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Metformin hydrochloride-loaded biodegradable microspheres employing the Box Behnken design for local delivery in periodontitis: design, optimization, and characterization

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ABSTRACT

In the current research, periodontitis was treated by filling periodontal pockets with metformin hydrochloride-loaded microspheres, either with or without grafts. In order to do this, chitosan was selected as the polymer and used in various drug/polymer ratios during the emulsion cross-linking process to create microspheres. Utilizing a three-factor, three-level Box–Behnken architecture allowed for optimization. Regression analysis was used to create mathematical models for the responses of particle size (PS) and entrapment efficiency (EE). The experimental design considered the economical reduction of chemical usage and formulation time to develop an optimized formulation with highest %EE and minimal PS under optimal process conditions for the microsphere formulation. Based on the desirability function, the optimal formulation was chosen, and it was then assessed in terms of particle size, entrapment efficiency, drug release in vitro, differential scanning calorimetry (DSC), fourier transform infrared (FTIR) spectroscopy, and surface morphology investigations. Kinetic and statistical analyses were performed on the release study findings. The chosen batch's particle size and entrapment effectiveness were determined to be between 40.2 and 59.6 µm and 85 and 95%, respectively. The drug's molecular dispersion and transformation into an amorphous state were shown by the DSC investigations. By using scanning electron microscopy (SEM) to examine the surface morphology of the microspheres, it was discovered that they had a smooth, spherical surface.

Introduction

Type 2 diabetes is treated with metformin hydrochloride, a second-generation biguanide, as a hypoglycemic medication.[1] Metformin hydrochloride has been demonstrated to have osteogenic activity in addition to its established use in the treatment of diabetes. It has shown a dose-dependent rise in the proliferation of two osteoblast-like cells (MC3T3E1 and UMR106). Additionally, it has increased the synthesis of type-I collagen in both cell lines and encouraged. Activity of alkaline phosphatase in osteoblasts MC3T3E1.[2] A group of inflammatory illnesses known as periododontitis affect the tissues that support and surround the teeth, called the periodontium. It results in the gradual loss of the alveolar bone around the teeth by destroying the attachment system of the teeth, which creates the periodontal pocket and normal osseous structure.[3] Treatments for periodontitis may be divided into two categories: regenerative and anti-infective. The regenerative therapy method is the foundation of the present investigation. Bone regeneration is necessary for the treatment of periodontitis since the condition causes a gradual loss of alveolar bone. Bone transplants are used in conventional treatment for bone repair. These solid bone grafts are inserted into the affected area to promote bone regrowth. As a regenerative treatment for periodontitis, chitosan

microspheres containing metformin hydrochloride may be used in addition to or instead of bone transplants. Drug delivery at the intended place may be regulated thanks to microspheres.[4] Choosing the proper preparation technique, copolymer concentration, and polymer with the optimum molecular weight can let you use microspheres to produce the required release profile. They may be utilized for regulated administration and, as a result, for prolonged release of metformin hydrochloride for the treatment of periodontitis because of their tiny particle size and large surface-to-volume ratio.
Natural chitosan is a biodegradable polymer that has a number of benefits, including mucoadhesion, high charge density, non-toxicity, and biocompatibility. Chitosan microspheres may be made using a variety of methods, including solvent evaporation, chemical reactions, thermal cross-linking, and interactions with anions.[5] Box Behnken Design (BBD), which helps statistically assess the impact and interaction among factors, requires fewer treatment combinations. By creating polynomial equations and response surface graphs, BBD aids in process optimization by reducing the number of experimental runs.[6]
The purpose of this work was to use the Box Behnken design to determine the ideal formulation parameters, such as the drug to polymer ratio, surfactant concentration, and rotation speed, in order to manufacture metformin hydrochloride-loaded chitosan microspheres with sustained drug release.

Materials and Methods

Materials

I got a complimentary sample of metformin hydrochloride from USV in Mumbai, India. Chitosan (molecular weight of 10,000 Da, 85% deacetylation) was purchased from Annlab Fine Chemicals in Mumbai. Glutaraldehyde, glacial acetic acid, and light liquid paraffin were acquired from Research Lab Fine Chem Industries, Mumbai. We purchased Span 80 from Molychem Industries in Mumbai.

Setting Up Microspheres[7]

Emulsion cross-linking was used to create the chitosan microspheres loaded with metformin hydrochloride. The process parameters were optimized by the use of Box Behnken design.

A mere 1% of glacial acetic acid was used to dissolve the chitosan. To create an aqueous phase, the medication was dissolved in an excessive quantity of distilled water and combined with a chitosan solution. Using a needle, the aqueous phase was gradually introduced to the oily phase—light liquid paraffin with span 80 acting as a surfactant—drop by drop. After 40 minutes and 1.5 mL of stirring, the w/o emulsion was added to at intervals of 10, 20, and 30 minutes, along with glutaraldehyde solution. After being separated by centrifugation at 5000 rpm for 30 minutes, the produced microspheres were air-dried and chloroform-washed to get rid of any remaining light liquid paraffin.

Statistical Analysis and Experimental Design

If conventional experimental techniques are used during the development of a complicated formulation, more work, time, and resources are needed. As a result, a variety of experimental designs that take less work and provide estimates of the relative importance of various factors are being adopted.[8–12] The study's dependent variables were particle size (μm) and entrapment efficiency (%), whereas the independent variables (Table 1) chosen were drug: polymer (A), surfactant concentration (%) (B), and speed of rotation (RPM) (C). Preliminary research was the foundation for choosing the factor levels (Table 2). Variations were made to the polymer content between 100 and 300 mg, the surfactant concentration between 1 and 2%, and the homogenizer's rotation speed between 3000 and 4000 rpm. Every dependent variable had a mathematical model created using Design Expert 7.1 software, which was then statistically examined.

Choosing the Optimal Formulation

The design expert program recommends combinations of the independent variable that should be utilized to distribute the best batches with the desired entrapment efficiency and particle size rather than actually producing and assessing them based on the data collected from the trial runs. Particle size, entrapment efficiency, in-vitro drug release, scanning electron microscopy (SEM), DSC, and FTIR tests were assessed for the improved microsphere formulation.

Assessment of Formulation

Size of Particles[13]

The size of the particles was measured using an optical microscope (Micron, Optik). The particle size was determined by fitting the eyepiece with a micrometer scale. On a microscope slide, the microspheres were distributed. In every measurement, a minimum of 100 particles were analyzed inside a scanned microscopic field. The typical size of the particles

Table 1: Levels of chosen independent variables for the formulation of microspheres

Factors	*Levels*		
	-1	*0*	*+1*
(A) Drug: Polymer	1:1	1:2	1:3
(B) Surfactant concentration (%)	1	1.5	2
(C) Speed of rotation (rpm)	3000	3500	4000

The microspheres' volume surface diameter (μm) was used to represent the microspheres, and the standard deviation was computed for every batch of microspheres. Efficiency of Entrapment and Drug Loading[14,15] The drug contents were ascertained using spectrophotometric techniques. At room temperature, 0.1M HCl was used to dissolve microspheres that weighed, crushed, and contained 10 mg of metformin HCl. 200 μg/mL of the samples were diluted. A UV-visible spectrophotometer for metformin HCl was used to assess the solution at 233 nm after it had been spun for 15 minutes at 13000 rpm. Using the following formulas, the drug loading and entrapment efficiency (%) were determined.

$$\text{Drug loading (\%)} = \frac{M_{actual}}{\text{Weighed quantity of powder of microsphere}} \text{ X } 100$$

$$\text{Entrapment efficiency (\%)} = \frac{M_{actual}}{M_{theorical}} \text{X } 100$$

In-vitro Drug Release[16]
The drug release from the formulations was investigated in vitro using phosphate buffer saline pH 7.4. To put it briefly, the drug-loaded microspheres were sealed in a dialysis bag (molecular weight cut off at 10,000 D) and suspended in 1 mL of pH 7.4 PBS. After that, the bag was suspended in a beaker filled with 50 mL PBS and 0.5% sodium azide as a preservative, and it was continuously swirled at 50 rpm at 37 ± 0.5°C. Additionally, 3 mL aliquots were taken out at intervals of 1, 2, 3, 4, 5, 6, 12, 24, 48, 72, 96, 120, 144, 168, 192, and 240 hours, and each time a corresponding amount of buffer was added. After that, the material was passed through a 0.45 μ membrane filter and examined at 233 nm using a UV spectrophotometer. Every experiment was carried out three times.

Table 2: An experimental design arrangement of a Box Behnken

Formulation code	Coded level (Independent variable)		
	A	B	C
F1	-1	-1	0
F2	+1	-1	0
F3	-1	+1	0
F4	+1	+1	0
F5	-1	0	-1
F6	+1	0	-1
F7	-1	0	+1
F8	+1	0	+1
F9	0	-1	-1
F10	0	+1	-1
F11	0	-1	+1
F12	0	+1	+1

Release Kinetics
The following four mathematical models were fitted with data to calculate the release kinetics of the in-vitro release investigations, where Mt/M∞ represents the proportion of medication released at time t. The zero-order release constant, first-order release constant, Higuchi constant, and Korsmeyer-Peppas constant are denoted by the symbols k0, k1, kH, and k, respectively.

The drug release mechanism is indicated by the release exponent, or n, in the Power-law model. Equations corresponding to k0, k1, kH, k, and n values were fitted to the release data. In pharmacological dose forms, W0 is the starting dosage of the medication. What is the drug's residual dosage form quantity at time 't'?

Zero order $\frac{Mt}{M\infty} = k_0\, t$

1st order $\frac{Mt}{M\infty} = 1 - \exp(k_1 t)$

Higuchi model $\frac{Mt}{M\infty} = K_H\ t^{1/2}$

Korsmeyer Peppas $M_t / M_\infty = kt^n$

Hixon-crowell $W_0^{\frac{1}{3}} - W_t^{\frac{1}{3}} = kt$

Results and Discussion

Preparation of Metformin HCl Loaded Chitosan microspheres
In this work, the emulsion cross linking technique was used to create metformin HCl loaded microspheres. Chitosan was used as a bioadhesive carrier, and glutaraldehyde was used as a cross linking agent. Smaller size microspheres (less than 60 µm) with a narrow size distribution and high entrapment effectiveness were consistently produced using this approach.

Statistical Analysis and Experimental Design

Because box Behnken designs need fewer treatments than complete factorial studies, they were chosen. Twelve batches were made using three components at three different levels. The 12 batches were assessed in terms of percentage entrapment efficiency (%EE) and particle size (µm). The findings of the %entrapment effectiveness and particle size (µm) varied between 85 and 95% and 40.2 to 59.6 µm, respectively (Table 3). Thus, it is possible to say that the chosen independent variables determine the outcomes. Y1 and Y2 response variables were fitted using a quadratic model. The negative sign denotes an antagonistic connection, whereas the positive sign indicates a synergistic influence of the elements on the response. Given that the r2 values for particle size and percentage EE are both rather high—0.96993 and 0.9693, respectively—the polynomial equations provide a very good fit to the experimental data and have a strong statistical foundation.

Multiple regressions were used on practically collected dependent variables in order to produce a quadratic equation. The following equation was used to get the particle size findings.

$$PS = +46.38+4.55*A+1.20*B-0.91*C$$
$$+4.32*A*B+5.54*A*C+0.21*B*C+3.85*A^2-2.47*B^2$$

Table 3: Values of Entrapment Efficiency and particle size of and 86.29%, respectively. microspheres as per Box Behnken design

Batch no.	*Entrapment Efficiency (%)*	*Particle size (µm)*
F1	87 ± 0.89	44.54 ± 2.69
F2	89.4 ± 0.76	46.28 ±3.82
F3	86.5 ± 1.27	40.6 ± 2.91
F4	90.5 ± 1.53	59.6 ± 3.12
F5	94.3 ± 1.82	52.43 ± 4.26
F6	85.0 ± 0.83	49.18 ± 3.65
F7	85.0 ± 1.42	40.2 ± 4.14
F8	95.0 ± 1.08	59.1 ± 2.87
F9	87.1 ± 1.74	45.3 ± 2.43
F10	87.9 ± 0.77	45.0 ± 1.92
F11	88.0 ± 1.91	42.4 ± 2.28
F12	90.0 ± 1.63	42.92 ± 1.08

Optimization and Validation

Four of the most desirable options, out of the many that the Design Expert program recommended, were developed. The factor combinations that yielded the necessary results were identified and then processed into batches M1, M2, M3, and M4. The experimental and anticipated values of the chosen responses are contrasted in Table 4. The low percentage error figure indicates how well the chosen design can be predicted.

Assessment of Microspheres

Size of Particles

The twelve microsphere formulations had varying particle sizes, ranging from 40.2 to 59.6 µm. It was discovered that the mean particle sizes for batches M1, M2, M3, and M4 were, respectively, 43.57, 54.04, 51.4, and 52.64.

Drug Loading and Effectiveness of Entrapment

Batches M1, M2, M3, and M4 were found to have entrapment efficiencies of 91.2, 88.35, 92.5, and 90.94, respectively.

Studies on In-vitro Release

A pH 7.4 phosphate buffer was used for the in-vitro release research (Fig. 3). On the tenth day, the in-vitro release values for batches M1, M2, M3, and M4 were determined to be 86.83, 82.68, and 79.8. Table 3 shows the particle size and entrapment efficiency values, which are 86.29% and 86.33%, respectively.

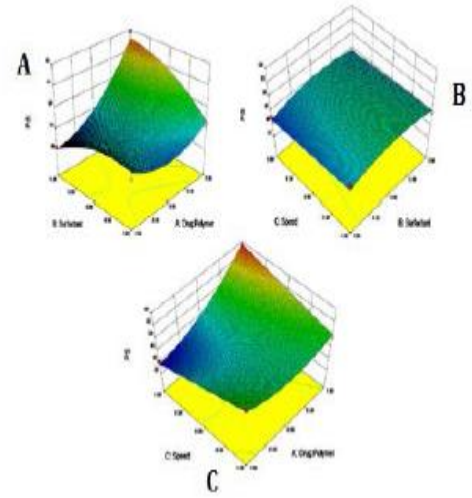

Fig. 1: Three dimensional graphs showing effect of A- Surfactant concentration and drug: polymer, B- Speed and Drug: polymer, and C- Surfactant concentration and Speed on Particle size

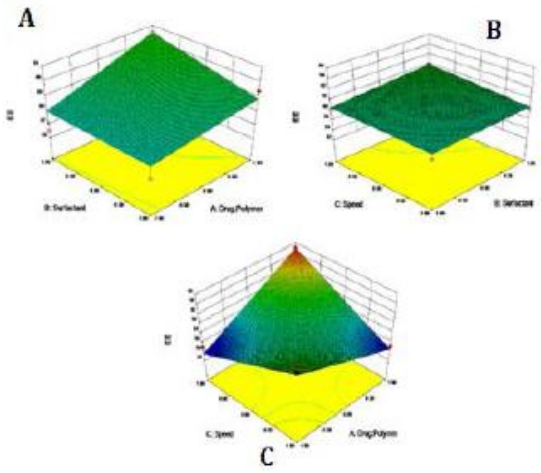

Fig. 2: Three dimensional graphs showing effect of A- Surfactant concentration and drug: polymer, B- Speed and Drug: polymer, and Surfactant concentration and Speed on Entrapment efficiency

Table 4: Comparison of experimental results with predicted responses

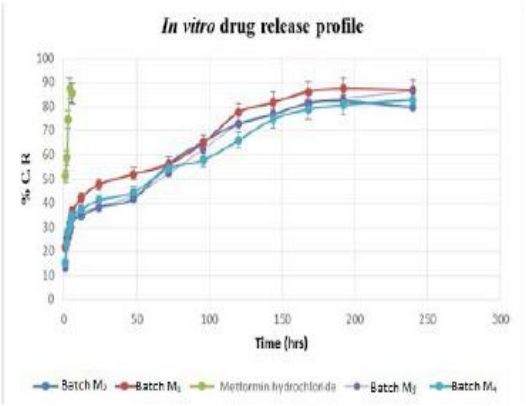

Fig. 3: *In-vitro* drug release in phosphate buffer saline pH 7.4 (n=3)

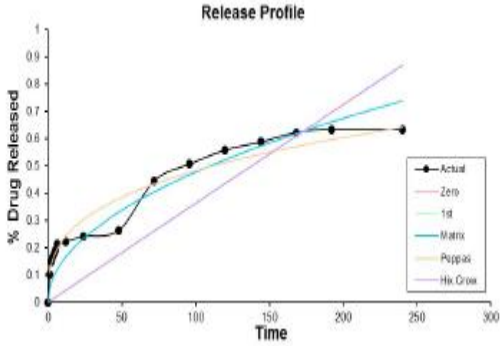

Fig. 4: Kinetic model fitting for drug release data of optimized batch M3

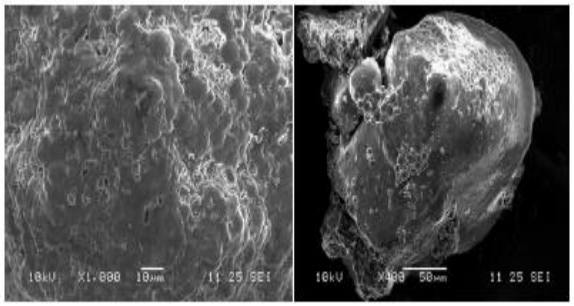

Fig. 5: SEM images of metformin hydrochloride loaded microspheres

Discussion

This research aimed to maximize the impact of formulation factors on response parameters and manufacture metformin hydrochloride microspheres using the emulsion cross-linking technique. An equation derived from BBD was used to forecast the values of the response variables at certain factors. It is possible to get the best formulation using ranges between the chosen independent variable values.

The study's findings on particle size analysis make it abundantly evident that polymer and surfactant concentrations significantly affect particle size. Particle size rises with increasing polymer content while decreasing with increasing

surfactant concentration. An increase in viscosity and cross-linking in the formulation may be associated with a rise in particle size when the polymer concentration is raised. Particle size is negatively impacted by rotational speed. As the rotational speed was increased, a reduction in particle size was seen. The percent age entrapment efficiency was positively impacted by the polymer concentration and rotational speed. This may be connected to the fact that a higher concentration of polymer causes the formulation to become more viscous and cross-linked, which increases the amount of drug entrapment in the polymer. The greater solubility of metformin hydrochloride in the dispersed phase may further contribute to the increased entrapment efficiency. The dispersion phase in this case is aqueous, and metformin hydrochloride dissolves entirely in aqueous solution. Given that glutaraldehyde is added right once and cross-linking takes place, the medication may stay in the dispersed phase.

One of the possible explanations for the drug's high entrapment efficiency is its insolubility non light liquid paraffin, or continuous phase, as the drug does not diffuse in continuous phase during the production of microspheres. However, a higher surfactant content resulted in a lower entrapment efficiency. This may be understood as a decrease in particle size, a rise in surfactant concentration, and a fall in entrapment efficiency.

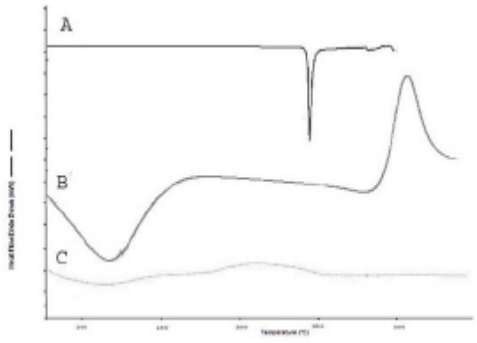

Fig. 6: DSC thermogram of A) metformin HCl B) chitosan and C) optimized batch M3

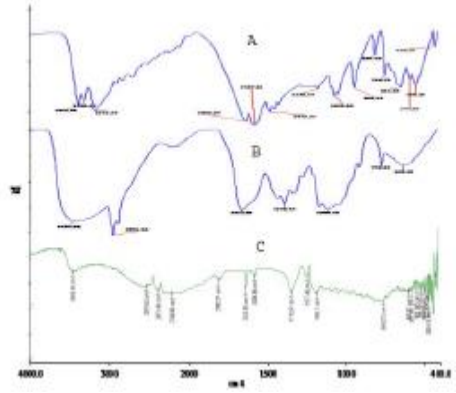

Fig. 7: FTIR spectra of A) metformin HCl B) chitosan and C) optimized batch M3

Conclusion

The current study suggested using biodegradable microspheres loaded with metformin hydrochloride to fill periodontal pockets, either with or without grafts. The emulsion cross-linking process was used to create the microspheres, which were then assessed for in-vitro release, trapping effectiveness, and particle size. Every measurement was found to be within a reasonable range. It was determined that the local distribution of biodegradable microspheres laden with metformin hydrochloride might successfully cure periodontitis. However, in order to assess the risk:benefit ratio, clinical data are still required.

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