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FORMULATION AND EVALUATION OF LIPOSOMAL GEL CONTAINING KETOCONAZOLE FOR TOPICAL APPLICATION

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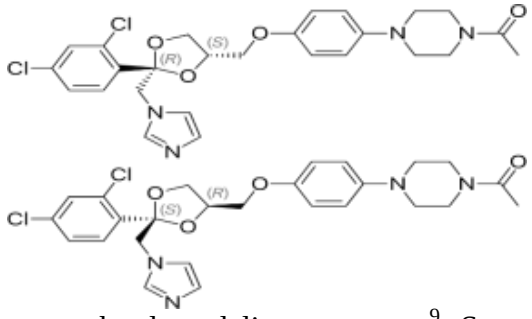
ABSTRACT

The present investigation was to prepare and evaluate the Carbopol gel formulation containing ketoconazole for topical application. Liposomes are used because have many of the requirements for good drug delivery systems as they are relatively non-toxic and bio-degradable. And they help the ketoconazole to pass through the skin layers. Phosphatidyl choline and cholesterol were taken in different concentration. Liposomes are prepared by using thin film hydration technique. Gel containing Carbopol 934 was prepared and characterization tests were performed for liposomal dispersion and liposomal gel. Liposomal gels were prepared with 1%, 1.5% and 2% Carbopol gels which gave the clear idea for the efficient concentration of Carbopol in topical gel.

INTRODUCTION:

Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shell(s) consisting of lipids arranged in a bilayer configuration. The potential use of liposomes as drug carriers was recognized more than 40 years ago and, since that time, liposomes have been used in a broad range of pharmaceutical applications. Liposomes are considered as superior carriers due to their capability to encapsulate both hydrophilic and lipophilic molecules[1]. Liposomes are also found to have important medical, cosmetic, and industrial applications [2]. Liposomes can substantially improve drug loading, drug delivery and sustained release, thereby offering advantages over traditional dosage form. Topical drug delivery is an attractive route for local and systemic treatment. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs[5] and protect them from degradation. When applied on the skin, liposomes act as a solublizing matrix for poorly soluble drugs, penetration enhancer and local depot at the same time reducing the side effects of these drugs. Thereby topical liposome formulations could be more effective and less toxic than conventional formulations. Liposomal formulations are widely used in the pharmaceutical field as drug delivery systems due to their versatility and clinical efficacy and they have been used

to administer drugs by several routes such as the oral, parenteral, and topical. Among these, topical delivery of drugs carried by liposomes exhibits interesting applications, not only for promoting dermal delivery of drugs which have toacttopically,suchaslocalanaesthetics,butals oforenhancingtransdermaldelivery of drugs intended for systemic use, thus more effectively exploiting this non-invasive alternative route to oral administration. Due to the aforementioned advantages, in this study liquid-state liposomes were chosen to



serve as the drug delivery system [9]. Several antifungal agents are available in the market in different topical preparations e.g., creams, ointments and powders for the purpose of local dermatological therapy. One of the antifungal agents is Ketoconazole, a substituted imidazole; it is having broad spectrum activity against systemic and superficial mycoses and is marketed as creams and tablets. Ketoconazole is usually effective for topical infections such as athletes foot, ringworm, candidiasis and jock itch. Common side effects associated with Ketoconazole therapy include mild burning at the application site, severe allergic reactions (rash, itching, and swelling of the mouth, face, lips or tongue), blisters, irritation, pain or redness. It was hypothesized that incorporation of Ketoconazole into liposome will improve the amount and time of the drug retention within the skin, so as to increase the therapeutic index of the drug. It was also expected that the dose and frequency of topical application of the drug will reduces, hence toxicity and cost of product. Encapsulation of Ketoconazole in liposome increases the half life providing prolonged drug delivery and minimizes the commonly occurring side effects as well as drug accumulation will be high. So, in the present investigation we are planning to prepare and evaluate the liposomal gel of Ketoconazole.

MATERIALS AND METHODS

Materials: Ketconazole was obtained gift sample from Sun Pharmaceuticals industries Ltd. Cholesterol from HiMedia Chem. Pvt. Ltd. Mumbai, Sorbitan esters from Colorcon Asia Pvt .Limited, Verna, India. Carbopol was purchased from S.D. Fine Chemicals, Mumbai. All other chemicals and solvents were of analytical or pharmacoepical grade.

Ketoconazole, sold under the brand name Nizoral among others, is an antifungal medication used to treat a number of fungal infections.[10] Applied to the skin it is used for fungal skin infections such as tinea, cutaneous candidiasis, pityriasis versicolor, dandruff, and seborrheic dermatitis. Other uses include treatment of excessive hair growth and Cushing's syndrome.

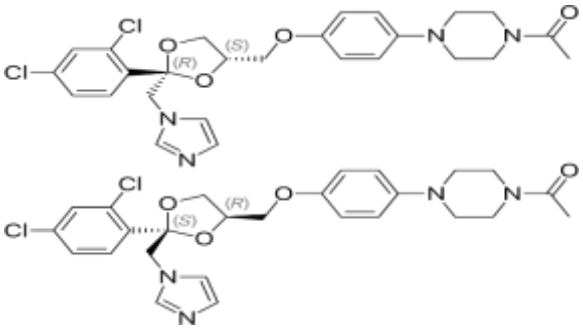


Fig.1 Chemical Structure of Ketoconazole

Preparation of liposomes [11,12] : Liposomes were prepared by a thin film hydration method using a lipid mixture consisting of surfactant (span 40, span 60 and tween 60) and CHO, at different specified ratios as given in Table 3. Surfactant, CHO and drug were dissolved in 10ml of chloroform. The lipid mixture was then transferred to a 100

ml round bottom flask, and the solvent was evaporated under reduced pressure at a temperature of 55-65°C, using a rotary flash evaporator until the formation of a thin lipid film. The formed film was hydrated with 20 ml of Phosphate buffer saline pH 7.4. The hydration was continued for 1 h, while the flask was kept rotating at 55-65°C in the rotary evaporator. The hydrated niosomes were sonicated for 20 min using a bath sonicator to obtain liposomal dispersion containing both free and entrapped drugs of varying size.

Evaluation of Ketoconazole Liposomes:

Morphological characterization: The vesicle formation was confirmed by optical microscopy in 45× resolution. The liposomal suspension placed over a glass slide and fixed over by drying at room temperature, the dry thin film of liposomal suspension observed in the formation of vesicles. The microphotography of the liposome also obtained from the microscope by using a digital camera. [Figure 1]. The detailed surface characteristic of the selected Ketoconazole liposome formulation was observed using a scanning electron microscope.

Morphology of formulated liposomal vesicles: The liposomal dispersion after hydration was stored less than 4°C for congealing and a drop of dispersion was viewed under an optical microscope to observe the shape and lamellar nature of the vesicle.

Entrapment efficiency[110]: Entrapment efficiency of liposomes was determined by exhaustive dialysis method. The measured quantity of liposomes suspension was taken into a dialysis tube to which dialysis membrane was securely attached on one side. The dialysis tube was suspended in 100 ml PBS pH 7.4 containing 10% v/v methanol, which was stirred on a magnetic stirrer. The un-entrapped drug was separated from the liposomes suspension into the medium through the membrane. At every hour, entire medium (100 ml) was replaced with fresh medium (for about 6-7 h) until the absorbance reached a constant reading indicating no drug is available in an un-entrapped form. Inclusion of CHO in niosomes increases its hydrodynamic diameter and entrapment efficiency.

It stabilizes liposome and decreases the permeability of vesicles to entrapped solute preventing leakage. An increase in CHO content of the bilayer resulted in a decrease in the release rate of encapsulated material and therefore, an increase of the rigidity of the bilayer obtained. Presence of charge tends to increase the inter lamellar distance between successive bilayer and leads to greater overall entrapped volume. The withdrawn samples were analyzed at 225 nm using a UV spectrophotometer. Amount of entrapped drug was obtained by subtracting amounts of un-entrapped drug from the total drug incorporated.

Percent entrapment = Total drug-Diffused drug/Totaldrug×100

***In- vitro* drug release study**[111,112]

The release of Ketoconazole from liposome formulations were determined using membrane diffusion technique. The liposome left after removal of un-entrapped drug were dialyzed into a beaker containing 100 ml of PBS pH7.4 containing10%v/v methanol (to maintain sink condition), which acted as receptor compartment.

The temperature of the receptor medium was maintained at 37 ± 0.5°C and agitated using a magnetic stirrer. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal, same volume of the medium was replaced. The collected samples were analyzed using a UV spectrophotometer at 225 nm. The tests were carried out in triplicate.

Formulation of liposomes entrapped Ketoconazole gel[113, 114] Promising liposomal suspension (formulation prepared by thin

hydration film method containing span 60 as surfactant (KLG4) Formulations of liposomes prepared using span 60 containing Ketoconazole equivalent to 2% w/w was incorporated into the gel base composed of Carbopol 934 (150 mg), glycerol (250 mg) Triethanolamine (quantity sufficient) and distilled water up to 15 g.

EVALUATION OF LIPOSOMAL GEL

a) Physical appearance: The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles.

b) pH: 2.5g of gel were accurately weighed and dispersed in 25 ml of distilled water. The pH of the Dispersion was measured by using a digital pH meter.

c) Rheological study: Viscosity measurement: Viscosity was determined by Brookfield programmable DV III ultra viscometer. In the present study, spindle no. CP52 with an optimum speed of 0.01rpm was used to measure the viscosity of the preparation.

d) Content uniformity: The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 10 mg of the drugin100 ml volumetric flask and volume was made up to 100 ml with methanol. The content was filtered through Whatman filter paper No. 41. 5 ml of above solution was taken into a 25 ml volumetric flask and volume was made up to mark with methanol. The content of Ketoconazole was determined at 225 nm against blank by using the Shimadzu UV/visible spectrophotometer. The drug content was determined from the calibration curve of Ketoconazole.

e) *In-vitro* drug diffusion study:[115, 116] The apparatus consists of a glass cylinder open at both ends. A dialysis membrane soaked in distilled water (24 h before use) is fixed to the one end of the cylinder with the aid of an adhesive. Gels equivalent to 10 mg of Ketoconazole is taken inside the cell (donor compartment) and the cell is immersed in a beaker containing 100 ml of PBS pH7.4 containing 10%v/v methanol (to maintain sink condition), act as receptor compartment. The whole assembly is fixed in such a way that the lower end of the cell containing gel is just above the surface of the diffusion medium (1-2 mm deep) and the medium was agitated using a magnetic stirrer at the temperature 37 ± 0.5°C. Aliquots (5ml) are withdrawn from the receptor compartment periodically and replaced with same volume with fresh buffer. The samples were analyzed by using UV-visible spectrophotometer at 225 nm. The tests were carried out in triplicate.

RESULTS AND DISCUSSION

Ketoconazole liposomal formulation: The Liposomes were prepared by dried thin film hydration technique using rotary evaporator with drug and carrier. The formulation containing Ketoconazole were prepared with different stabilizers like Dicetylphosphate and Stearylamine and all other parameters like temperature, vacuum and RPM were kept constant. The composition and ratios of compounds showed in Table No: 1. among those compositions KLG4 Formulations are selected as optimized batches for further evaluation.

Physicochemical characterization

a) Particle size distribution: The particle size distribution was analyzed for KLG4 formulations of ketoconazole Liposomes by wet method. The optimum particle size was obtained 5.94 µm in KLG4 Formulation, when compared to KLG2 and KLG8; the results were shown in Table No: 3.

b) Scanning Electron Microscopy: The Morphology and surface appearance of Liposomes were examined by using SEM. The SEM photographs of KLG4 formulation showed that the particles have smooth surface. The SEM images were shown in Figure No: 7 and 8.

c) Percent Entrapment: The percent entrapment of drug is determined for all the formulations. The percent entrapment drug

was 79.39±0.94, which is optimum in KLG4 formulation, and was as shown in the table no: 3.

d) *In-vitro* Dissolution data: The in-vitro dissolution profile of prepared formulations was determined by membrane diffusion method. The dissolution was carried out for a period of 12 hrs in 7.4 pH phosphate buffer. The cumulative percent release of KLG1 to KLG9 formulations at various time intervals was calculated and tabulated in Table No: 12-14 the cumulative percent drug release in all formulations was plotted against time in Graph No: 9, 10 and 11. The Maximum percent of drug release was found in KLG4 formulation about 88.78% at the end of 12 hrs. & also showed maximum drug entrapment.

e) Release Kinetics:

The release kinetics of all the formulations was studied. All formulations follow Zero order release kinetics and follow case II transport when it applied to the Korsmeyer-Peppa's Model for mechanism of drug release. KLG4 formulation has better kinetic results when compared to KLG2 and KLG6 formulations. The results are shown in Table No: 15.

SUMMARY AND CONCLUSION

The main objective of this work was designed to prepare and evaluate the Ketoconazole Liposomal Gel. This formulation will target the site of action with effect of various stabilizers on drug entrapment efficiency, and to reduce the side effects by formulating non-pegylated Liposomes. This liposomal formulation was formulated using the tween, spans and cholesterol which have lesser toxicity. The Liposomes were prepared by dried thin film hydration technique using rotary evaporator with drug, carrier, ammonium sulphate and stabilizers. The parameters like temperature, vacuum and RPM were maintained accordingly.

After preparation, the Liposomes were stored in freezed condition, and given for further evaluation. The prepared Liposomes of ketoconazole formulations were evaluated for physical and chemical characteristics like average vesicle size, and shape. The evaluated batches showed good physicochemical characteristics in KLG4 formulation when compared other formulations. This developed liposomal drug delivery system was also evaluated for dissolution study by pH 7.4 phosphate buffer using membrane diffusion method. The release of drug from KLG4 formulation was found to be sustained to certain extent when compared to other formulations. The release kinetics of all the Formulations was studied.

All formulations follow Case II transport when it applied to the Korsmeyer – Peppa's model for mechanism of drug release. KLG4 formulation has better kinetic results when compared to other formulations. The stability of the ketoconazole Liposomal gel was evaluated after stored at 40^0c and room temperature for 90 days. The assay of the samples was determined as a function of the storage at different time intervals. The Liposomal gel stored at 40^0 c was found to be stable for duration of three months. From the results of physical characterization, in-vitro evaluation, release kinetics and stability studies, it was found that charged Liposomes containing ketoconazole might be used for the treatment of a fungus when compared to the normal.

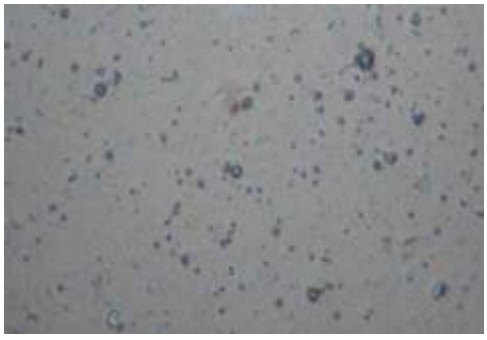


Fig 2: Microphotograph of liposome

Ingredients	Formulation code								
	KLG1	KLG2	KLG3	KLG4	KLG5	KLG6	KLG7	KLG8	KLG9
Ratios (Drug: Surfactant: Cholesterol)	1:1:0.2	1:1.5:0.3	1:2:0.4	1:1:0.2	1:1.5:0.3	1:2:0.4	1:1:0.2	1:1.5:0.3	1:2:0.4
Ketoconazole*	100	100	100	100	100	100	100	100	100
Span 40*	100	150	200	---	---	---	---	---	---
Span 60*	---	---	---	100	150	200	---	---	---
Tween60*	---	---	---	---	---	---	100	150	200
Cholesterol*	20	30	40	20	30	40	20	30	40
Chloroform-#	10	10	10	10	10	10	10	10	10
PBS(pH 7.4)#	20	20	20	20	20	20	20	20	20

Table 1: Formulations Table of Ketoconazole Liposomes

Table 2. Evaluation parameters of Ketoconazole liposomal gel (KLG4 =Formulations of liposomes prepared using span 60)

Parameter	Result
Appearance	Off-white
Homogeneity	Good
pH*	5.56±0.057
Percent drug content*	Viscosity*(cps)8370
Viscosity*(cps)	8370

Fig. -3: IR spectra of pure Ketoconazole & Fig. 3-a - IR Spectra of KLG4 formulation

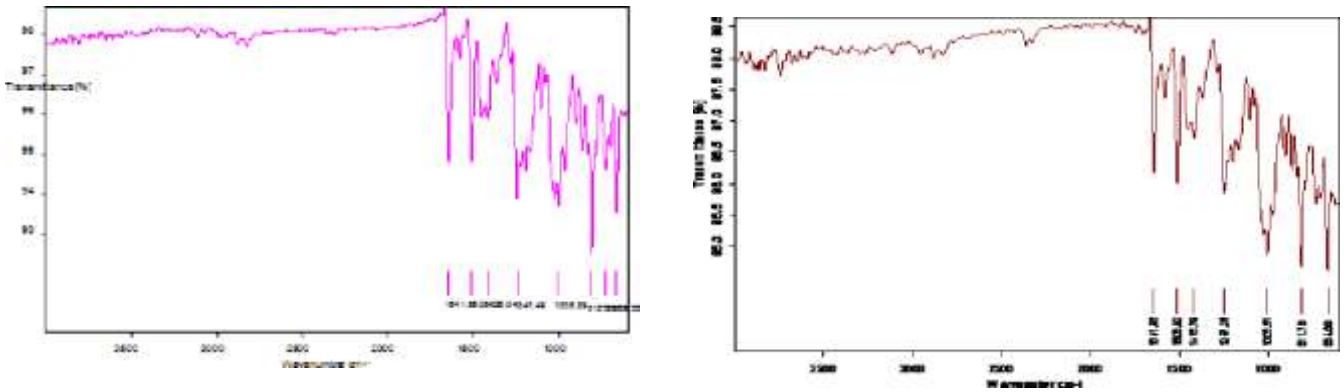


Table 3: Evaluation of Ketoconazole liposomes for particle size

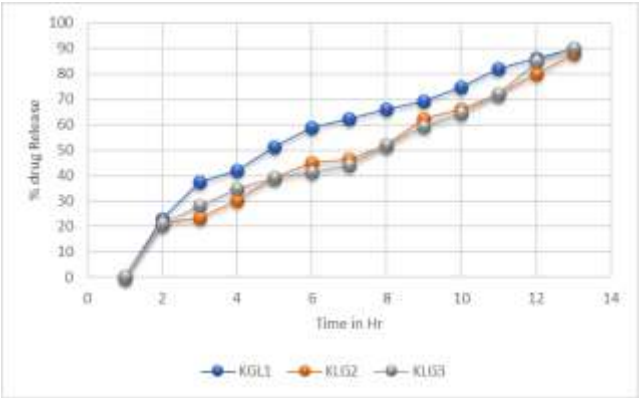
Formulation code	Particle size± SD(µm)	Percentage entrapment efficiency
KLG1	6.12±2.20	55.14±2.29
KLG2	5.76±2.06	59.08±3.27
KLG3	5.4±1.83	63.02±2.24
KLG4	5.94±2.14	79.39±0.94
KLG5	5.22±1.68	73.48±0.69
KLG6	4.86±1.24	76.96±1.89
KLG7	7.38±3.64	71.66±0.69
KLG8	6.84±2.93	76.36±2.27
KLG9	6.3±2.25	78.63±0.91

Table No: 4 Interpretations of FTIR Spectra for pure drug

Functional Groups	Range of Groups cm^{-1}	Assessment peak of pure drug cm^{-1}	Assessment peak of KGL4 cm^{-1}	Assessment peak of KGL8 cm^{-1}
C=C Streching (Aromatic)	1450 – 1600	1463.86 1524.51	1466.09 1524.38	1466.26 1524.48
O-H Bending (Alcohol)	1050 -1150	1072.51	1071.62	1071.69
C=O Streching	1705 -1735	1729.84	1729.80	1729.93
N-H Bending	1500 – 1650	1617.83	1617.67	1617.80
C-O Streching	1100 -1120	1114.46	1113.83	1113.89

Table 5 : *In-vitro* release studies of liposomal gel formulations

Time (h)	KLG1	KLG2	KLG3	KLG4	KLG5	KLG6	KLG7	KLG8	KLG9
0	0	0	0	0	0	0	0	0	0
1	33.63	31.6	30.55	18.77	17.32	16.04	18.71	17.43	15.98
2	49.75	46.22	42.25	28.07	27.05	26.42	35.42	34.14	30.41
4	64.21	58.72	53.07	37.09	36.45	35.22	51.47	50.24	44.94
6	78.35	70.21	64.86	46.12	45.32	43.06	66.57	65.79	57.13
8	90.4	81.08	74.12	55.58	53.03	50.09	80.09	78.38	69.19
10	95.39	91.1	84.55	63.82	60.33	56.59	91.01	88.12	79.01
12	99.43	98.39	93.99	88.78	81.44	86.15	99.24	95.83	88.22



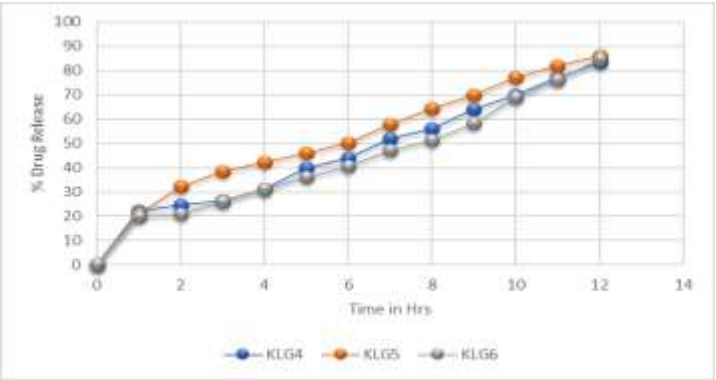


Fig. 9: *In-vitro* release of liposomal Gel KLG1- KLG3 &

Fig.**10: *In-vitro release* Gel KLG4- KLG6**

Fig. 11: *In-vitro* release profile of Ketoconazole from liposomal Gel KLG7- KLG9

Formulation code	Zero order	First order	Higuchi	Korsmeyer-Peppas		Hixon-Crowell	Erosion
	r^2	r^2	r^2	r^2	n	r^2	r^2
KLG 1	0.996	-0.725	0.998	0.998	0.829	0.910	-0.910
KLG2	0.982	-0.817	0.995	0.994	0.803	0.987	-0.987
KLG 3	0.995	-0.788	0.998	0.998	0.809	0.975	-0.975
KLG 4	0.996	-0.914	0.992	0.995	0.712	0.996	-0.996
KLG 5	0.995	-0.925	0.990	0.991	0.724	0.997	-0.997
KLG 6	0.996	-0.915	0.985	0.981	0.757	0.996	-0.996
KLG 7	0.996	-0.802	0.997	0.999	0.907	0.971	-0.971
KLG 8	0.988	-0.849	0.996	0.995	0.953	0.980	-0.980
KLG 9	0.999	-0.827	0.994	0.999	0.955	0.971	-0.971

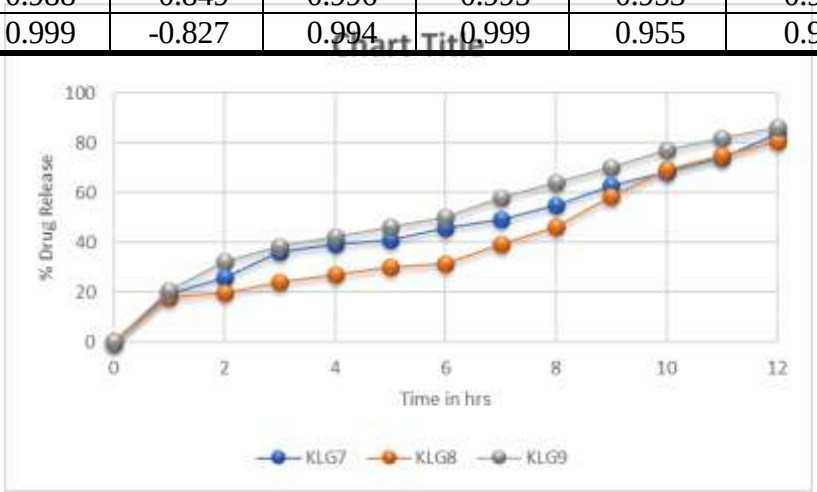


Table 6: Regression analysis of the *in vitro* release data according to various release kinetic models

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