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DESIGN AND EVALUATION OF ZERO-ORDER DRUG-RELEASING RIVASTIGMINE TRANSDERMAL SYSTEM

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Keywords:

Rivastigmine, Alzheimer's disease, Maltodextrin, Polycaprolactone, Microspheres, Transdermal System.

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ABSTRACT: Rivastigmine is used for the treatment of mild to moderate dementia of the Alzheimer's type. Transdermal delivery of Rivastigmine is preferable to improve gastrointestinal tolerability. A novel transdermal system with zero-order release kinetics was developed following a hybrid technique in a combination of the micro reservoir and adhesive dispersion system. The transdermal system was prepared by incorporating rivastigmine in adhesive matrix layer in which rivastigmine-loaded microspheres were dispersed. Microspheres were prepared by spray drying using poly-e-caprolactone and maltodextrin (1:1 ratio) as carriers in various drug: polymer ratios. Microspheres with1:2 drug: polymer ratio (A1) showed desired particle size, yield, assay and *in-vitro* drug release and were found to be suitable for designing the transdermal system. A1 was dispersed into silicon adhesive layer during the preparation of the patch with a calculated amount of rivastigmine. Transdermal patch with 18 mg rivastigmine was optimized by evaluating ratios of adhesive matrix and microsphere content following DOE where 13 formulations were evaluated for various physical and chemical properties. 5 among 13 formulations were found to be satisfactory and subjected for *in-vitro* drug release studies. 4 among 5 formulations have shown satisfactory drug release; hence, they were also subjected for *ex-vivo* permeation studies. *In-vitro* release kinetic data and *ex-vivo* permeation data resulted best with the formulation comprising 150 mg of silicon adhesive containing 11.33 mg of Rivastigmine and 20 mg of microspheres containing 6.67 mg of rivastigmine (F5). F5 was subjected for stability as per ICH guidelines and was found stable up to six months at accelerated conditions.

INTRODUCTION: Rivastigmine is a parasympathomimetic agent or cholinergic agent used in the treatment of mild to moderate dementia of the Alzheimer's type.

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Rivastigmine is a cholinesterase inhibitor that acts by inhibiting both butyrylcholinesterase and acetylcholinesterase [1-3]. Alzheimer's disease a progressive brain disorder that decreases the patient's mental ability and causes loss of mental, behavioural, functional, and learning abilities [4].

The first cholinesterase inhibitor approved as a transdermal patch was the rivastigmine transdermal patch. The pharmacokinetic profile of rivastigmine transdermal patch from the clinical data suggested that smooth and continuous drug delivery translates

into improved tolerability profile compared with the oral administration [5, 6]. Additional benefits include improved adherence, increased convenience, ease of use, and a reduced impact on daily activities. Gastrointestinal adverse effects were observed after oral administration of rivastigmine owing to rapidly achieved maximum concentrations in the CNS [7-10].

A zero-order drug release from rivastigmine patch provides a stable concentration of the drug in the CNS, thus improves gastrointestinal tolerability, and thus, maximum therapeutic dose can be administered. Trans-dermal drug delivery systems are extensively recognized ways of drug delivery and are used in the treatment of various diseases and disorders. Controlled release transdermal formulations may offer stable systemic drug level concentrations avoiding first-pass metabolism and improved bioavailability. Therapeutic benefits of trans dermal systems reflected higher marketing potential. The utilization of transdermal drug delivery systems improves treatment, especially in the treatment of Alzheimer's disorder. Various transdermal formulations were successfully developed with the drugs of therapeutic category like anaesthetics, anti-inflammatory agents and hormones, *etc*. In this research, an attempt was made to develop a controlled release transdermal system of rivastigmine following a novel method of designing a transdermal patch.

MATERIALS AND METHODS:

Materials: Rivastigmine USP (free base) was received as a gift sample from Aarti Chemicals, Mumbai, India. Poly-e-caprolactone of (Mw 14000) was procured from Sigma Aldrich, Germany. Vitamin-E TPGS USP-NF grade was procured from BASF, Germany. Maltodextrin (Glucidex IT17) of USP-NF grade was procured form Roquette Pharma, France. Sodium metabisulfite of Ph. Eur. grade was procured from Merck India. Aluminum vapor-coated pigmented polyethylene polyester backing membrane (3M Scotchpack 1109) and Fluoropolyester coated release liner (3M Scotchpack 9744) were received as gifts samples from 3M Corporation, USA. Silicon adhesive USP-NF grade (7-4302 BIOPSA) was procured from DOW Corning, USA. Solvents like methanol, ethyl acetate, heptane, and dichloromethane of HPLC grade were procured from Merck, India. Ethanol 99.9% was procured from fisher scientific, India.

Methods: A novel controlled transdermal release system was developed with the aim of zero-order release kinetics, which was a combination of micro reservoir system and adhesive dispersion system. The novel transdermal system development was done in two steps.

Preparation of Rivastigmine Loaded Microspheres:

Preparation of Transdermal System: by incorporating rivastigmine drug in adhesive matrix layer which was dispersed with rivastigmine microspheres.

Preparation of Rivastigmine Microspheres: The spray drying technique was used to prepare rivastigmine microspheres where poly-e-caprolactone and maltodextrin (in 1:1 ratio) are used as polymeric drug carriers. Different ratios of drug to polymer were used to prepare the microspheres, *i.e.,* 1:2, 1:4, 1:6, 1:8, 1:10 & 1:12. The preparation was initiated by dissolving poly-e-caprolactone in methanol, maltodextrin, vitamin-E TPGS, and sodium metabisulfite in water and rivastigmine in ethanol. Thus three clear solutions were homogenized (Kinematica® Polytron PT 3100D) at 3000 rpm where an opaque dispersion appeared.

Then the homogenized mixture was spray-dried to form microspheres in a spray drier of 4 inches internal diameter chamber (JISL, LSD-48) at a flow rate of 2.5 mL to 3.0 mL per min using 1 mm spray nozzle maintaining inlet temperature, outlet temperature, and product temperature at 110 °C to 120 °C, 60 °C to 70 °C and below 55 °C respectively. The atomization pressure was maintained at 30 ± 3 PSI and aspiration rate of -140 mmwc (1400 rpm and in the machine). The dried microspheres were collected sealed in glass containers and were stored in desiccators until analysis [11, 12]. The composition of microspheres is presented in **Table 1**.

Evaluation of Rivastigmine Loaded Microspheres: The prepared rivastigmine microspheres were evaluated for various physical, chemical and drug release parameters. The methods used for the evaluation were explained below.

Description and Appearance of Microspheres: Optical microscopy was used to evaluate the description of rivastigmine microspheres, where the microspheres were analyzed for their spherical appearance and existence as separate entities.

Particle Size Distribution Analysis: Optical microscopy was used to determine the particle size of rivastigmine microspheres [13]. The average size and size distribution of rivastigmine microspheres was determined using an optical microscope (Olympus CX21i) fitted with an eyepiece micrometer which was pre-calibrated using a stage micrometer. The mean diameter was calculated after evaluating the size of 100 microspheres.

Mean particle size = (Σ (Mean particle size X Weight fraction) / (Σ(Weight Fraction)

Surface Morphology by Scanning Electron Microscopy: A scanning electron microscope (Zeiss Sigma HDVP, Switzerland) was used to evaluate the surface morphology of rivastigmine microspheres. Small quantities (5 to 10 mg) of microsphere samples were dispersed on a standard half-inch diameter by half-inch tall aluminium SEM specimen mount stubs aided with double stick carbon tape and were placed in the sputter coater chamber. The chamber was ensured a vacuum of 0.08 mbar, and samples were coated with gold for 10 min. The processed samples were placed in an SEM chamber and examined for surface morphology by magnifying up to 1KX to 10KX at EHT 15.0 kV.

Percentage Yield: The percentage yield of the rivastigmine microspheres was calculated using the weight of microspheres obtained in relation to the sum of the weights of starting materials used for the preparation of microspheres [14].

% Yield = (The amount of microspheres obtained) / (The theoretical amount of materials used × 100

Drug Content by Assay: Percentage drug content in rivastigmine microspheres was determined by extracting the drug from the microspheres where 10 mg equivalent rivastigmine microspheres were taken in 25 mL volumetric flask to which 5 mL of dichloromethane and 5 mL 1% v/v acetic acid was added, and microspheres were allowed to disintegrate completely, and then the volume was adjusted to 25 mL with a solvent mixture of Methanol: Acetonitrile (1:1 ratio). The solution was sonicated for 15 min and was then filtered using 0.45-micron nylon filter. The filtered samples were analyzed for drug content using HPLC.

Encapsulation Efficiency: The filter flush technique was used to evaluate encapsulation efficiency. 25 mg of rivastigmine equivalent microspheres (weight compensated after the determination of drug content by assay method) were taken in a sintered glass filter covered with zirconium beads of 0.5 mm and 1.0 mm diameter in 1:1 ratio by weight. 5 mL of ethanol was flushed over the surface of the microsphere placed over the beads, and the solvent was allowed to wash and flow down. The filtrate was collected from the bottom and evaluated for the free drug content eluted in it by the HPLC method. Encapsulation efficiency was determined by deducting free drug content from actual drug content in microspheres and dividing it with the actual drug content, and multiplied with 100 [15].

% Encapsulation Efficiency = (Actual drug content-Free drug content in eluent) / (Theoritical drug content) × 100

***In-vitro* Drug Release Studies:** USP type II dissolution apparatus was used to determine *in-vitro* drug release from the rivastigmine microspheres. 0.9% sodium chloride solution was used as a medium, and the paddle speed was maintained at 50 rpm. 25 mg drug equivalent rivastigmine microspheres were filled in a size 1/ size 2 capsules and were inserted in USP-type spiral sinker.

The capsule inserted into the sinker was dropped into the dissolution media (0.9% sodium chloride 750 mL) before starting the agitation. The samples were collected at three-time points *i.e*. at 1 h, 2 h and 3 h. 5 mL of the sample was collected at each time point and was filtered using 0.45 µ nylon filter and was then analyzed for drug content using HPLC.

Preparation of Transdermal Patches: Novel zero-order release transdermal systems of rivastigmine were developed by performing design of experiment studies. Optimization of the formulation was performed by using central composite design in the application Statease Design Expert® USA.

Study Design:

Central Composite Design: Central composite design was selected for the evaluation where the selected design model was pre-evaluated for design acceptability, and the design was executed in 13 runs [16-18]. The experiments were executed as per the design runs generated by the tool where two factors having three levels (0, ±1) of formulation variables were considered *i.e.* X1: Silicon adhesive (125 mg, 150 mg and 175 mg), X2: rivastigmine loaded microsphere base (10 mg, 20 mg, and 30 mg). The influence of the selected variables was evaluated on four critical responses, *i.e.*, Y1 (Thickness), Y2 (Folding endurance), Y3 (Weight of the patch content) & Y4 (*In-vitro* dissolution at 180 min). The selected responses were considered for optimization and evaluated for design space establishment. The details of the experiments generated is mentioned in **Table 2**.

TABLE 1: THE COMPOSITION OF RIVASTIGMINE MICROSPHERES

Formulation Code	Drug: Polymer	Drug (g)	Polymeric carrier(g)	Total drug and polymer mixture	Vitamin E TPGS * (mg)	Sodium Meta Bisulfite * (mg)
A1	1:2	0.9	1.8	2.7	6.75	6.75
A2	1:4	0.9	3.6	4.5	11.25	11.25
A3	1:6	0.9	5.4	6.3	15.75	15.75
A4	1:8	0.9	7.2	8.1	20.25	20.25
A5	1:10	0.9	9	9.9	24.75	24.75
A6	1:12	0.9	10.8	11.7	29.25	29.25

Note: *Vitamin E TPGS and Sodium Metabisulfite were incorporated at 0.25% concentration with respect to the weight of drug and polymeric carrier mixture.

TABLE 2: DETAILS OF RIVASTIGMINE PATCH COMPOSITIONS EVALUATED USING CENTRAL COMPOSITE DESIGN

Std.	Run	Factor X1: Silicon Adhesive (mg)	Factor X2: Microsphere Base (mg)
1	1	150	20
6	2	175	20
13	3	150	20
4	4	150	10
5	5	150	20
10	6	125	20
3	7	125	10
11	8	125	30
12	9	175	30
9	10	175	10
8	11	150	20
7	12	150	20
2	13	150	30

Preparation of Transdermal Systems of Rivastigmine: Drug containing adhesive layer was prepared by mixing silicon adhesive with anti-oxidant sodium metabisulfate, a part of API, methanol, silicon oil, and heptane. To the above clear solution weighed amount of microspheres were added and mixed. Then above mixture was cast over a backing membrane and dried for 24 h. Then release liner was applied on the adhesive side, and the system was cut into the suitable size [19].

Evaluation of Microspheres Loaded Adhesive Dispersion Patches:

General Appearance: The appearance and surface integrity of the patches were observed with naked eye.

Thickness: The thickness of the whole patch including release liner was determined at six different points using digital micrometer. The thickness of the backing membrane and release liner were measured before casting drug-containing adhesive solution on to it [19]. The actual thickness of the drug containing adhesive matrix was calculated with the following formula:

Thickness of the Drug Containing Adhesive Matrix = Thickness of the whole patch system-(Thickness of the backing membrane + Thickness of the release liner)

Weight Variation: Six patches were selected randomly and weighed individually on a semi-micro balance (Sartorius TE-124, Germany), then

the release liner and backing membrane (by washing out the drug matrix with solvent) from each patch system were separated and weighed individually. The together weight of the release liner and backing membrane was subtracted from the whole weight of the patch system for obtaining the drug matrix layer weight. Accordingly, after 6 determinations, the average weight of the patch was calculated [19].

Weight of the drug matrix layer = Weight of the whole patch system - (weight of the backing membrane + Weight of the release liner)

Determination of the Drug Content of the Patch: A circular patch of 3.569 cm diameter or 3.163 cm L × B having a surface area of 10 cm^{-2} was extracted in 100 mL of ethanol. Then the solution was filtered and analyzed by the RP-HPLC method (Waters 2695, USA). Three replicate measurements were performed for each formulation [19].

Moisture Uptake Studies: The prepared patches were evaluated for moisture absorbing capacity. Six patches were selected randomly and weighed individually, kept in a desiccator containing 100 mL of a saturated solution of aluminium chloride at room temperature for 24 h which maintains the RH of 79.5%. The patch was weighed periodically till it showed a constant weight. % moisture uptake can be calculated using the following formula [19].

% Moisture up take = (Final weight of the patch-Initial weight of the patch) / (Initial weight of the patch × 100

Moisture Content Determination: Six patches from each formulation were selected and weighed individually. Then release liners from each patch were removed and weighed separately. The liner-separated patches were spread on a mesh and were kept in a desiccator containing anhydrous calcium chloride at room temperature, the weight of each patch was monitored after every 6 h till they showed a constant weight. % moisture content was determined using the following equation [19].

% Moisture content = (Initial weight of the patch-Final weight of the patch) / (Final weight of the patch × 100

Flatness: The flatness of the patch was determined to evaluate the constriction behaviour of the patch after application to the skin. The transdermal patches were cut into the length of the longitudinal strip of each strip was measured initially and after keeping aside. Variation in the length of the patch was determined using formula for % constriction. Zero % constriction is equivalent to 100% flatness.

% Constriction = (Initial length of the patch-Final length of the patch) / (Final length of the patch × 100

Folding Endurance: Folding endurance is a value indicating the number of times the patch could be folded at the same place without any crack/break. Six patches of each formulation (2 cm × 2 cm) were selected randomly and were folded in a repeated manner at the same place till it broke. The number of times the film was folded at the same place without braking gave the folding endurance value or folded up to 200 times if the patch does not show any crack [19].

Measurement of Mechanical Properties: Mechanical properties of the patches were evaluated using a fabricated tensile strength testing apparatus shown in **Fig. 1.** A film strip with dimensions of 60 × 10 mm was held in between two clamps. Then weights were added to the pan led to elongation of the patch. The force required for breaking the patch is noted F [19].

FIG. 1: FABRICATED INSTRUMENT TO DETERMINE MECHANICAL PROPERTIES OF THE PATCH

Mechanical properties of the patch were calculated using the following formulae.

Tensile Strength (kg/ mm^2 = (Force at break (kg)/ (Initial cross sectional area of the sample (mm^2)

Elongation at break (% mm^2) = (Increase in lenght (mm) / (Original Length (mm) × 100 / (Cross sectional area)

Evaluation of Hardness of Patch: The hardness of the patch was determined using the fabricated instrument for detecting the hardness of the patch shown in **Fig. 2**. The hardness testing apparatus consists of a wooden stand of 8 cm in height and a top area of 8 × 8 cm. A hole of 0.2 cm diameter is made in the center of the wooden top. A small metallic pan of a diameter 6 cm is fixed on one end of a 2 mm thick smooth iron rod. Rod had the pan on its upper end is inserted into the hole of the wooden top, and its lower sharp end was placed on a metal plate.

The battery of 3V was used to make an electric circuit. Assembly was set in such a way that the bulb lights up only when the circuit is complete via the contact of the metal plate and the sharp end of the rod. The patch (release liner removed) was placed (drug matrix layer facing towards rod) between the metal plate and the sharp end of the iron rod and the weights were gradually added on to the pan. The total weight required to penetrate the patch is indicated by the lighted bulb, which was noted as observation [19].

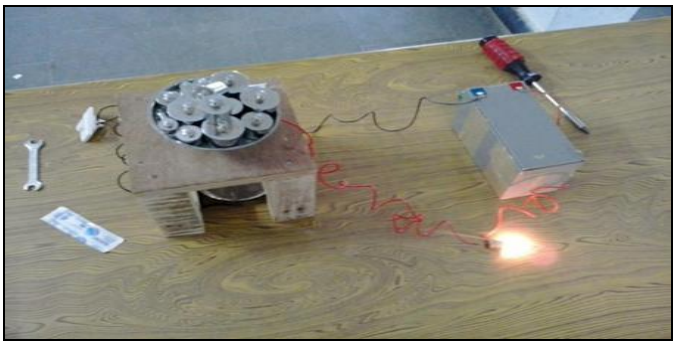

FIG. 2: FABRICATED INSTRUMENT TO DETERMINE HARDNESS OF THE PATCH

***In-vitro* Dissolution Studies:** *In-vitro* dissolution study was conducted using USP Type 5 apparatus *i.e.* paddle over disc. Transdermal patch holder with 17 mesh screen was used to hold the release liner separated patch, and it was dropped in the dissolution medium (0.9% sodium chloride 500 mL) at a temperature 32 ±0.5 ºC. Apparatus was maintained with a paddle speed of 50 rpm and samples were withdrawn periodically *i.e.* after 1, 2, 4, 7, 9 and 12 h.

***Ex-vivo* Permeation Study:** *Ex-vivo* permeation study was performed using Franz diffusion cell apparatus using rat skin as a membrane. 25 mL of phosphate buffer pH 7.4 was taken in the receiver compartment of the diffusion cell and is covered by rat skin over which transdermal patch was placed. Temperature and speed of the magnetic bead were maintained at 32 ± 3 °C and 100 rpm, respectively. 0.5 mL of media was collected as a sample at time intervals 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18 & 24 h, and samples were analyzed using RP-HPLC.

Mathematical modelling of release kinetics: To understand the release kinetics from the formulated patches, the permeation data was fitted into various kinetic equations [20].

Zero Order Model (Cumulative Percentage Drug Permeated Vs Time): The equation that represents zero-order kinetics

$$Q_t = Q_0 + k_0 t$$

Where Qt is the amount of drug released at time 't'
Q_o is the initial amount of drug and
K_o is the zero-order release rate constant

First Order Model (Log Cumulative Percentage of Drug Remaining *vs.* Time: The equation representing first-order kinetics

$$\text{Log } C = \text{Log } Co - k1\ t/2.303$$

Where Co is the initial concentration of the drug, C is the concentration after time t, K1 is the first-order release rate constant, and t indicates the time.

Higuchi Model (Cumulative Percentage Drug Release *vs.* Square Root of Time: This model can be expressed using equation

$$F_t = Q = A\sqrt{(D(2C-Cs)\ Cst)}$$

Where Q is the amount of drug released in time t per unit area A, C is the initial concentration of the drug, and Cs is the drug solubility in the matrix media, D is the diffusivity of the drug molecules in the matrix substance

Korsmeyer - Peppas Model: The permeation data were fitted into the Korsmeyer-Peppas model to know the mechanism of drug release. The equation that describes this model is

$$M_t/M\infty = Ktn$$

Where $Mt/M\infty$ is the fraction of drug released at time t, K is the release rate constant is the release exponent.

TABLE 3: DRUG RELEASE KINETIC PARAMETERS LIMITATIONS

Release Exponent (n)	Drug Transport Mechanism
0.5	Fickian diffusion
0.45-0.89	Non-Fickian transport
0.89	Case II transport
Higher than 0.89	Super case II transport

Stability Study: The stability study was conducted as per ICH guidelines Q1A (R2). The formulations F2, F5, F6 & F9, were subjected for stability at the below-mentioned conditions for six months as presented in **Table 4**.

TABLE 4: STABILTY CONDITIONS AS PER ICH GUIDELINES

S. no	Study	Temperature Condition	Room Humidity Condition	Time period
1	Long term/ Real time	25ºC ±2ºC	60% ± 5%	6 Months
2	Intermediate	30ºC ±2ºC	65% ± 5%	6 Months
3	Accelerated	40ºC ±2ºC	75% ± 5%	6 Months

After stability period the patches were evaluated for physical appearance, assay, % impurities and % drug release.

RESULTS AND DISCUSSION:

A Rivastigmine Loaded Microspheres:

Description: Rivastigmine microspheres were prepared with varying drug to carrier ratio (carrier-polycaprolactone: maltodextrin in 1:1 ratio). The prepared microspheres were found to be discrete and spherical in shape. The microsphere was noticed to be free-flowing and existed as an individual entity without any agglomerates.

Particle Size Distribution: Average particle size distribution of the prepared rivastigmine loaded micro spheres were found to be ranging from 18.65 µm to 41.65 µm. An increase in the polymer ratio increased the particle size linearly. The results are presented in **Table 5**.

Surface Morphology by Scanning Electron Microscopy: The SEM images of rivastigmine loaded microspheres were found to be satisfactory description in compositions prepared at 1:6 ratio D: CB (Carrier base) whereas, the compositions prepared with increased D: CB resulted in losing sphericity and increase in PSD. Pictograms of evaluated microspheres are presented in **Fig. 3** to **Fig. 5.**

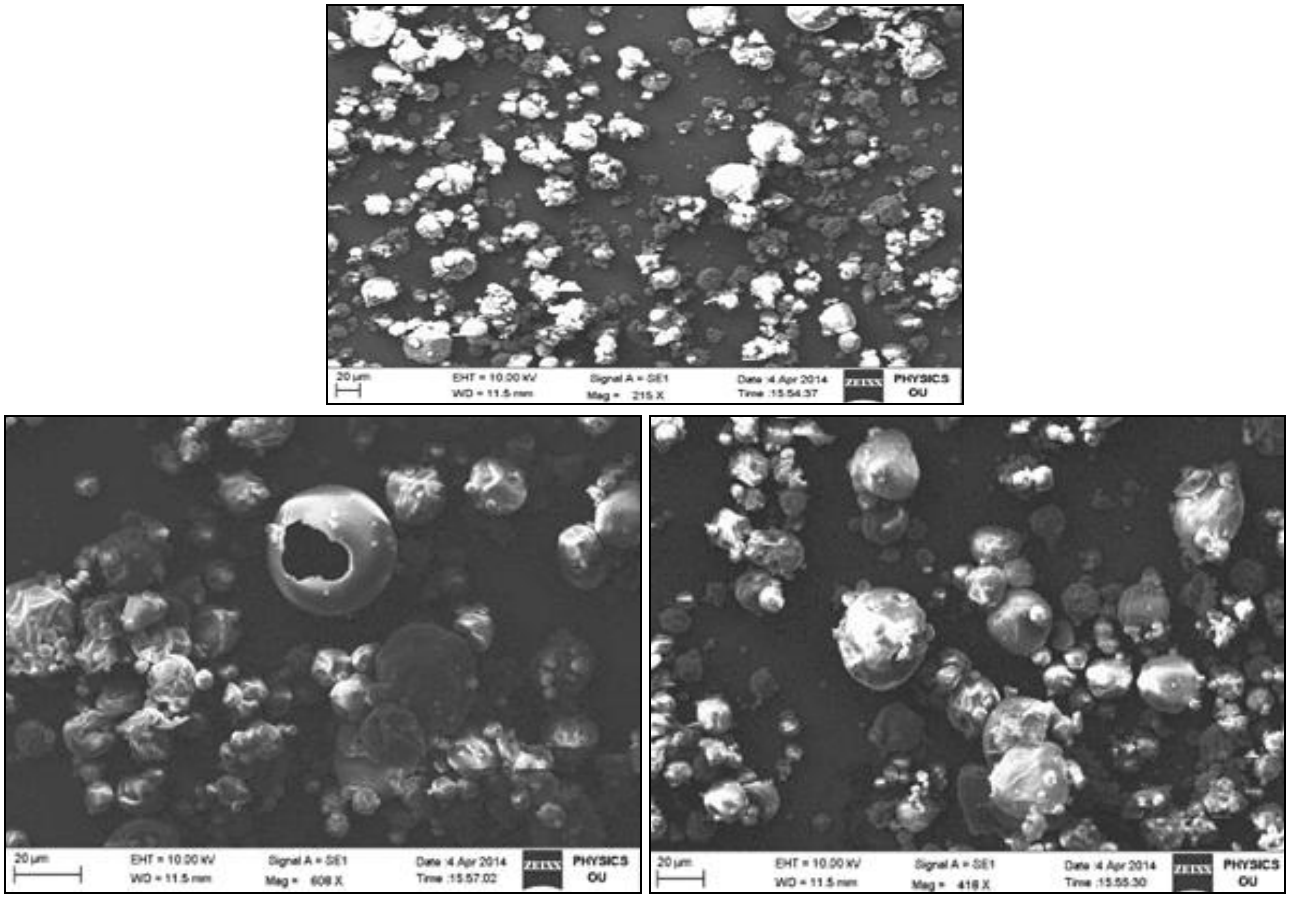

FIG. 3: PICTOGRAMS OF RIVASTIGMINE LOADED MICROSPHERES WITH UNIFORM AND DESIRED PSD

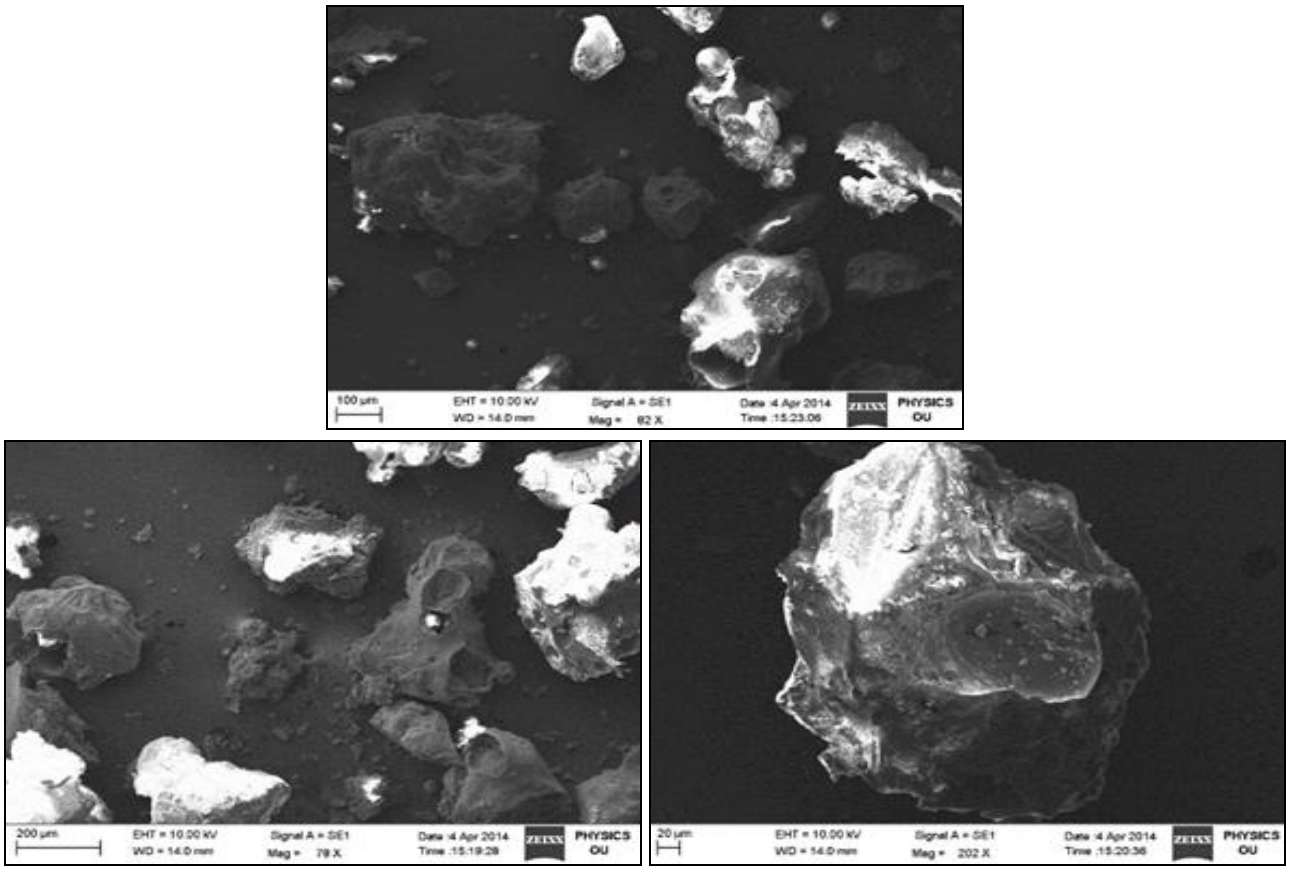

FIG. 4: PICTOGRAMS DEPICTING DEPRIVATION OF SPHERICITY OBSERVED IN FEW COMPOSITIONS UPON INCREASING D: CB RATIO

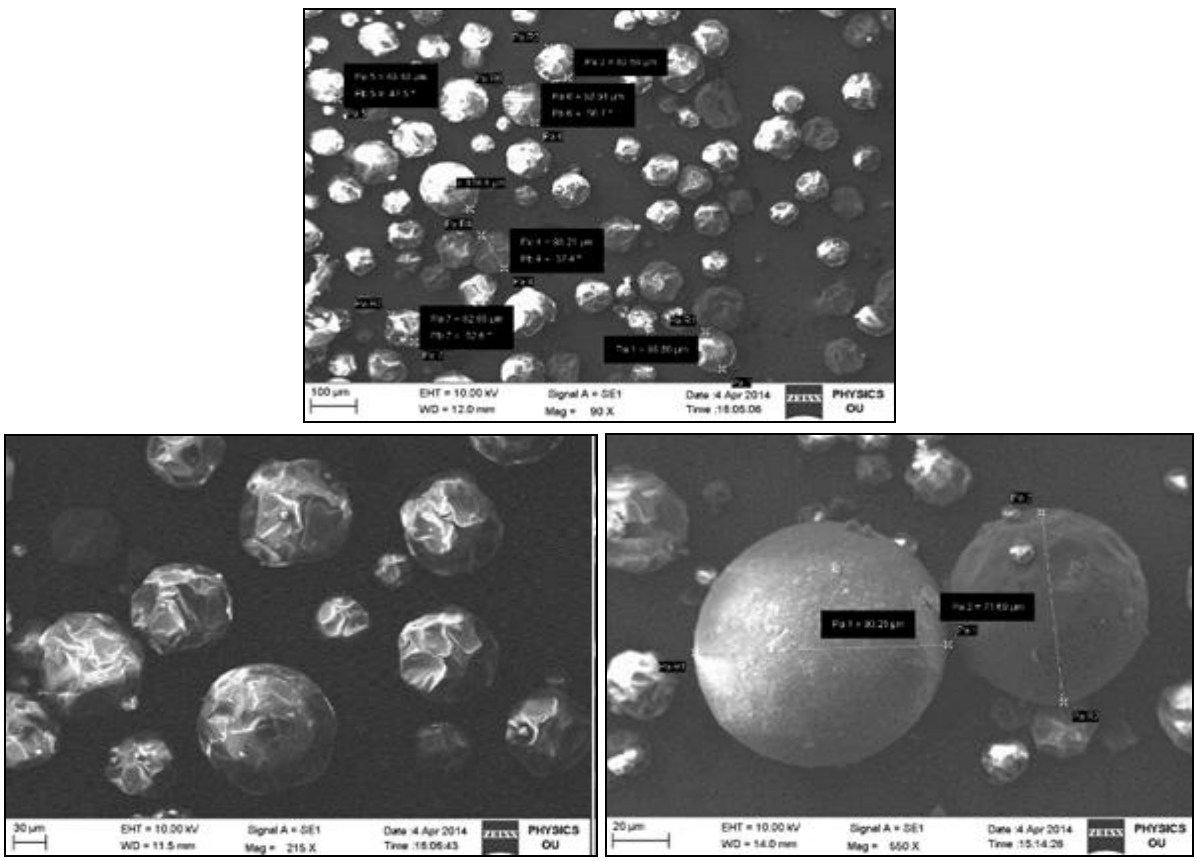

FIG. 5: PICTOGRAMS DEPICTING INCREASE IN PARTICLE SIZE OBSERVED IN FEW COMPOSITIONS UPON INCREASING D: CB BASE RATIO

Percentage Yield: Practical yield of various rivastigmine loaded microsphere formulations were found to be ranged around 69.71 to 86.12. Among the prepared microsphere formulations, the formulation (A5) prepared at drug (rivastigmine): carrier ratio of 1:12 was found to be with maximum yield *i.e.* around 86.12% and the least yield was obtained in the formulation prepared at drug (rivastigmine): carrier of 1:2 ratio. The data is presented in **Table 5**.

Drug Content by Assay: % drug content in the rivastigmine loaded microsphere formulations were evaluated by assay method following HPLC technique.

The % drug content in various microsphere formulations was found to ranging from 92.54% to 99.87%. The maximum % drug content was obtained in the formulation (A3) prepared at 1: 6 Drug (rivastigmine): carrier base ratio. The results are presented in **Table 5**.

Encapsulation Efficiency: Encapsulation efficiency in various microsphere formulations was found to be ranging from 76.51% to 84.58%. The formulation prepared at a drug: carrier mixture ratio of 1:8 and 1:12 was found to be with high encapsulation efficiency, but compositions were ruled out considering PSD requirement for the patch. Results are presented in **Table 5**.

***In-vitro* Drug Release Studies:** Among the prepared rivastigmine loaded microspheres, formulations prepared at Drug: Carrier base mixture ratio of 1:2, 1:4 & 1:6 (considering desired PSD and assay) were evaluated for *in-vitro* drug release where the maximum release of rivastigmine within 3 h was found to be in formulations prepared with Drug: Carrier base mixture ratio of 1:2 (A1) and 1:6 (A6), *i.e.*, around 99.79% & 99.46% respectively. Whereas, the formulation prepared at Drug: Carrier bae mixture rati of 1:4 (A2) showed less release, 98.36%, after 3 h. Results were depicted in **Table 5**.

TABLE 5: CHARACTERIZATION DATA OF RIVASTIGMINE LOADED MICROSPHERES

Code (D:CB)	Avg. particle size (μ)	Yield (%)	Assay (%)	%EE	*In vitro* drug release at 3 h (%) @ 32°C
A1 (1:2)	18.65	69.71	99.19	82.31%	99.79
A2 (1: 4)	19.15	71.26	96.32	80.13%	98.36
A3 (1:6)	21.64	70.17	99.87	77.61%	99.46
A4 (1:8)	29.23	73.26	94.32	83.74%	Ruled Out
A5 (1:10)	32.35	74.51	93.16	76.51%	Ruled Out
A6 (1:12)	41.65	86.12	92.54	84.58%	Ruled Out

From the results of the parameters evaluated, the microspheres from the A1 batch were considered to be optimized and used for incorporation into the adhesive layer of the transdermal patch following the design of experiments.

B. Rivastigmine Micro Reservoirs Loaded Adhesive Dispersion Patches:

General Appearance/Description: The patch was found to be thin, translucent, smooth homogeneous, and flexible in nature when observed with the naked eye.

Thickness: The thickness of the patch was found to be uniform. The thickness of the various transdermal systems was ranged from 0.324 ± 0.03 mm to 0.487 ± 0.035 mm. Results were presented in **Table 6.**

Weight variation: The weight of trans dermal patches was found to be in the range of 0.197 ± 0.0011 g to 0.226 ± 0.0015 g. the standard deviation values of the patches were low, indicating weight variation among the prepared transdermal systems was found to below. Results were presented in **Table 6.**

Drug Content Uniformity: The drug content of the patches was determined using RP-HPLC technique which was found to be in the range of 93.19 ± 2.449 % to 101.78 ± 3.02%. Less standard deviation values indicate more uniformity in the drug content of the patches. Results were presented in **Table 6.**

Moisture Absorption: The average moisture uptake was found to be in the range of 2.005 ± 0.195% and 4.64 ± 0.146 %. Absorption of the moisture by the patch upon exposure to 84% RH did not influence much, but the transdermal patch must be stored in moisture barrier foil since the mild increase in moisture gain was observed. Results were presented in **Table 6.**

Moisture Content: The average % moisture content of the transdermal patches was found to be in the range of 0.65 ± 0.116 % to 1.91 ± 0.105 %.

Less moisture content indicates higher stability of the patches. Results were presented in **Table 6.**

Flatness: Flatness indicates the patch's level of immediate constriction. The flatness study proved that all the formulations had the same strip length before and after cutting/separation, which indicates 100% flatness of the patch. Thus the patch has no level of immediate constriction, and the same could be maintained when the patch has applied to the skin. Results were presented in **Table 6.**

Folding Endurance: The study showed that all the formulations found to be having folding endurance values above 250 except F4, F7, and F8. It suggests that the patches were having good strength, elasticity and can their integrity when applied on to the skin. Results were presented in **Table 6.**

TABLE 6: EVALUATION DATA OF RIVASTIGMINEMICROSPHERES LOADED TRANSDERMAL PATCHES:

Code	Weight variation (g)	Thickness (mm)	% Moisture content	%Moisture uptake	Folding endurance	Drug content (%)	% Flatness
F1	0.203 ± 0.0014	0.355 ± 0.024	1.35 ± 0.112	2.882 ± 0.895	> 250	98.06 ± 3.56	100%
F2	0.213 ± 0.0009	0.367 ± 0.032	1.63± 0.031	3.786 ± 0.141	> 250	94.33 ± 2.67	100%
F3	0.202 ± 0.0011	0.341 ± 0.035	1.26 ± 0.127	3.484 ± 0.411	> 250	101.78 ± 3.02	100%
F4	0.226 ± 0.0015	0.402 ± 0.034	1.91 ± 0.105	2.005 ± 0.195	< 250	100.32 ± 3.31	100%
F5	0.208 ± 0.0014	0.337 ± 0.028	0.65 ± 0.116	3.248 ± 0.184	> 250	99.29 ± 4.03	100%
F6	0.198 ± 0.0016	0.324 ± 0.031	1.46 ± 0.287	3.052± 0.246	> 250	99.56 ± 2.82	100%
F7	0.217 ± 0.0017	0.397 ± 0.022	1.79 ± 0.167	4.64 ± 0.146	< 250	93.19 ± 2.449	100%
F8	0.219 ± 0.0019	0.372 ± 0.015	1.59 ± 0.219	3.015± 0.181	< 250	97.65 ± 4.46	100%
F9	0.197 ± 0.0011	0.324 ± 0.031	1.06 ± 0.249	3.019 ± 0.19	> 250	95.59 ± 2.64	100%
F10	0.219 ± 0.0021	0.413 ± 0.023	1.35 ± 0.341	3.235 ± 0.152	> 250	94.29 ± 3.29	100%
F11	0.205 ± 0.0008	0.341 ± 0.034	1.23 ± 0.125	3.204 ± 0.284	> 250	99.56 ± 4.28	100%
F12	0.209 ± 0.001	0.371 ± 0.035	1.59 ± 0.214	3.641 ± 0.154	> 250	97.78 ± 2.08	100%
F13	0.218± 0.0014	0.487 ± 0.035	1.86 ± 0.248	2.458 ± 0.208	> 250	95.48 ± 3.46	100%

Note: ± = SD

Mechanical Properties: Mechanical properties indicate the strength and elasticity of the transdermal patches. A suitable transdermal patch must have high tensile strength and elongation at break. The results indicate that as the polymer concentration increased, there was an increase in tensile strength but a decrease in elongation at break. The optimized formulation was found to have high mechanical strength. Results are presented in **Table 7.**

TABLE 7: MECHANICAL PROPERTIES OF RIVASTIGMINE TRANSDERMAL FORMULATIONS

Formulation	Tensile strength(kg/mm^2)	Elongation at break(%mm^2)
F2	3.66±0.029	13.81±0.56
F5	4.01±0.037	14.26±0.72
F6	2.89±0.082	11.51±0.63
F9	2.59±0.054	13.69±0.42

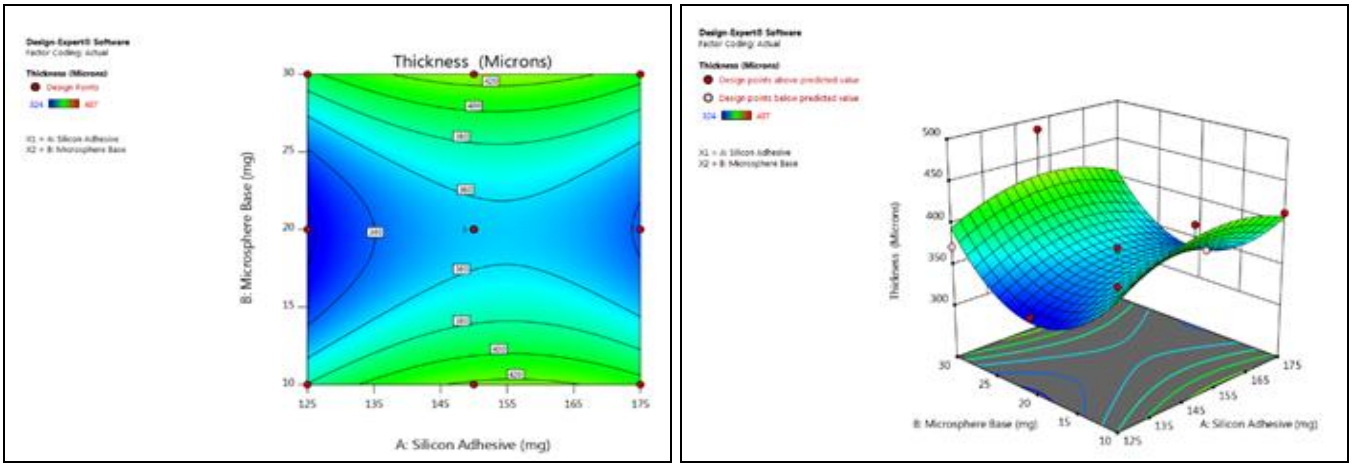

FIG. 6: CONTOUR PLOT AND RESPONSE SURFACE PLOT SHOWING THE RELATIONSHIP BETWEEN VARIOUS LEVELS OF TWO FACTORS ON THICKNESS

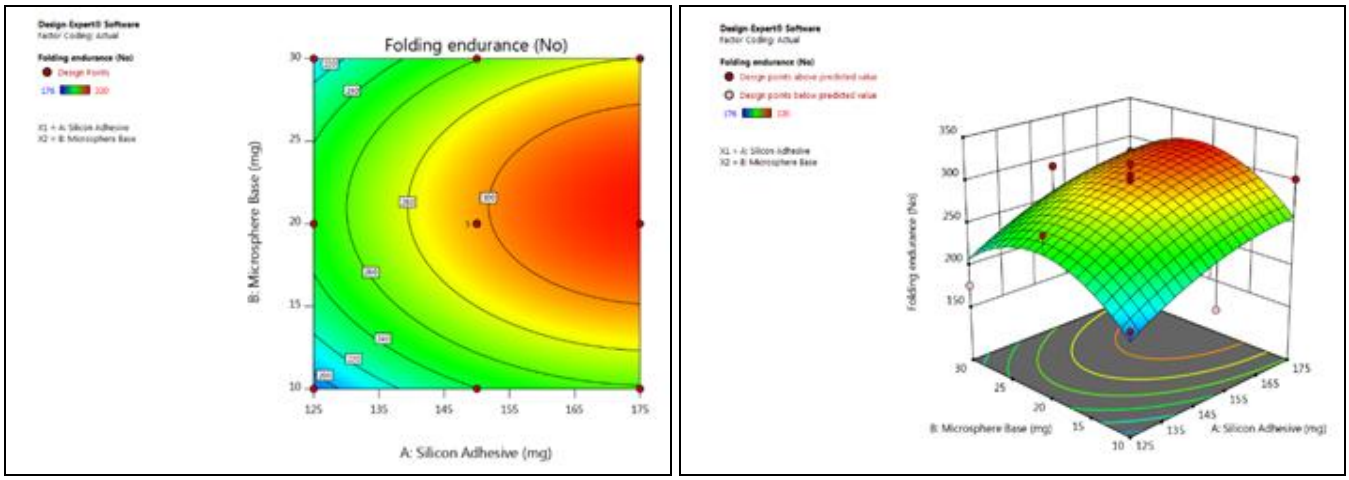

FIG. 7: CONTOUR PLOT AND RESPONSE SURFACE PLOT SHOWING THE RELATIONSHIP BETWEEN VARIOUS LEVELS OF TWO FACTORS ON FOLDING ENDURANCE

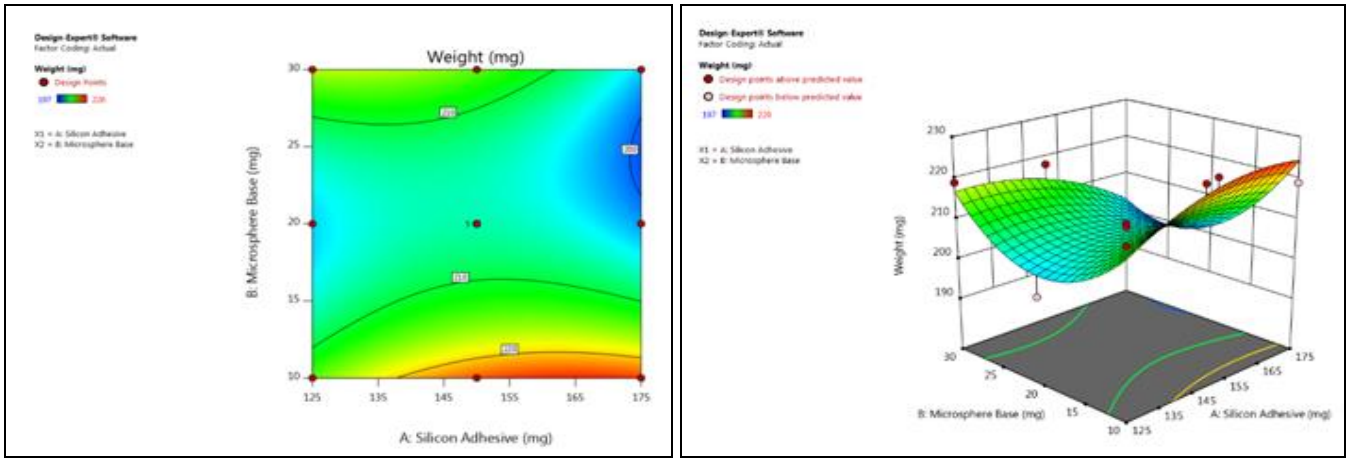

FIG. 8: CONTOUR PLOT AND RESPONSE SURFACE PLOT SHOWING THE RELATIONSHIP BETWEEN VARIOUS LEVELS OF TWO FACTORS ON WEIGHT OF THE PATCH

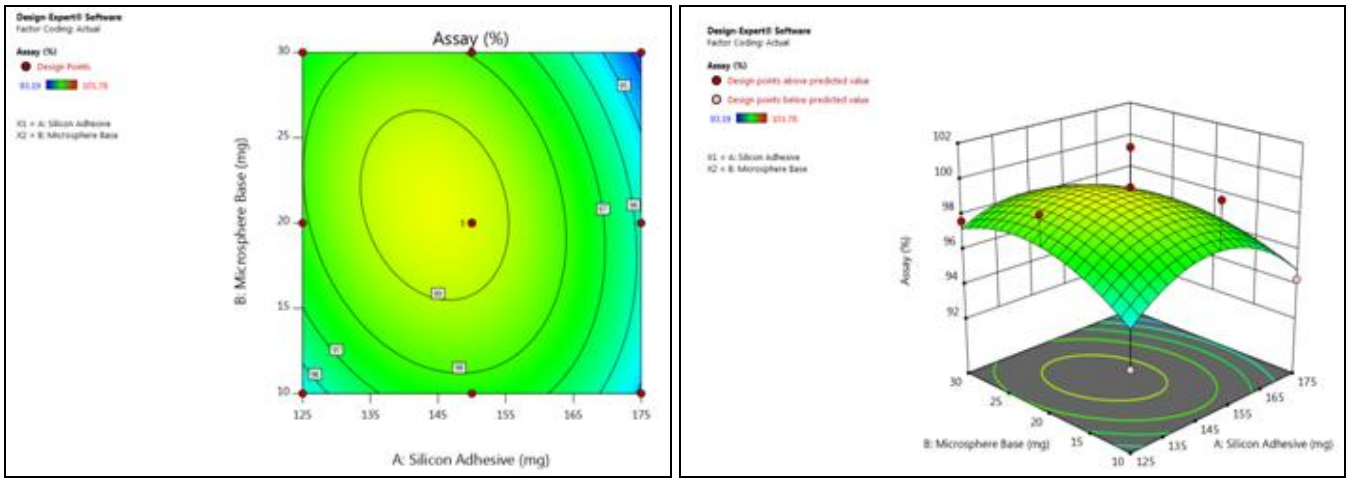

FIG. 9: CONTOUR PLOT AND RESPONSE SURFACE PLOT SHOWING THE RELATIONSHIP BETWEEN VARIOUS LEVELS OF TWO FACTORS ON WEIGHT OF THE PATCH

Experimental Design Analysis: The impact of two factors, *i.e.,* Silicon adhesive and microsphere base, on thickness, folding endurance, the weight of the patch, and assay of the patch is presented in **Fig. 6** to **Fig. 9.**

Assay and folding endurance of the formulation were found to be high upon taking the two factors in medium concentration, whereas the thickness and weight of the patch were found to be decreased.

Design Optimization: The impact of silicon adhesive and microsphere base was found to be varied on various responses like thickness, folding endurance, weight and assay of the formulation. To get an Optimum ratio of silicon adhesive and microsphere base for obtaining best fit satisfactory responses, numerical optimization was performed where the optimum ratios of factors were identified with maximum desirability *i.e.*, upon taking 150 mg of silicon adhesive and 18.428 mg of microsphere base produces a formulation with 99.28 % of assay, 207.69 mg of weight, 357.36-micron thick patch and with a folding endurance of 294.29 times **Fig. 10.** with maximum desirability of 0.778. The formulation F5 results were found to be correlating with the numerical optimization outcome. Hence, the best formulation found with satisfactory physical parameters and in vitro drug release is subjected to *ex-vivo* permeation studies.

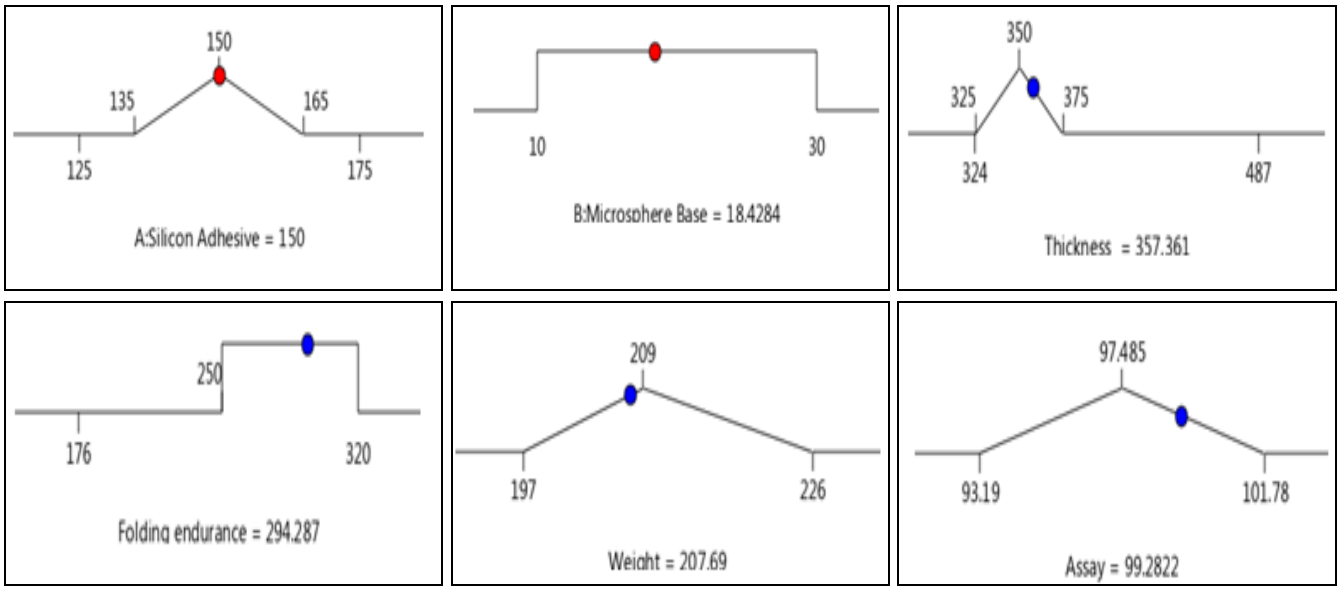

FIG. 10: OUTCOME OF NUMERICAL OPTIMIZATION OF THE RIVASTIGMINE FORMULATIONS SCREENED BY CENTRAL COMPOSITE DESIGN

***In-vitro* Dissolution Studies:** *In-vitro* dissolution study was conducted using USP-5 dissolution test apparatus *i.e.*, paddle over disc apparatus using 500 mL of 0.9% Sodium chloride solution as the dissolution media. Among the four formulations (F2, F5, F6 & F9), the maximum release was observed from the F5 patch. Drug release plots were presented in **Fig. 11.**

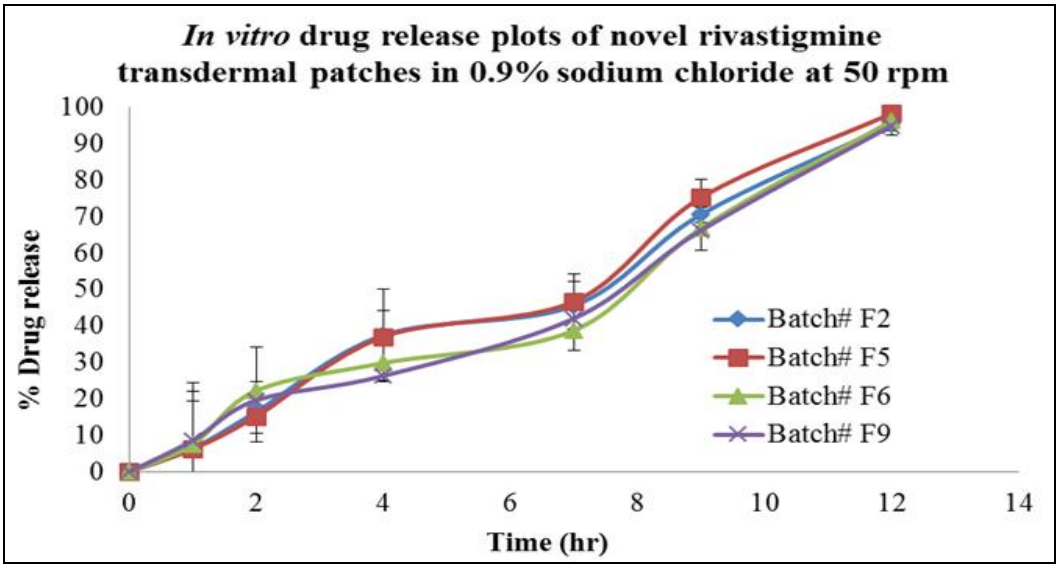

FIG. 11: *IN-VITRO* DRUG RELEASE PLOTS OF RIVASTIGMINE TRANSDERMAL PATCHES IN 0.9% SODIUM CHLORIDE SOLUTION

Mathematical Modelling of Release Kinetics: The *Ex-vivo* drug permeation data was fit into various release kinetic models, and the results were presented in **Table 8.** The formulation F5 released the drug from the formulation following zero-order release kinetics with Super Case II transport mechanism.

TABLE 8: *EX-VIVO* PERMEATION KINETICS DATA OF BEST IDENTIFIED RIVASTIGMINE TRANSDERMAL FORMULATIONS:

Formulation code	Zero-order		First-order		Higuchi		Best fit	Korsemeyer - Peppas		Mechanism
	R^2	k_o	R^2	k_1	R^2	k_H		R^2	n	
F2	0.986	2.548	0.995	0.016	0.971	14.902	First order	0.987	0.998	Super Case II Transport
F5	0.998	2.518	0.989	0.017	0.966	14.642	Zero order	0.994	1.007	Super Case II Transport
F6	0.931	2.312	0.968	0.014	0.987	13.938	First order	0.954	0.779	Case II Transport
F9	0.997	2.457	0.991	0.016	0.965	14.312	Zero order	0.974	1.128	Super case II Transport

Stability Study: Thermal stability of formulations were evaluated for 6 months in accelerated, intermediate, and real-time conditions. The patches subjected for stability were then evaluated for description, % assay, % impurities, and % drug release. Results are presented in **Table 9.**

TABLE 9: STABILITY DATA OF FORMULATIONS F2, F5, F6 AND F9

S. no	Formulation	Parameter	Temperature					
			25 ±2˚C/ 60 ±5% RH		30 ±2˚C/ 65 ±5% RH		40 ±2˚C/ 75 ±5% RH	
			Initial	6 M	Initial	6 M	Initial	6 M
1	F2	Description	Good	Good	Good	Acceptable	Good	Acceptable
		Assay (%)	94.33	93.18	94.33	93.02	94.33	92.86
		%DR (12 H)	95.2	94.85	95.2	93.21	95.2	92.68
		% Total Impurities	0.71	0.89	0.71	0.93	0.71	1.74
2	F5	Description	Good	Good	Good	Good	Good	Good
		Assay (%)	99.29	98.93	99.29	99.17	99.29	98.63
		%DR (12 H)	98.31	98.76	98.31	97.65	98.31	97.77
		% Total Impurities	0.68	0.88	0.68	0.91	0.68	1.39
3	F6	Description	Good	Good	Good	Acceptable	Good	Acceptable
		Assay (%)	99.56	98.32	99.56	97.56	99.56	96.51
		%DR (12 H)	96.3	94.56	96.3	93.71	96.3	92.44
		% Total Impurities	0.81	0.96	0.81	1.05	0.81	2.17
4	F9	Description	Good	Good	Good	Good	Good	Good
		Assay (%)	95.59	95.56	95.59	94.81	95.59	94.02
		% DR (12H)	94.9	93.82	94.9	94.04	94.9	93.12
		% Total Impurities	0.78	0.97	0.78	1.19	0.78	2.85

CONCLUSION: Transdermal system of Rivastigmine with zero-order release kinetics was developed using a novel technique which is a combination of micro reservoir and adhesive dispersion system. Optimization of trans dermal system was performed by central composite design (using application Statease Design Expert®).

The optimized formulation (F5) was subjected to ex vivo permeation study and the data was fit into various release kinetic models. The optimized formulation (F5) released the drug from the formulation following zero order release kinetics with Super Case II transport mechanism. The optimized formulation was found to be stable up to six months when subjected for stability as per ICH guidelines.

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