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Research Article

Formulation and in-vitro Evaluation of Baclofen loaded Transferosomal Gel

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Abstract

Baclofen is a class of medications called skeletal muscle relaxant. It is a class III drug having low solubility and high permeability used in treating multiple sclerosis symptoms such as spasticity, stiffness, and pain. The current study involves the development and in-vitro evaluation of a Baclofen-loaded transfersomal gel to reduce dosage frequency. Drug and excipient interactions were not detected in the FTIR spectra. Hand shaking modified thin film hydration method was used to developed transfersome formulations, which then consolidated at 0.5% carbopol gel. Optimized BF7 formulation provides higher Entapment efficiency and maximum drug delivery. The prepared formulation had been examined for FTIR, Organoleptic evaluation, Viscosity, Spread-ability, Entrapment Efficacy, Drug content, SEM analysis, Particle size, Zeta potential, Stability testing, and other parameters. The optimised formulation BF7 confirmed Entapment Efficiency ranging from 80.04 to 87.42%, Drug content ranging from 90.26 -95.37%, Vesicle Size of 167.8nm & In-vitro diffusion of 97.48%. The drug release data from the chosen gel demonstrated a good fit with the Higuchis and Zero order release kinetics.

Keywords: Transferosomes, vesicular drug delivery system, Thin film hydration method

1. INTRODUCTION:

Transdermal delivery of drug through the skin to the systemic circulation is a beneficial technique of administration for a range of therapeutic reasons. Transdermal delivery is currently rising in interest due to several benefits over the traditional oral method[1]. Because TDDS does not need transit via the gastrointestinal system, there is no loss due to first-pass metabolism, and medications can be administered without interference by pH, enzymes, or intestinal microbes[2]. Furthermore, TDDS may be utilized to limit drug release based on usage constraints, which contributes to the method's high persistence. Most significantly, because TDDS is a noninvasive administration approach with low discomfort and stress on the patient, medications may be safely and readily provided to youngsters or the elderly[3]. Transfersomes are composed of phospholipids and edge activator (EA), which is a membrane-softening agent (such as Tween 80, Span 80 and sodium cholate) that facilitates the ultra-deformable property of the transfersomes. This is the so-called self-optimizing deformability[4]. Moreover, transferomes are extremely deformable; therefore, they easily cross even the very narrow pores.

2. MATERIALS AND METHODS:

a. Procurement of drug and polymers

Baclofen was provided by R.L.Fine chem Banglore , lecithin, span-80, carbopol, cholesterol, sodium deoxycholate, sodium hydroxide,tri-ethanolamine.

b. Pre-formulation studies

SOLUBILITY

Excess amount of drug was added to distilled water, phosphate buffer (pH 6.8), methanol, and ethanol in a sealed 10 mL Erlenmeyer flask. The flasks were mechanically shaken at a speed of 37±0.5, 50 rpm for up to 24 hours. The solution was filtered using filter paper with a pore size of 0.22 µm. Measure the absorbance and determine the concentration of each solvent[5].

MELTING POINT

Using a melting point equipment, the drug's melting point was determined by the capillary technique. The temperature at which the medication began to melt is ascertained.

DETERMINATION OF ABSORPTION MAXIMA:

1mL of the working solution was transferred to a 10mL volumetric flask. 6.8 phosphate buffer solution was used to fill the capacity to 10ml. The generated solution had a concentration of 10 µg/ml and was scanned between 200-400nm. Then the maxima of absorption were established.

CALIBRATION CURVE OF BACLOFEN:

A pH 6.8 phosphate buffer was used to quantify the concentration of baclofen in 5, 10, 15, 20, and 25 µg/ml, respectively. Every concentration was tested for absorbance at 220 nm. graph was then utilised to produce the caliberation curve foe baclofen.

DRUG – EXCIPIENT COMPATIBILITY STUDY: FTIR Studies

FTIR spectroscopy can be utilized to explore and anticipate any physicochemical intuitive between distinctive components in a detailing. The initial spectra were at that point compared with original.

FORMULATION OF TRANSFERSOMES

Transfersome preparation by improved lipid membrane hydration technique by hand shaking:

Thin film hydration was used to synthesize transfersomes from baclofen, soya lecithin, cholesterol, and various surfactant concentrations (span-80, sodium deoxycholate). All formulations comprise the same propotion of drug (50mg). Different formulations were created by combining different phospholipid and surfactant ratios. The table contains information on the surfactants utilized, as well as the amount of lecithin and surfactant used in each formulation. 10ml of organic solvent (ethanol: chloroform 1:2) is used to dissolve lecithin, surfactants, and the medication. The organic solvent is then evaporated during hand shaking at temperatures exceeding the lipid transition temperature. Under vacuum, the remaining traces of solvent are eliminated. By rotating at 60 rpm, the deposited lipid film is hydrated with the phosphate buffer (ph 6.8). At normal temperature, the resultant vesicles swell for 2 hours. The multilamellar lipid vesicles (mlv) are then sonicated for 30 minutes with a sonicator.

Table 1: Formulation code for preparation of transferosomes

Formulation	Drug(mg)	Soya lecithin	Cholesterol	Span80(mg)	Sodium deoxycholate (mg)
BF1	50	90	10	10	--
BF2	50	85	10	15	--
BF3	50	80	10	20	--
BF4	50	75	10	25	--
BF5	50	70	10	30	--
BF6	50	90	10	--	10
BF7	50	85	10	--	15
BF8	50	80	10	--	20

Preparation of transfersome gel:

The topical gel was made using an aqueous dispersion of transfersomes. Transferosome gel was made using a polymer such as carbopol 934. To generate 0.5% gel, 0.5g of carbopol-934 powder was rapidly swirled (stirred by magnetic stirrer remi 5mlh) in 100 ml distilled water (taking care not to create indispersible lumps) and left to hydrate for 24 hours. Similarly, 1.5% carbopol gels were made. The dispersion was neutralized with tri-ethanolamine to fix the ph [6.8] using a PH meter.

Table 2: Formulation code for preparation of gel

INGREDIENTS	FORMULATION CODE		
	BTG1	BTG2	BTG3
Baclofen Transferosome	0.1%	0.1%	0.1%
Carbopol 934	0.5%	1%	1.5%
Propylene glycol	0.15%	0.15%	0.15%
Methyl paraben	0.05%	0.05%	0.05%
Triethanolamine	0.1%	0.1%	0.1%
Distilled water	qs	qs	qs

EVALUATION OF TRANSFEROSOMES:

Determination of entrapment efficiency percentage: The amount of drug entrapped in transfersomes was estimated by centrifugation method. 1gm of Transferosome gel was taken and diluted with 10ml phosphate buffer (pH 6.8). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted approximately and absorbance was measured using UV spectrophotometer at 220nm[6,7]. Entrapment efficiency is expressed as the percentage of drug trapped.

$$\% \text{ Entrapment} = \frac{\text{Total drug} \_ \text{diffused drug}}{\text{Total drug}} \times 100$$

Determination of Particle Size and Zeta Potential: Every produced baclofen Transfersome was subjected to zeta potential and particle size measurements utilizing the Particle Size System at 25°C[8].

Surface morphology of transferosomes: (SEM) is utilized to decide the shape and estimate of defined baclofen stacked transferosomes by scanning electron-microscope[9].

EVALUATION OF TRANSFEROSOMAL GEL:

Physical appearance: The presence of particles in the produced gels was also assessed. Gel smears were made on glass slides and examined under a microscope for the presence of particles or grittiness[10].

Viscosity determination: The viscosities of the gels were measured using a Brookfield Viscometer (model- RVTP). RV-7 spindle at 100 revolutions per minute[11].

Spreadability: To determine spreadability, a modified apparatus was developed. The slip and drag properties of the gels were used to calculate spreadability. The modified apparatus was made of two glass slides, the bottom of which was fastened to a wooden plate and the top of which was attached to a balance via a hook[12,13]. The spreadability was calculated using the formula:

$$s=ml/t,$$

where s is the spreadability, m is the weight in the pan attached to the upperslide, t is the duration, and l is the distance traveled. The mass, length, and 't' were maintained constant for practical purposes.

***In-vitro* diffusion:** A diffusion analysis of Transfersomes formulations was performed utilizing a Franz diffusion cell with a dialysis membrane. For 24 hours, the dialysis membrane was immersed in distilled water. The Franz diffusion cell is divided into two compartments: the upper donor compartment and the bottom receptor compartment. The receptor compartment was filled with 6.8 pH buffer, and the donor compartment contained 5ml of transferosome suspension on dialysis membrane with a 2cm2 exposure area to receptor medium. The entire assembly was kept on a magnetic stirrer at 600rpm for 10 hours, and samples were withdrawn at 1 hour intervals and replaced with equal volume of buffer[14,15]. Samples were diluted with buffer and analyzed using a UV spectrophotometer at 220nm.

Stability studies: Stability is examined by evaluating structure, size of formulation over time. The improved formulas stored at various temperatures in firmly sealed amber vials. Pharmaceutical products intended for refrigeration should be subjected to long-term storage at 5 ± 3 °C for 1 month and accelerated testing at 25 ± 2 °C/60% RH ± 5% RH for 1 month. Failure to meet the drug's criteria is defined as a substantial change[16].

3. RESULTS AND DISCUSSION

PREFORMULATION RESULTS

A. Organoleptic properties (Colour, Odor, Taste and Appearance)

COLOUR =white or off white crystalline powder
ODOUR = odourless
STATE = crystalline

B. MELTING POINT:

Table 3: Melting point

SNO.	PURE DRUG	REFERENCE RANGE	OBSERVED VALUE
1.	Baclofen	181 - 191⁰C	185⁰C

The data presented as mean value ± S.D (n = 3).

Observation: The observed melting point was found to be 185ºC.

C. SOLUBILITY:

Table 4: Baclofen solubility

SOLUBILITY	CONCENTRATION (µg/ml)
Water	23.1 µg/ml
Methanol	3.54 µg/ml
Ethanol	3.1 µg/ml
Phosphate buffer 6.8	15.6 µg/ml

OBSERVATION: Baclofen was found to be very slightly soluble in Methanol, Ethanol and soluble in phosphate buffer and distilled water.

ABSORBPTION MAXIMA OF BACLOFEN:

An array of 200–300 nm wavelengths was used to scan a solution containing 10 ug/ml of Baclofen. The 220 nm wavelength was where the API was found to exhibit significant absorbance. A maximum wavelength of 220 nm was observed in the sharp absorption spectrum.

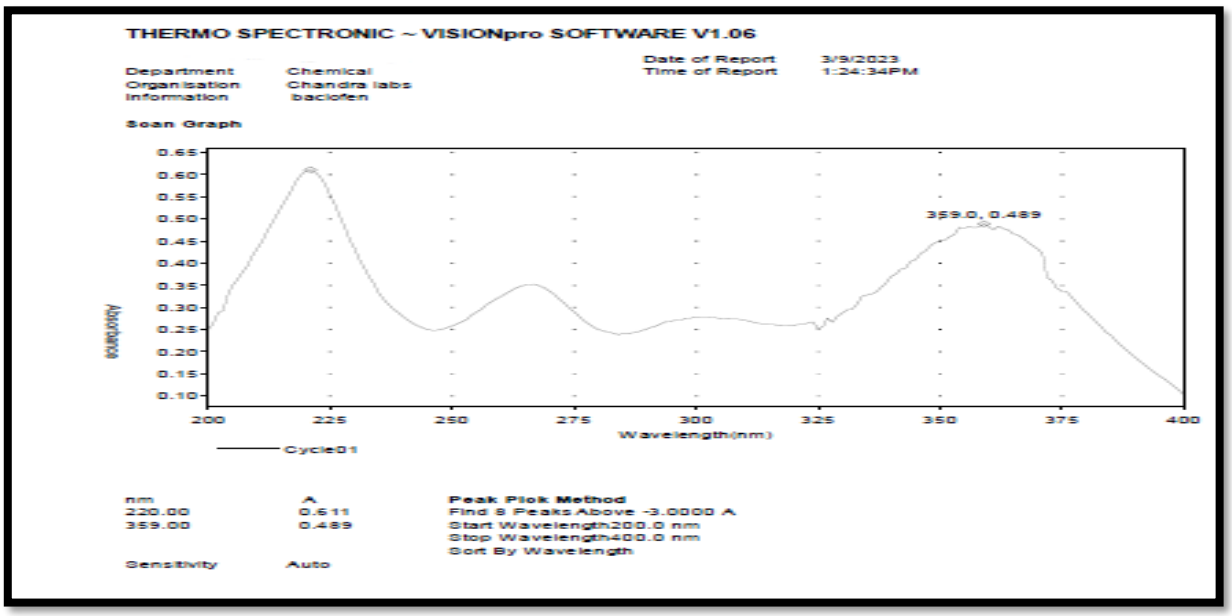

Figure 1: lambda max of drug (baclofen)

d. CALIBERATION CURVE:

Table 5: graph data of Baclofen in Phosphate Buffer pH6.8

S.no	Conc (µg/ml)	Abs
1	0	0
2	5	0.205±0.21
3	10	0.416±0.14
4	15	0.618±0.31
5	20	0.819±0.20
6	25	0.987±0.15

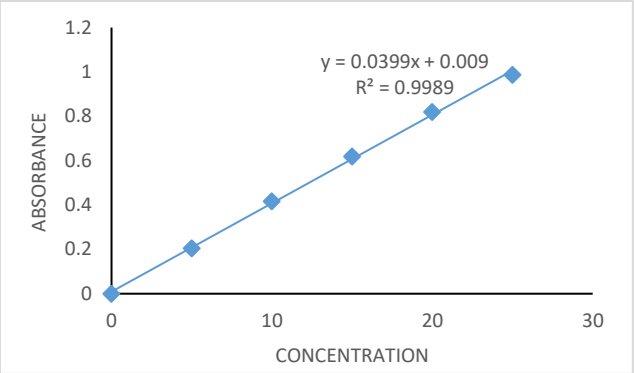

Figure 2: Standard Graph of Baclofen -Phosphate Buffer pH 6.8

3.3. BACLOFEN AND POLYMER COMPATIBILITY STUDIES:

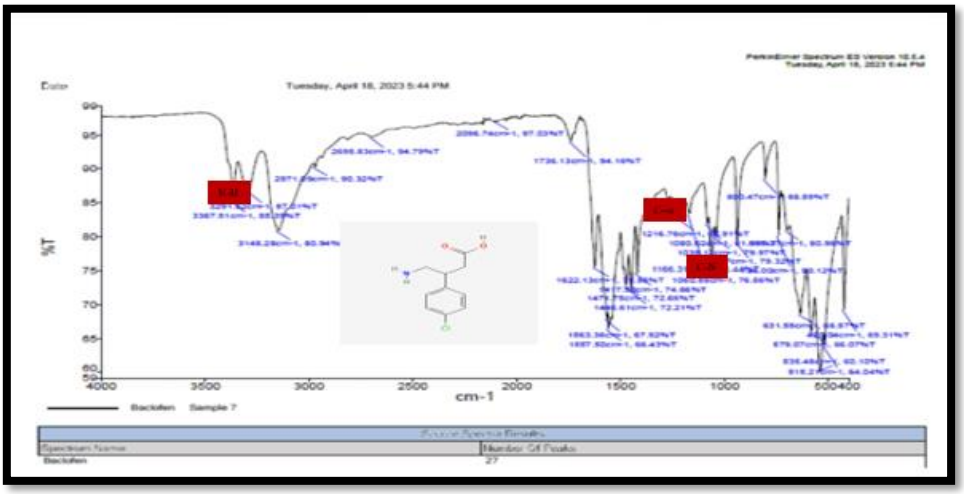

Figure 3: FTIR of pure drug

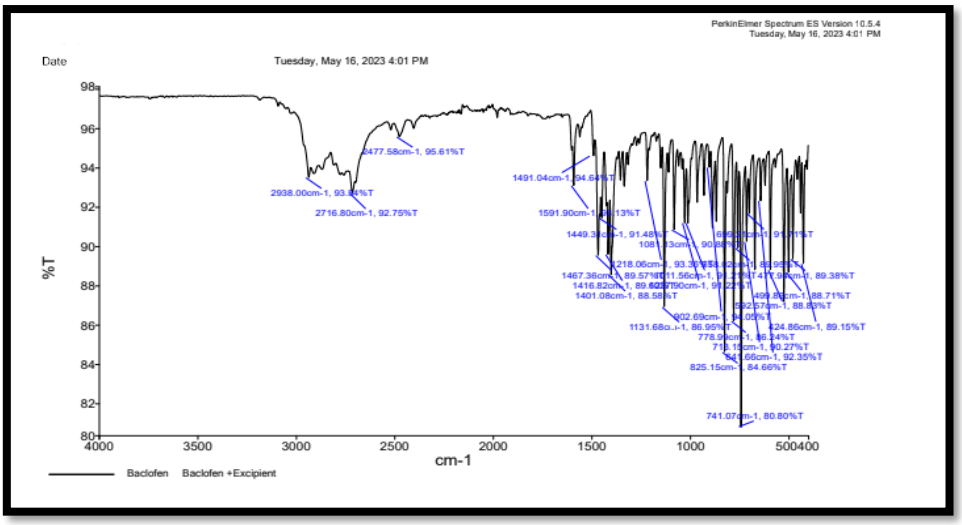

Figure 4: FTIR of optimised

The drug, mixture, and chosen carriers do not interact with one another., as shown by spectra. As a result, it was discovered that the chosen carrier and the chosen Baclofen could be captured together without causing any mutual interactions.

Characterization of prepared Baclofen Transferosomes:

Entrapment efficiency: According to Table 4.6, the percent entrapment efficiency of the deformable vesicles formulations ranged from 80.04 to 87.42. The Bf7 formulation's entrapment efficiency was high (maximum 87.42).

Table 6: % Drug entrapment Efficiency

Formulation	% Entrapment Efficiency
Bf1	80.4±0.16
Bf2	82.14±0.14
Bf3	84.26±0.25
Bf4	82.31±0.12
Bf5	84.18±0.28
Bf6	85.21±0.27
Bf7	87.42±0.19
Bf8	84.232±0.18

Particle size analysis:

The produced Bf7 formulation had the smallest particle size, measuring 167. 8 nm.

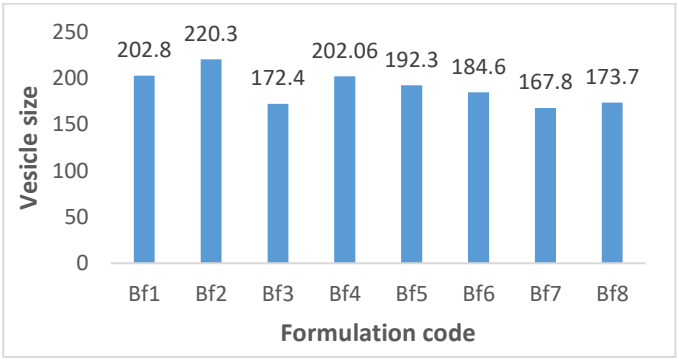

Figure 5: Particle Size graph of all (Baclofen) formulation

Zeta Potential and PDI:

Table 7: All formulation PDI and Zeta potential

Formulation code	PDI	Zeta potential
Bf1	0.48±0.12	-22.9±1.45
Bf2	0.37±0.18	-23.9±1.65
Bf3	0.42±0.11	-27.6±1.16
Bf4	0.±0.013	-24.8±1.45
Bf5	0.461±0.017	-25.6±1.78
Bf6	0.49±0.15	-28.1±1.27
Bf7	0.32±0.10	-30.2±1.04
Bf8	0.40±0.15	-26.9±1.34

***3.7 In-vitro* diffusion study:**

Table 8: percentage release of transfersomes

Time(hr)	Bf1	Bf2	Bf3	Bf4	Bf5	Bf6	Bf7	Bf8
0	0	0	0	0	0	0	0	0
1	12.41±0.6	13.32±0.11	15.21±0.6	18.19±0.1	20.47±0.10	17.42±0.4	19.34±0.2	16.27±0.1
2	22.15±0.10	27.48±0.2	30.26±0.4	33.52±0.2	35.75±0.11	32.18±0.1	37.27±0.2	29.51±0.4
3	36.48±0.5	37.54±0.11	42.4±0.1	45.18±0.1	47.42±0.3	45.14±0.8	48.41±0.5	41.28±0.1
4	41.61±0.4	40.42±0.15	48.26±0.4	51.26±0.5	59.17±0.1	53.45±0.2	52.52±0.6	50.61±0.2
5	56.52±0.25	55.18±0.14	52.28±0.2	62.41±0.3	65.19±0.4	64.19±0.6	69.16±0.2	59.28±0.4
6	64.27±0.6	67.52±0.8	69.51±0.8	70.14±0.2	70.28±0.2	71.42±0.4	75.28±0.1	72.42±0.1
8	70.41±0.4	74.64±0.4	77.29±0.5	75.28±0.4	74.14±0.4	75.32±0.4	83.2±0.1	76.28±0.4
10	79.28±0.2	80.44±0.1	82.75±0.4	81.41±0.1	80.37±0.1	79.37±0.2	88.15±0.6	79.11±0.6

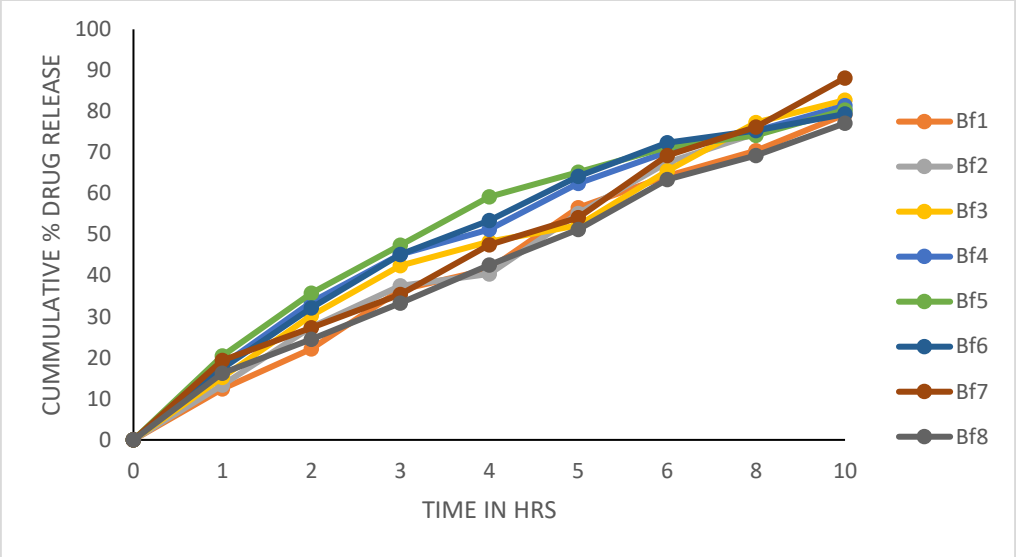

Figure 6: Comparative *IN-VITRO* drug release of formulations Bf1-Bf8

CHARACTERISTICS OF OPTIMIZED FORMULATION:

SEM OF OPTIMIZED FORMULATION

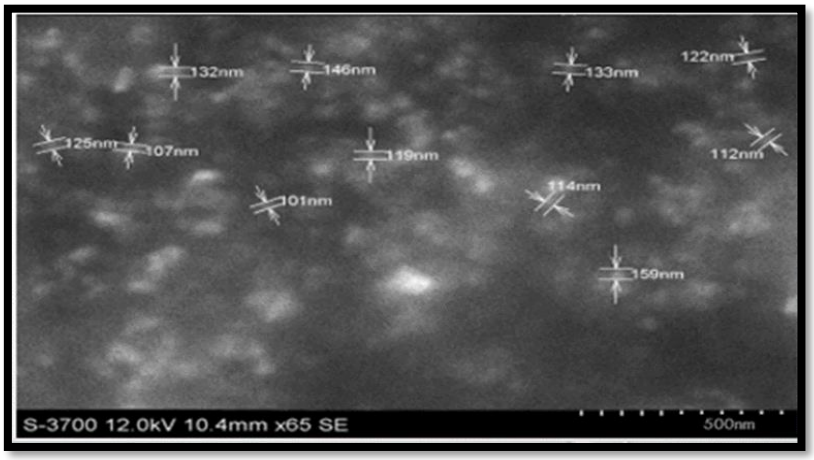

Figure 7: SEM analysis of optimized Gel

Particle Size:

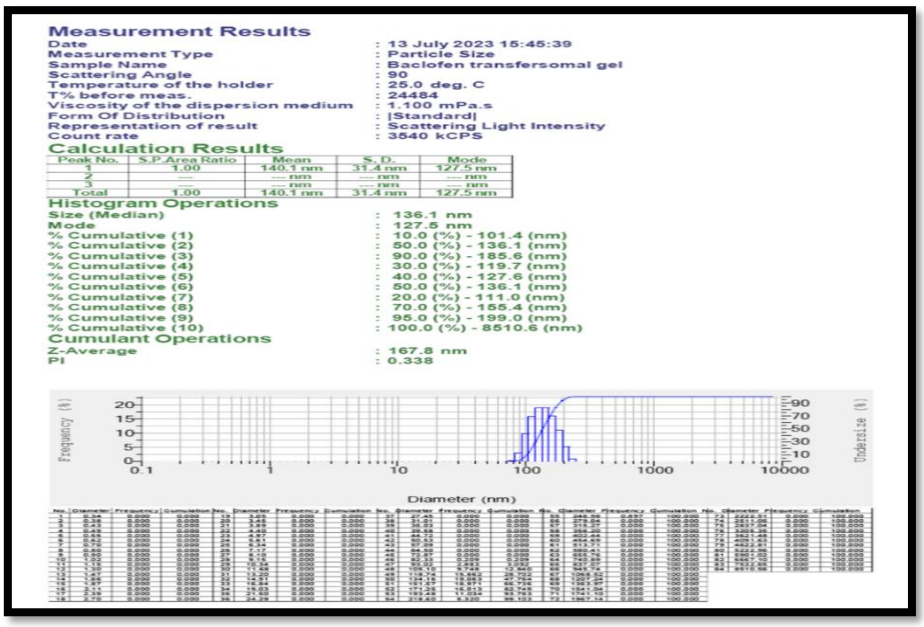

Figure 8: Particle Size of BF7 formulation

Zeta Potential:

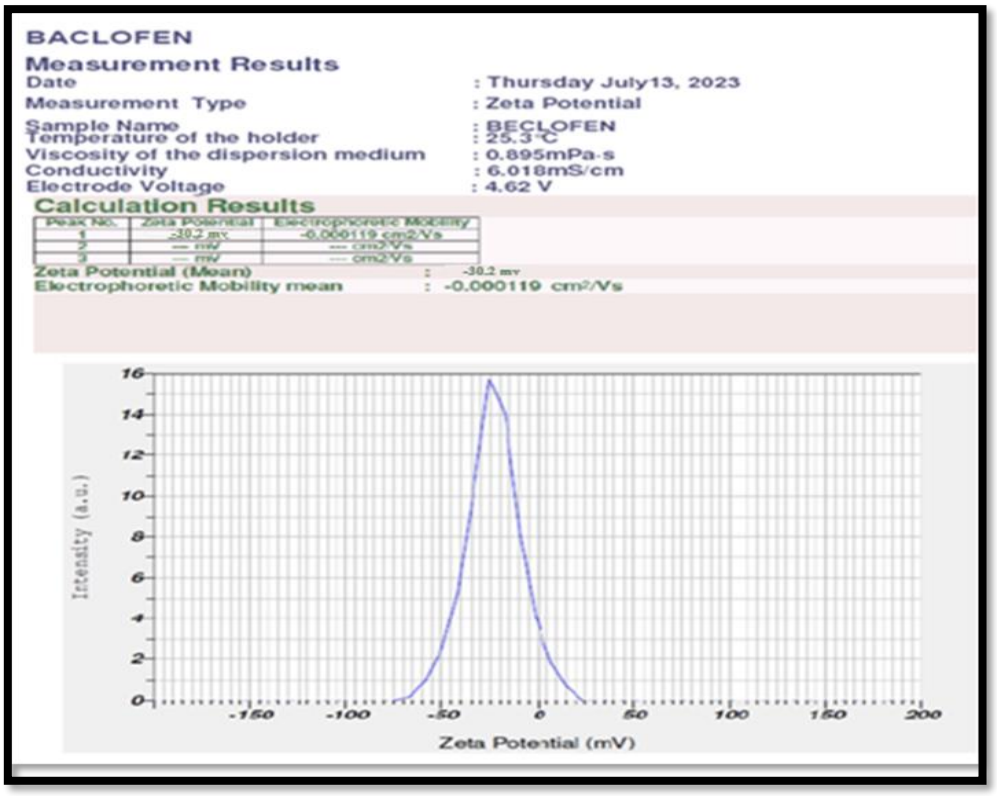

Figure 9: BF7 formulation – zeta potential

EVALUATION OF GEL:

Table 9: Gel evaluation parameters

Formulation code	Carbopol 934 gel	PH	Viscosity(cps)	Spreadability	%Drug-content	Skin irritation
BTG1	0.5%	5.21±0.26	4200	4.0 ± 0.21	96.19±0.24	NO
BTG2	1.0%	5.41±0.68	4540	3.2 ± 0.12	90.37±0.18	NO
BTG3	1.5%	5.18±0.24	5100	2.8± 0.31	89.18±0.22	NO

Table 10: *IN-VITRO* DIFFUSION STUDIES:

Time(hr)	BTG1 0.5% Carbopol gel	BTG2 1% Carbopol gel	BTG3 1.5% Carbopol gel
0	0	0	0
1	15.26	12.37	9.78
2	22.37	23.28	19.41
3	34.61	30.37	28.56
4	48.28	42.39	39.05
5	53.26	49.42	43.77
6	64.37	55.64	51.34
8	76.15	62.37	58.64
10	90.34	65.32	62.71

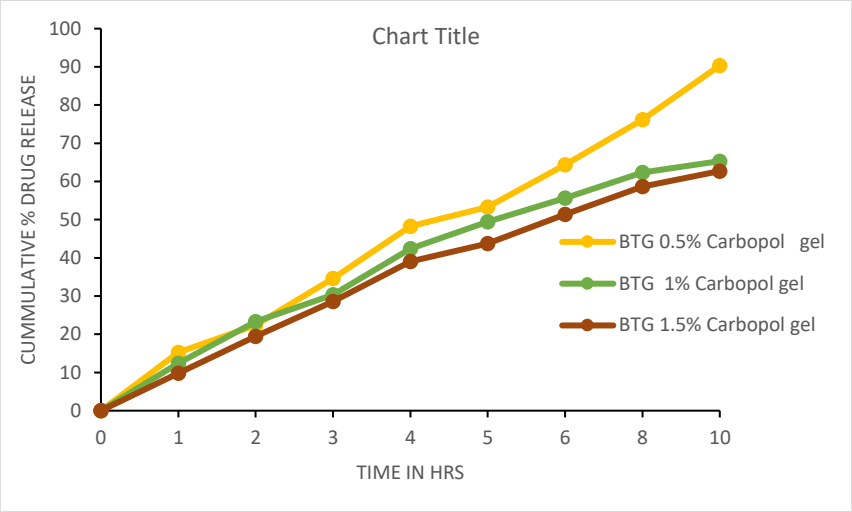

Figure 10: *In-vitro* diffusion for Transfersome gel with different concentrations of Carbopol 934.

RELEASE KINETICS OF DRUG:

percentage drug release from the optimal formulation, fit into Higuchi's plot and showed zero order followed by diffusion and/or erosion mechanisms. Table No.11 displays the results.

Table 11: Release kinetics of optimized formulation

Time(hr)	cumulative % drug release	sqr time	log % cdr	log time	% Drug remaining	log % Drug remaining
0	0	0			100	2.000
1	18.26	1.000	1.262	0	81.74	1.912
2	22.37	1.414	1.350	0.301	77.63	1.890
3	34.61	1.732	1.539	0.477	65.39	1.816
4	42.28	2.000	1.626	0.602	57.72	1.761
5	48.26	2.236	1.684	0.699	51.74	1.714
6	53.37	2.449	1.727	0.778	46.63	1.669
8	64.15	2.828	1.807	0.903	35.85	1.554
10	76.34	3.162	1.883	1.000	23.66	1.374
12	96.42	3.464	1.984	1.079	3.58	0.554

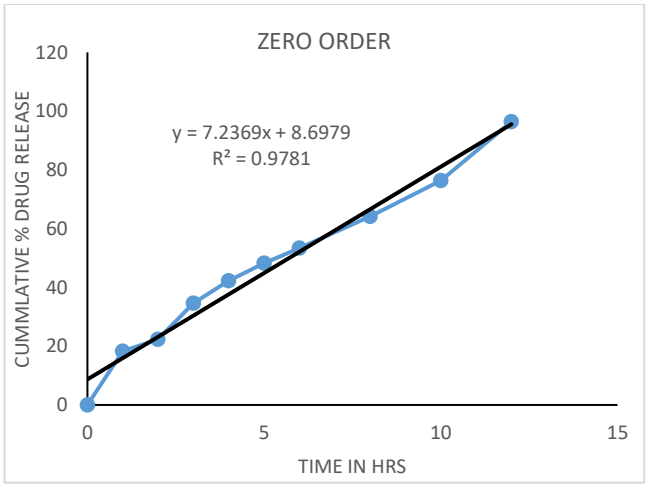

Figure 11: Zero order release kinetic

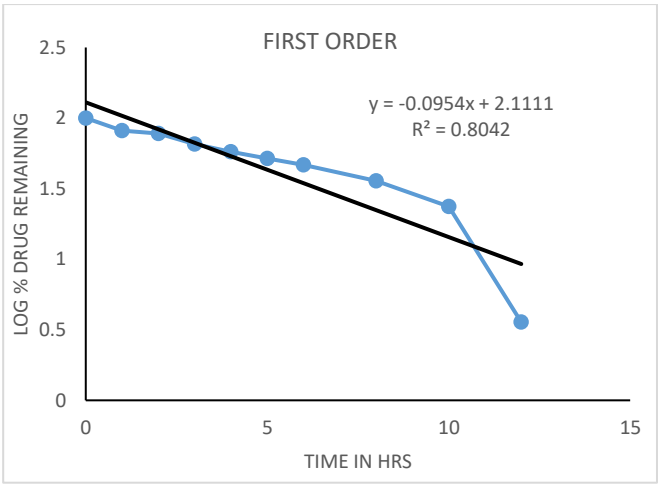

Figure 12: First order release kinetic

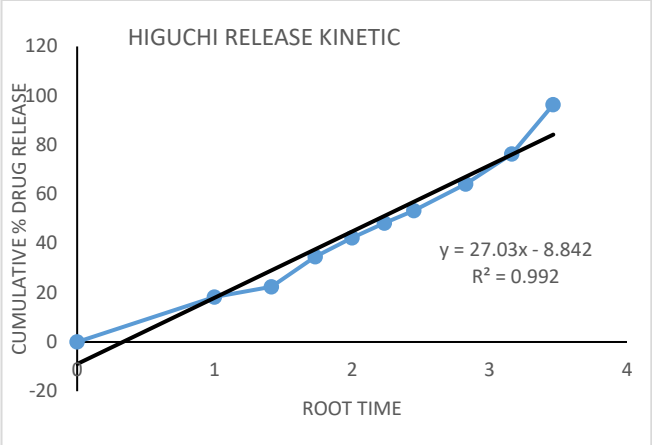

Figure 13: Higuchi release kinetic

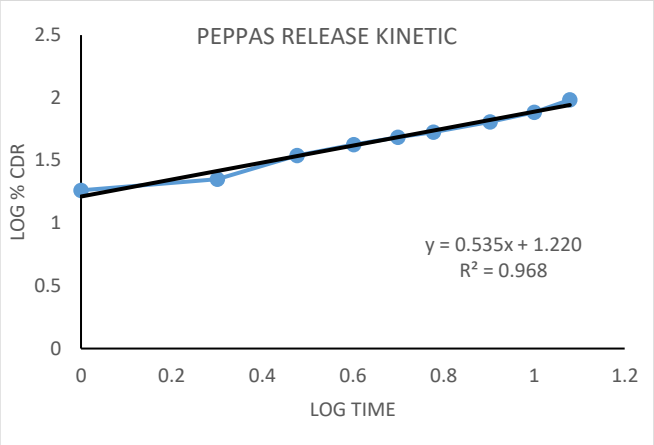

Figure 14: Peppas release kinetic

Observation: The drug release mechanism. the best fit model for selected formulation were found to be Higuchi kinetics with correlation coefficient value (0.992).

Stability Studies:

Table 12: Stability studies of BTG3 optimized 0.5% carbopol 934 transferome gel

No. of Days	% Entrapment Efficiency at temperatures		% Drug Content at temperatures	
	25±2°C (room temp)	4±2°C (Refrigerator temp)	25±2°C (room temp)	4±2°C (Refrigerator temp)
15	87.42	87.47	95.37	96.37
30	87.34	87.38	95.21	95.64
45	86.52	85.48	94.19	93.42
90	86.19	85.17	94.8	93.27

Observation: Stability studies were carried out as per ICH guidelines at 25±2°C and 4±2°C.

CONCLUSION:

In order to reduce the frequency of dosage, the current study aims to develop a transferosomal gel loaded with Baclofen and assess it in vitro. Baclofen is only slightly soluble in methanol and has good solubility in phosphate buffer and distilled water, according to preformulation tests. The drug's absorption maxima in phosphate buffer 6.8 was found to be 220 nm, and FTIR analyses that there was no interaction between the drug and its excipients. Using a modified hand shaking thin film hydration process, eight formulations of transferosomes were created; the best percent CDR (96.42), entrapment efficiency (87.42), and vesicle size (167.8 nm) were found in the optimized BF7. SEM analysis demonstrated the spherical nature of optimized Baclofen transferosomes. The BTG1 formulation, which has 0.5 percent carbopol 934 transferosomal gel is selected as optimised one and Baclofen transferosomal gel has 90.34% drug release is 90.34. Produced transferosomes are more stable at low temperatures, according to stability studies for optimized gel formulations.. The findings of the present investigation allow us to draw the final conclusion that transferosomal gel enhances transdermal drug delivery and overcomes the drawbacks of oral dosage forms.

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CONFLICTS OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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