**RESEARCH PAPER**

# Optimization Of Metformin Pulsatile Drug Delivery System Using Central Composite Design

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**ABSTRACT**
Objective: The aim of the study was to create a pulsatile multiparticulate system of metformin to be optimized in the delivery of chronotherapeutic drugs.
Methods: Metformin pulsatile beads were made and optimized based on the BoxBehnken design with polyvinylpyrrolidone (PVP, $X_1$) and ethyl cellulose (EC, $X_2$) as the independent variables. The particle size, polydispersivity index (PDI), entrapment efficiency, lag-time and drug release were analyzed.
Results: EC had a substantial effect on particle size, lag time and drug release whereas PVP primarily had an effect on entrapment efficiency and matrix hydration. Per cent percentage of drug release was 68.4 ± 0.31 and 88.4 ± 0.48, and lag time 0.5 ± 0.02 h to 4.5 ± 0.06 h. The MP4 formulation, which was optimized (PVP 1.0 mg, EC 6 mg) was found to have a particle size of 1336.0 ± 3.6 nm, PDI of 0.794 + 0.06 and entrapment efficiency of 98.50 + 0.52, lag time of 0.5 + 0.03 h, and drug release of 88.4 + 0.48.
Conclusion: The optimized pulsatile system exhibited a short lag time with a rapid release of the drug, therefore, it can be considered an effective chronotherapeutic delivery system of metformin
**Keywords**: Metformin; Pulsatile drug delivery; Chronotherapy; Ethyl cellulose; Polyvinylpyrrolidone; Response surface methodology; Multiparticulate beads; Box–Behnken design
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## INTRODUCTION

Diabetes mellitus is a persistent metabolic disorder, which is defined by the inability of normal regulation of blood glucose level. Type 1 diabetes is caused due to autoimmune destruction of the pancreatic 0-cells resulting in absolute insulin deficiency, but Type 2 diabetes is mostly caused by insulin resistance combined with gradual 0-cell dysfunction. Diabetes is a significant chronic issue in the world and poses significant challenges to the health of the masses because of its long-term effects, such as retinopathy, neuropathy, nephropathy, and cardiovascular diseases. Diabetes needs to be well managed with an accurate blood glucose level. Nevertheless, optimum glycemic control is a clinical challenge, which cannot be achieved and sustained using the available therapies [1].

The traditional antidiabetic management plans involve the use of oral hypoglycemic compounds alongside injectable treatments like the use of insulin and glucagon-like peptide-1 (GLP-1) receptor agonists. Although most commonly used, these therapies do not always offer a steady glycemic control and in most cases have to be taken a number of times each day. These regimens predispose patients to adverse effects, such as hypoglycemia, glycemic variation, and poor adherence on the part of the patient. As a result, there is a strong demand that superior drug delivery systems that can decrease the number of dosing per patients, improve their compliance, and attain a more stable and predictable blood sugar regulation [2].

One of the oral hypoglycemic agents prescribed extensively to treat Type 2 diabetes mellitus is Metformin. Its clinical efficacy, however, has a number of pharmacokinetic disadvantages. Due to its short biological half-life of 4-8.7h, little protein binding and partial absorption into the gastrointestinal tract (about 50-60%), the constant level of glycemic regulation is frequently achieved by taking many doses per day. This causes varying levels of plasma drug concentrations and uneven therapeutic results. Pulsatile drug delivery is an important approach to address these weaknesses through the ability to adjust the release of metformin to the physiological changes in glucose levels, thereby improving therapeutic response, hemoglobin levels, and decreasing the dosing interval [3].

The goal of pulsatile drug delivery systems is to reproduce the physiological rhythmic activity of the body by the time-programmed usage of drugs in discrete pulses. These strategies have a number of benefits as compared to traditional delivery systems to manage diabetes. To begin with, the release of the drugs can be synchronized with the natural patterns of insulin secretion, especially during postprandial glycemic spikes, which allows the achievement of better glycemic control. Second, pulsatile administration decreases the probability of adverse effects, including hypoglycaemia,

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by eliminating excessively elevated peak plasma concentrations. Lastly, the systems enhance patient compliance by reducing the dosing schedule and providing a more regular uptake of the drug [4].

There are several studies on pulsatile delivery platforms to treat diabetes. As shown by Rajput et al. (2024), chronicomodulated drug delivery provided better therapeutic effects and emphasized the combination of chronobiology with pharmaceutical technologies [1]. Pandey and Selvamurthy (2020) found better in vitro results of a pulsatile system to administer biguanide antidiabetic drugs, though additional in vivo confirmation was suggested [2]. Hu et al. (2006) prepared sustained-release metformin pellets and showed the improved bioavailability and therapeutic efficacy [4]. Moreover, Wanasawas et al. (2022) used a different approach based on the use of calcium pectinate-based coatings to deliver colon-specific drugs, which provides another solution to the controlled release of metformin [5].

Taken altogether, these studies suggest the possibility of pulsatile systems to improve the bioavailability of drugs and optimize the therapeutic efficacy of anti-diabetic agents, especially metformin. To this end, the current paper will focus on designing and testing a pulsatile delivery system of metformin that will seek to enhance the ability to manage the glycaemic process by making sure that the drugs are released with the endogenous glucose changes and, therefore, will offer a more efficient and patient-friendly method of managing diabetes.

The use of pulsatile delivery of metformin is one of the measures that have a promising approach to eliminating the shortcomings of traditional diabetes treatments. Maintaining stable blood glucose levels during the day is one of the major issues in the management of diabetes. Conventional metformin treatment can require various doses a day leading to variable glycaemic regulation and alternating hyperglycaemic and hypoglycaemic spikes. The pulsatile drug delivery systems can alleviate these problems by administering metformin at specific times, which is related to physiological glucose peaks, to provide the drug with the metabolic needs of the body more accurately to control the glycaemic level.

In addition, the bioavailability of metformin by mouth is restricted to about 50-60 percent, which also adds to the inconsistent treatment effects. Pulsatile systems can be used to improve and stabilize bioavailability through the delivery of controlled release of drugs in discrete pulses, which will allow more consistent absorption and predictable glycaemic control. Moreover, metformin has a short biological half-life (4-8.7 h) and, therefore, requires a combination of truly frequent dosing to maintain therapeutic plasma levels. Pulsatile systems can be used to extend an effective exposure of drugs, decrease the frequency of drug administration, and enhance the convenience of patients, by releasing drugs at appropriate times.

The other benefit of pulsatile delivery is that it can coordinate the release of drugs with the postprandial excursions of glucose. Traditional metformin preparations are usually at their peak plasma concentrations 23 h following intake; this could not correspond to these glucose surges. Conversely, pulsatile systems may be designed to produce peak drug levels at some of the most critical times of increased blood glucose, enhancing the efficacy of the therapy. The current study on this basis assumes that a pulsatile metformin delivery system will lead to better glycaemic control, bioavailability and provide a more effective and patient friendly method to the management of Type 2 diabetes mellitus [1–6]. This study should therefore maximize pulsatile metformin formulations, assess excipient and drug compatibility, and determine the release kinetics in vitro, and determine the right polymers to use in controlling the release and assess the scalability and manufacturability of the optimized system which will ultimately lead to better clinical outcomes and quality lives of patients.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Metformin was obtained at Swarnoop Pharmaceutical, Mumbai and utilized as active pharmaceutical ingredient. All other excipients were of HiMedia Pvt. Ltd., Mumbai, i.e., microcrystalline cellulose (MCC) beads, polyvinylpyrrolidone (PVP), Aerosil, ethyl cellulose (EC7 CPS), di-octyl phthalate (DOP) and isopropyl alcohol, except 5% soluble starch. These materials were chosen according to the fact that they comply with the requirements of pharmaceutical quality, and they are suitable to develop a formulation and achieve stability of the products. A binding agent was soluble starch, and MCC beads were the core material on which the beads are made. Polyvinylpyrrolidone was added to increase the drug encapsulation ability and Aerosil was added to enhance the powder flow characteristics. The ethyl cellulose, which was used together with DOP as the coating agents, was used to control drug release, the isopropyl alcohol was the solvent used in the coating process. Together, these ingredients led to the attainment of the desired physicochemical characteristics and optimization of performance of the pulsatile bead formulation.

### 2.2. Preformulation Studies

Preformulation tests were conducted to gauge the physicochemical properties of metformin hydrochloride and its interaction with excipients of choice and this is a crucial step in the development of a stable and effective dosage form.

**Drug and Excipient Compatibility Studies**

Different scanning Calorimetry (DSC) and Fourier Transform Infrared (FTIR) spectroscopy were used to determine the compatibility of metformin HCl with formulation excipients. DSC analysis was conducted to examine thermal behaviour of the pure drug and physical mixtures of the drug with selected excipients, namely, hydroxypropyl methylcellulose (HPMC), micro crystalline cellulose (MCC), and polyvinylpyrrolidone (PVP). The samples were placed in heating at a rate of

30-300°C, and the resulting thermograms were obtained. The melting endotherms of the drug excipient mixtures were compared to the pure metformin HCl. Any notable change, loss or expansion of the typical melting point of the drug was taken to be a pointer of potential incompatibility. Without the changes, the excipients were considered as compatible and suitable in a formulation [10-14].

**Fourier Transform Infrared Spectroscopy (FTIR)**

FTIR was conducted to estimate potential chemical reactions between metformin hydrochloride and the excipients chosen. With an FTIR spectrophotometer, samples of pure metformin HCl, its individual excipients and physical mixtures, were scanned over the wavenumber range 4000-400cm$^{-1}$. The metformin HCl characteristic absorption peaks, especially those of NH and CN were determined and compared with the physical mixture spectra. Any difference in the location or strength of these typical peaks in the mixtures was taken to represent possible drug-excipient interactions. Otherwise, the excipients were deemed to be chemically compatible with metformin HCl [10-16].

**Differential Scanning Calorimetry (DSC)**

DSC was used to examine the thermal behaviour of metformin HCl and its physical mixtures with the selected excipients so as to reveal potential incompatibility that may cause formulation instability. About 5-10 mg of pure metformin HCl and physical mixtures of metformin with other excipients like HPMC, MCC and PVP were accurately weighed and put on aluminium pans. Analysis of the DSC instrument was performed by the use of standard reference materials to calibrate it. The samples were heated between 30°C and 300°C at a gradual pace of 10°C min$^{-1}$. A temperature dependent heat flow was measured to get thermograms. The resulting melting endotherms of the mixtures were compared to that of the pure drug and any serious shift, loss or new peaks were viewed as any evidence of possible incompatibility. Stable thermal behaviour was taken to ensure the appropriateness of the excipients to be used in the further development of formulations [10-16].

**2.3. Preparation and Optimization of Metformin Pulsatile Beads**

Metformin pulsatile beads were prepared by loading immediate release (IR) granules and pulsatile-covered beads into one hard gelatin capsule to obtain a biphasic drug release profile. The IR granules were made by weighing first, 250 mg metformin hydrochloride, then mixed with 5 percent of w/w soluble starch as a binding agent to form a homogeneous mixture. The mixture was dampened using purified water to create a uniform damp mass, which was then screened using a sieve with a diameter of 16 and dried using a hot air oven at a temperature of 60°C until all the weight was constant. The dry granules were then sieved using a 20 sieve to get even particle size and stored in airtight containers awaiting further use.

Preparation of pulsatile core beads was done using microcrystalline cellulose (MCC) as an inert carrier. Dispersal of MCC in polyvinylpyrrolidone (PVP) solution was prepared in isopropyl alcohol and dropwise the dispersion was enabled under non-solvent constant stirring to create spherical beads. The shaped beads were filtered and washed well and dried at room temperature. In the case of drug layering, PVP containing isopropyl alcohol dissolved metformin hydrochloride and the solution was sprayed onto the MCC beads using a coating pan. The temperature of the pan was kept lower than the melting point of the drug and drying at intervals of 60°C was done to avoid agglomeration of the drug until the desired drug loading was obtained.

The beads that were drug-layered were then subjected to seal-coating with hydroxypropyl methylcellulose (HPMC K4M) and controlled-release coating using ethyl cellulose (EC 7 cps). The EC coating solution had 2.5 percent w/w di-octyl phthalate as a plasticizer and isopropyl alcohol was the solvent. Application of the seal-coating and controlled-release coating was done in that order with sufficient drying time between layers to maintain homogenous film formation and mechanical stability.

To optimize, PVP concentration was coded on three levels: -1 (0.50 mg), 0 (1.00 mg) and +1 (1.50 mg) but EC 7 cps concentration angled on 6-18mg levels as outlined in the experimental design. Each formulation had a different amount of HPMC K4M seal-coating layer that was used, ranging between 2 and 18 mg. There were 13 formulations (MP1 to MP13) that were prepared according to the design matrix in Table 1. The weight of the pulsatile bead to be used in all the formulations was kept constant at 250mg, and 250mg of IR granules was put in the capsules, making the total weight of the capsules 500mg.

The formulations were all assessed on the basis of particle size, polydispersity index, entrapment efficiency, lag time, and in vitro university drug release behavior. The experimental results have been applied to determine an optimized value of PVP and EC 7 cps to achieve an unambiguous lag phase with a quick release of a drug, which is suitable in pulsatile delivery. Such systematic optimization methods resulted in the formulation reproducibility, content uniformity and the accurate control of the pulsatile release properties. Table 1 summarises the formulation design and optimization strategy [7-15].

**2.4. Pellet Size and Shape**

Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM) were used to determine the size and morphology of the prepared pellets.

Dynamic Light Scattering (DLS)

The mean particle size, PDI and size distribution of the pellets were determined using DLS. The pellets were put into a clean cuvette in a diluted suspension in distilled water. Analysis of the sample was done through DLS instrument which quantified the fluctuations of the intensities of the scattered laser light caused by the Brownian movement of the particles. The size

distribution of the particles was determined based on the data of the scattering, which gave data about the uniformity of the pellets which is a significant parameter that determines drug release behaviour and bioavailability [3,4–7].

### 2.5. Entrapment Efficiency

The entrapment efficiency of the Metformin in the pellets was determined by the amount of drug included in the pellets vis-a-vis the amount of the drug intake in the formulation. The pellets were dissolved in an appropriate solvent and the concentration of Metformin was determined by computing the UV- visible spectrophotometry. The entrapment efficiency was in form of a percentage [16-19].

**Table 1: Formulation Design Matrix Showing Coded and Actual Levels of Polyvinylpyrrolidone (PVP) and Ethyl Cellulose (EC 7 CPS) with Composition Details for Metformin Pulsatile Beads (MP1–MP9)**

| Run | Formulation | PVP (Coded / mg) | EC 7 cps (Coded / mg) | MCC Beads (mg) | Metformin Pulsatile (mg) | Aerosil (mg) | HPMC K4M Seal Coat (mg) | Di-octyl Phthalate (% w/w of EC) | IPA | Total Pulsatile Beads (mg) | IR Metformin (mg) | Total Capsule Weight (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MP1 | 0 / 1.00 | 0 / 12 | 9.57 | 239.25 | 0.24 | 2 | 2.5 | QS | 250 | 250 | 500 |
| 2 | MP2 | 0 / 1.00 | +1 / 18 | 9.57 | 239.25 | 0.24 | 4 | 2.5 | QS | 250 | 250 | 500 |
| 3 | MP3 | +1 / 1.50 | +1 / 18 | 9.57 | 239.25 | 0.24 | 6 | 2.5 | QS | 250 | 250 | 500 |
| 4 | MP4 | 0 / 1.00 | −1 / 6 | 9.57 | 239.25 | 0.24 | 8 | 2.5 | QS | 250 | 250 | 500 |
| 5 | MP5 | 0 / 1.00 | 0 / 12 | 9.57 | 239.25 | 0.24 | 10 | 2.5 | QS | 250 | 250 | 500 |
| 6 | MP6 | +1 / 1.50 | −1 / 6 | 9.57 | 239.25 | 0.24 | 12 | 2.5 | QS | 250 | 250 | 500 |
| 7 | MP7 | +1 / 1.50 | 0 / 12 | 9.57 | 239.25 | 0.24 | 14 | 2.5 | QS | 250 | 250 | 500 |
| 8 | MP8 | −1 / 0.50 | −1 / 6 | 9.57 | 239.25 | 0.24 | 16 | 2.5 | QS | 250 | 250 | 500 |
| 9 | MP9 | 0 / 1.00 | 0 / 12 | 9.57 | 239.25 | 0.24 | 18 | 2.5 | QS | 250 | 250 | 500 |
| 10 | MP10 | −1 / 0.50 | 0 / 12 | 9.57 | 239.25 | 0.24 | 10 | 2.5 | QS | 250 | 250 | 500 |
| 11 | MP11 | 0 / 1.00 | 0 / 12 | 9.57 | 239.25 | 0.24 | 10 | 2.5 | QS | 250 | 250 | 500 |
| 12 | MP12 | 0 / 1.00 | 0 / 12 | 9.57 | 239.25 | 0.24 | 10 | 2.5 | QS | 250 | 250 | 500 |
| 13 | MP13 | −1 / 0.50 | +1 / 18 | 9.57 | 239.25 | 0.24 | 6 | 2.5 | QS | 250 | 250 | 500 |

### 2.6. Lag Time Determination

Lag time was tested by adding a fixed amount of Metformin-coated pellets in a USP dissolution apparatus under simulated physiological conditions (37°C) with the help of a suitable dissolution medium i.e. phosphate buffer (pH 7.4). Samples were taken after fixed time intervals, and Metformin concentration set free was spectrophotometrically analyzed at 232 nm. The lag time

was defined as the time interval, which began with the beginning of the experiment up to a time when a definite concentration of Metformin could be detected. This parameter gave essential data regarding the drug release initiation and the functioning of the pulsatile delivery system in comparison to therapeutic goals [20–22].

### 2.7. In vitro Drug Release

The percent of Metformin released through pulsatile beads that have immediate-release (IR) granules were estimated to establish the conditions of physiological conditions that would be used to analyze the drug release over time, the USP basket method was used. In a USP apparatus type I, specific amount of Metformin pulsatile beads that offer both immediate and sustained release were added in dissolution basket, and phosphate buffer of pH 7.4 was the dissolution medium, which was preheated to 37°C to simulate body temperature. The equipment was used to rotate at 100 rpm, to ensure consistency with regard to the dissolution medium. Samples were taken without disturbing the pellets at pre-defined intervals of 1, 2, 4, 8 and 12 hours with the help of a pipette. The Metformin concentration of the samples was determined through UV, visible spectrophotometry at 232 nm on the basis of a calibration curve that was previously prepared to ensure the samples were accurately quantified. The amount of Metformin that had been released in the beads at 12 hours was noted. This experiment gave information on the release profile which was the instantaneous release of the granules as well as the regulated release of the pulsatile system, thus allowing the release kinetics of the formulation to be characterized and its ability to sustain levels of therapeutic drugs to control diabetes [26-29].

## 3. Results and Discussion:

### 3.1. DSC Analysis

DSC pattern of pure Metformin showed sharp endothermic peak at 236.7°C and is a sign of high purity and great thermal stability. The melting point of the physical mixture of Metformin and the chosen excipients was 238.45°C. The absence of any other thermal happenings and only one, sharp peak point indicates that there was very little interaction of the drug and excipients and no major degradation was experienced. The fact that the melting point has marginally increased can be explained by the fact that it has some slight interactions yet it does not affect the overall stability of drug. Such findings reveal that there is a good thermal compatibility of Metformin to the excipients, which indicates that the physical mix can be formulated as long as processing temperatures are kept under 238.45°C to maintain stability (Figure 1).

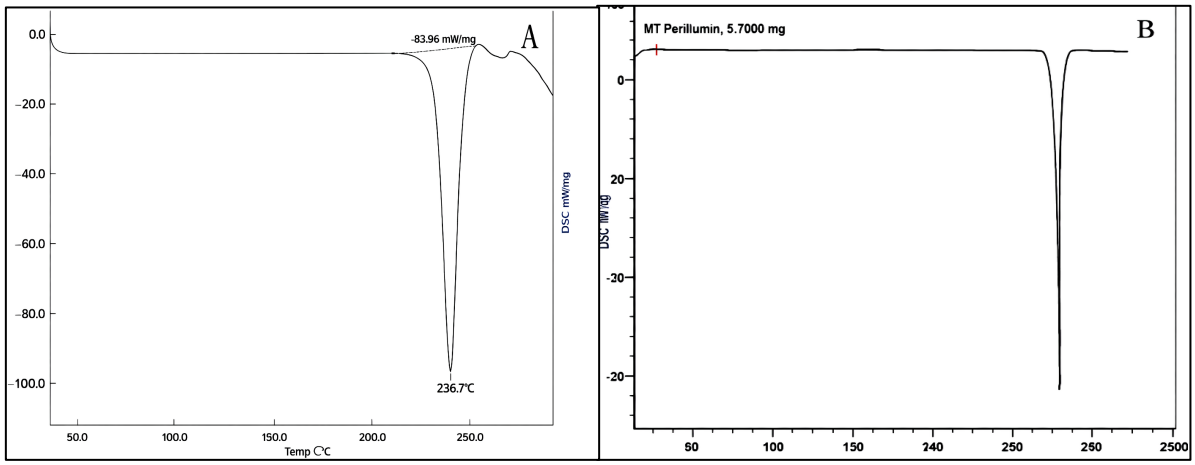


**Figure 1:** DSC thermograms showing thermal behavior of (A) pure Metformin and (B) formulation, with changes in the peak due to excipient interaction.

### 3.2. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of Metformin were used to determine that the characteristic functional groups were present in the pure drug and the pellet formulation. The N-H stretching vibrations were found to be at 3433.85 $cm^{-1}$ and 3344.70 $cm^{-1}$ and the C-N stretching vibrations were seen as 1255.09 $cm^{-1}$, 1175.64 $cm^{-1}$, 1109.24 $cm^{-1}$, 1099.37 $cm^{-1}$ and 1051.52 $cm^{-1}$. The fact that these peaks were reproducible during the formulation implies that no major chemical reactions took place between Metformin and the excipients, and hence, it can be said that the pellets were well-compatible with each other (Figure 2).

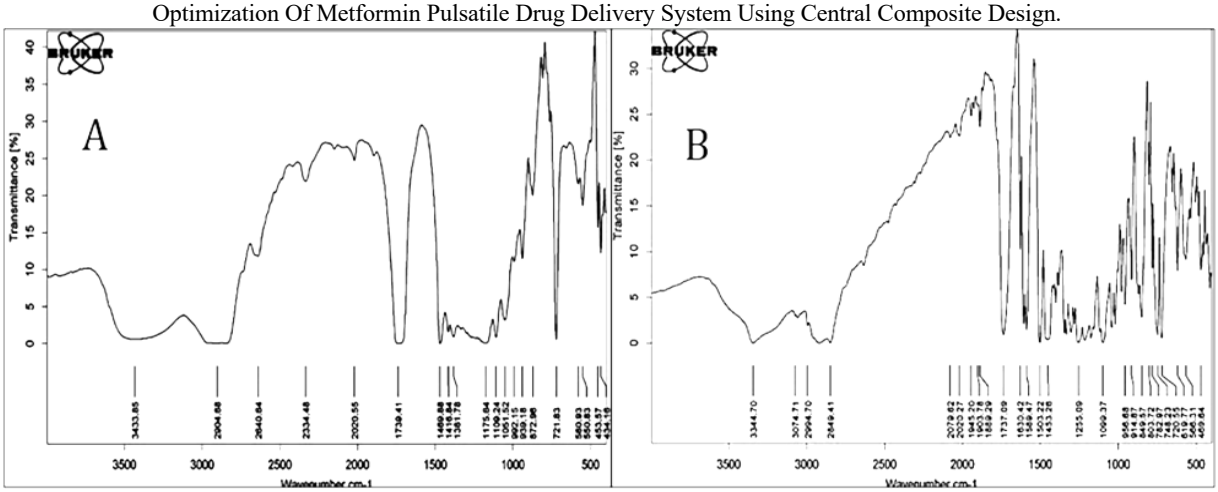


**Figure 2:** FTIR spectra of (A) pure Metformin and (B) formulation, showing possible interactions with excipients.

**Table 2: Formulation Design Matrix Showing Actual and Coded Levels of Polyvinylpyrrolidone (PVP) and Ethyl Cellulose (EC) for Metformin Pulsatile Beads (MP1–MP9)**

| Run | Formulation | PVP (mg) | EC (mg) | Particle Size (nm) | PDI | Entrapment Efficiency (%) | Lag Time (h) | Drug Release (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | MP1 | 1.00 | 12 | 1896.4 ± 4.2 | 1.268 ± 0.04 | 82.54 ± 0.38 | 3.5 ± 0.05 | 78.8 ± 0.42 |
| 2 | MP2 | 1.00 | 18 | 2245.6 ± 5.1 | 1.045 ± 0.02 | 86.00 ± 0.44 | 4.0 ± 0.07 | 70.6 ± 0.36 |
| 3 | MP3 | 1.50 | 18 | 2106.2 ± 4.8 | 1.087 ± 0.04 | 88.00 ± 0.41 | 4.5 ± 0.06 | 68.4 ± 0.31 |
| 4 | MP4 | 1.00 | 6 | 1336.0 ± 3.6 | 0.794 ± 0.06 | 98.50 ± 0.52 | 0.5 ± 0.03 | 88.4 ± 0.48 |
| 5 | MP5 | 1.00 | 12 | 1926.8 ± 4.3 | 0.985 ± 0.04 | 84.38 ± 0.39 | 3.5 ± 0.04 | 76.8 ± 0.40 |
| 6 | MP6 | 1.50 | 6 | 2258.4 ± 4.9 | 0.864 ± 0.02 | 79.00 ± 0.35 | 0.5 ± 0.02 | 74.0 ± 0.33 |
| 7 | MP7 | 1.50 | 12 | 2456.6 ± 5.5 | 0.988 ± 0.04 | 82.00 ± 0.37 | 3.5 ± 0.05 | 70.0 ± 0.34 |
| 8 | MP8 | 0.50 | 6 | 1424.8 ± 3.9 | 0.875 ± 0.06 | 90.00 ± 0.46 | 0.5 ± 0.02 | 82.6 ± 0.41 |
| 9 | MP9 | 1.00 | 12 | 1892.6 ± 4.1 | 1.254 ± 0.05 | 80.44 ± 0.36 | 3.5 ± 0.05 | 79.4 ± 0.43 |
| 10 | MP10 | 0.50 | 12 | 2182.2 ± 4.7 | 0.926 ± 0.02 | 77.00 ± 0.33 | 3.2 ± 0.04 | 76.0 ± 0.39 |
| 11 | MP11 | 1.00 | 12 | 1806.2 ± 3.8 | 0.804 ± 0.02 | 79.48 ± 0.34 | 3.5 ± 0.05 | 80.4 ± 0.44 |
| 12 | MP12 | 1.00 | 12 | 1945.6 ± 4.4 | 0.819 ± 0.02 | 82.14 ± 0.38 | 3.5 ± 0.04 | 79.6 ± 0.42 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 13 | MP13 | 0.50 | 18 | 2212.4 ± 4.9 | 1.264 ± 0.02 | 79.00 ± 0.35 | 0.5 ± 0.03 | 75.0 ± 0.36 |
| | Metformin IR | | | | | | | 99.54 ±5.4 at 1.5 hr |

**3.3. Optimization and Best Formulation Discussion.**

The response surface design used was a Box-Behnken in that the polyvinylpyrrolidone (PVP, $X_1$) and ethyl cellulose (EC 7 cps, $X_2$) were used together to determine their combined effect on the critical quality attributes of the metformin pulsatile beads which included particle size ($Y_1$), polydispersity index (PDI, $Y_2$), entrapment efficiency (EE, $Y_3$), lag time ($Y_4$) and percent drug release at the end of the dissolution process ($Y_5$). Experimental runs (MP1-MP13), were obtained with repeated center points (1.0mg PVP and 12mg EC) to determine the error of the experiment and to provide robustness in the design.

**3.3.1. Effect on Particle Size**

The size of the particles was between 1336.0 ± 3.6 nm (MP4) to 2456.6 ± 5.5 nm (MP7). It led to significant changes in particle size (MP4: 1336 nm MP3: 2106 nm MP7: 2456 nm), with EC concentration gradually increasing to 18 mg as the concentration of a coating dispersion led to the creation of a polymeric barrier that was thicker and an increase in viscosity. PVP also worked in the favor of particle size. The increased PVP (1.5 mg) led to increased beads, which is likely due to the tendency of PVP to Increase hydration of the matrix and the entanglements between the polymer chains, which causes the bead to expand. Table 3 and Figure 3 represent the Results.

**Table 3: ANOVA for Quadratic model Response 1: Particle Size**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 1.173E+06 | 5 | 2.346E+05 | 19.37 | 0.0006 | significant |
| A-PVP | 1.555E+05 | 1 | 1.555E+05 | 12.84 | 0.0089 | |
| B-EC | 4.616E+05 | 1 | 4.616E+05 | 38.11 | 0.0005 | |
| AB | 2.208E+05 | 1 | 2.208E+05 | 18.23 | 0.0037 | |
| $A^2$ | 2.763E+05 | 1 | 2.763E+05 | 22.81 | 0.0020 | |
| $B^2$ | 29411.66 | 1 | 29411.66 | 2.43 | 0.1631 | |
| Residual | 84791.01 | 7 | 12113.00 | | | |
| Lack of Fit | 73337.21 | 3 | 24445.74 | 8.54 | 0.0326 | significant |
| Pure Error | 11453.81 | 4 | 2863.45 | | | |
| Cor Total | 1.258E+06 | 12 | | | | |

A quadratic polynomial model was developed to study the influence of PVP (A) and EC (B) on particle size and was expressed as:

**Particle Size (nm)=1893.52+139.43A+240.22B−234.95AB+199.28$A^2$−65.02$B^2$**

The value of the model was found to be very important (F = 19.37, p = 0.0006), which validates the fact that the model is sufficient in the explanation of the experimental results. The strongest influence on the size of the particles was EC (F = 38.11, p = 0.0005) which showed that with the increased EC concentration the bead size increased significantly because there was a thicker polymeric coating layer and the viscosity of the coating dispersion increased. PVP too had the significant positive effect (p = 0.0089), which indicates that the greater the PVP, the greater the polymer hydration, and chain entanglement, which resulted in bead swelling. The large negative interaction term (AB, p = 0.0037) demonstrated that high polymers at the same time hindered excessive particle growth, perhaps because of a limitation on the spreading of the viscous coating solution. The large A 2 value (p = 0.0020) was a confirmation of curvature in the response that showed the increase in the particle size at high and high levels of PVP was non-linear, yet, the B 2 was minor, which was an indication of the nearly linear effect of EC in the experimental range. These results were further justified by the contour and 3D surface plots which indicated the smallest size of a particle at low PVP and low EC that represents formulation MP4 (1336 ± 3.6 nm). Therefore, the study concluded that EC is the most important factor of particle size, then that of PVP and both polymers should be kept at a low concentration using the polymers

to end up with uniformly sized, optimized pulsatile beads.

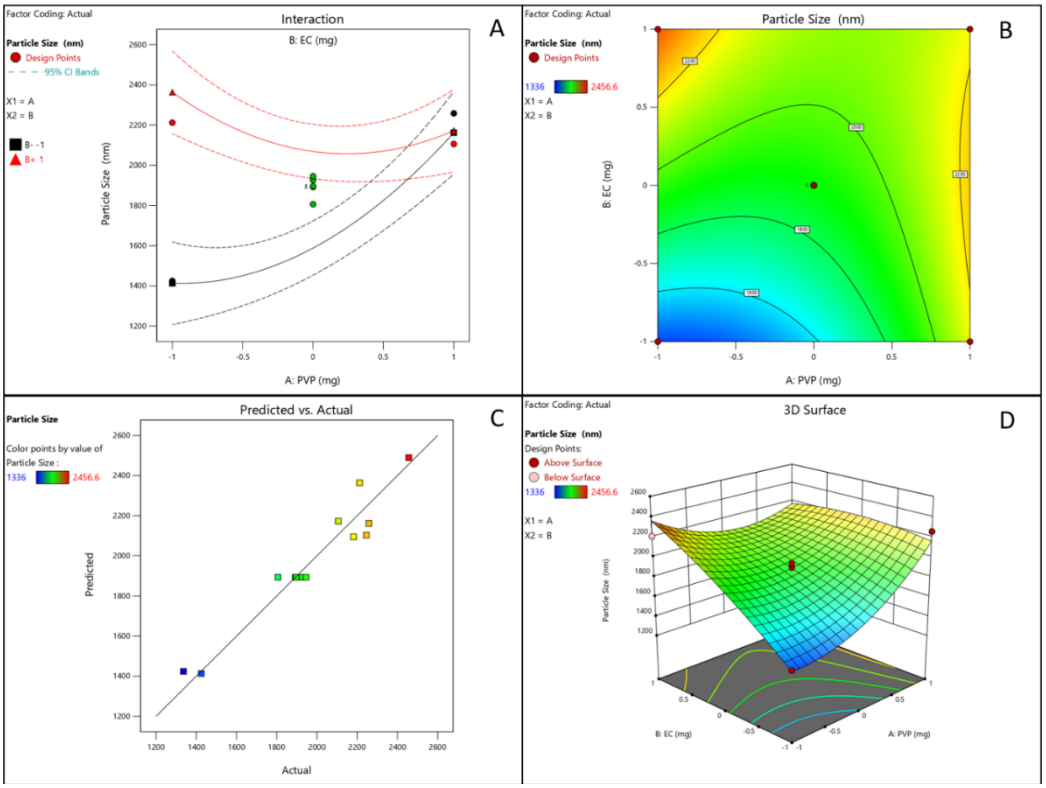


**Figure 3:** Response Surface Methodology (RSM) Analysis Showing the Influence of Polyvinylpyrrolidone (PVP, A) and Ethyl Cellulose (EC, B) on Particle Size of Metformin Pulsatile Beads: (A) Interaction Plot with 95% Confidence Bands, (B) Contour Plot, (C) Predicted vs. Actual Values, and (D) Three-Dimensional Response Surface Plot.

### 3.3.2. Effect on Entrapment Efficiency (EE)

The efficiency of entrapment was between 77.00 ± 0.33 and 98.50 ± 0.52. Low EC (6 mg) showed the maximum EE (MP4: 98.5% MP8: 90.0%). High EC (18 mg) caused lower EE (MP2, MP3, MP13) which may be caused by diffusion of the drug into the external phase in long coating and hardening period. EE was barely affected by increasing PVP because of enhancing drug polymer affinity, but this was masked by EC concentration. Table 4 and Figure 4 indicate the Results.

**Table 4: ANOVA for Quadratic model Response 2: Entrapment Efficiency**

| **Source** | **Sum of Squares** | **df** | **Mean Square** | **F-value** | **p-value** | |
|---|---|---|---|---|---|---|
| **Model** | 344.54 | 5 | 68.91 | 7.71 | 0.0091 | significant |
| A-PVP | 3.21 | 1 | 3.21 | 0.3595 | 0.5677 | |
| B-EC | 48.40 | 1 | 48.40 | 5.41 | 0.0529 | |
| AB | 100.00 | 1 | 100.00 | 11.18 | 0.0123 | |
| $A^2$ | 18.18 | 1 | 18.18 | 2.03 | 0.1969 | |
| $B^2$ | 157.50 | 1 | 157.50 | 17.61 | 0.0041 | |
| **Residual** | 62.59 | 7 | 8.94 | | | |
| Lack of Fit | 48.04 | 3 | 16.01 | 4.40 | 0.0931 | not significant |
| Pure Error | 14.55 | 4 | 3.64 | | | |
| **Cor Total** | 407.14 | 12 | | | | |

### 3.3.3. Effect of PVP and EC on Entrapment Efficiency (EE%) with Polynomial Model Interpretation

Quadric polyvinylpyrrolidone (PVP, A) and ethyl cellulose (EC, B) and their effect on entrapment efficiency of metformin pulsatile beads were assessed by using quadratic polynomial. The model was statistically significant (F = 7.71, p = 0.0091), the lack-of-fit was non-significant (p = 0.0931), which implies sufficiently good model adequacy. The coded polynomial equation for entrapment efficiency was:

$$EE(\%)=81.80+0.63A-2.46B+5.00AB-1.62A^2+4.76B^2$$

The positive coefficient of A, which is small (+0.63) and the p-value of A (0.5677) means that PVP in itself did not have any significant linear effect on entrapment efficiency, but EC had a moderate negative linear effect (-2.46, p = 0.0529) meaning that an increase in EC slightly reduced drug entrapment because a denser hydrophobic barrier would form and block drug incorporation. Interaction term AB was very significant and positive ( +5.00, p = 0.0123), which indicated a strong synergistic effect between PVP and EC: the entrapment efficiency rose significantly at the optimum intermediate levels of the two polymers, which is reflected in the increase of the surface in the response plots (EH). Of the quadratic terms, B2 was extremely significant (+4.76, p = 0.0041) and the curvilinear effect of EC is present with the highest entrapment being noted at medium ECs and not at extremes. The predicted vs. actual plot showed that the experimental values and predicted values were quite close which provided evidence to the reliability of the model. Generally, the response surface analysis showed that the most entrapment efficiency (~98.5% MP4) was reported at intermediate PVP (1 mg) and low EC (6mg), which is important to note in optimizing drug loading efficiency because of the polymer interaction and the optimal level of EC.

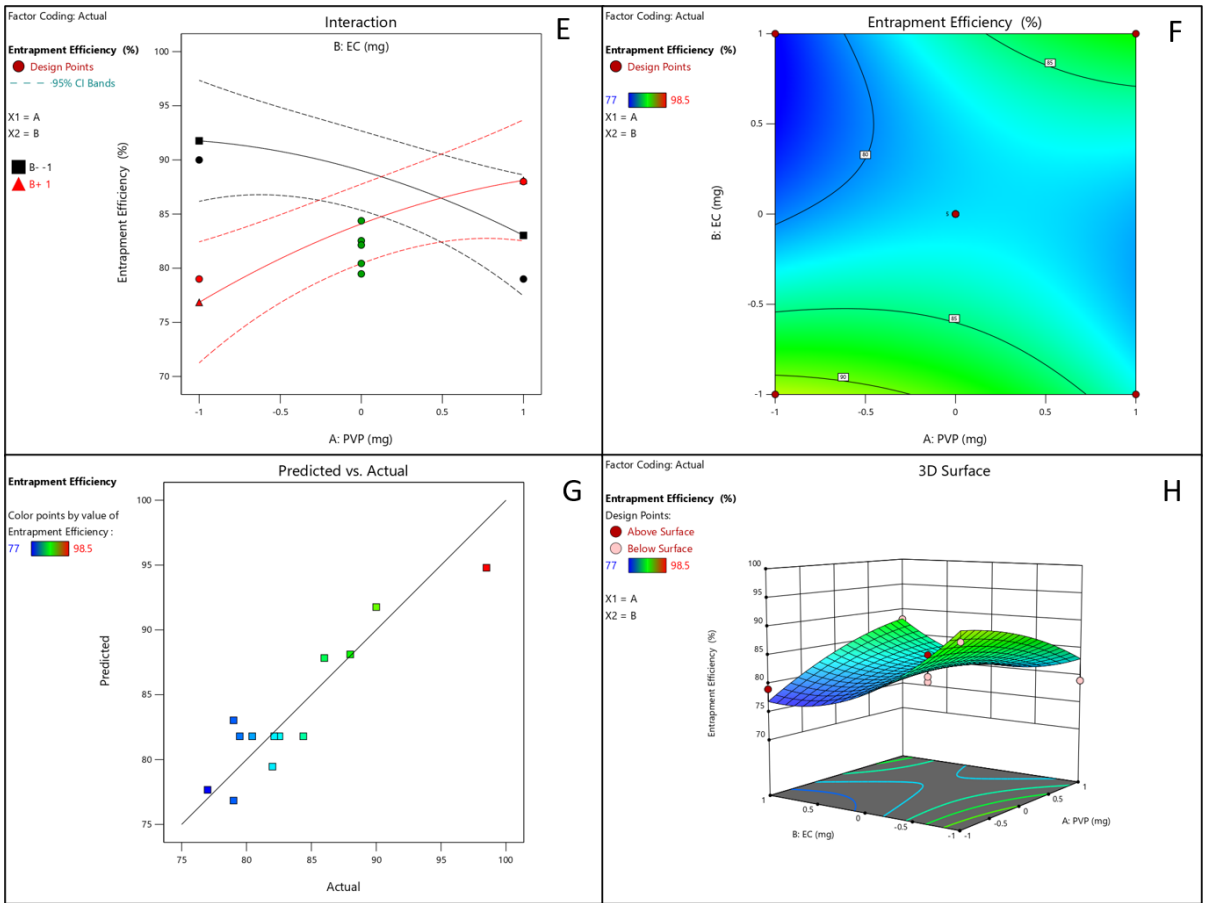


**Figure 4:** Response Surface Methodology (RSM) Plots Depicting the Effect of Polyvinylpyrrolidone (PVP, A) and Ethyl Cellulose (EC, B) on Entrapment Efficiency of Metformin Pulsatile Beads: (E) Interaction Plot, (F) Contour Plot, (G) Predicted vs. Actual Values, and (H) Three-Dimensional Response Surface Plot.

### 3.3.4. Effect of PVP and EC on Lag Time with Polynomial Model Interpretation

A good predictive model (F = 6.40, p = 0.0152) was found to explain the effect of polyvinylpyrrolidone (PVP, A) and ethyl cellulose (EC, B) on lag time of metformin pulsatile beads in terms of a significant quadratic model. An expressed lag time equation that was coded was:

Lag Time (h)=3.50+0.55A+1.12B+1.00AB−0.40$A^2$−0.95$B^2$.

The ANOVA results indicated that the positive effect of EC was very significant on lag time (B, p = 0.0076) and

as such, it is clear that an increment in EC concentration significantly increased the lag time as a result of the creation of a stronger, more hydrophobic polymeric coating that slowed water uptake and erosion of the coating layer. PVP had a non-significant positive effect (A, p = 0.1093) which showed that it has a secondary effect on regulating lag time. The interaction term AB was slightly significant (p = 0.0515), implying that PVP and EC had a joint influence that altered the properties of the coating as a barrier. The quadratic terms were also significant, with the B 2 value of 0.0218 (p = 0.0218), indicating non-linearity of lag time in relation to EC concentration with excessive amounts of EC level leading to plateauing response. The response surface and contour plots (I-L) clearly designed that the lag time continued to increase with increasing EC level, i.e. at low EC (6 mg) to medium EC (12 mg) to high EC (18 mg), without respect of PVP level. The comparison of the predicted and actual plot revealed that there was a close agreement between the experimental and predicted value which proved the strength of the model. In general, EC concentration emerged to dictate the behavior of pulsatile behavior and formulations with increased EC could deliver a clear-cut programmable lag phase required in the delivery of metformin in a chronotherapeutic manner. Table 5 and Figure 5 present the Results.

**Table 5: ANOVA for Quadratic model Response 3: Lag Time**

| **Source** | **Sum of Squares** | **df** | **Mean Square** | **F-value** | **p-value** | |
|---|---|---|---|---|---|---|
| **Model** | 23.28 | 5 | 4.66 | 6.40 | 0.0152 | significant |
| A-PVP | 2.45 | 1 | 2.45 | 3.36 | 0.1093 | |
| B-EC | 10.01 | 1 | 10.01 | 13.77 | 0.0076 | |
| AB | 4.00 | 1 | 4.00 | 5.50 | 0.0515 | |
| $A^2$ | 1.11 | 1 | 1.11 | 1.53 | 0.2559 | |
| $B^2$ | 6.28 | 1 | 6.28 | 8.63 | 0.0218 | |
| **Residual** | 5.09 | 7 | 0.7273 | | | |
| Lack of Fit | 5.09 | 3 | 1.70 | | | |
| Pure Error | 0.0000 | 4 | 0.0000 | | | |
| **Cor Total** | 28.37 | 12 | | | | |

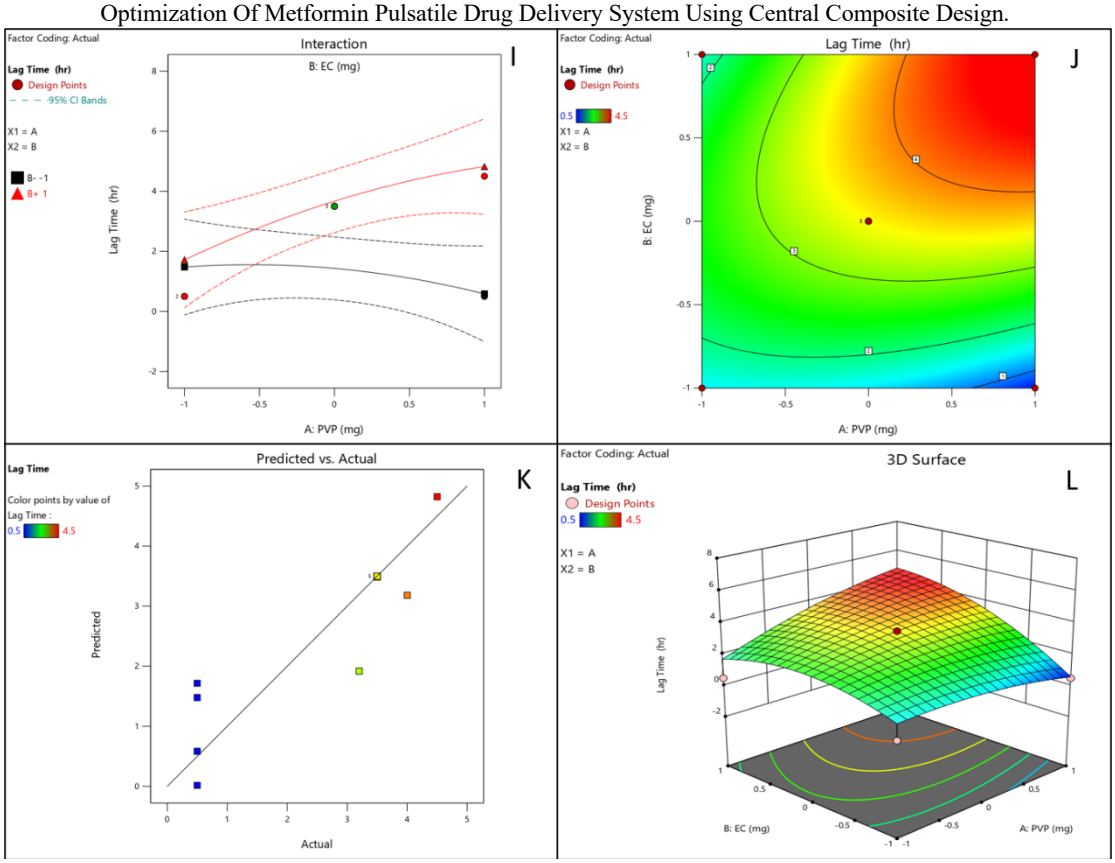


**Figure 5:** Response Surface Methodology (RSM) Plots Illustrating the Influence of Polyvinylpyrrolidone (PVP, A) and Ethyl Cellulose (EC, B) on Lag Time of Metformin Pulsatile Beads: (I) Interaction Plot, (J) Contour Plot, (K) Predicted vs. Actual Plot, and (L) Three-Dimensional Response Surface Plot

### 3.3.5. Effect of Formulation Variables on Drug Release (%)

The extent of drug release of metformin pulsatile beads was found to range between 68.4 ± 0.31 % (MP3) to 88.4 ± 0.48 % (MP4), depending on the concentrations of polyvinylpyrrolidone (PVP, A) and ethyl cellulose (EC, B). EC increase significantly decreased drug release owing to the development of a thick less permeable polymeric barrier, but moderate PVP increased release because it facilitated hydration of polymer and creation of pores. The interaction, contour and 3D surface plot (M, N, P) show that the greatest drug release was obtained at low EC and intermediate PVP levels and high EC resulted in incomplete release at high PVP. The actual versus the predicted plot (O) was found to be in good agreement indicating that the model is adequate. The quadratic polynomial equation describing the effect of formulation variables on drug release in coded terms is:

$$\textbf{Drug Release (\%)} = 78.45 + 2.96A - 5.53B - 0.35AB + 3.08A^2 - 0.06B^2$$

A represents the coded level of PVP and B represents the coded level of EC. ANOVA was used to affirm the importance of the model ($F = 13.67$, $p = 0.0017$). PVP (A, $p = 0.0067$) and EC (B, $p = 0.0005$) were both considerably linear, but EC was the most dominant factor that controlled drug release. PVP ($A^2$, $p = 0.0054$) was a nonlinear effect, but AB and $B^2$ were not significant. Lack-of-fit was significant ($p = 0.0811$) which confirmed the model. All in all, the peak drug release was high at low EC and moderate concentrations of PVP, which guarantees effective diffusion of metformin following the intended lag time. Table 6 and Figure 6 present the Results.

**Table 6: ANOVA for Quadratic model - Response 4: Drug Release**

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 332.48 | 5 | 66.50 | 13.67 | 0.0017 | significant |
| A-PVP | 70.12 | 1 | 70.12 | 14.42 | 0.0067 | |
| B-EC | 184.06 | 1 | 184.06 | 37.85 | 0.0005 | |
| AB | 1.00 | 1 | 1.00 | 0.2056 | 0.6639 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| $A^2$ | 76.33 | 1 | 76.33 | 15.70 | 0.0054 | |
| $B^2$ | 0.0272 | 1 | 0.0272 | 0.0056 | 0.9425 | |
| **Residual** | 34.04 | 7 | 4.86 | | | |
| Lack of Fit | 26.68 | 3 | 8.89 | 4.83 | 0.0811 | not significant |
| Pure Error | 7.36 | 4 | 1.84 | | | |
| **Cor Total** | 366.52 | 12 | | | | |

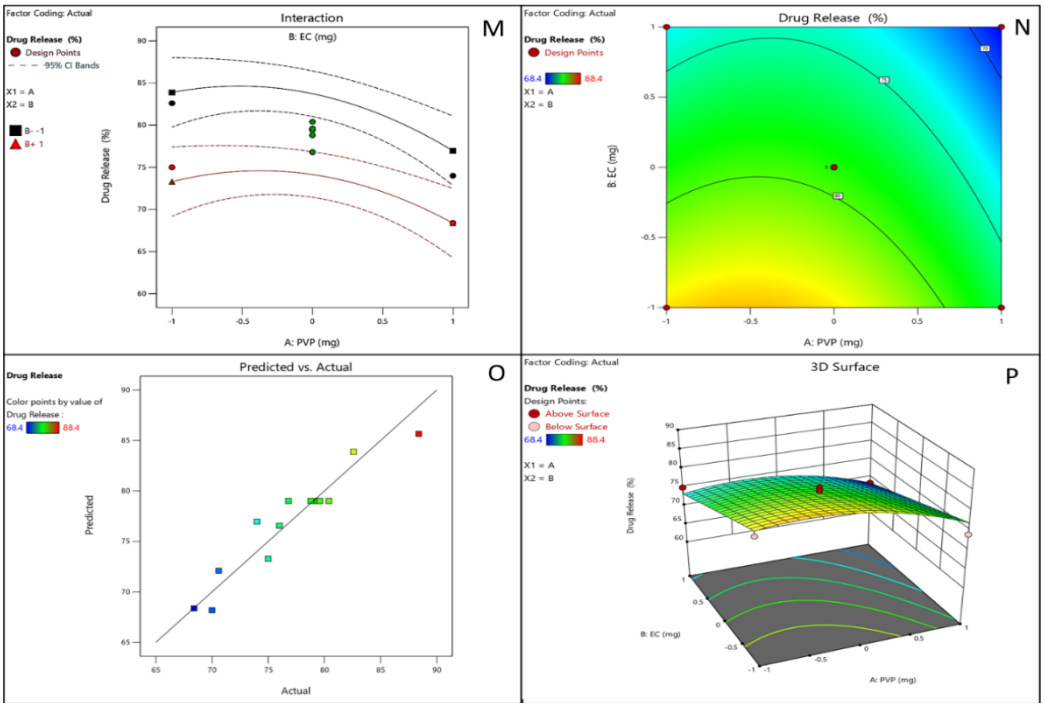


**Figure 6:** Response Surface and Interaction Plots Illustrating the Effect of PVP (A) and Ethyl Cellulose (B) on Drug Release from Metformin Pulsatile Beads: (M) Interaction Plot, (N) Contour Plot, (O) Predicted vs. Actual Plot, and (P) 3D Surface Plot.

The optimized formula MP4 which includes PVP 1.0 mg and EC 6 mg was considered due to the capacity to comply with all the predetermined requirements of an effective pulsatile delivery system, such as high entrapment efficiency (>90%), controlled particle size (<1500 nm), low PDI (<0.9), a definite lag time of about 0.51 h, and high drug release (>85%). The particle size of Mp4 was 1336.0 ± 3.6nm, PDI 0.794 ± 0.06, entrapment efficiency 98.50 ± 0.52, lag time 0.5 ± 0.03 h, drug release 88.4 ± 0.48. Response surface analysis showed that EC concentration was the key variable that controlled lag time, particle size and drug release whereas PVP was the main factor that influenced matrix integrity and drug entrapment. Due to its superior encapsulation, low size distribution, small lag phase and rapid drug release, MP4 was thus declared as the optimized formulation and it was chosen to undergo further correlation and stability analysis in in-vivo.

## 4. Discussion

The suitability of the formulation process was supported by the fact that DSC and FTIR analyses showed that metformin was thermally and chemically stable and did not show any degradation or excessive drug-excipient interaction with the chosen excipients. The Box-Behnken response surface design was effectively used to optimize the formulation variables which are polyvinylpyrrolidone (PVP) and ethyl cellulose (EC) and to investigate their effect on optimizing the particle size, PDI, entrapment efficiency, lag time and drug release. As EC and PVP concentration rose, the particle size rose to a significant degree and EC was the one that showed the greatest influence as it also affected the coating viscosity and thickness. The trapment efficiency was maximum at low EC and moderate and middle level of PVP and interaction between the two polymers was critical in maximizing drug loading. The EC concentration was the main factor in control of lag time and the higher the EC concentration the longer and better-defined delay in drug release, which is vital to pulsatile delivery. The release rate was reduced significantly with higher EC as a result of the formation of a solid dense and hydrophobic barrier, whereas moderate PVP increased the release rate by increasing the hydration of the polymers and formation of holes. MP4 (PVP 1.0 mg, EC 6mg) was found to possess the best of features that included: optimum size of the particle, high

entrapment efficiency, short and clear lag time, and optimum release of the drug. In general, EC was confirmed as the decisive factor governing pulsatile behavior, and PVP helped to regulate the matrix integrity and entrapment which resulted in the choice of MP4 as the optimized formulation to be used in the further in-vivo and stability experiments.

**5. Conclusion**

The current work has been able to optimize metformin pulsatile beads by response surface methodology and determined that ethyl cellulose was the factor that majorly affected the lag time, bead size and drug release and that polyvinylpyrrolidone was the factor that mainly affected the integrity of the matrix and entrapment efficiency. The optimization formulation MP4 (PVP 1.0 mg, EC 6 mg) was highly entraped (98.50 ± 0.52 percentage), had controlled particle size (1336.0 ± 3.6 nm) and acceptable PDI (0.794 ± 0.06), a distinct lag time (0.5 ± 0.03 h), and good drug release (88.4 0.48 percentage), meeting all the predetermined optimization parameters. These results affirm that the rational balance of hydrophilic and hydrophobic polymers can effectively be used to adjust pulsatile release behaviour and the optimized MP4 system is a promising system to deliver metformin chronotherapeutically, which requires additional in-vivo correlation and stability analysis.

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