As seen from data in tables 5 and 6, the value of A is far greater than the value of B which suggests that drug release occurred mainly as a result of Fickian diffusion. There are literature reports wherein the release of a thin topical application of a drug is modeled by transient diffusion between the application, the underlying stratum corneum, and a receptor [13,14]. However the diffusion of EN was slower when compared to F0 the hydrogel made with pure drug and served as the reference formulation. Thus the incorporation of EN nanosphere in hydrogel served the purpose of sustaining the drug release. On further analysis of the release data, the parameter c of the Makoid-Banakar model almost equaled to zero for all the formulations and in this situation this model approaches Korsmeyer-Peppas power law ($e^{-0t}=1$)[15]. Thus the mechanism of drug release from nanosphere loaded hydrogel was found to be effected by drug-polymer interaction and followed Makoid-Banakar model.

CONCLUSION

A sustained release topical drug delivery system of econazole nitrate developed as a nanosponge hydrogel offered solubilizing matrix for poorly soluble drug, served as a local depot for sustained drug release and provided a rate-limiting matrix barrier for modulation of drug release. Further investigations using ex vivo models are being carried out to substantiate the *in vitro* results.

Table 5: Results of model fitting of drug release of nanosponge loaded hydrogel in phosphate buffer pH 6.8

Kinetic model	Para meter	Formulation code							
		F0	F1	F2	F3	F4	F5	F6	F7
Zero order	R^2	0.887	0.812	0.752	0.746	0.828	0.820	0.767	0.742
	K_0	0.105	0.032	0.037	0.042	0.057	0.065	0.068	0.077
	SSR	1539	258.5	477.2	644.9	731.8	1013	1507	2213
Higuchi	R^2	0.983	0.951	0.923	0.921	0.986	0.966	0.933	0.915
	K_H	3.036	0.950	1.107	1.269	1.685	1.932	2.040	2.324
	SSR	236.1	68.17	148.0	200.9	136.2	192.9	437.1	731.3
Korsmeyer-Peppas	R^2	0.989	0.958	0.959	0.988	0.968	0.987	0.968	0.947
	K_{KP}	5.716	1.669	3.556	4.148	4.176	5.063	6.679	7.141
	N	0.408	0.418	0.334	0.331	0.370	0.362	0.331	0.340
	SSR	147.3	58.35	79.74	106.7	59.58	75.78	207.9	452.9
Kopcha	R^2	0.912	0.968	0.966	0.933	0.904	0.966	0.933	0.904
	A	3.036	0.950	1.107	1.389	1.685	1.932	2.040	2.564
	B	0.0001	0.0001	00001	0.0001	00001	0.0001	00001	0.0001
	SSR	236.1	68.17	148.0	222.9	136.2	192.9	437.2	824.2
Makoid-Banakar	R^2	0.990	0.992	0.978	0.980	0.992	0.994	0.980	0.975
	K_{MB}	6.943	0.431	2.035	2.265	2.812	3.241	4.238	3.250
	N	0.362	0.734	0.478	0.487	0.465	0.470	0.447	0.537
	C	-0.0002	0.0012	0.0007	0.0007	0.0004	0.0006	0.0007	0.0007
	SSR	131.6	11.76	43.02	50.03	34.90	33.45	129.0	211.6

Table 6: Results of model fitting of drug release of nanosponge loaded hydrogel in phosphate buffer pH 7.4

Kinetic model	Para meter	Formulation code							
		F0	F1	F2	F3	F4	F5	F6	F7
Zero order	R^2	0.864	0.846	0.783	0.762	0.838	0.826	0.785	0.763
	K_0	0.093	0.028	0.036	0.039	0.057	0.064	0.066	0.069
	SSR	1479	160.6	380.3	513.6	680.2	927.4	1296	1592
Higuchi	R^2	0.980	0.959	0.943	0.930	0.970	0.969	0.947	0.935
	K_H	2.712	0.829	1.068	1.175	1.676	1.888	1.981	2.079
	SSR	220.9	42.84	99.74	150.8	125.9	167.8	317.5	435.3
Korsmeyer- Peppas	R^2	0.990	0.961	0.963	0.957	0.979	0.984	0.975	0.973
	K_{KP}	5.738	1.162	2.639	3.277	3.197	4.392	5.778	6.931
	N	0.3914	0.4507	0.3698	0.3532	0.4063	0.3782	0.3468	0.3285
	SSR 1	05.7	40.47	64.58	92.76	89.12	83.95	148.1	184.4
Kopcha	R^2	0.979	0.959	0.942	0.921	0.970	0.969	0.947	0.935
	A	2.803	0.829	1.096	1.288	1.676	1.888	1.981	2.079
	B	0.0001	0.0001	00001	0.0001	00001	0.0001	00001	0.0001
	SSR	234.5	42.84	101.1	171.5	125.9	167.8	317.5	435.3
Makoid-Banakar	R^2	0.9914	0.979	0.991	0.984	0.995	0.996	0.992	0.990
	K_{MB}	5.190	0.415	1.062	1.519	1.272	2.337	3.140	3.901
	N	0.415	0.691	0.590	0.546	0.619	0.527	0.497	0.473
	C	0.0010	0.0009	0.0009	0.0009	0.0008	0.0006	0.0007	0.0007
	SSR 1	02.1	21.57	16.47	34.33	22.51	23.57	50.55	66.07

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