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Preformulation Studies of PLGA Encapsulated Metformin Hydrochloride Microsphere

Priyanka Chauhan*, C S Chauhan

Corresponding author: pri3aug84@gmail.com
*Research scholar, Faculty of Pharmacy, B N University, Udaipur

Abstract Preformulation stage is a critical step in the development of new dosage forms, ensuring a comprehensive understanding of the physicochemical properties of the active pharmaceutical ingredient (API or drug molecules) and other excipients . This step is essential for establishing a solid foundation for the subsequent formulation and development stages. The focus of preformulation studies is on assessing the fundamental physicochemical properties of the drug molecules. A key aspect of this research is the exploration of how varying concentrations of the PLGA polymer affect the properties of the resulting microspheres. By systematically adjusting the polymer concentration, the study aims to understand its impact on crucial microsphere characteristics such as size, morphology, drug loading efficiency, and release kinetics. To ensure a comprehensive analysis, the microspheres undergo meticulous characterization. This involves employing various techniques such as SEM, DLS, FTIR, and drug release studies. Through these analyses, the researchers aim to gain a detailed understanding of how the inclusion of the PLGA polymer influences the overall behaviour and performance of the microspheres. Furthermore, the study seeks to elucidate the release kinetics and pharmacological activity of Metformin when delivered via these PLGA-based microspheres. By examining how different polymer concentrations affect drug release patterns, the research aims to optimize the formulation to achieve sustained release of Metformin over an extended period.

Keywords: Preformulation stage, PLGA, PLGA-based microspheres

Introduction

Diabetes mellitus encompasses several disorders indicated either due to inadequate insulin generation because of damaged islet cells of the pancreas or due to lack of sensitivity of host cells (1). Diabetes mellitus has been published to be linked with the incidences of heart failure, leading to the death of individuals. Insulin is pancreatic produced hormone which exhibits a crucial role in controlling blood sugar levels. When there is a deficiency in insulin or when the cells become unaffected by its effects, it leads to the development of diabetes mellitus (2, 3). This condition has been related with an amplified chances of heart failure, a serious cardiovascular complication that can have severe consequences, including the death of affected individuals. The intricate interplay between diabetes and heart failure is multifaceted. Diabetes can contribute to the development and progression of heart failure through various mechanisms, including the impact of prolonged high blood sugar levels on the cardiovascular system. The connection between diabetes and heart failure accentuates the significance of comprehensive management and preventive strategies for people with diabetes to alleviate the danger of cardiovascular complications (4, 5).

Microspheres are three-dimensional spherical particles with diameters (1 to 1000 μm). Its inception can be traced back to 1959 when Fox et al. introduced them during research on the proteinoid dissolution and reassembly. Initially, the microspheres found utility as fillers in plastics, paving the way for their adoption in various industrial sectors such as aerospace, abrasives, and transportation, etc (6, 7). The utility of microspheres in the biomedical industry emerged in the mid-1990s, marking a significant shift in their use towards life sciences and healthcare. In the realm of biomedical engineering, microspheres play a pivotal role with two primary applications: serving as scaffolds for tissue engineering and acting as local delivery systems (8). One of the key advantages of microspheres in biomedical applications is their suitability for tissue engineering. Microspheres can function as scaffolds that provide a supportive structure for the regeneration of tissues. Their unique properties, including size, shape, and flowability, make them well-suited for filling bone defects with complex shapes (9, 10).

Microspheres loaded with anti-diabetic drugs

Microspheres are among the versatile carrier used for the delivery of various active pharmaceutical ingredients for altering the delivery characteristics, protection against degradation, targeted delivery, improving palatability, etc. Several recent pieces of research have focussed upon surface treatment of microspheres for the localized release of active molecules (11, 12). One of the recent studies attempted the fabrication of exenatide containing PLGA microspheres by employing the W/O/W emulsion method, fabricated using the microfluidic technique. The *in vitro* characterization indicated that the optimized formulation showed a quick and steady initial release because of large pores and a thin exterior.

The antidiabetic microsphere formulation was also tried out by a group of scientists who prepared floating microspheres containing curcumin. The main idea was to improve gastric retention of the formulation which will reduce plasma concentration variations. The development of floating microspheres were done using the emulsion-solvent diffusion method by employing various concentrations of polymers, such as hydroxypropyl methylcellulose, Eudragit S 100, and Chitosan. The drug entrapment efficiency was higher for the batches with high polymer concentration, possibly due to higher viscosities of polymers. The release of the drug was high in the beginning because a drug to polymer ratio is high and the drug adhered on the surface starts to dissolve rapidly. Curcumin-containing microspheres showed satisfactory antidiabetic activity which was retained up to 120 h after administration (13).

The primary objective of the research work is to develop novel PLGA microspheres utilizing a modified double-emulsion technique for the encapsulation of the anti-diabetic drug, Metformin. This research focuses on exploring the impact of varying polymer concentrations on the characteristics of the microspheres. The microspheres are meticulously characterized to comprehensively analyse how the incorporation of the polymer affects their properties. The study aims to gain insights into the release kinetics and pharmacological activity of Metformin when delivered via PLGA-based microspheres. By systematically investigating the relationship between polymer concentration and microsphere characteristics, the research aims to provide a deeper understanding of how these parameters influence drug release behaviour and therapeutic efficacy.

Preformulation stage is a critical step in the development of new dosage forms, ensuring a comprehensive understanding of the physicochemical properties of the active pharmaceutical ingredient (API or drug molecules) and other excipients (14, 15). This step is essential for establishing a solid foundation for the subsequent formulation and development stages. The focus of preformulation studies is on assessing the fundamental physicochemical properties of the drug molecules (16,17).

a) Preparation of stock solution and serial dilutions

The stock and serial dilutions were prepared by taking 100 mg of MH by weighing them precisely and dissolving in phosphate buffer solution at pH 6.8 (PBS), with the final volume adjusted to 100 ml (Solution 1). Subsequently, 10 ml of Solution 1 was taken and further diluted with PBS to reach a final volume of 100 ml (Solution 2). From Solution 2, various volumes were measured and transferred into five separate 25 ml volumetric flasks. These volumes were then adjusted with 1 ml of 0.1 N HCl acid and supplemented with sufficient PBS to generate distinct standard solutions, exhibiting the concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 μg/ml.

c) Scanning using UV/Visible spectrophotometer

In order to determine the wavelength of maximum absorption (λ_{max}), a standard solution from the prepared series was subjected to UV/Visible Spectrophotometry. The scan covered a wavelength range from 190 nm to 400 nm. The absorbances of all standard solutions were subsequently measured at the observed λ_{max}. To ensure accuracy and reliability of the results, this process of scanning and absorbance measurement was repeated three times (18).

Figure 5.1. (A) Overlain UV spectra for linearity of MH (2–20 µg/mL), and (B) Standard curve of MH in pH 6.8 PBS at λ max of 233 nm.

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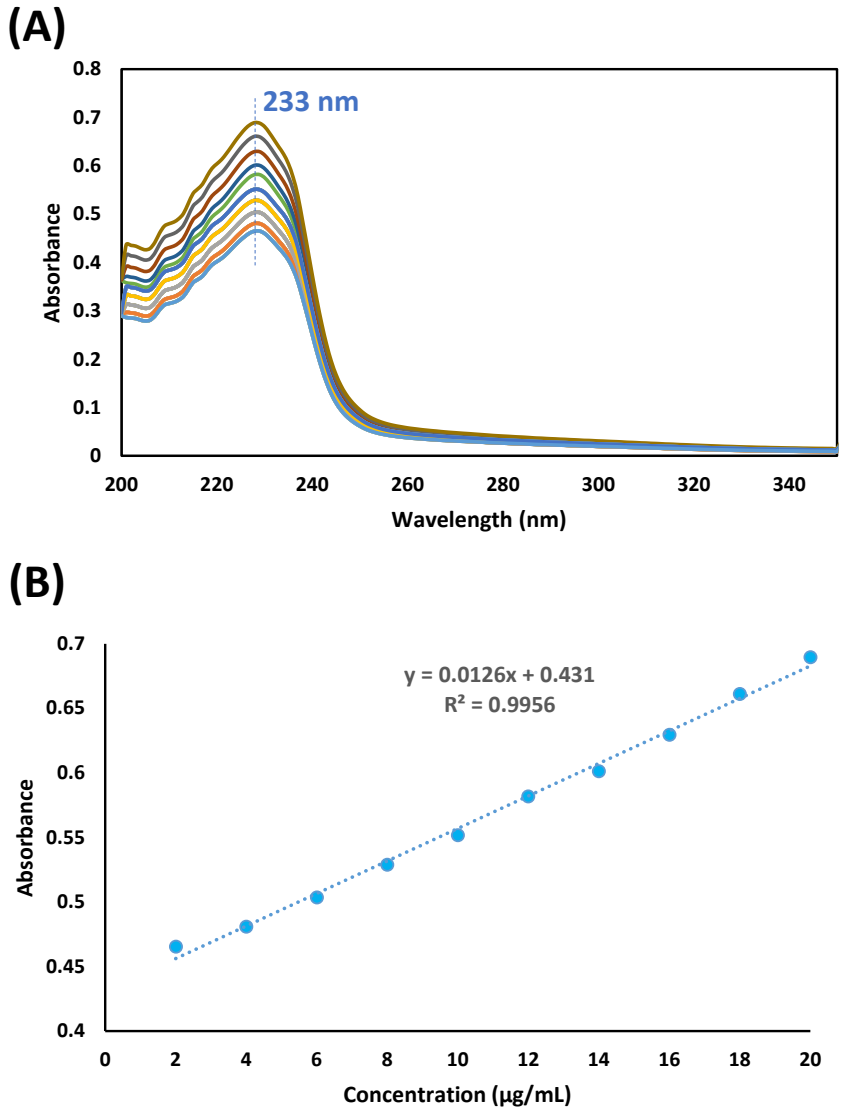

Figure 1: (A) Overlain UV spectra for linearity of MH (2–20 µg/mL), and (B) Standard curve of MH in pH 6.8 PBS at λ max of 233 nm.

d) HPLC Analysis

The various dilutions of MH, prepared according to previous section, were subjected to analysis using a reversed-phase column (Hyperisil ODS, 4.6 mm × 150 mm, 5 µm, C18-AR; Thermo Scientific, Lithuania). The absorption wavelength was adjusted at 233 nm. Mobile phase, employed for chromatographic separation, comprised

acetonitrile and a 0.02 mol/L KH_2PO_4 solution, incorporating 2 mmol/L sodium dodecyl sulphate in a proportion of 75:25. The pH of the mobile phase was attuned to 3.5, and the flow rate was consistently maintained at 1 mL/min.

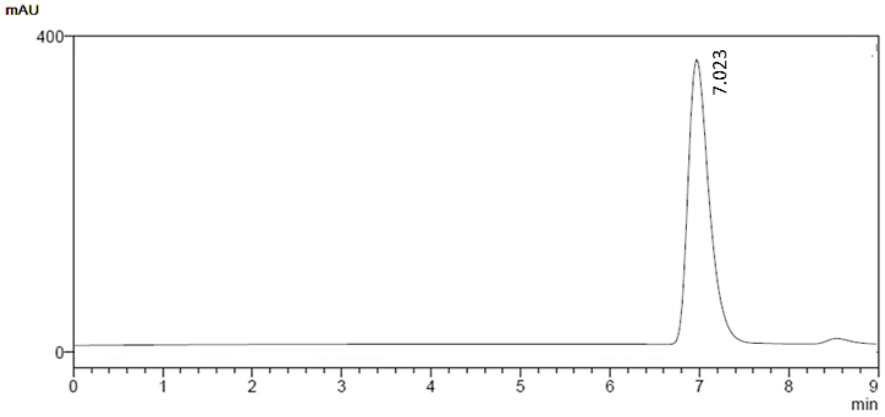

Figure 2: Chromatogram of Standard MH solution (10 µg/mL)

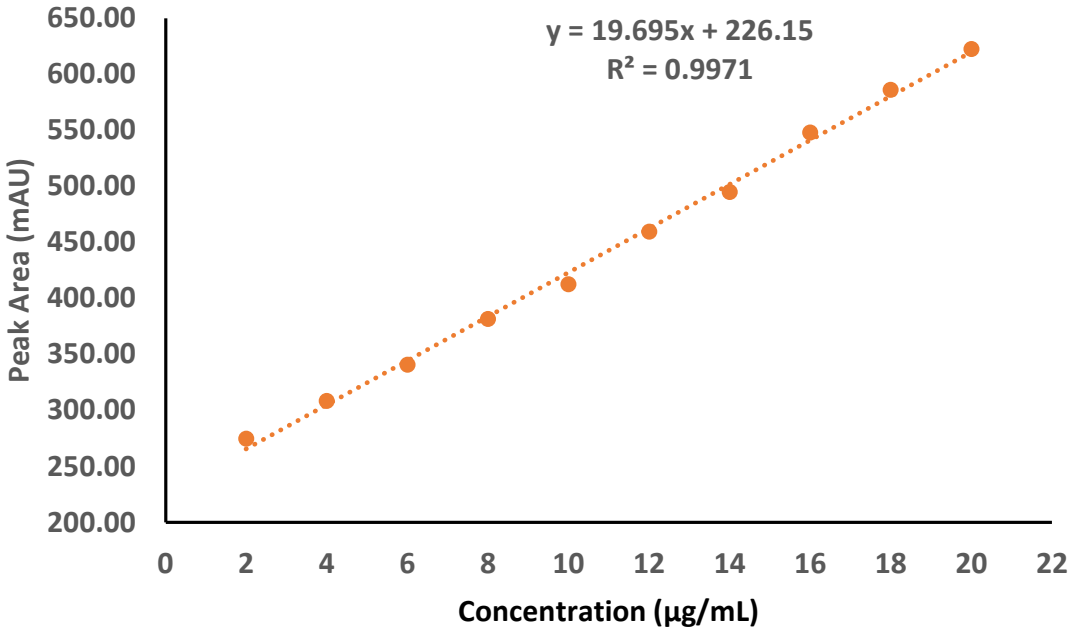

Figure 3: HPLC calibration plot of pure MH solution (2 -20 µg/mL).

Melting point determination

The estimation of the melting point holds significant importance in pharmaceutical studies, particularly during preformulation, with a primary focus on crystalline solubility. This emphasis stems from the challenge of accurately determining solubility due to the limited availability of drug powder. Melting point and solubility are intricately associated through the latent heat of fusion, representing the heat produced throughout the process. The melting point of the drug was assessed using a digital melting point apparatus. This analytical method provides a precise and reproducible means of assessing the temperature wherein the solid drug transitions to a liquid state, offering valuable insights into its physical characteristics and behaviour (19,20).

The melting point of the pure MH was estimated by employing a MP apparatus, and the recorded value was compared with the literature value. The observed melting point was found to be in the range of 231-233 ℃, closely

matching the reported value (21). This congruence between the practical and theoretical values suggests that the MH is in a pure state.

Estimation of solubility

The solubility of MH was investigated following the protocol outlined by Gracin et al (22). Excess MH was suspended in 20 mL of methanol within glass vessels, each capped with glass stoppers, and tightly sealed. The sealed vessels were then stirred for 24 hours at the specified temperature, using a stir device able to operate at a specific temperature, such as magnetic stirring in a temperature-control water bath. Subsequently, the contents were allowed to rest for 1 hour to reach equilibrium. Following this, the vessel contents were centrifuged, and the resulting supernatant solutions were filtered through a Whatman filter paper (No. 1) to ensure the absence of particulate matter before sampling. After appropriate dilution with the relevant aqueous/buffer solution, the solubility of MH was determined using an HPLC method. Solubility assessments were conducted in various aqueous media with different pH conditions, including water, methanol, pH 1.2 (HCl buffer), 50 mM acetate pH 4.5 buffer, and 50 mM PBS pH 6.8 buffer.

Table 1: Solubility of MH in solvents at different temperatures.

Solutions	Equilibrium Solubility of MH (mg/mL)		
	25 ℃	**30 ℃**	**37 ℃**
0.1 N HCl (pH 1.2)	305.2±3.39	336.42±2.48	349.18±2.99
50 mM acetate pH 4.5 buffer	301.96±2.91	331.81±2.56	347.83±3.82
50 mM PBS pH 6.8 buffer	299.12±4.19	325.38±3.51	341.02±4.47
Double Distilled Water	309.71±5.03	342.86±3.88	358.86±4.28
Methanol	265.15±2.86	299.16±2.74	311.04±5.25

The solubility of MH, was investigated in the pH of 1.2–6.8, revealing a consistent solubility of about 300 mg/mL at 25 °C. This indicates that the pH-based dissolution behaviour of MH does not ascribe to variations in equilibrium solubility or the absence of sink. Surprisingly, despite its satisfactory pH-independent solubility, MH does not necessitate the occurrence of a surfactant in the dissolution medium. The analysis studies demonstrated that the solubility of MH exhibited minimal change with increasing pH and reached its maximum solubility in double distilled water. Furthermore, MH was found to be freely soluble in methanol. These outcomes contribute valued considerations into the dissolution characteristics and solubility profile of MH, essential considerations for its pharmaceutical applications.

Determination of Partition Coefficient

The determination of the partition coefficient holds significant importance in preformulation activities, particularly due to its relevance in passive diffusion—the predominant mechanism of drug absorption (23) The partition coefficient of the drug was assessed through a laboratory shake flask experiment following OECD guidelines 107 (24), utilizing 1-octanol and water as the two phases. The drug was dissolved in 1-octanol to achieve a concentration of 0.5 mg/mL. The partitioning process involved mechanical shaking of equal volumes of both phases for 24 hours to ensure equilibrium, followed by a sufficient duration for the phases to separate. The aqueous solution was appropriately diluted, and the drug concentration was determined using an HPLC method. The study was undertaken at laboratory temperatures, specifically 25±1°C. This approach provides valuable insights into the drug's partitioning behavior between 1-octanol and water, offering relevant information for understanding its potential absorption mechanisms and aiding in the optimization of formulation strategies. The log P was estimated from the following equation:

$$\textbf{Log P} = \textbf{Log } (C_O/C_W)$$

where, C_O and C_W are the concentrations of drug in octanol and water.

The log P of MH, determined using the shake flask method in accordance with OECD guideline 107 (199) was found to be -2.92 ± 0.16. which suggests that MH is more hydrophilic in nature. The MH characterized by its hydrophilic chemical structure, exhibits a low log P value, indicating challenges in its rapid passive diffusion through biological membranes. The hydrophilic nature of MH contributes to its relatively slow absorption. Notably, the primary mode of absorption for MH is through several organic cation transporters (OCTs), emphasizing the importance of active transport mechanisms in its absorption process.

Estimation of Ionization constant (pKa)

The ionization behaviour of weak acids in solution, a crucial aspect influenced by pH, is quantified by the ionization constant, pKa (25). This thermodynamic parameter is of paramount importance in pharmaceutical dosage form design due to its impact on various aspects of drug behaviour. The pKa value is known to influence solubility, absorption, distribution, metabolism, protein binding, etc (26,27). To determine the pKa of weak acids with high precision, a commonly employed technique is potentiometric titration using a glass pH electrode. In this method, a solution of a fixed concentration of the acid is titrated with small increments of a standardized concentration of a strong base. The pH of the solution is measured after each increment of base, allowing for accurate determination of the pKa value. The drug solution was meticulously prepared by introducing a precisely weighed drug sample into a volumetric flask containing 100 mL of a 0.15 M KCl solution. The flask underwent a 15-minute sonication process followed by placement in a shaker bath at 25±1°C for a day. Subsequently, the mixture was allowed to equilibrate at ambient temperature for an additional 24 hours. To eliminate any undissolved drug particles, the mixture underwent centrifugation at 3000 rpm.(28,29). The supernatant solutions (free of particulate matter) were subjected to filtration through Whatman filter paper (No. 1) for further refinement and were subsequently utilized for titration. The electrode was immersed in a 20 mL solution of MH, and titration was initiated slowly using 0.005 M NaOH solutions. The controlled volumes of the titrant were incrementally added with continuous stirring, and the corresponding pH values were estimated. The equivalence point was assessed graphically by identifying the burette value corresponding to the maximum alterations in pH per unit volume change. To confirm the equivalence point, an alteration of curvature in pH against the titrant volume was constructed. A differential curve was plotted, pinpointing the equivalence point as a peak. The pKa of the drug was determined using the half-neutralization method, relying on the buffer equation as follows:

$$\mathbf{pKa = [H+]\,[A+] / [HA]}$$

The pH of the solution was recorded using pH meter with equivalence of base to the acid concentration and estimated as the pKa of the compound (30,31).

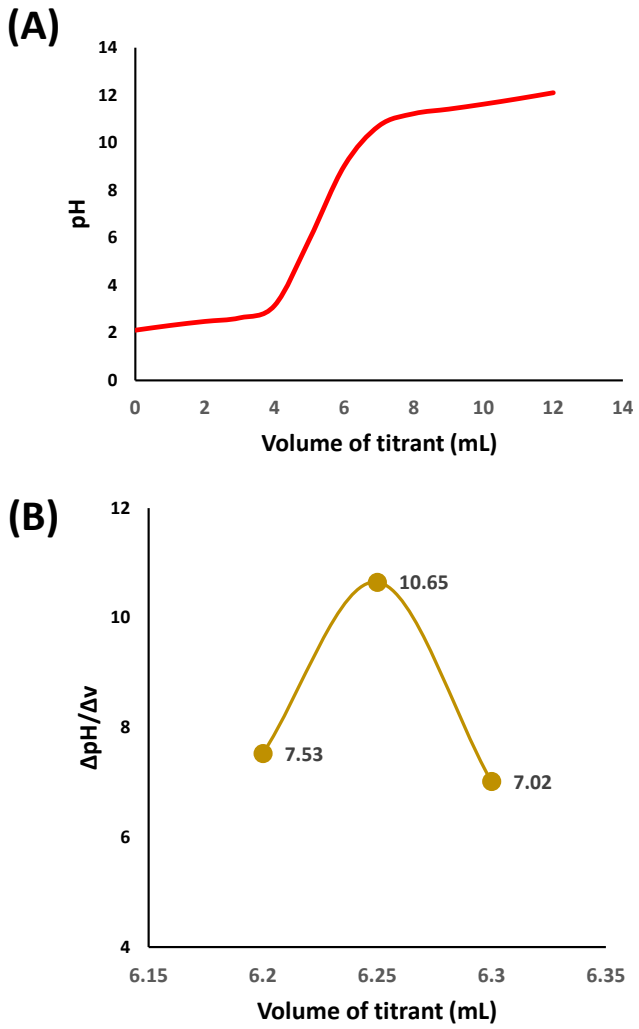

Figure 4: (A) Potentiometric titration curve of MH for the estimation of equivalence point, and (B) Potentiometric differential titration curve (25±1°C).

Identification using FTIR, DSC, and XRD

The identification of a drug sample in research involves a systematic process to confirm the identity of a substance and ensure its purity. Various analytical techniques and methods can be employed for drug identification, depending on the nature of the compound (32,33). The obtained drug sample was examined by infrared absorption spectral analysis, thermal analysis, and X-ray diffraction pattern and compared the spectrum with that of reference standard.

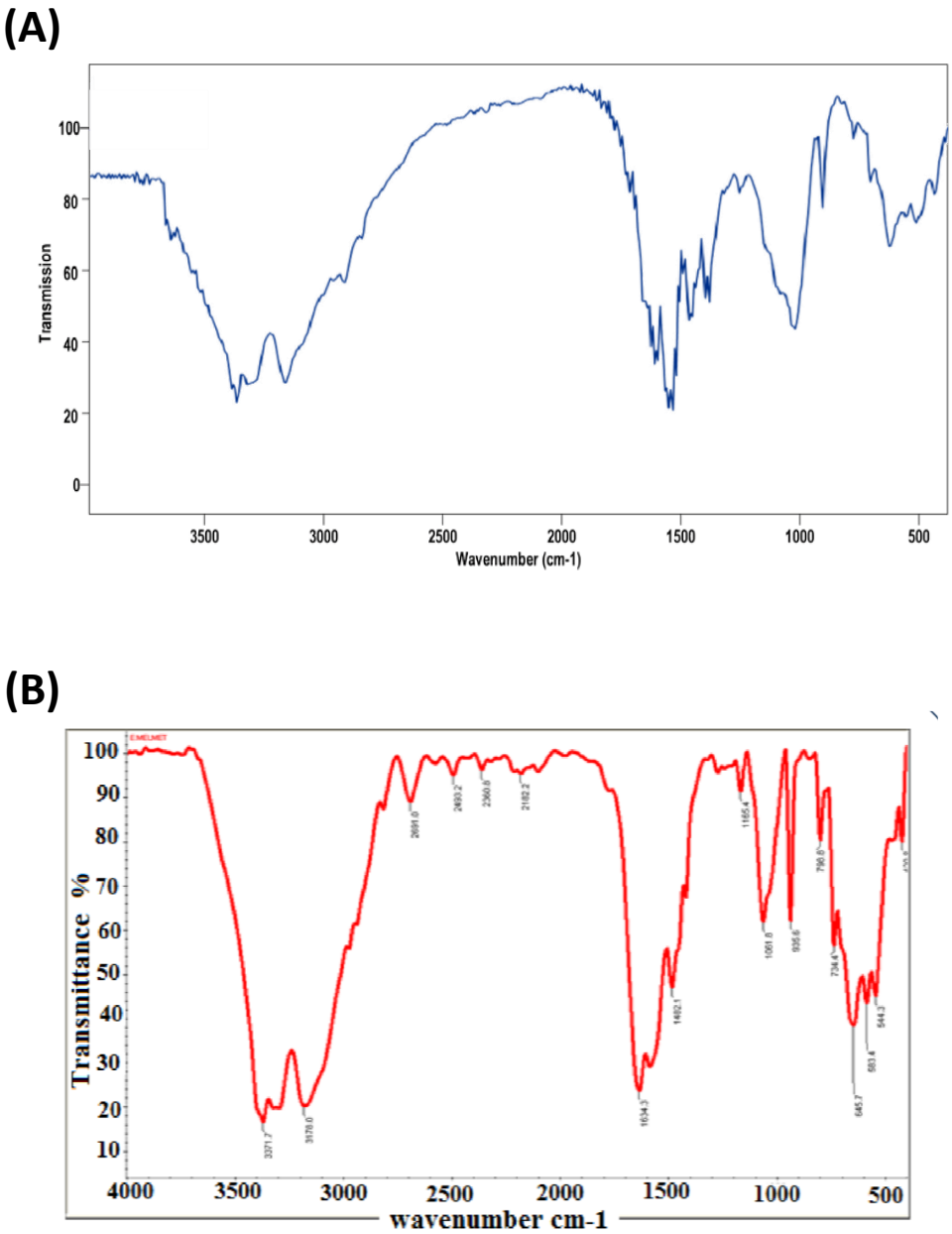

Figure 5: Comparison FTIR spectra of pure MH (A) with standard FTIR spectra (B) obtained from Rani et al (34)

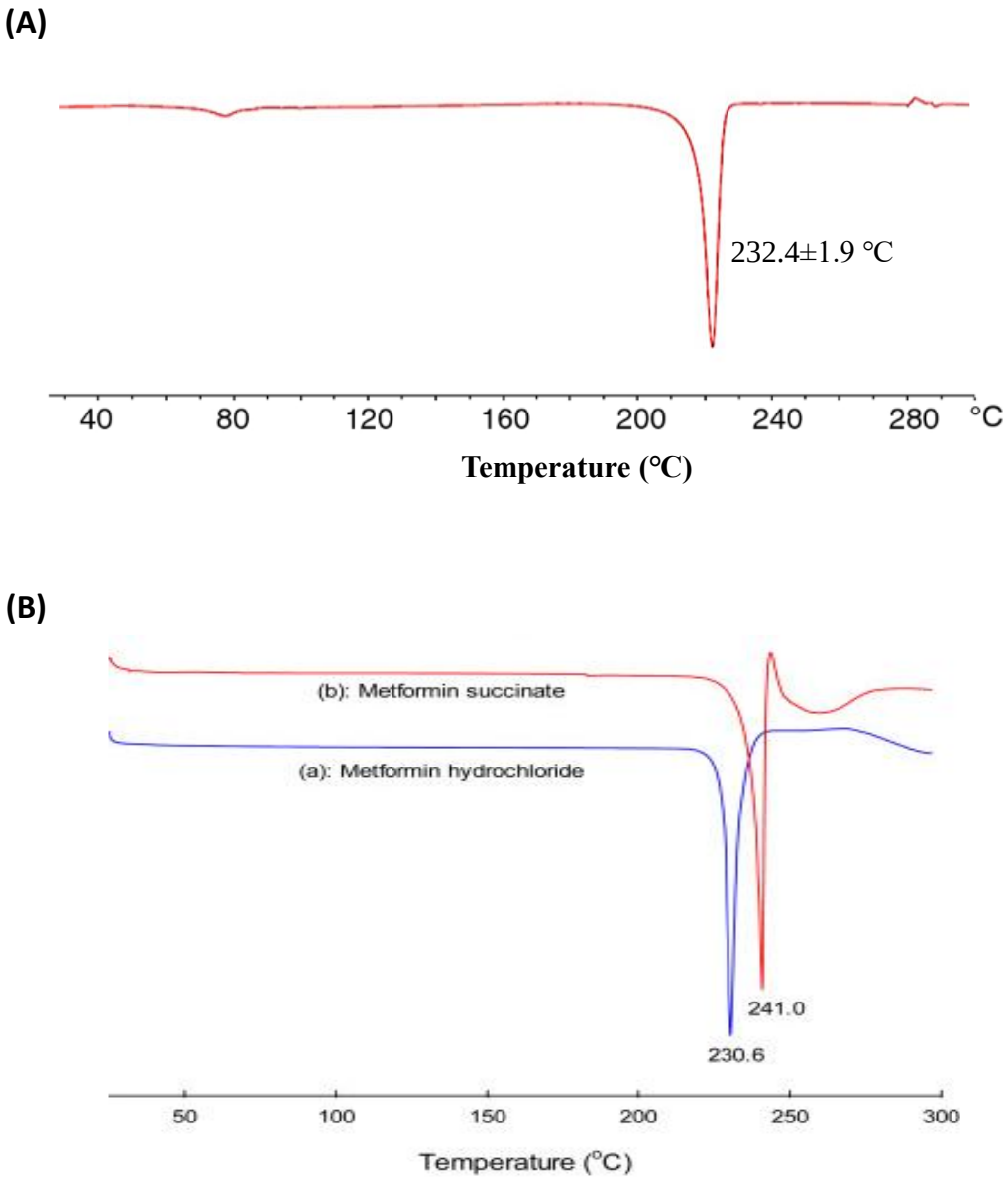

Figure 6: DSC Curve of pure MH (A) in comparison with curve (B) obtained by Kim et al(35).

The obtained FTIR spectra, DSC curve and XRD pattern were found to be in compliance with the reference spectra and curves (36,37). Thedistinctive peaks of MH were found to appear in Figure 5, 6, and 7, thus indicating purity of the drug which is used in the research for developing formulation and conducting characterization study.

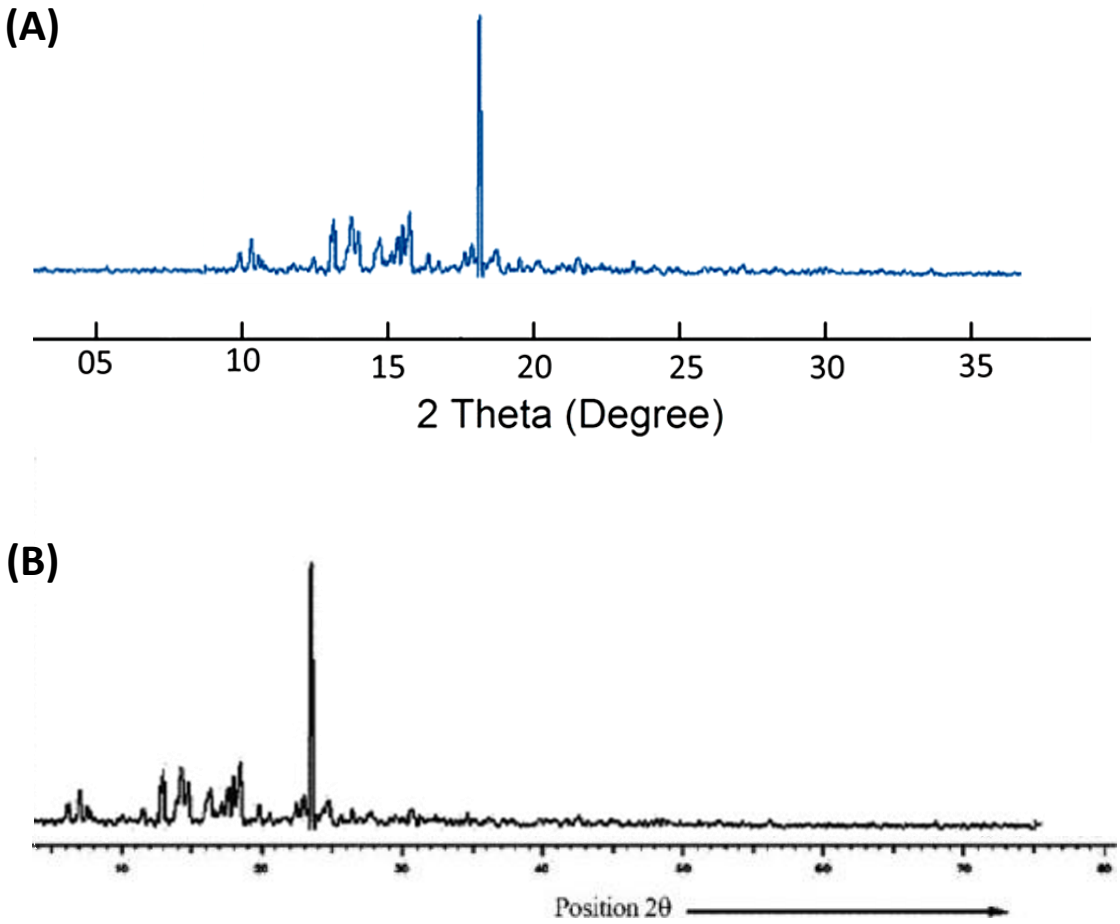

Figure 7: X-ray Diffraction Pattern of pure MH (A) in comparison with XRD pattern obtained by Ige et al (B) (38).

Determination of polymer viscosity

The impact of the polymer strength and estimated viscosity (particularly, o-phase) upon the entrapment of the drug into the microspheric formulatoin is presented in Figure 8. The increment in concentration of polymer contributes to the augmentation in the viscosity of the o-phase. The increase in viscosity of the o-phase owing to increasing polymer concentration may also contributed to higher encapsulation. This results in formation of significant barrier avoiding leakage of MH in to the Wb. Although, extremely high viscosity o-phases generates bigger particles owing to the requirement of strong shear forces to disrupt the globules. Certainly, the solutions with high viscosity needs the stronger requisite stirring forces, which results in generation of larger emulsion globules, followed by microsphere with large size.

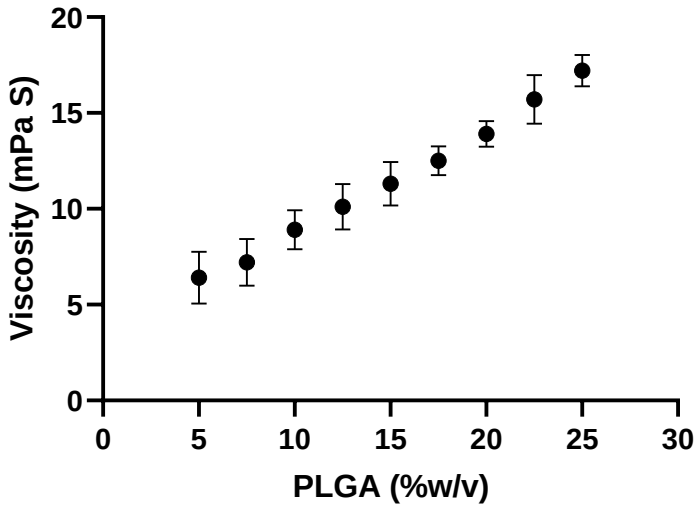

Figure 8: Effect of polymer concentration in oil phase on the viscosity

Conclusion

The primary objective of this research work is to advance the development of controlled drug delivery systems by focusing on the creation of novel PLGA (Poly(lactic-co-glycolic acid)) microspheres. These microspheres are designed to encapsulate Metformin, a widely used anti-diabetic medication. To achieve this, a modified double-emulsion technique is employed, allowing for precise control over the encapsulation process. A key aspect of this research is the exploration of how varying concentrations of the PLGA polymer affect the properties of the resulting microspheres. By systematically adjusting the polymer concentration, the study aims to understand its impact on crucial microsphere characteristics such as size, morphology, drug loading efficiency, and release kinetics. To ensure a comprehensive analysis, the microspheres undergo meticulous characterization. This involves employing various techniques such as SEM, DLS, FTIR, and drug release studies. Through these analyses, the researchers aim to gain a detailed understanding of how the inclusion of the PLGA polymer influences the overall behavior and performance of the microspheres. Furthermore, the study seeks to elucidate the release kinetics and pharmacological activity of Metformin when delivered via these PLGA-based microspheres. By examining how different polymer concentrations affect drug release patterns, the research aims to optimize the formulation to achieve sustained release of Metformin over an extended period.

The obtained FTIR spectra, DSC curve, and XRD pattern were thoroughly analyzed and found to align closely with the reference spectra and curves. This indicates that the materials used in the study, including the drug MH, maintain their expected chemical composition and structural characteristics, ensuring the reliability of the research findings. Specifically, the distinct peaks corresponding to MH were clearly identifiable in the resulting graphs, affirming the high purity of the drug utilized for formulating and conducting characterization experiments. Additionally, an interesting observation was made regarding the viscosity of the oil phase. It was noted that as the concentration of polymer introduced into the oil phase increased, there was a corresponding rise in viscosity. This phenomenon suggests that the polymer molecules are interacting with the oil phase, leading to increased molecular entanglement and a thicker consistency. This increase in viscosity is significant as it can potentially influence various aspects of the formulation process, such as emulsification and encapsulation efficiency. The higher viscosity of the oil phase resulting from increased polymer concentration may contribute to improved encapsulation of the drug within the microspheres.

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