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Formulation Designing And Optimizing Acetazolamide Microspheres Using A Box-Behnken Method

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A Box-Behnken experimental design is used in this work to find the best way to make Acetazolamide-loaded microspheres. Eudragit RS100 is used as the polymer, and the liquid evaporation method is used. The manufacturing yields, particle size distribution, shape, entrapment effectiveness percentage, and drug release properties of the microspheres that were made were all looked at. The Box-Behnken design was used to make fifteen formulas with known values for the drug: polymer ratio (X1), the concentration of surfactant, and the speed of stirring (rpm) (X3). Particle size (µm) (Y3), entrapment effectiveness, and drug release after six hours (%) were the things that were being looked at. The acetazolamide-filled microspheres were smooth on the outside

and round on the inside. It was found that the microspheres had a production rate that ranged from 70.45% to 92.98% per batch. Entrapment efficacy rates for the microspheres that were made ranged from 34.84% to 76.19%, which is a good result. We used surface and contour plots, regulated drug release, and the computer optimization method to guess the values of independent factors X1, X2, and X3 that would lead to the best particle size and the highest EE% response.

Keywords: Drug delivery, microspheres, Box-Behnken, microencapsulation.

1. Introduction

The therapeutic efficiency of a certain drug could be improved by a carefully planned controlled drug delivery system. This would also help fix some problems with current treatment methods. It is very important to get the drug to the right cells at the right time and in the right amount so that it works as well as possible while also causing as few side effects as possible [1-3]. Long-term controlled release techniques give you a lot of options for getting a medicine to the right place. One way to do this is to use microspheres to carry medicines. A microsphere is a type of multiparticulate drug delivery system that can be used to exactly deliver a drug to a certain location, make it more bioavailable or stable, and allow for long-term drug administration [2-4].

Small, solid particles called microspheres have a width of 1 to 1000 μm and are shaped like spheres. Medications that come in either a fluid or a microcrystalline form are among these particles [3-5]. Emulsion-solvent evaporation is a microencapsulation method used to cover a drug that doesn't dissolve in water with a polymer that doesn't dissolve in water. This makes the drug last longer. By evaporating an organic solvent, the spread of oil drops that contain both polymer and medicine is changed into microspheres. There are different grades of acrylate and methacrylate polymers in the Eudragit polymer line, and they all have different physical and chemical qualities [5-7].

Eudragit types don't dissolve in water or digestive juices. Despite this, they can get bigger and make holes in them, which lets drugs pass through them. For some grades, they dissolve quickly at certain pH levels. Acetazolamide is given by mouth in large amounts to people with glaucoma in order to lower eye pressure [6-8]. Systemic side effects of this medicine include central nervous system depression, renal failure, diuresis, vomiting, anorexia, and metabolic acidosis. This means that new drug delivery methods need to be developed right away. Different experimental methods can help you figure out how important different factors are and come up with a formula that needs fewer experiments. The goal of this study was to use a Box-Behnken design to make acetazolamide-containing microspheres work better [7-9].

In this study, the Box-Behnken design and the polymer and emulsion solvent evaporation method were used to create a precise drug delivery system for giving acetazolamide by mouth. Finding the best formulation that would allow controlled release of the drug and increase its ability to be trapped was the goal.

2. Material and Methods

2.1. Materials

The Pharmaceutical Company graciously offered a complimentary sample of acetazolamide, while Sigma Aldrich and Loba Chemical Mumbai kindly supplied eudragit. We procured sodium lauryl sulphate, ethanol, and dichloromethane from Loba Chemical, a supplier based

in Mumbai, India. All the chemicals utilized in this experiment were of utmost purity and met the standards of analytical-grade quality.

2.2. Methods

2.2.1. Acetazolamide-loaded Microsphere Preparation

To make Eudragit microspheres with acetazolamide inside, the usual method of emulsion fluid evaporation was used. The right amount of Eudragit was broken down with 10 milliliters of ethanol and dichloromethane. The desired amount of dissolved ACZ powder was present in the polymeric solution. The dispersion that was made was carefully added to a 100 ml water solution that had sodium lauryl sulfate in it. To make an emulsion, use a three-blade motorized stirrer to stir the mixture very quickly while it is at room temperature. The medicine and polymer spread out right away and turned into very small drops. The small drops turned into hard microspheres as the solvent evaporated [8-10].

2.2.2. Designing Box-Behnken experiments

Often, pharmaceutical formulations are made in a time-consuming process that involves changing one variable at a time and not taking into account how the separate parts affect each other. As a result, factorial design is an important way to find out how medication formulations work together. Using the design of tests method, the emulsion solvent evaporation method for making microspheres was made more efficient. DOE makes it possible to quickly and thoroughly look at the cause-and-effect links between different process factors and the end result. A series of studies were done to learn as much as possible about the parts and how they work together while reducing the number of tests that had to be done. A quadratic model that is interactive was used to evaluate both response factors. Expression in math notation: This is the polynomial equation that this experimental setup in Microsoft Excel produced [9-11].

$$\mathbf{Y_i = b_0 + b1X1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^{\,2} + b_{22}X_2^{\,2} + b_{33}X_3}$$

The dependent variable is labeled as Yi, and the intercept. The pilot tests were used to choose the independent factors, which are X1, X2, and X3. The experiment's data were used to find the terms that had a statistically significant effect on the regression coefficients. A 3-factor 3-level factorial Box-Behnken experimental design method was used to look at the factors.

2.3. Evaluation of prepared formulation

2.3.1. Morphology of Microspheres

A light microscope with different magnification settings was used to look at the shape of the made microspheres. A single drop of the freshly made microsphere solution was put on a slide, and then a cover glass was put on top of the slide. Researchers looked at the microspheres' shape, how evenly their sizes were spread out, and how likely they were to combine or stick together [10-12].

2.3.2. Particle size analysis

To keep the microspheres from sticking together, they were spread out in pure water that had

2% weight/volume tween 80 added to it. After that, sonication was done on the suspension in a water bath, and laser diffraction was used to find the average width in micrometers [11-13].

2.3.3. % yield

The microsphere yield was found by splitting the weight of the microspheres that were made by the amount of polymer and medicine that was used at the start. The following equation was used to turn the numbers into a percentage [12-14].

$$\% \textbf{ Yield} = \frac{\textbf{Actual weight}}{\textbf{Total weight of drug and polymer}} \textbf{ X 100}$$

2.3.4. % Entrapment efficiency

A changed form of this method was used to figure out the entrapment efficiency percentage of the microspheres that were made. A fine powder made from about 25 mg of the microspheres that were made was used. The powder was then mixed with 100 milliliters of PBS until it was totally gone. After that, it was shaken very hard in an automatic shaker for six hours. Following that, the solution was kept for twenty-four hours. Spectrophotometry at a wavelength of 265 nm was used to find the concentration of the drug after 5 ml of the fluid was run through a filter. To find out the exact drug loading and entrapment efficiency, the following formulae were used [14-17].

$$\% \textbf{ EE} = \frac{\textbf{Calculated Drug Conc.}}{\textbf{Theorotical Drug Conc.}} \textbf{ X 100}$$

2.3.5. Acetazolamide-loaded microsphere dissolution in-vitro

The USP dissolving Apparatus II was used to dissolve the microspheres in vitro. It rotated at a speed of 100 revolutions per minute (rpm). Because Eudragit can pass through medicines regardless of pH, a liquid that dissolves at 7.4 was chosen for tests. The medicine ACZ was taken out of the microspheres using microspheres that had 125 mg of the drug in them. 500 ml of PBS was used as a dissolving medium and kept at the same level. To keep the volume the same, 3 ml samples of the dissolving medium were taken at different times and replaced with the same amount of newly hot material. We used spectrophotometry to find the drug concentration and amount of drug released over time. The wavelength of maximum absorbance was lambda max 265 nm [18-20].

3. Results and Discussion

3.1. Acetazolamide-loaded Microsphere Preparation

The desired amount of dissolved ACZ powder was present in the polymeric solution. The dispersion that was made was carefully added to a 100 ml water solution that had sodium lauryl sulfate in it. To make an emulsion, use a three-blade motorized stirrer to stir the mixture very quickly while it is at room temperature. The medicine and polymer spread out right away and turned into tiny drops. The small drops turned into hard microspheres as the solvent evaporated [21-24].

3.2. Box-Behnken Design

The goal of this project was to find the best way to make acetazolamide microspheres. The goal of the adjustment was to get the highest percentage of drug entrapment, the best particle size, and controlled drug release. In this case, the Box-Behnken experimental form was used. The Box-Behnken design was chosen for this task because, in situations with three or four elements, it can use fewer treatment choices than other designs. In Table 1, you can see the converted numbers for each formulation along with the answers that go with them.

Table 1: Acetazolamide microsphere box-Behnken experiment

Batch	Independent Variables			Dependent Variables		
	X_1 (Drug: polymer ratio)	**X_2 (Surf. conc.)**	**X_3 (Stirring)**	**Y_1 (EE %)**	**Y_2 (% release after8 hrs.)**	**Y_3 (MD)**
1	1:0	0.5	800	56.25	40.12	420.66
2	1:5	1.0	800	50.80	45.33	317.41
3	1:0	0.5	800	52.25	50.64	295.02
4	1:0	2.0	800	35.75	60.34	252.20
5	1:5	1.0	1000	75.34	90.65	1054.82
6	1.6	1.0	1400	55.96	58.44	253.36
7	1:2	1.0	800	54.13	50.78	774.82
8	1:2	1.0	1400	42.96	69.74	180.69
9	1:4	0.5	1000	71.89	40.33	1000.70
10	1:4	1.0	1400	51.22	52.01	255.60
11	1:4	2.0	1000	53.33	53.25	620.40
12	1:4	2.0	1400	48.66	58.97	171.60

3.3. Evaluation of prepared formulation

3.3.1. Acetazolamide Microsphere Morphology

Figure 1 shows the particles that were made when the emulsion fluid evaporated. They were mostly spherical and had a smooth surface.

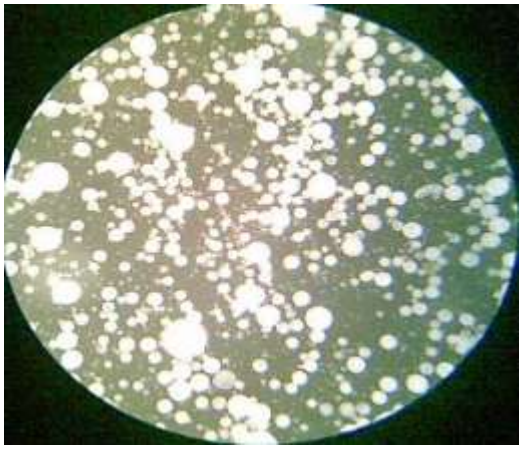

Figure 1: Photomicrographs of microspheres

Production yield

The quantity of the ACZ microspheres that were made ranged from 75.29%±1.27 to 93.79±1.35. Formulation 5, which had less detergent and slower stirring, made the most microspheres. Also, formulation 8 made the fewest microspheres, even when the stirring rate and surfactant content were at their highest. This was because the smallest and lightest particles were removed during the filtration and washing steps [25-29].

Particle size distribution:

A laser diffractometer was used to find the average width of the microspheres. The diameter was between 180.65±6.25 m and 940.80±12.46 m on average. The rate of stirring is the main thing that sets the best settings for managing the biggest drug/matrix droplets in the continuous phase during the microsphere formation process. The experiment demonstrated that increasing the stirring rate led to a noticeable decrease in the size of the microspheres when higher shear forces were used to make the emulsion droplets smaller. The experiment used high stirring speeds to make Eudragit RS100 microspheres with small particles, while lower stirring speeds made particles with larger sizes (figure 2) [30-34].

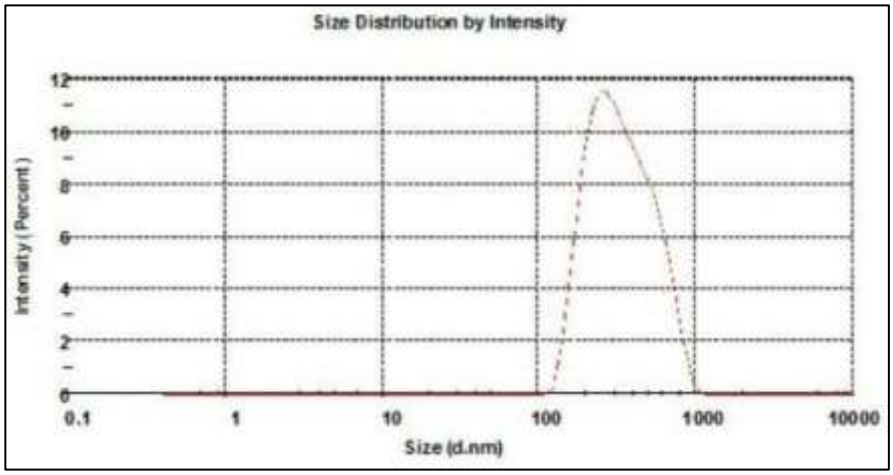

Figure 2: Particle Size of the prepared formulation

Entrapment efficiency (%):

There was a wide range in the entrapment efficiency percentage numbers for the different microsphere formulations, from 35.91±1.14% to 75.85±1.26%. Formulation 5 had the best acetazolamide trapping rates in microspheres, at 76.19%. When the drug concentration and stirring rate changed, these numbers changed by a large amount. Formulation 5 is the most effective at entrapment because it has the highest quantity of ACZ per unit polymer. A higher EE% was seen in microspheres that were made with less detergent and less stirring. When you mix ingredients faster and use more surfactant, however, the drug encapsulation efficiency (EE %) is lower in the mixtures you make [35-39].

Acetazolamide-loaded microsphere dissolution in-vitro:

After eight hours, the amount of drug that was released varied from 40.28±2.35% to 90.06±1.24%. Figure 3 shows how much ACZ is released from different formulas as a percentage. Over the course of six hours, the drugs slowly came out of each of the mixtures. As the rate of agitation went up, so did the rate at which the medicine was released. Microspheres made with a faster stirring rate released acetazolamide more quickly than microspheres made with a slower stirring rate. The reason for this is that microspheres made with a faster stirring rate had smaller particles and more surface area that was exposed to the medium where the medicine is released. Because of this, the drug came out faster. At different stirring speeds, there were statistically significant changes in the amount of drug released [40-47].

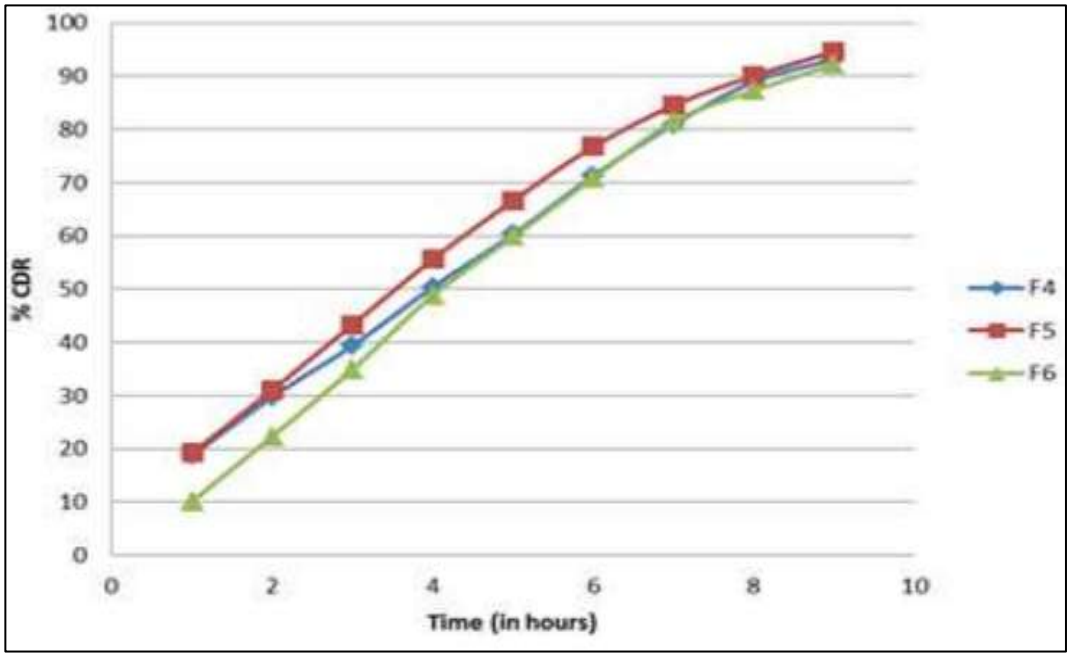

Figure 3: In-vitro release of microspheres formulation

CONCLUSION:

An emulsion solvent evaporation method can be used to safely make Eudragit RS microspheres that contain acetazolamide. As you can see, the microspheres' surfaces are smooth and perfectly round. Eudragit microspheres had a lower release rate and a high percentage of entrapment effectiveness in the microspheres that were made. We used regression analysis, contour plots, and the Box-Behnken experimental method to find the best values for the formulation variables used to make acetazolamide microspheres. Finding the best mixture based on the expected amounts of variables gave us the entrapment effectiveness%, released percent, and mean particle size numbers for Y1, Y2, and Y3 as 90.14%, 40.20%, and 1050.20 µm, respectively.

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Conflict of Interest:
None

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