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DEVELOPMENT AND CHARACTERIZATION OF TRASTUZUMAB ETHOSOMAL DRUG DELIVERY SYSTEM

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Abstract:

Trastuzumab-loaded ethosomes were developed and characterized to enhance transdermal delivery and provide controlled drug release. Eight formulations containing varying concentrations of lipid were prepared and evaluated for Preformulation parameters, vesicle characteristics, in vitro release behavior and stability. Trastuzumab was identified as a white to off-white, tasteless and odorless crystalline powder with a melting point of 70–80 °C and good aqueous solubility. FT-IR studies confirmed the absence of drug–excipient interactions. The ethosomal vesicles exhibited spherical morphology with particle sizes ranging from 186 nm to 253 nm and a negative zeta potential (–23 mV to –30 mV), indicating good colloidal stability. Entrapment efficiency was high (76.86 %–85.10 %), with formulation F8 showing the highest value and achieving 97.98 % cumulative drug release within 12 h. Drug-release kinetics for the optimized formulation followed Higuchi's diffusion model (R^2 = 0.973). Accelerated stability testing (40 ± 2 °C/75 ± 5 % RH for 3 months) revealed no significant loss of drug content, which remained above 94 %. The results demonstrate that ethosomes can effectively encapsulate trastuzumab and provide a stable, sustained-release transdermal delivery system suitable for further therapeutic development.

Keywords: *Trastuzumab, Ethosomes, FTIR Studies, In vitro drug release studies, Stability studies*

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INTRODUCTION:

In recent years, nanotechnology-based drug delivery systems have emerged as promising strategies to improve the delivery of macromolecular drugs, including monoclonal antibodies. Among these systems, ethosomes have gained considerable attention due to their unique composition and superior permeation-enhancing properties.[1] Ethosomes are soft, malleable lipid vesicles composed mainly of phospholipids, a high concentration of ethanol, and water. The presence of ethanol imparts high deformability to ethosomal vesicles, enabling them to penetrate deep into the skin layers and cross biological membranes more effectively than conventional liposomes.[2]Ethosomal drug delivery systems offer several advantages, including enhanced drug encapsulation efficiency, improved stability of sensitive biomolecules, controlled drug release, and increased bioavailability.[3]Trastuzumab, a humanized monoclonal antibody targeting the HER2 receptor, has significantly improved survival and prognosis in patients with HER2-positive breast cancer by inhibiting receptor signalling, inducing antibody-dependent cellular cytotoxicity, and preventing tumour cell proliferation.[4]The development of a trastuzumab-loaded ethosomal drug delivery system represents a novel approach to overcome the limitations of conventional dosage forms.[5] By encapsulating trastuzumab within ethosomal vesicles, it is possible to protect the drug from degradation, enhance its permeation across biological barriers, and achieve sustained and targeted delivery at the site of action.[6] Moreover, such a delivery system may improve patient compliance by offering a non-invasive alternative to injectable therapy.Therefore, the present research focuses on the development and characterization of a trastuzumab ethosomal drug delivery system.[7] This research is expected to provide valuable insights into the potential of ethosomal carriers as an effective and patient-friendly delivery platform for monoclonal antibody-based cancer therapy.[8]

MATERIALS

Trastuzumabwas Procured from Hetero Labs, Hyderabad. Phosphotidyl choline wasobtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

METHODOLOGY:

Formulation development

Table-1: Composition of Trastuzumab Ethosomes (F1 to F4)

S.No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
1	Trastuzumab	8	8	8	8	8	8	8	8
2	Phosphotidyl choline	100	200	300	400	500	600	700	800
3	Ethanol	5	5	5	5	5	5	5	5
4	Water	10	10	10	10	10	10	10	10

Procedure

Ethosomal formulations were prepared by using the cold method. This is the most common and widely used method for the ethosomal preparation. Phospholipid and drug and other pharmaceutical ingredient listed in table were dissolved in Ethanol in a covered vessel at room temperature with vigorous stirring. This mixture was heated to 30^0C ± 10C and a fine stream of distilled water was added slowly, with constant mixing at 100 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 30^0 C ± 10C. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 40 C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator.[9]

CHARACTERIZATION

Particle size:

All the prepared batches of ethosomes were viewed under microscope to study their size. Size of Ethosomes from each batch was measured at different location on slide by taking a small drop of ethosomes on it and average size of ethosomes were determined.[10]

SEM analysis

The morphology of Ethosomes was studied by a scanning electron microscope. For this purpose, the sample was lyophilized and placed on aluminum stubs and the surface was coated with a layer of gold particles using a sputter coater. The shape of the Ethosomes was determined by scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA.[11]

Zeta Potential:

The zeta potential means the charges which are present on the surface of Ethosomes. The many time the charge is present on the surface of Ethosomes. This charge is come due to the component or ingredient which was used during the manufacturing. Some charge is must be required on surface of all transferosome present in formulation, due to some charge all Ethosomes particle repeal to each other and coagulation of particle are avoided. The zeta potential of Ethosomes was taken in zeta sizer instrument having Malvern software. The analysis of sample was carried out at 25°c with the angle of detection 90°. The ideal zeta potential value must be required in range between +30 to -30mV. These ranges prevent the aggregation of Ethosomal particle.[12]

Entrapment efficiency

To determine entrapment efficiency, a known amount of the prepared formulation (for example, a nanoparticle or vesicular suspension) is first separated into two fractions: the free or unentrapped drug and the carrier-associated (entrapped) drug. Separation is usually achieved by centrifugation, ultrafiltration or dialysis; for vesicular systems like liposomes or niosomes, high-speed centrifugation is common. The supernatant, which contains the unentrapped or free drug, is carefully collected and analyzed—typically by UV–visible spectrophotometry to quantify the amount of free drug. The total drug content of the formulation is determined separately by lysing or disrupting the vesicles or particles and measuring the total drug present. Entrapment efficiency is then calculated using the formula:[13]

Entrapment efficiency (%)=Total drug−Free drug/Total drug×100

This percentage expresses how much of the original drug dose is actually entrapped within the delivery system, providing a measure of the formulation's loading capacity and overall effectiveness.

***in-vitro* Drug release studies:**

The *in-vitro* study of drug permeation through the Dialysis membrane was performed using a modified Franz type glass diffusion cell. The modified cell having higher capacity is (10 ml) is used to maintain sink condition. The samples were analyzed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal.[14]

Percentage of drug release was determined using the following formula.

$$\text{Perentage drug release} = \frac{\text{Da}}{\text{Dt}} \times 100$$

Where, Dt = Total amount of the drug in the patch

Da = The amount of drug released

Conditions:

Medium: Phosphate buffer pH 7.4

RPM: 200

Temperature: 37 ± 0.5^0C

Time intervals: 1, 2, 3, 4, 5, 6, 8 , 10 and 12 hours

Release kinetics: [15]

The release kinetics can be understood basically by applying the obtained data to the release kinetics models.

Zero order kinetics

$$C = K0t$$

K0 - rate constant for Zero-order (concentration/time) t - Time (h).

First orderkinetics

$$Log\ C = LogC0 – Kt / 2.303$$

Where C0 - Initial concentration of drug K = constant first order and t = Time (h)

Higuchi Model

$$Qt = Kt1/2$$

Where Qt - Amount of the drug release drug in time t K- Kinetic constant and t- is time in hrs**Korsmeyer Pappas** Model

$$Mt / M = Kt\ n$$

Where, Mt - amount of the released drug at time t, M- Overall drug amount released after 8 hrs. K- Diffusion constant n- Diffusion exponent mechanism of release of drug.

Stability studies:

Stability studies are performed to determine how the quality of a drug substance or drug product varies with time under the influence of environmental factors such as temperature, humidity and light, and to establish a suitable shelf life and recommended storage conditions. According to ICH Q1A(R2) guidelines, the study begins with preparation of a stability protocol that specifies the number of batches, packaging type, storage conditions, testing intervals and acceptance criteria. Marketed or pilot batches are packed in the intended commercial container and placed in stability chambers maintained at defined conditions—commonly long-term (25 ± 2 °C / 60 ± 5 % RH for 12–24 months), accelerated (40 ± 2 °C / 75 ± 5 % RH for 6 months) and, if required, intermediate (30 ± 2 °C / 65 ± 5 % RH).[16]

RESULTS AND DISCUSSION:

Drug - excipient compatibility studies (FT-IR):

Thecompatibilitybetweenthedrugandtheselectedlipidandotherexcipientswasevaluatedusing FTIR peak matching method.There was no appearanceor disappearance of peaks in thedrug-lipid mixture, which confirmed the absence of any chemical interaction between thedrug,lipid andother chemicals.

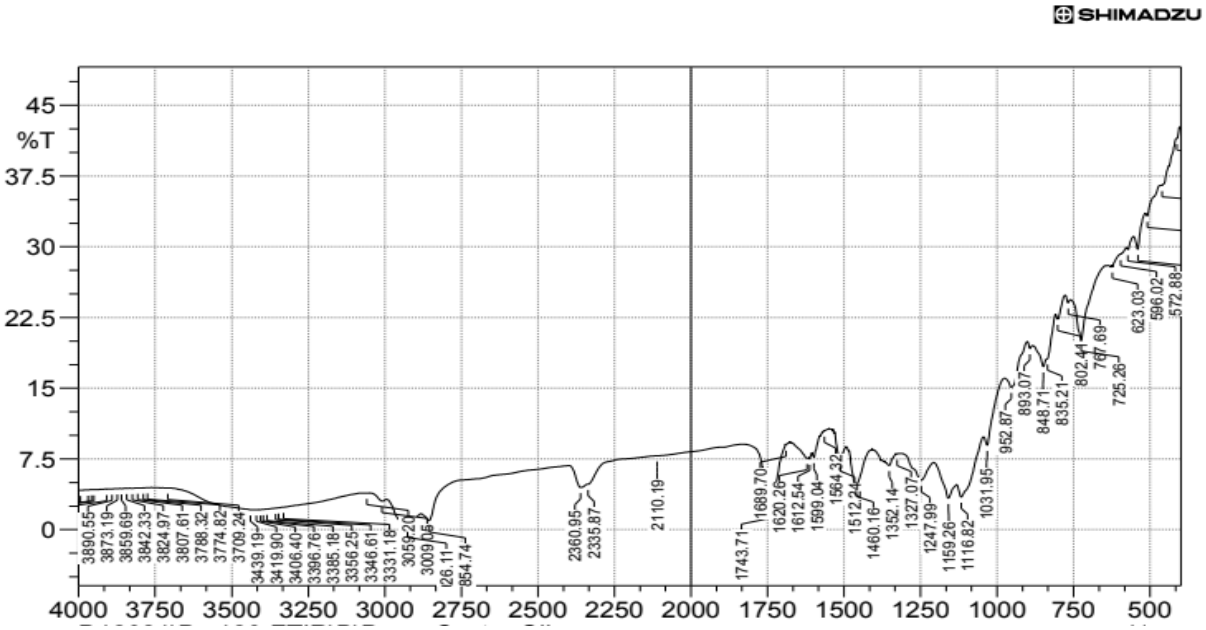

Fig-1: FTIR Studies of Trastuzumab

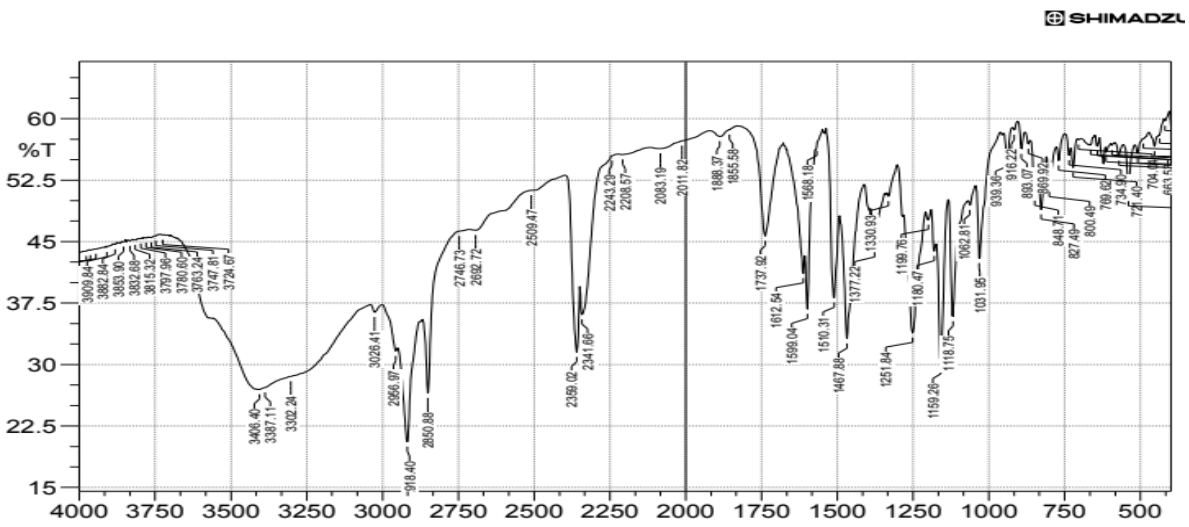

Fig-2: FTIR Studies of optimized formulation

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of Pure drug and physical mixture of drug and excipients were studied. The characteristic absorption of peaks was obtained as above and as they were in official limits (±100 cm^{-1}) the drug is compatible with excipients.

Determination of Vesicle morphology and Size

Themorphologicalcharacteristicsofformulated ethosomes werecarriedbyusingScanningelectron microscopy (SEM). A small drop of Ethosomes was placed between two rivets fixedon a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85^{0}C for 30 min to sublime the surface moisture. Finallythesample was coated with gold and allowed the SEM to capture the images at a temperature of - 120^{0}c and voltage of 5kV.

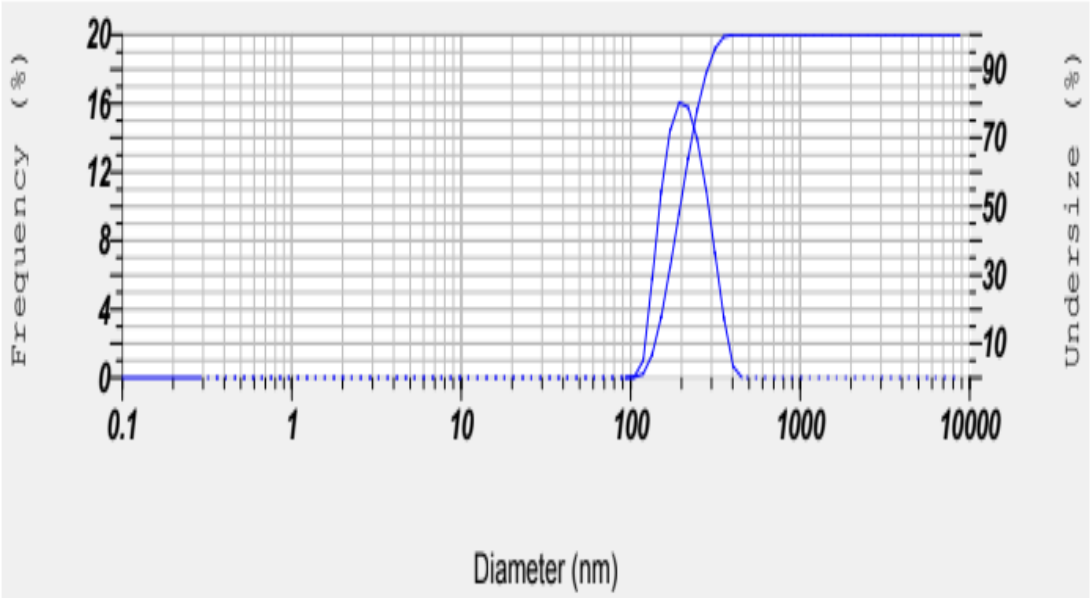

Fig-3: Particle size analysis of Optimized ethosomes

Zeta potential

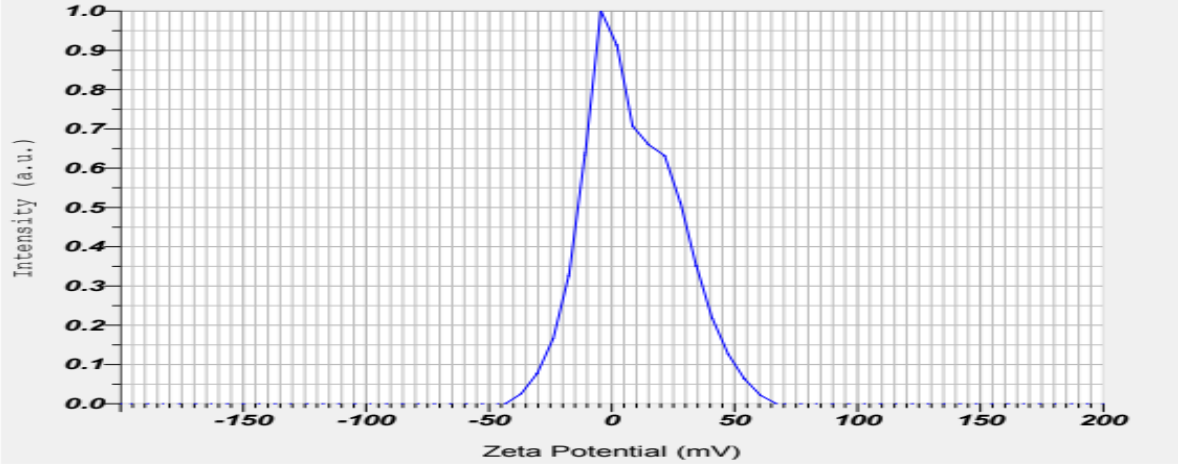

Fig-4: Zeta potential of Optimized formulation

Table-2: Evaluation Studies of particle size and Zeta potential Ethosomes

F. No	Particle size (nm)	Zeta potential
F1	232	-26
F2	253	-28
F3	222	-23
F5	219	-25
F6	201	-29
F7	226	-30
F8	186	-24

SEM Analysis

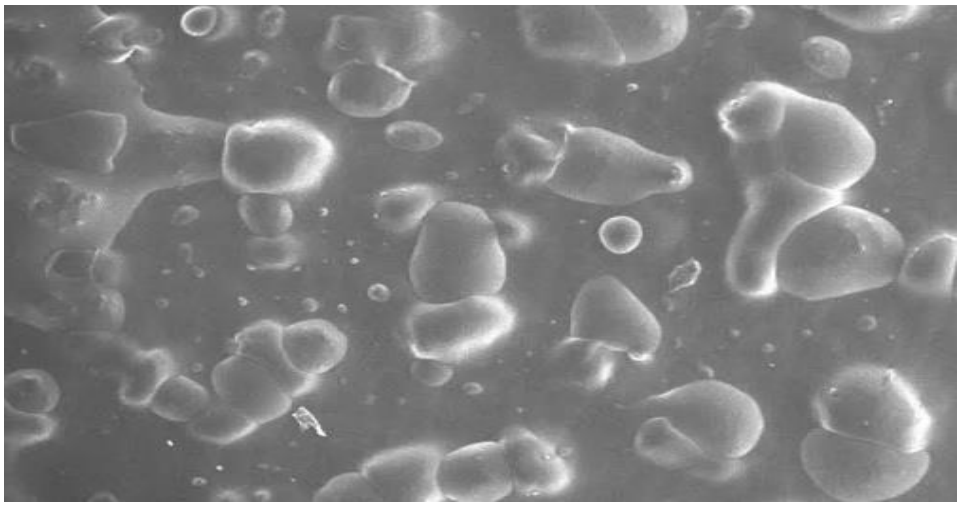

Fig-5: SEM Analysis of Ethosomes

Entrapment Efficiency:

Table-3: Drug entrapment efficiency

F. code	Drug entrapment efficiency
F1	82.63
F2	79.50
F3	80.20
F4	81.36
F5	80.36
F6	76.86
F7	84.42
F8	85.10

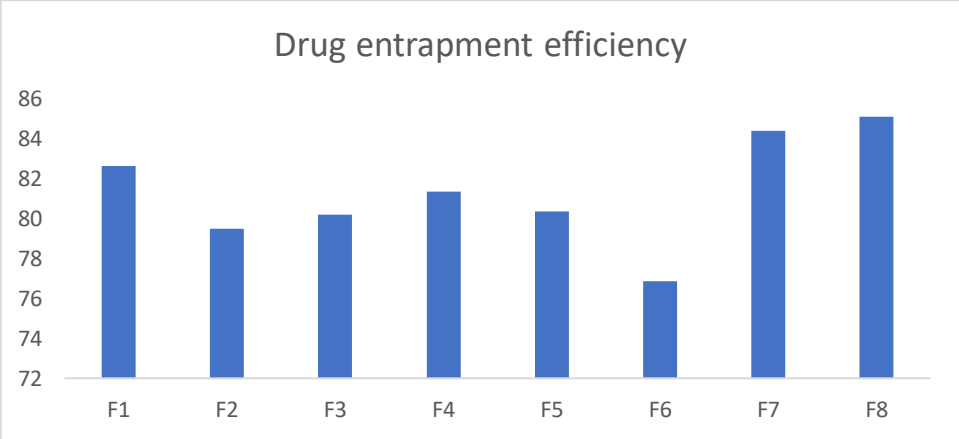

Fig-6: Drug entrapment efficiency of all formulation

***In vitro* release study:**

Phosphate buffer pH 7.4 was used as medium for the release studies and good linearity was observed in the plotted standard graph with a correlation coefficient of 0.998.

Table-4: *In vitro* drug release profiles of Ethosomes (F1-F8)

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0		0	0
1	16.10	16.54	17.25	16.82	17.410	17.12	18.51	19.20
2	27.09	28.65	29.66	27.10	28.40	28.10	28.96	29.55
3	36.38	37.05	38.05	39.96	36.25	35.10	35.96	38.10
4	42.55	44.92	45.25	46.96	48.10	47.82	48.85	48.47
6	54.98	55.94	56.50	58.20	59.30	54.94	56.98	58.92
8	67.10	68.69	69.10	70.50	71.52	72.56	73.99	78.21
10	81.23	81.34	82.10	83.64	85.84	86.22	86.36	85.82
12	92.69	93.20	95.32	94.66	96.82	96.50	96.75	98.32

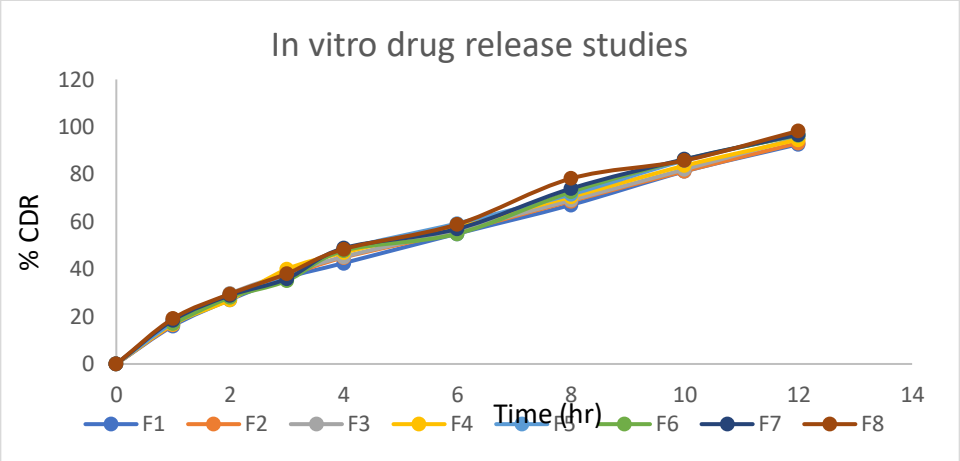

Fig-7: In vitro drug release studies of F5-F8 formulations

Kinetic modelling of drug release

All the 8 formulation of prepared ethosomes were subjected to in vitro release studies these studies were carried out using diffusion apparatus.

The dissolution medium consisted of 900 ml of Standard buffer pH 6.8 period of time.

The results obtaining in vitro release studies were plotted in different model of data treatment as follows:

1. Cumulative percent drug released vs. time (zero order rate kinetics)
2. Log cumulative percent drug retained vs. time (First Order rate Kinetics)
3. Cumulative percent drug released vs. square root of time (Higuchi's Classical Diffusion Equation)
4. Log of cumulative % release Vs log time (Pappas Exponential Equation)

Zero order kinetics

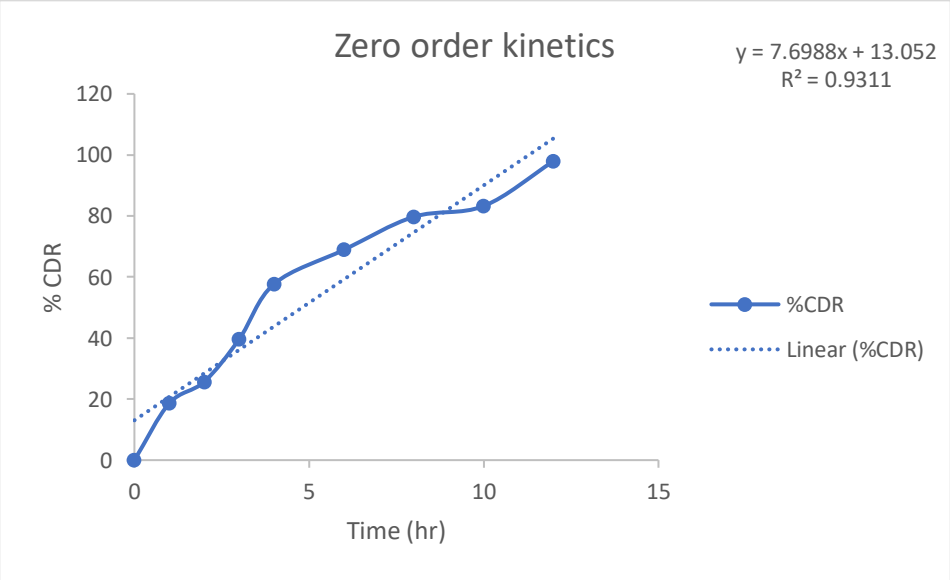

Fig-8: Zero order kinetics of optimized formulation

First order kinetics

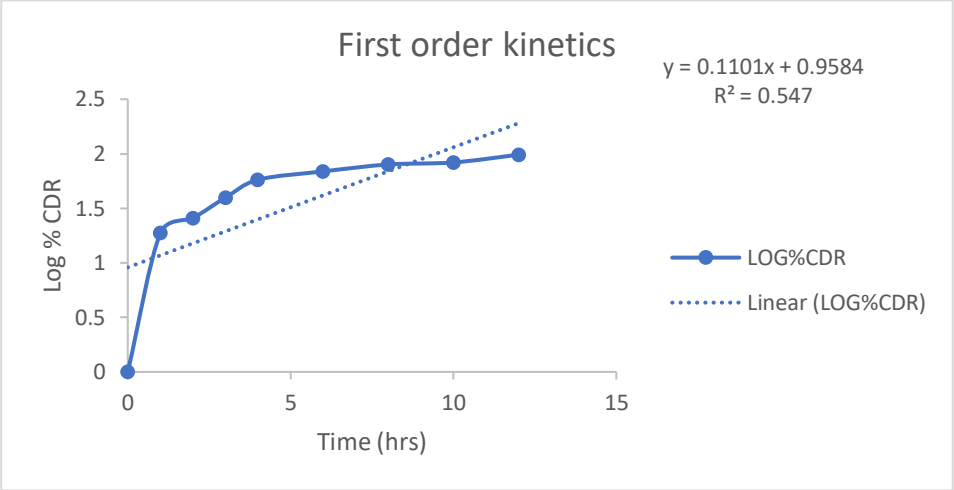

Fig-9: First order kinetics of optimized formulation

Higuchi model

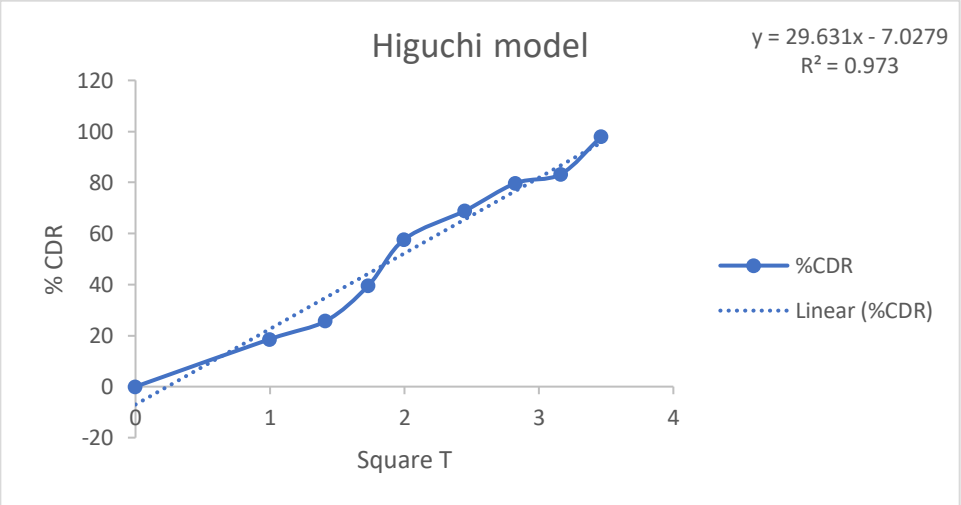

Fig-10: Higuchi model of optimized formulation

Korsmeyer peppas

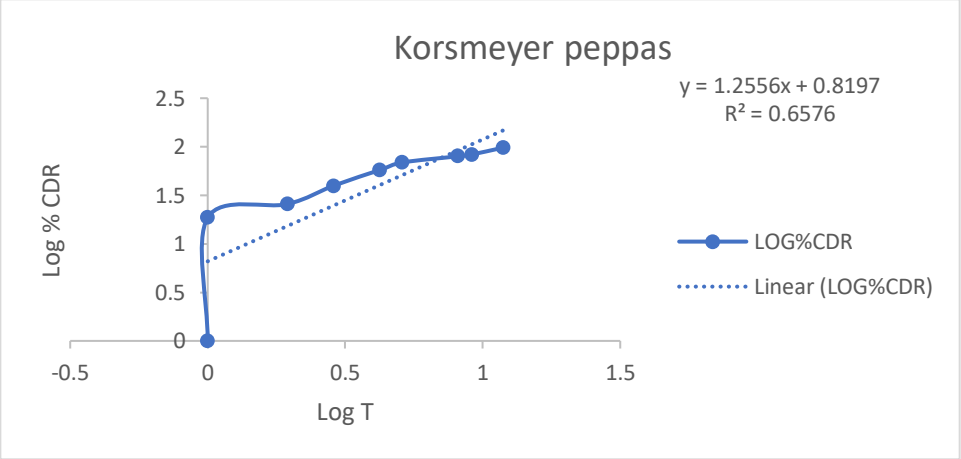

Fig-11: Korsmeyer peppas of optimized formulation

The kinetic values obtained for formulation F8 were shown. The values of in vitro release were attempted to fit into various mathematical models.

Stability studies

Optimized formulations F8 was selected for accelerated stability studies as per ICH guidelines.

Table-5: Stability studies of optimized formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

Formulation Code	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-8	97.98	96.38	95.12	94.32	Not less than 85 %
F-8	97.98	96.25	95.33	94.02	Not less than 85 %
F-8	97.98	96.30	95.10	94.25	Not less than 85 %

CONCLUSION:

The study successfully developed a stable ethosomal delivery system for trastuzumab. Pre-formulation tests verified the drug's purity and compatibility with selected excipients, while SEM, particle size and zeta potential analyses confirmed the formation of stable nanosized vesicles. The optimized formulation (F8) showed high drug entrapment and a controlled diffusion-based release profile with excellent stability under accelerated conditions. These findings indicate that ethosomes are a promising carrier for trastuzumab, offering efficient encapsulation, sustained release and good shelf-life potential for targeted drug delivery applications.

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