Formulation And Charctrization of Pioglitazone Loaded Ethosomal Gel

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Abstract*— This study aimed to develop and characterize a Pioglitazone-loaded ethosomal gel for enhanced transdermal delivery. Ethosomes, lipid-based vesicles containing high ethanol concentrations, were formulated to encapsulate Pioglitazone, a drug used in diabetes management. The ethosomal gel was prepared using a modified thin-film hydration method, optimizing lipid and ethanol concentrations to achieve stable vesicles with desirable particle size and drug encapsulation efficiency. Characterization studies included evaluating the ethosomal gel's physicochemical properties such as particle size distribution, morphology using scanning electron microscopy (SEM), and drug release kinetics. Stability studies over various storage conditions confirmed the formulation's robustness and shelf-life suitability. Results indicated that the ethosomal gel exhibited uniform spherical vesicles with a mean particle size suitable for effective skin penetration. In vitro release studies demonstrated sustained drug release characteristics, suggesting potential for prolonged therapeutic action. This formulation represents a promising approach to enhance Pioglitazone's bioavailability through transdermal delivery, potentially improving patient compliance and therapeutic outcomes in diabetes treatment. Further research is warranted to explore its clinical efficacy and safety in human trials, paving the way for its application in diabetes management as an innovative drug delivery system.

Index Terms- Pioglitazone, Ethosomes, Transdermal Delivery, Diabetes Mellitus, Nanocarriers

I. INTRODUCTION

Diabetes mellitus (DM) It is commonest endocrine disorder that affects more than 100 million people worldwide (6% population). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. It is found to damage many of body systems particularly blood vessels, eyes, kidney, heart and nerves[1]. Diabetes mellitus has been classified into two types i.e. insulin dependent diabetes mellitus (IDDM, Type I) and non-insulin dependent diabetes mellitus (NIDDM, Type II). Type I diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin secreting cells whereas Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion[2]. Pioglitazone is a thiazolidinedione antidiabetic with actions similar tothose of rosiglitazone. Pioglitazone depends on the presence of insulin for its mechanism of action [3]. It is used to treat diabetes mellitus type 2. It may be used alone or with other medicines such as insulin, metformin, or sulfonylurea agents. Pioglitazone is used together with a proper diet and exercise to help control blood sugar levels. It decreases insulin resistance in the periphery and in the liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output. In animal models of diabetes, pioglitazone reduces the hyperglycemia, hyperinsulinemia, and hypertriglyceridemia characteristic of insulin-resistant states such as type 2 diabetes. The metabolic changes produced by pioglitazone result in increased responsiveness of insulin-dependent tissues and are observed in many animal models of insulin resistance. Pioglitazone is not intended for treating type 1 diabetes [4]. Ethosome Ethosomes are ethanolic liposomes". Non-invasive delivery carriers enabling drugs to reach to bottom into the skin layers are known as ethosome. These are the small changes of well-established drug carrier liposomes. Ethosomes are lipid or soft vesicles having phospholipids, alcohols in greater concentration and water. Ethosomes ranges from 10 nm to few microns (μm) that are able to penetrate across the skin layers more quickly and have fundamentally higher

transdermal transition available for circulation. These are delicate, flexible vesicles ideal for improved conveyance of active agents. A schematicrepresentation of ethosomes is in the figure no. 1 [5]

II. MATERIAL AND EQUIPMENTS

Table No 1: List of Chemicals

Sr.No.	Chemicals	Manufacturer
1	Pioglitazone	Angel Biopharma
2	Soya lecithin	RESEARCH-LAB FINE CHEM INDUSTRIES
3	Cholesterol	DEEPEN DRUG PVT LTD
4	Ethanol	LOBA CHEM PVT.LTD
5	Carbopol-934	THERMOSILE FINE CHEM INDUSTRIES
6	Triethanolamine	LOBA CHEM PVT.LTD
7	Propylene glycol	THERMOSILE FINE CHEM INDUSTRIES

Table No 2 List of Instruments

Sr. No.	Instruments	Manufacturer
1	Electronic analytical weighing balance	Wesnar
2	Sonicator	Labman
3	Magnetic stirrer	Labman
4	Electronic Water bath	Lab Equipment
5	UV-Spectrophotometer	Shimadzu UV visible spectrophotometer 1900i
6	Refrigerator	Wesnar

III. EXPERIMENTAL WORK

3.1. Preformulation Study

A preformulation study is an essential step in drug development where the physical and chemical properties of a drug substance are evaluated before formulating it into a dosage form. This helps in understanding its behavior, stability, and compatibility with excipients, aiding in the design of an effective and stable formulation.

3.1.1. Solubility: Soluble in DMF, DMSO (79 mg/mL); slightly soluble in ethanol (4 mg/mL), acetone, or acetonitrile; practically insoluble in water; insoluble in ether. Soluble in 25 mM of DMSO [18]; in water, 46.85 mg/L at 25°C [6]. Pioglitazone hydrochloride very soluble in dimethyl formamide; slightly soluble in ethanol; very slightly soluble in acetone, acetonitrile. Practically insoluble in water and ether [7,19]. Tao et al. [7] measured the solubility of pioglitazone hydrochloride (form I) in methanol, ethanol, 1-propanol, acetic acid, and N,Ndimethylacetamide between 278.15 and 323.15 K at atmospheric pressure. The solubility of pioglitazone hydrochloride (form I) increases with increasing temperature and the order is N,N-dimethylacetamide>methanol>acetic acid>ethanol>1-propanol.

3.1.2. Melting Point: To melt Pioglitazone, pack a small amount into a capillary tube and fill it with oil. Attach a thermometer to the side arm of the tube, ensuring the bulb is at the same level as the sample. Secure the tube with the sample to the thermometer and heat the sidearm with a Bunsen burner. Gradually heat the tube to the expected melting point of 183°C. Carefully observe the sample as it heats up, recording the temperature at which it begins to melt and when it completely melts. The precise melting point is the temperature at which the sample transitions from solid to liquid.

3.1.3. CALIBRATION BY UV SPECTROPHOTOMETER

To prepare pioglitazone standard solutions, use a stock solution of known concentration and dilution with a solvent. Warm up a UV-Vis spectrophotometer and prepare a blank solution using the same solvent. Set the absorbance to zero and transfer aliquots of each solution into separate cuvettes. Measure the absorbance at a wavelength known to correspond to pioglitazone's absorption maximum. Choose the wavelength based on the spectrophotometer's specifications and the compound's absorbance characteristics. Record the absorbance readingsfor each standard solution. Plot a graph of absorbance versus concentration for the pioglitazone standard solutions using a suitable software tool. Perform linear regression analysis to determine the calibration curve

equation (y = mx + c), where 'y' is absorbance,'m' is the slope, 'x' is concentration, and 'c' is the y-intercept.

3.1.4. Fourier Transformed Infrared Spectroscopy: The KBr Pellet method involves weighing and grinding a small amount of pioglitazone with 100 mg of KBr to create a homogeneous mixture. The mixture is then transferred into a pellet die and compressed using a hydraulic press to form a thin, transparent pellet. The Bruker IR spectrometer is set up, and the software is initialized. A background spectrum is measured with no sample in place, or using a clean KBr pellet. The sample is placed in the sample holder and the pioglitazone is placed directly onto the ATR crystal. Parameters such as resolution, number of scans, and wavenumber range are set. The data acquisition process begins to collect the IR spectrum of the pioglitazone.[8]

3.2. Method of preparation:

3.2.1. Formulation of Ethosomes :

In order to optimize the formulation, the Box-behnken design, with 3 factors and 3 levels, was employed based on initial trial batches. Three independent factors up to three levels were applied for the preparation and optimization of ethosomes by factorial design using Design of Experiment (DOE) software version 11.0. Factorial design and DOE formulation batches of ethosomes

Design of experiments of factors of ethosomes:

Table No. 3: Factorial Design of Ethosomes

Factor	Name	Type	Min.	Max.	Coded Low	Coded High	Mean	Standard Deviation
A	Soya lecithin	Numeric	3	5	-1 ↔ 3.00	+1 ↔ 5.00	4.00	0.7559
B	Cholesterol	Numeric	2	4	-1 ↔ 2.00	+1 ↔ 4.00	3.00	0.7559
C	Sonication	Numeric	10	30	-1 ↔ 10.00	+1 ↔ 30.00	20	7.560

DOE Formulation of Ethosomes:

Table No. 4: Formulation of DOE batches of Ethosomes

DOE of experiment	Percent of components						Sonication time (sec)
	Pioglitazone (w/v)	Soy lecithin (w/v)	Cholesterol (w/v)	Ethanol (v/v)	Propylene Glycol (v/v)	Purified water (v/v)	
DOE1	1.0	4	2	30.0	15.0	q.s.to100	30
DOE2	1.0	3	3	30.0	15.0	q.s.to100	30
DOE3	1.0	4	4	30.0	15.0	q.s.to100	30
DOE4	1.0	4	4	30.0	15.0	q.s.to100	10
DOE5	1.0	4	3	30.0	15.0	q.s.to100	10
DOE6	1.0	4	3	30.0	15.0	q.s.to100	20
DOE7	1.0	4	3	30.0	15.0	q.s.to100	30
DOE8	1.0	4	2	30.0	15.0	q.s.to100	10
DOE9	1.0	5	4	30.0	15.0	q.s.to100	20

DOE10	1.0	5	3	30.0	15.0	q.s. to100	30
DOE11	1.0	3	3	30.0	15.0	q.s. to100	10
DOE12	1.0	3	2	30.0	15.0	q.s. to100	20
DOE13	1.0	5	3	30.0	15.0	q.s. to100	10
DOE14	1.0	3	4	30.0	15.0	q.s. to100	20
DOE15	1.0	5	2	30.0	15.0	q.s. to100	20

3.2.2. Preparation of Pioglitazone-Loaded Ethosomes:

Ethosomes were prepared by using a cold method. Tazarotene was dissolved in ethanol in a dried and cleaned round bottom flask. Then soy-lecithin and cholesterol were accurately weighed and transferred into the ethanol. The ethanol mixture was mixed with the help of mechanical stirrer. Then propylene glycol was added to the above lipid mixture under stirring and mixed. In a separate vessel, took purified water and maintained the temperature at 30° C by using a water bath. On the other side ethanolic mixture was also heated to 30° C temperature in a water bath. Then aqueous phase was added slowly to the lipid phase in a fine stream with constant mixing at 700 rpm by using a mechanical stirrer. After the complete addition of the aqueous phase, mixing was continued for 5 minutes and ethosomal suspension was obtained. The ethosomal suspension was then sonicated for 0.5 to 3 minutes to reduce the size of ethosomes by using a probe sonicator. After sonication, the ethosomal suspension was stirred continuously for 30 minutes to obtain stabilized ethosomes. The formulation was stored at 4-8 °C until further use. [9]

3.3. Evaluation Parameter:

3.3.1. Size Distribution and Zeta Potential:

The formulated ethosome vesicles for size, zeta potential and PDI values analysis was performed using dynamic light scattering (DLS) through computerized system (Zeta Sizer, Nano-ZS, Malvern, UK). The ethosomes samples were diluted to avoid aggregation and analyzed with constant angle of 90º [5]. The investigations were performed in triplicate for all samples at 25 ºC.

3.3.2. Entrapment Efficiency (% EE):

The entrapment efficiency of the Pioglitazone loaded ethosomes suspension stored overnight at 4 ºC was determined by the ultra-centrifugation technique. In eppendorf tubes 1 mL Pioglitazone loaded ethosomes formulation was added to PBS (pH 7.4) [10]. Further, tubes were kept in cold centrifuge Beckman model F1202 supplied with fixed angle rotor and centrifuged at 15,000 rpm for 2 h [10]. Further, separated uppermost clear layer, diluted with PBS (pH 7.4) and analyzed using UV–Vis spectrophotometer for recording absorbance at wavelength 223 nm [8]. The % EE was expressed as below:

$$EE\ (\%) = \frac{T - C}{T} \times 100$$

3.3.3. DSC Analysis:

The drug-excipient compatibility was confirmed by analysis of thermal behavior of pure TH and its physical mixture run on DSC. DSC (model Mettler TA 4000) was used to measure the transition temperature of lipids. The thermogram of optimized formulation was recorded for analysis of changes in thermal behavior evidenced due to changes in physical characteristic. The energy scale of the instrument was calibrated using blank aluminum crucible [11]. Accurately weighed samples were transferred to an aluminum pan and heated in the range 30-350 ℃, at 5 ℃/min of under constant nitrogen purge to obtain thermogram.

3.4. Preparation of transdermal ethosomal gel:-

In the Carbopol gel of various compositions (1%, 1.5%, 2% w/w) the best achieved Ethosomal suspension, of formula was selected and was incorporated specific amounts of Carbopol 934 powder which were added slowly to ultra pure water and kept for 20mins at a temperature of 100OC. Then later on, drop wise Triethanolamine was added into it. Then incorporation of an accurate quantity of formula which contains Carvedilol (1.5% w/w) was done in the

gel base and then water of sufficient quantity was added with continuous stirring to the other ingredients of the formulation until a formulation of homogenous nature was obtained which were christened as (G-1, G-2, G-3).By the use of 1.5%w/w Carbopol, a gel constituting free Carvedilol was prepared with the aid of similar method.[12]

Table No. 5: Composition of different Ethosomal Gel formulation

Gel formulation	Carvedilol Ethosomal suspension (ml)	Carbopol (%)	Triethanolamine (ml)	Phosphate buffer (pH 7.4)
G-1	20	1	0.5	q.s
G-2	20	1.5	0.5	q.s
G-3	20	2	0.5	q.s
*G-4	0.025g	1.5	0.5	q.s

*G-4 Drug free gel

3.5. Evaluation of ethosomal gel based on gel characteristics:

The drug loaded ethosomes incorporated gels along with plain drug gel were evaluated for pH, spreadability, gel strength , extrudability and drug release and permeation studies based on the methods in literatures.

3.5.1. Measurement of pH of the ethosomal gel:

1 g Pioglitazone ethosomal gel was paired with a homogenizing agent in 100 mL of distilled water. Electrodes are then submerged in the solution and readings from the optical pH meter were recorded in three times and the average value was determined.

3.5.2. Spreadability:

It is defined as the extend of area covered when gel is applied over the skin or affected part, as the spreading value effects the therapeutic efficacy of a formulation. When gel is placed between the slides against a certain load, the two slides slip off over the gel and this time is noted down to calculate the spreadibilty. Minimum time taken by the slides for separation, shows better spreadability. [13] Spreadability was calculated by using the following formula:

$$S = M.L / T$$

Where, S = Spreadability, M = weight attached to upper slide, L = length of spread, T= time taken.

3.5.3. Viscosity study: Viscometer spindle was dipped into the preparation of about 50 gm which was placed in suitable beaker up to an immersion mark on the spindle shaft. The motor rotates the spindle at definite speed in rpm and the resistance to rotation gives the viscosity value. Readings were utilized for obtaining viscosity.

3.5.4. Homogeneity and grittiness: Minimal volume of ethosomal gel between the index finger and thumb has been pressed. The presence of coarse particles was tested for gel consistency between the statistics. In addition, homogeneity may be observed if a tiny amount of ethosomal gel was rubbed on the back of the hand skin. The scraping of prepared ethosomal gel was similarly

In vitro release studies: Franz diffusion cell was used for trials of permeation of the skin. Study with prepared rat skinm was performed. The receptor bath took 28 ml of PBS pH7.4 and constantly rubbed with a magnetic ring and held temperature at a temperature of 37°±1°C. In the donor compartment was the primed skin facing stratum corneum upwards. In the donor compartment, 1 g of ethosomal gel formulation was put. A 5 ml sample was collected over 8 hours through the sampling port and each sample was substituted with equivalent volume of the fresh dissolution media. Then the pharmaceutical material was measurement by examining the samples using a 246 nm UV-visible double-beam spectrophotometer phosphate buffer as null. Related analysis with branded Pioglitazone gel was carried out. In-vitro permeation trials have been performed with all laboratory batches of ethosomal gel, cumulative substance emission and permeation fluid. [14]

IV. RESULT AND DISCUSSION

4.1. Preformulation Study

Following results are obtained in the preformulation of drug by melting point and spectral analysis.

4.1.1. Organoleptic Properties:

Table No. 6: Result of Organoleptic Properties of Pioglitazone

Sr. No.	Parameters	Observations
1	Physical State	Crystalline powder
2	Colour	White to off white
3	Odour	Odourless

4.1.2. Solubility of Pioglitazone:

Table No.7: Result of Solubility of Pioglitazone

Sr. No.	Solvent	Solubility
1	Water	Insoluble
2	Ethanol	Soluble
3	Methanol	Soluble

4.1.2. Melting Point: The reported melting point of pioglitazone was in range of 183-184^0 C. The observed melting point was found at 183^0 C. It confirms that the given powdered drug is pure in nature and it complies that powder is a Pioglitazone.

4.1.4. Characterization by UV Spectrophotometer

Determination of λ Max

The spectra of pioglitazone were obtained by using 10µg/ml solution of pioglitazone in ethanol, where an absorbance maximum was found to be at 272.0 nm

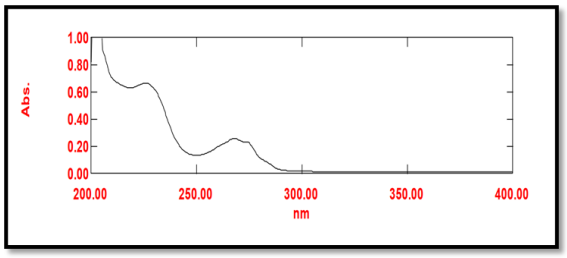


Fig. No.2: UV absorption spectrum of Pioglitazone in ethanol

Calibration curve of Pioglitazone in ethanol

Table No.8: Calibration curve of Pioglitazone in ethanol

Concentration (µg/ml)	Absorbance
5	0.2228
10	0.3608
15	0.5171
20	0.6908
25	0.8328

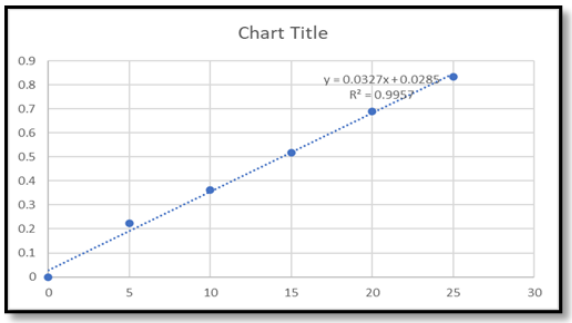


Fig. No.3 : Calibration Curve of Pioglitazone in Ethanol

Table No. 9: Various constants for calibration curve of pioglitazone in Ethanol

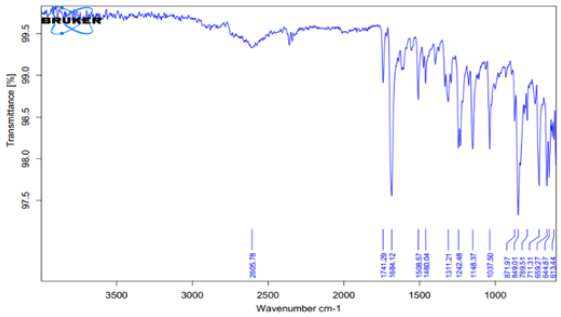
Parameters	Slope	Intercept	R^2
Value for calibration curve in pioglitazone	0.0327	0.0285	0.9957

4.1.5. Charecterization By IR

Drug FTIR: Common peaks observed include a broad peak around 3300 cm^-1 corresponding to the N-H stretching vibration, peaks around 1700-1750 cm^-1 indicating the presence of carbonyl groups, and peaks around 1500-1600 cm^-1 corresponding to aromatic C=C stretching vibrations. These results confirm the identity and structural features of pioglitazone HCL, aiding in its characterization and analysis.

Fig. No.4: Fourier Transformed Infrared Spectrum of Pioglitazone

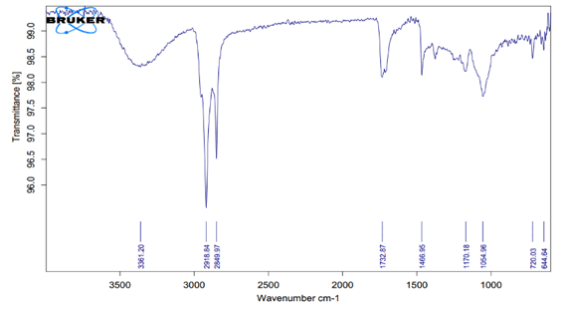
Table No.10.: Interpretation of data of FTIR



Functional Gorup	Standard Value (cm-1)	Observed Value (cm^{-1})
N-H	3509-3460	3489
C=O	1745-1680	1721
C-O-C	1250-1150	1210
S-NH	1159-1150	1152

4.1.6. Drug And Excipient FTIR: The infrared (IR) spectroscopy results of the combination of pioglitazone, soya lecithin and cholesterol indicate distinct peaks corresponding to the functional groups present in each compound. Common peaks observed include those for pioglitazone, such as the N-H stretching vibration around 3300 cm^-1, carbonyl groups around 1700-1750 cm^-1, and aromatic C=C stretching vibrations around 1500-1600 cm^-1. Peaks corresponding to soya lecithin and cholesterol functional groups will also be present, providing additional information about their chemical structures. These results aid in the characterization and analysis of the mixture, facilitating the identification and quantification of each compound present.

Fig. No. 5: FTIR Spectrum of physical mixture of Pioglitazone



4.2 Characterization of pioglitazone loaded ethosomes :

4.2.1. Physical appearance: The ethosomal suspension was found to be

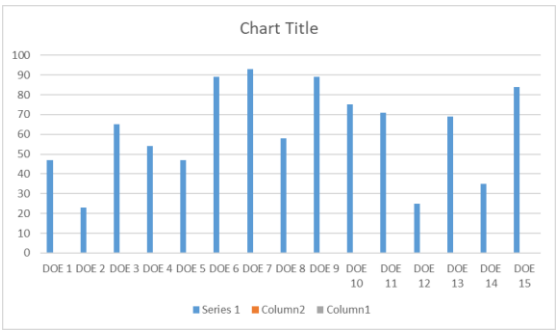
Table No.11: Physical appearance of Pioglitazone loaded ethosomes

Parameters	Observation
Appearance	Suspension
Color	Pale yellow to white creamy
Odor	Alcoholic

4.2.2. Drug entrapment efficiency (%) : Drug entrapment efficiency was determined by using UV spectrophotometer (UV-1800 Shimadzu) for all trial batches. Comparative results were presented in Table Noand Graph No

Table No.12: Entrapment efficiency of prepared ethosomal batches of pioglitazone

Batche code	Entrapment efficiency %
DOE1	47± 0.80
DOE2	23±1.02
DOE3	65±1.60
DOE4	54±0.90
DOE5	47±1.03
DOE6	89±1.06
DOE7	93 ±1.27
DOE8	58±0.70
DOE9	89±1.42
DOE10	75±1.07
DOE11	71±0.86
DOE12	25±1.54
DOE13	69±1.05
DOE14	35±0.9
DOE15	84±1.08



4.2.3. Average determination of pioglitazone of ethosomes:

The average particle size of pioglitazone loaded Ethosomes was found to be 350.9 nm. of optimum batch DOE 7. And The zeta values for Ethosomal formulations were found to be in range of -2.7 ± 0.45 mV to -31.04 ± 0.25 mV as shown in Table 3. The zeta potential of the phytosome under study was found to be 31.04 ± 2.7 mV as shown in Figure 1. The results revealed that the zeta values of the vesicles increase toward negative with increasing the HLB values of the surfactants. The effect of HLB values of surfactants on zeta potential could be explained in terms of surface energy, which tends to increase with increase in HLB values toward the hydrophilicity. Increase in surface energy of the vesicles

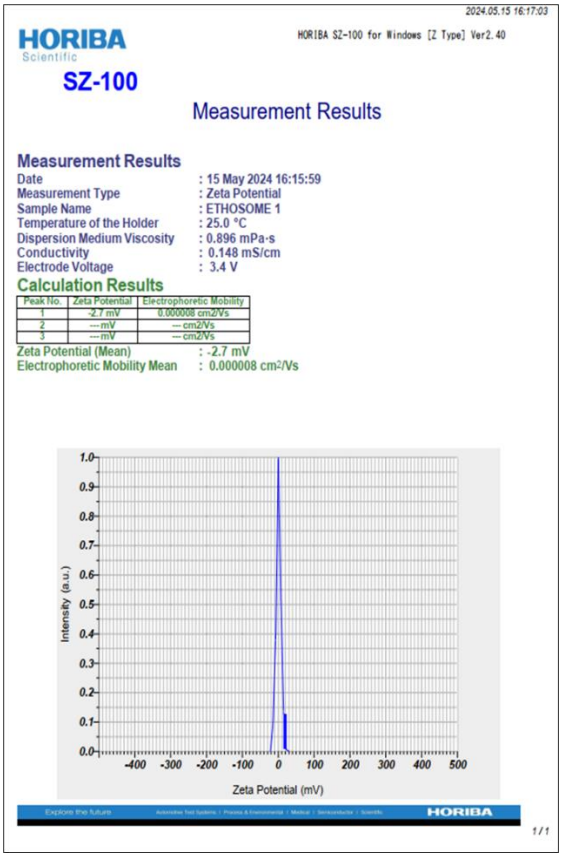


Fig. No. 6: Zeta Potential of Ethosomes

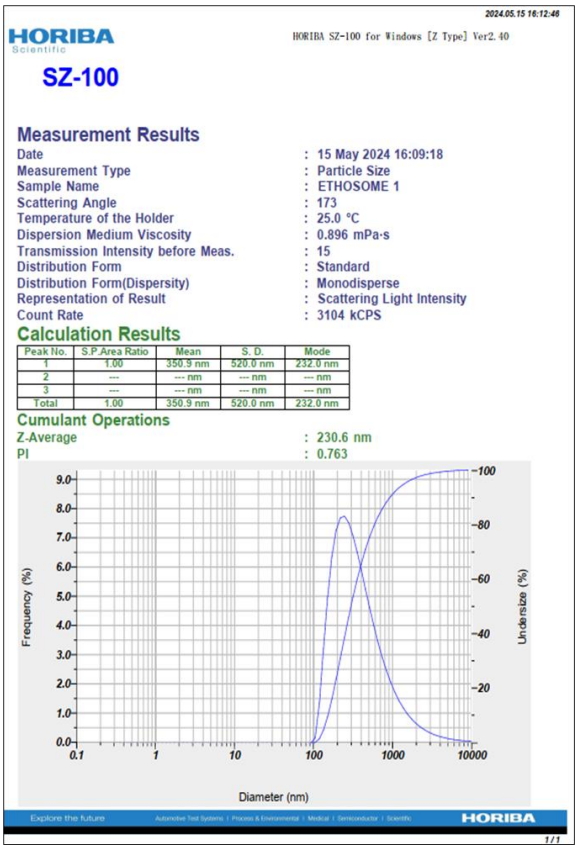


Fig. No. 7: Average particles size of ethsomes

CONCLUSION

In conclusion, the formulation and characterization of Pioglitazone-loaded ethosomal gel represent a significant achievement in pharmaceutical research focused on enhancing drug delivery efficiency. Ethosomes, characterized by their lipid bilayer structure and high ethanol content, have demonstrated superior skin penetration capabilities, which are crucial for effective transdermal drug delivery. This study successfully developed a stable ethosomal gel formulation of Pioglitazone, ensuring optimal drug encapsulation and retention.The comprehensive characterization of the ethosomal gel encompassed various parameters such as particle size, morphology, drug release kinetics, and stability profiles. These assessments confirmed the formulation's robustness and suitability for sustained drug delivery applications. Importantly, the ethosomal gel exhibited promising results in terms of enhancing Pioglitazone's permeation through the skin barrier, thereby potentially improving its bioavailability and therapeutic efficacy.The development of Pioglitazone-

loaded ethosomal gel holds significant promise for diabetic patients, offering a non-invasive and patient-friendly treatment option with controlled drug release characteristics. This formulation not only addresses the challenges of conventional drug delivery but also opens avenues for further research into optimizing diabetes management strategies. Future studies should focus on clinical trials to validate its effectiveness and safety profile in real-world applications, ultimately aiming to integrate this innovative formulation into clinical practice for enhanced patient care.

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