https://doi.org/10.33472/AFJBS.6.1.2024.2148-2159

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

# Elaboration and Characterization of Empagliflozin-Loaded Pharmacosomes: A Comprehensive Study

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**Article Info**

Volume 6, Issue 6, June 2024

Received: 05 April 2024

Accepted: 1 0 May 2024

Published: 06 June 2024

*doi: 10.33472/AFJBS.6.1.2024.2148-2159*

**ABSTRACT:**

The primary objective of the current research design was to develop pharmacosomes that could be linked to Empagliflozin (EMGF). Pharmacosomes represent a highly promising technique for delivering drugs in vesicular form, offering several advantages over conventional methods of vesicular drug administration. These specialized structures, known as phospholipid complexes or pharmacosomes, can potentially enhance the absorption of poorly soluble medications in both lipids and water. Given that the FDA and EMA classify Empagliflozin (EMGF) as a BCS class 3 drug, which typically exhibits limited permeability, the focus was on creating pharmacosomes to enhance its permeability and bioavailability. By utilizing an innovative technique known as anhydrous cosolvent lyophilization, Empagliflozin (EMGF) was combined with soya phosphatidylcholine in various ratios to generate the pharmacosomes. This approach allowed for a comprehensive assessment of the resulting pharmacosomes, encompassing factors such as drug content, zeta size and potential, FTIR (Fourier-transform infrared spectroscopy) analysis, DSC (differential scanning calorimetry) analysis, partition coefficient, and an in-vitro dissolution study. Among the various pharmacosome formulations, the most optimal one, designated as P5 and characterized by an Empagliflozin to lecithin ratio of 1:2, exhibited an impressive drug content of 96.4% w/w. The zeta size and potential measurements, indicating stability, were found to be 283 nm and -68.69, respectively. Notably, formulation P5 demonstrated a robust drug release rate of 94.43%. The FTIR analysis of the pharmacosomes revealed a noteworthy shift in the Alcohol OH bond to lower wave numbers, which suggested the formation of bonds between the drug and soya lecithin. The DSC results further confirmed the distinct peaks of the pharmacosomes compared to pure EMGF, serving as evidence of successful pharmacosome preparation. In conclusion, the process of complexing Empagliflozin (EMGF) with soya phosphatidylcholine through pharmacosome formation holds significant promise for significantly improving the drug's permeability and bioavailability. This innovative approach could potentially revolutionize the delivery of poorly soluble drugs, offering a more effective means of administration.

Keywords: Pharmacosomes; Vesicular Drug Delivery System; Amphiphilic; Phospholipid; Anhydrous Cosolvent Lyophilization Method

## 1. Introduction

The first naturally occurring SGLT (Sodium-Glucose Co-Transporter) inhibitor with high affinities, comparable, specificity inhibitory activity for SGLT1 & SGLT2 was phlorizin, which in 1835 was separated from an apple tree [1]. Phlorizin is the cause of glycosuria. Different analogues of phlorizin have been created, each with varying potencies and selectivities against SGLT. Dapagliflozin (Dapa), which has more than 1200 times more efficacy for SGLT2 than SGLT1 [2], was created in 2008. Another phlorizin derivative, Canagliflozin (Cana), has 400 times more potent anti-SGLT2 activity than anti-SGLT1 [3]. Compared to other SGLT2i that are commercially available, Empagliflozin (EMGF), The third drug in this category, favours SGLT2 over SGLT1 most strongly 2700 times [4]. The fourth phlorizin analogue to be created, ertugliflozin, SGLT2 exhibits a 2200-fold higher selectivity than SGLT1 [5].

SGLT inhibitors result in glycosuria, which has cardioprotective benefits and lowers the risk of cardiovascular consequences [6]. All of the filtered glucose is usually reabsorbed. When the plasma's glucose level exceeds 180 mg/dL, filter glucose is excreted in the urine. The "threshold for glycosuria" is the name given to this cutoff. Functional or morphological glomerular tubular imbalance explains the splay between the actual threshold of 180 mg/dL and the theoretical threshold of 300 mg/dL. A lower threshold for glycosuria, a rise in splay, or a reduced ability of the kidneys to reabsorb glucose are the causes of glycosuria [7].

In a typical situation, there is no glucose in the urine because all filtered glucose is re-absorbable in the renal tubules [8]. Eighty to ninety percent of the filtered glucose is reabsorption by SGLT2s in the S1 section of the proximal tubules, and ten to twenty percent is reabsorption by SGLT1s in the S2/S3 segment [7,9].

The CV safety of EMGF was investigated in the EMGF CV Outcome Event Trial in Type 2 DM Patients. In contrast to placebo, Incidences of nonfatal myocardial infarction, mortality from CV causes and nonfatal stroke were all reduced in the EMGF group. It also decreases heart rate without changing systolic or diastolic blood pressure [10].

In colloidal vesicles called pharmacosomes, drugs associated with lipids are distributed [11, 12]. Any medication containing an active carboxyl, hydroxyl, or amine group can form a covalent link with lipid molecules. Drugs that contain carboxyl groups can be esterified, while those that contain amine or hydroxyl groups need the help of a spacer linkage [13, 14].

The science of medication distribution makes extensive use of phospholipids. Due to quicker body disposal, it is a crucial carrier for therapeutic compounds that need sustained or controlled release in vivo. Developing pharmaceuticals as lipid complexes may prove to be a viable strategy to increase solubility and reduce GI toxicity. These stable and more accessible amphiphilic drug-lipid complexes have low interfacial tension between the system and the GI fluid, which makes it easier for the organism to transport the compounds across membranes, tissues, or cell walls [13].

Nanoemulsions, Chitosan derivatives, bile salt, spray freeze drying, straight chain fatty acids, Cyclodextrin inclusion complex, self-micro-emulsifying drug delivery systems, and saponins are just a few of the different methods used to increase intestinal permeability [15].

Numerous medications, including rifampicin, tamoxifen and curcumin, have reportedly enhanced oral bioavailability and solubility due to the phospholipid complexation phenomenon. Additionally, its short half-lives make it a crucial carrier for therapeutic compounds requiring a sustained/controlled release behaviour inside the body [16-18]. This occurrence was also ascribed to the ability of phospholipids to solubilize lipids, which allowed water-soluble medications, including vinorelbine, clarithromycin, and insulin, to pass more easily through lipidic membranes [19-21].

In our research, we use Empagliflozin, classified as being in BCS Class III (low intestinal permeability) by the FDA and EMA [22, 23]. By creating pharmacosomes (a phospholipid

complex), we attempt to tackle this difficulty in this research. The anhydrous cosolvent lyophilisation process is used to obtain the pharmacosomes of Empagliflozin or EMGF-PC in dry powder form, which offers stability and increases intestinal permeability. Additionally, evaluations were performed for different physical characteristics including FTIR, DSC, Solubility, Zeta sizer and potential, and in vitro dissolution studies.

## 2. Materials and Methods

### 2.1. Materials

Drug Empagliflozin is purchased from Bulat Pharmaceuticals Pvt. Ltd., and all other materials like Soya lecithin, Mannitol, PEG 4000, and DMSO from Central Drug House in New Delhi.

### 2.2. Formulation of EMGF-PC

Using the anhydrous cosolvent lyophilization method, we produce the pharmacosomes of EMGF. This method uses a tetrahydrofuran solvent to dissolve the medication EMGF and soya lecithin. Different cryoprotectants in varying doses should be added, such as mannitol, DMSO, and PEG 4000. Place this mixture in a lyophilizer for 6 hours at a temperature of –60 °C and a vacuum of –998 mmHg, as illustrated in Figure 1. Place it at room temperature for some time and pass it through sieve no. 80. Put it away in a closed container in a cool, dark place. Best formulations are obtained by using mannitol as a cryoprotectant instead of DMSO and PEG 4000. So, we use mannitol for further studies shown in Table 1.

### 2.3. Determination of the Content of EMGF in pharmacosomes

By using spectrophotometry, the amount of EMGF in the complex was identified. 10mg of pharmacosomes, the medication equivalent in powder form, was prep. in 10 ml acetonitrile + $H_2O$ (1:1), and then the mixture was agitated for two hours on a shaker. Dilute the mixture & measure the absorbance of the result at 224 nm; EMGF's absorption in the complex was identified.

### 2.4. In vitro d

### Rug release of EMGF pharmacosomes

The HICON USP dissolution test apparatus Type 2 was used to conduct an in-vitro release study for all formulations. EMGF-PC solution dispersion was positioned within the dialysis bag and sealed from both ends afterwards. These vials have a 900 ml volume of pH 6.8 release medium and are set to 37ºC and 100 rpm. External release media in 5 ml was taken out as planned and replaced with a new medium in the same amount. A UV spectrophotometer was then used to measure the drug release from the samples.

### 2.5. Kinetic models of EMGF pharmacosomes

Fitting the data of best formulation obtained from In Vitro drug permeation studies to several kinetic equations like zero order, first order, Higuchi, and Korsmeyer Peppas model.

### 2.6. Partition coefficient of EMGF & its pharmacosomes

The shaking flask method calculates the EMGF and its pharmacosomes partition coefficient. Mix 25 ml of octanol and 25 ml of water to dissolve the specified amount of compound. Measure the absorbance using a UV-visible spectrophotometer after 24 hours of dark storage and thorough shaking. Compared to hydrophilic medicines with partition coefficients below 1, lipophilic medications have partition coefficients above 1 ($\log P > 1$).

### 2.7. Particle size distribution & potential of EMGF pharmacosomes

The Malvern Zetasizer is used to examine particle diameter and size of pharmacosomes. To achieve the scattering intensity of 1lac counts/s, samples get diluted with $H_2O$. In the analysis, each sample was evaluated in triplicate. Pharmacosomes values were expressed as a range of mean sizes.

### 2.8. FTIR analysis of EMGF & its pharmacosomes

Prepare a physical mixture of drugs with KBr pellets per standards made by carefully combining 1mg of sample powder with 100mg of KBr. FTIR spectra for the EMGF pharmacosomes were acquired on a Jasco FT/IR 4100typeA with wave number area 4,000-400 1/cm.

### 2.9. DSC analysis of EMGF & its pharmacosomes

Using a Perkin Elmer Pyris 6 Differential Scanning Calorimeter, EMGF pharmacosome thermograms were captured. Each sample was heated to a temperature of 2.0±0.2 mg in a sample pan with N2 gas flowing to study the thermal behaviour. A heating rate of 10ºC /min was used during the experiments, which were conducted at temperatures between 40 and 400 ºC.

### 2.10. Scanning Electron Microscopy (SEM) of EMGF pharmacosomes

To detect the surface morphology of the pharmacosomes, SEM of EMGF pharmacosomes is determined by Scanning Electron Microscope ZEISS EVO 18.

### 2.11. Stability Study of EMGF pharmacosomes

Stability analyses of the ideal formulation P5 were conducted in a humidity control oven for 30 days at an accelerated temp. of 40°±2°C and RH of 70±5%RH. The sample was assessed 30 days later for its physical characteristics, drug content, and in-vitro drug release investigations.

## 3. Results and Discussion

We prepared EMGF pharmacosomes by anhydrous cosolvent lyophilization technique. Different quantity ratio of drug and soya lecithin 1:1 and 1:2 is taken, and various cryoprotectants like DMSO, PEG 4000, and Mannitol are used.


Figure 1. Lyophilizer display

Table 1. Formula of pharmacosomes

| Formulation | Drug (mg) | Soya Lecithin (mg) | Mannitol (%) |
|---|---|---|---|
| P1 | 50 | 50 | 5% |
| P2 | 50 | 50 | 10% |
| P3 | 50 | 50 | 15% |
| P4 | 50 | 100 | 5% |
| P5 | 50 | 100 | 10% |
| P6 | 50 | 100 | 15% |

### 3.1. Physical appearance of pharmacosomes

The physical appearance of pharmacosomes prepared from different cryoprotectants differs, as by using DMSO, pharmacosomes form but are sticky to the surface of the petri dish and hard to scrape, and by using PEG 4000, pharmacosomes form but are less sticky, easy to scrape, but unable to pass through the sieve, while using Mannitol, pharmacosomes form that are none sticky to the surface of the petri dish, easy to scrape, and also pass through the sieve as illustrated in Figure 2. So, we use mannitol for further study.

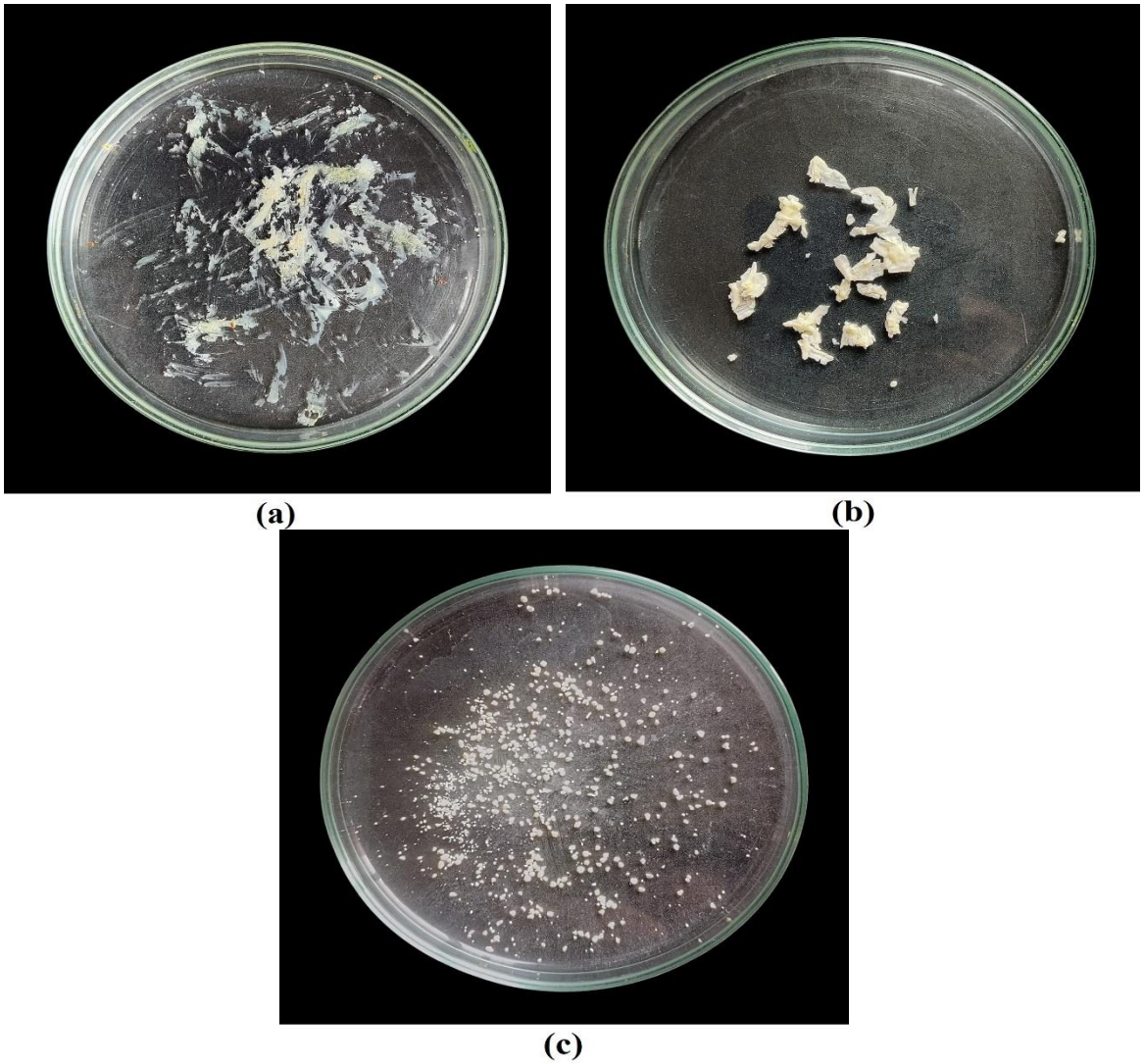


**Figure 2.** Physical appearance of pharmacosomes using different cryoprotectants (a) DMSO, (b) PEG 4000 and (c) Mannitol

### 3.2. Determination of the Content of EMGF in pharmacosomes

The complex's EMGF drug content was between 87 and 96.4%, showing that the formulations had an appropriate level of the medication. A major benefit over liposomes is the high proportion of drug loading in pharmacosomes. The largest amount of medication was present in formulation P5, at 96.4%.

### 3.3. In vitro drug release of EMGF pharmacosomes

Data on EMGF pharmacosomes for in-vitro release. The experiment was run utilizing the dialysis procedure for 24 hrs. The release of formulation P1 was 80.93%, that of Formulation P2 was 82.85%, that of Formulation P3 was 85.13%, that of Formulation P4 was 90.04%, that of Formulation P5 was 94.43%, and that of Formulation P6 was 92.32% as illustrated in Figure 3. Formulations P4, P5, and P6 received relatively higher soya lecithin additions than P1, P2, and P3. But overall, drug release from formulation P5 was high. The rate and proportion of drug release were shown to have dramatically decreased when the content of soya lecithin in the formulation dropped. The mechanism of drug release from EMGF-PC was determined by different mathematical models, e.g., zero order, first order, Higuchi kinetics and Korsmeyer-Peppas model, but the maximum value of $R^2$ found by zero-order kinetics as illustrated in Figure 4. So, the drug release is not depend on the concentration of the drug.

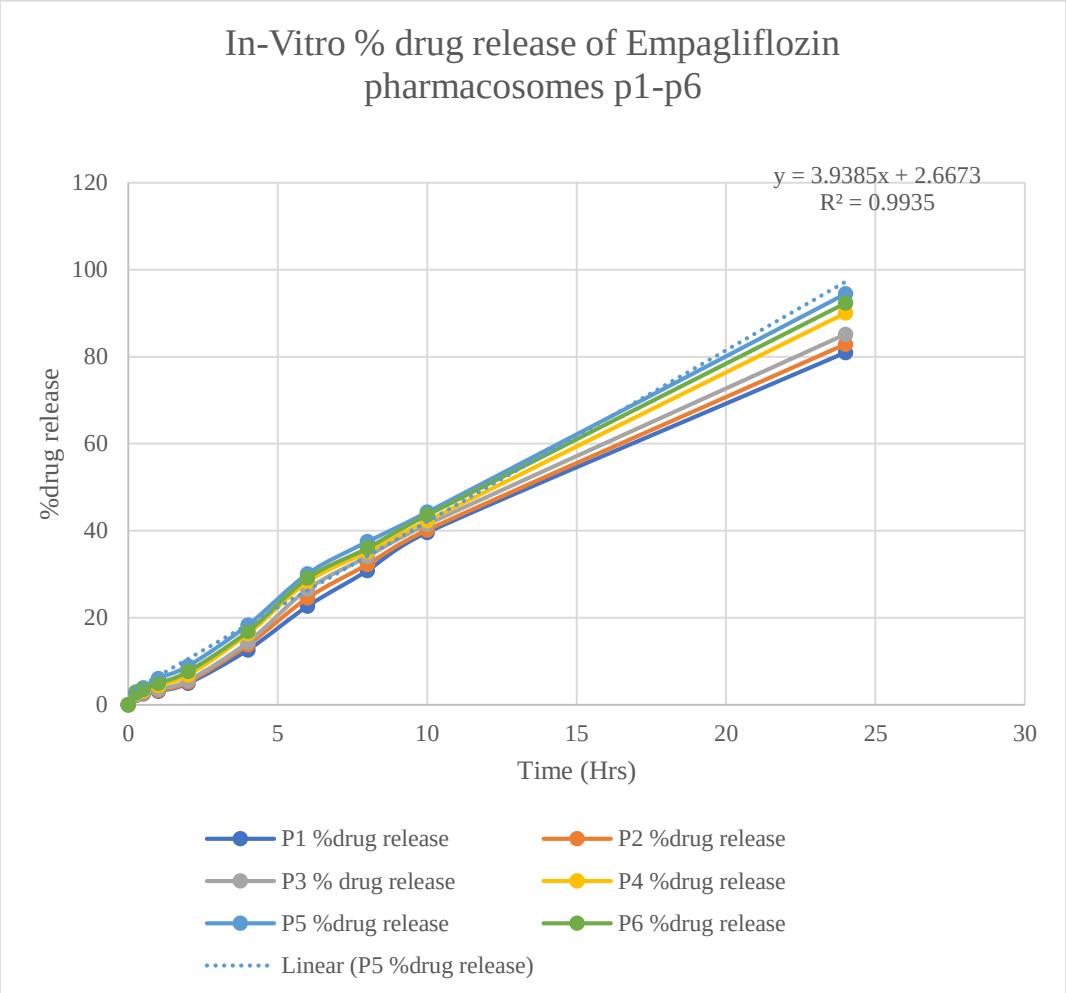


Figure 3. In vitro drug release of EMGF pharmacosomes P1-P6.

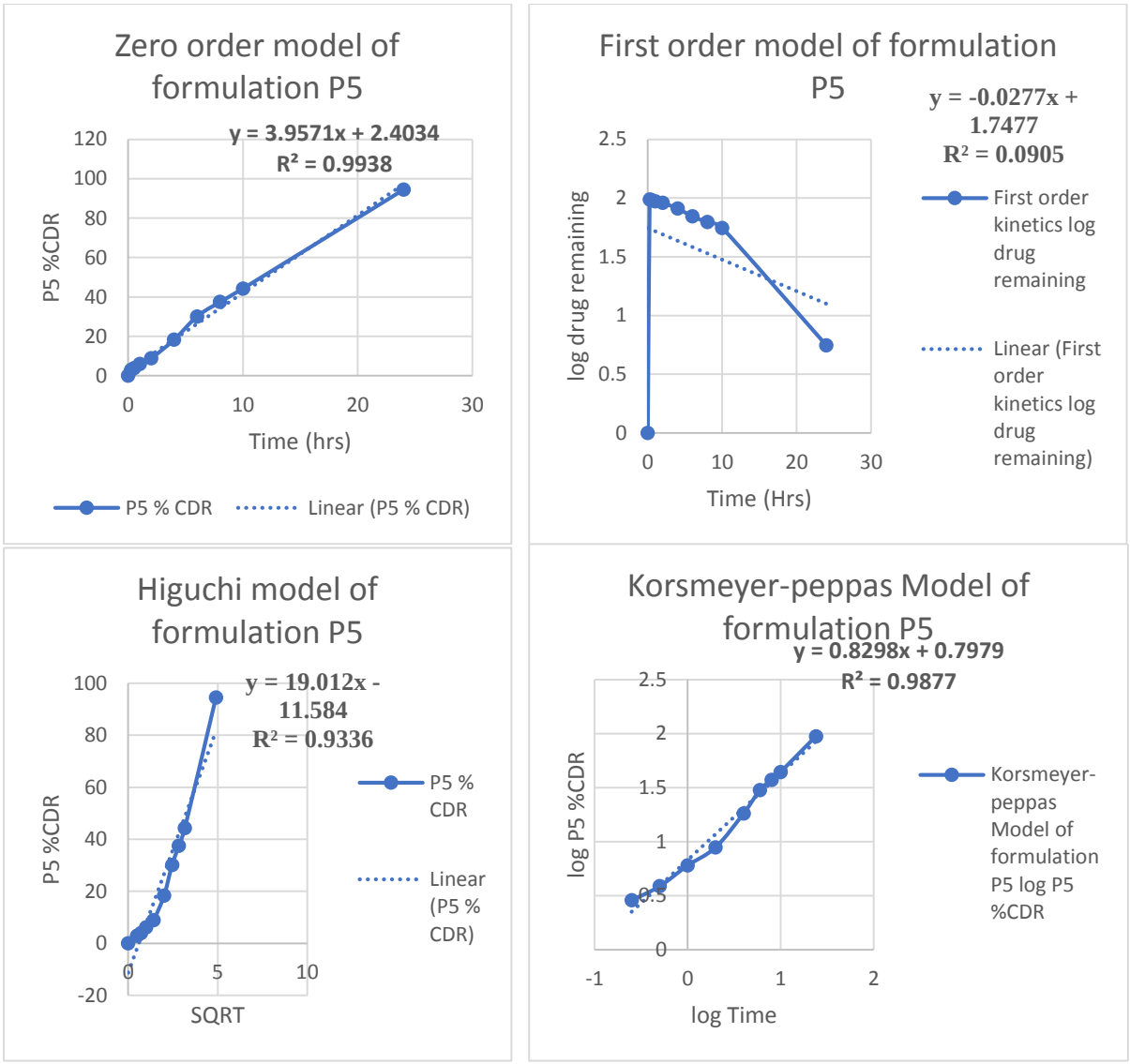


Figure 4. Drug release kinetics

### 3.4. Partition coefficient of EMGF & its pharmacosomes

The pharmacosomes of EMGF were discovered to be considerably more soluble than the drug itself (in water and n-octanol). According to the data, pharmacosomes considerably improved the lipophilicity of EMGF, as illustrated in Table 2. This was because of the pharmacosomes considerable dispersibility or/and amphiphilic character and because phospholipids covered up a polar group of the drug EMGF.

Table 2. Partition coefficient data of EMGF and Pharmacosomes P5.

| S.no. | Solvent | Empagliflozin (EMGF) | Pharmacosomes P5 |
|---|---|---|---|
| 1 | n-octanol | 0.681 mg/ml | 1.277 mg/ml |
| 2 | Water | 0.419 mg/ml | 0.569 mg/ml |
| 3 | P value | 1.625 | 2.244 |

### 3.5. Particle size distribution & potential of EMGF pharmacosomes P5

It shows that after being slightly shaken in purified water, displays the size and potential of the pharmacosomes; Malvern zeta sizer is used to determine the pharmacosome preparation's size

and zeta potential. The mean particle size and potential for the pharmacosome P5 samples are 283 nm and -68.69, respectively, when centred at the highest intensity peak, as illustrated in Figures 5 & 6.

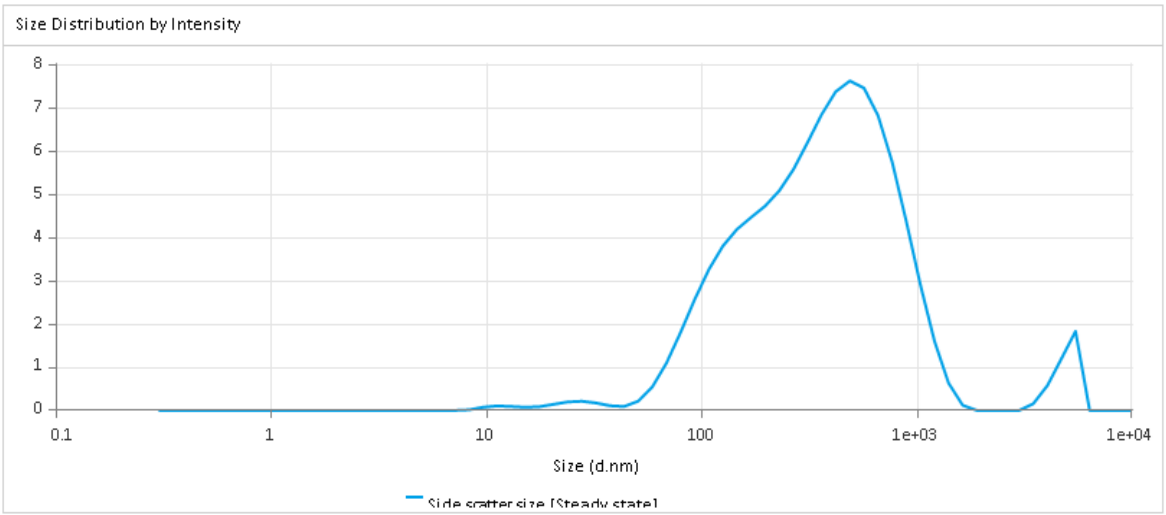


Figure 5. Particle size distribution of EMGF pharmacosomes

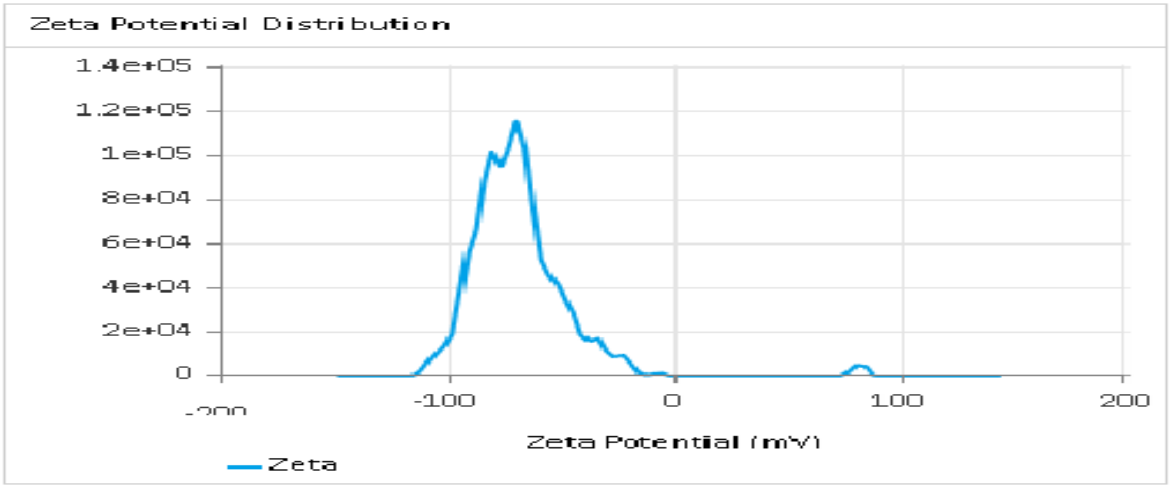


Figure 6. Zeta potential of EMGF pharmacosomes

### 3.6. FTIR analysis of EMGF & its pharmacosomes

The characteristic IR (KBr) peaks for EMGF were seen at 3598.52 $cm^{-1}$, 3062.41 $cm^{-1}$, 2989.12 $cm^{-1}$, 1060.66 $cm^{-1}$, 674.96 $cm^{-1,}$ which correspond to the Alcohol OH stretch, Aromatic C-H stretch, Aliphatic C-H stretch, C-O-C stretch & C-Cl stretch respectively. This can be found in the EMGF chemical structure. The characteristic IR (KBr) peaks for soy lecithin were seen at 3378.67 $cm^{-1}$, 2989.12 $cm^{-1}$, and 1689.34 $cm^{-1}$, which correspond to the N-H stretch, Aliphatic C-H stretch, and C=O stretch, respectively. This can be found in soya lecithin's chemical structure. The characteristic IR (KBr) peaks for a physical mixture of EMGF & Soya Lecithin show different peaks like 3598.52 $cm^{-1}$, 1060.66 $cm^{-1}$, 678.82 $cm^{-1,}$ which corresponds to Alcohol OH stretch, C-O-C stretch, C-Cl stretch respectively which are present in chemical structure of EMGF while peaks like 3378.67 $cm^{-1}$, 2989.12 $cm^{-1}$ which corresponds to N-H stretch, Aliphatic C-H stretch. This shows there is no bonding between EMGF & Soya lecithin. While the characteristic IR (KBr) peaks for pharmacosomes P5 were seen at 3471.24 $cm^{-1}$, 3019.98 $cm^{-1}$, 1060.66 $cm^{-1}$, 833.098 $cm^{-1}$ which correspond to Alcohol OH stretch (Intermolecular bond), Aromatic C-H stretch, C-O-C stretch, C-Cl stretch. Through this, we

understand that Alcohol OH stretching vibration at 3598.52 $cm^{-1}$ in EMGF has changed to the lower wave number in the EMGF pharmacosomes with absorption's band at 3471.24 $cm^{-1}$ which shows there would be bonding between the drug and soy lecithin. As in pure drug Alcohol, OH stretch is free, but in pharmacosomes, Alcohol OH stretch is an intermolecular bond, as illustrated in Figure 7.

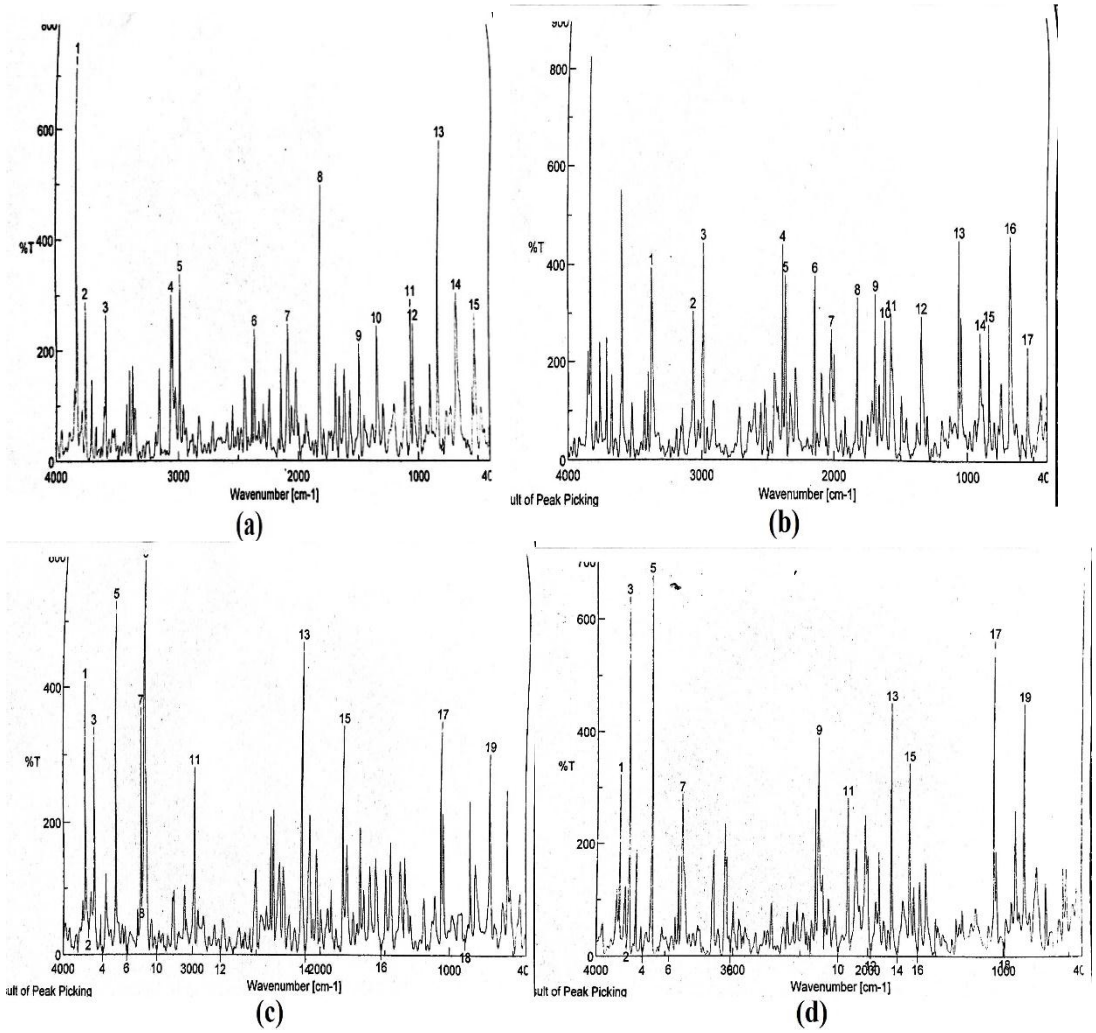


Figure 7. FTIR of (a) EMGF drug, (b) Soya lecithin, (c) Physical mixture of EMGF drug and soya lecithin, (d) EMGF Pharmacosomes.

### 3.7. DSC analysis of EMGF & its pharmacosomes

EMGF thermogram revealed a single, broad exothermic peak at 158.581°C. Various large exothermic peaks were visible on the soya lecithin thermogram at 79.288°C, 200.283°C, 300.950°C, and 332.953°C. Two large exothermic peaks at 157.991°C and 357.547°C were visible on the thermogram of the physical mixture of the drug EMGF and p'lipid. While a single peak at 144.196°C may be seen on the thermogram of EMGF pharmacosomes (complex), which differs from the EMGF peak. It is clear from the thermogram of Pharmacosomes P5 (complex) that the EMGF initial peaks vanish. Because of the development of hydrogen bonds and van der Waals forces, it was shown that phospholipids and EMGF may interact. The carbon-hydrogen chain in the phospholipids can easily rotate and enwrap the polar components of the phospholipid molecule thanks to the interaction between the polar components of EMGF and phospholipids. The second endothermal peak of phospholipids

disappears due to the sequential decrease between aliphatic hydrocarbon chains, which also lowers the phase transition temperature, as illustrated in Figure 8.

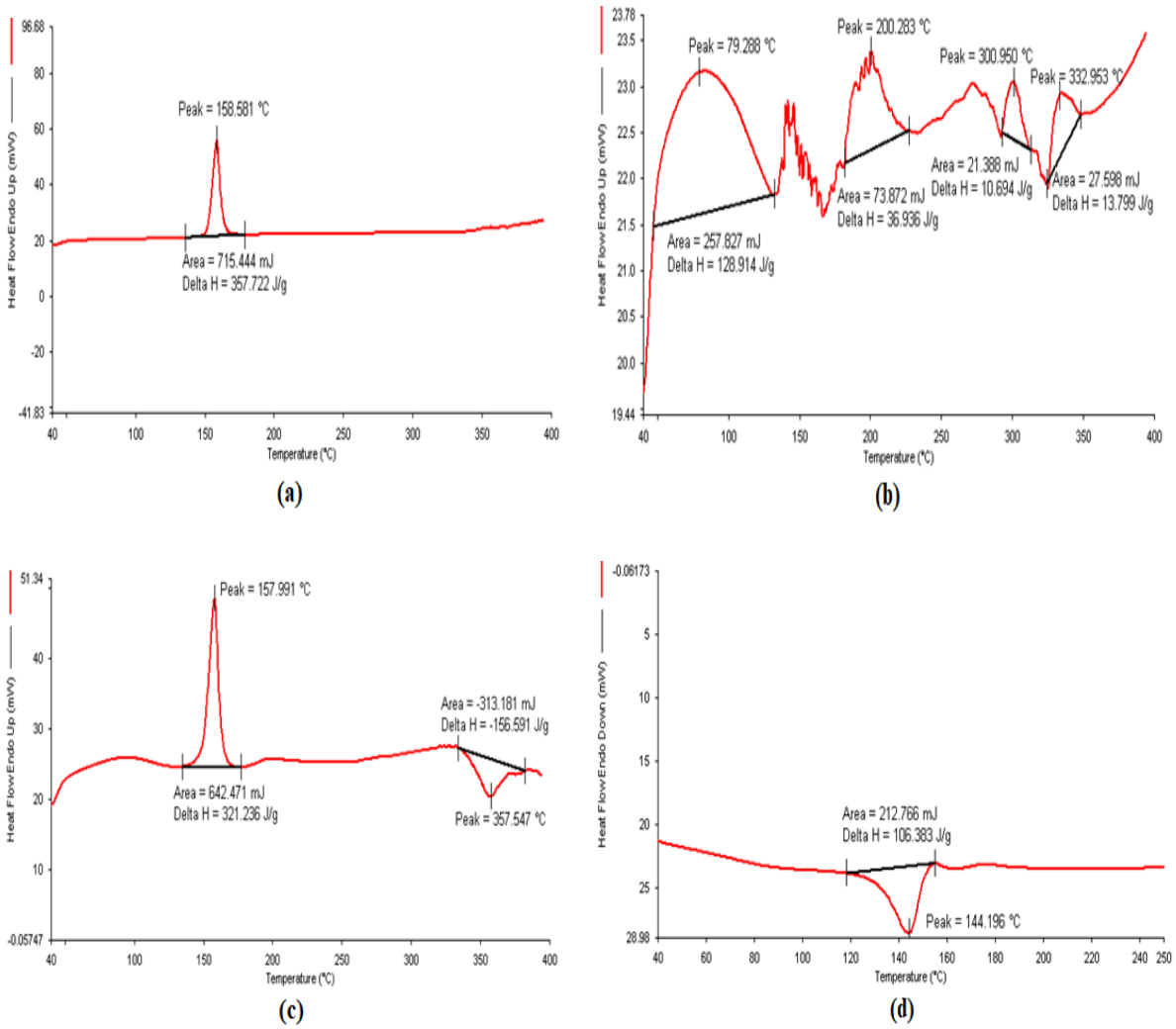


Figure 8. DSC of (a) EMGF drug, (b) Soya lecithin, (c) Physical mixture of EMGF drug and soya lecithin, (d) EMGF Pharmacosomes.

### 3.8. Scanning Electron Microscopy (SEM) of EMGF pharmacosomes

SEM of EMGF pharmacosomes are shown in Figure 9. It was discovered that EMGF pharmacosomes had a rough surface morphology and were disc-shaped. Pharmacosomes were discovered to be free flowing particles.

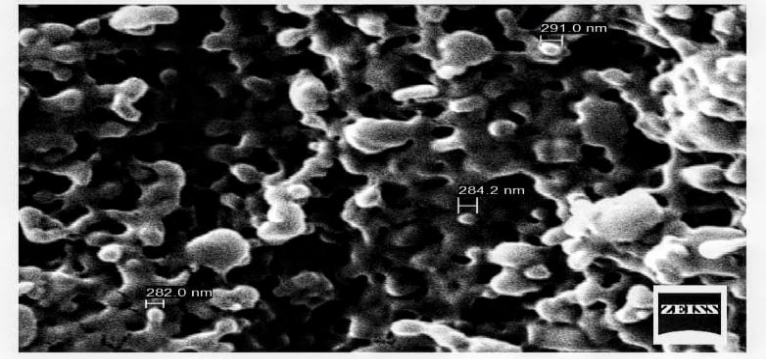


Figure 9. SEM of EMGF pharmacosomes.

### 3.9. Stability Study of EMGF pharmacosomes

It was established that there had been no significant changes in the various criteria assessed, such as physical appearance and the percentage of P5 drug content at 40°±2°C and RH of 70±5%, after the study for 30 days. As a result, it is possible to conclude that P5 is stable for 30 days at a temp. of 40° ±2°C and a RH of 70±5% as illustrated in Table 3.

Table 3. Stability study of EMGF pharmacosomes

| Parameters | Physical appearance | Drug content |
|---|---|---|
| 0 Day | Yellow colour powder | 96% |
| $30^{th}$ Day | Yellow colour powder | 95.2% |

## 4. Conclusion

The current study used the anhydrous cosolvent lyophilization method to organize an EMGF-Phospholipid complex (Pharmacosomes) and analyze it for several physicochemical properties. Overall, formulation P5 is the best one. EMGF and phospholipid interacted somewhat, as evidenced by the FTIR and DSC. In FTIR, Alcohol OH stretching vibration in EMGF has moved to the lower wave no. in the EMGF pharmacosomes, showing there would be bonding between the drug and soya lecithin. As in pure drugs, the Alcohol OH stretch is free, but in pharmacosomes, the Alcohol OH stretch is an intermolecular bond. In DSC, a single peak seen of pharmacosomes differs from pure drug EMGF and may create a covalent link, a hydrogen bond, or a van der Waals force. The lipophilicity of the drug is increased in pharmacosomes, which enhances the permeability of the pure drug. Using SEM, it was found that EMGF pharmacosomes had a rough surface morphology and were disc-shaped. The produced compound displayed amphiphilic characters and showed 94.43% drug release. The zero-order model explains how EMGF pharmacosomes release and permeate the body. Also, stability studies show that pharmacosomes are physically and chemically stable.

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