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Formulation & Evaluation of Meletin Microspheres

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Kishor Dholwani[1], Bharat Vashistha[2], Zankhana Pramodchandra Sheth[3] Rajesh Dodiya[4] , Vaishnav Sonal Hiralal[5], Divya Niranjan[6] Saurabh Sharma[7]

1Principal, Sardar Patel College of Pharmacy, Anand, Gujarat
2Associate Professor, Sardar Patel College of Pharmacy, Anand, Gujarat
3Associate Professor, Sardar Patel College of Pharmacy, Anand, Gujarat
4Associate Professor, Deptt. of Pharmaceutics, School of Pharmacy, Parul University Vadodara, Gujarat
5Assistant Professor, Sardar Patel College of Pharmacy, Anand, Gujarat
6Research Scholar, School of Pharmacy, ITM University, Gwalior M.P.
7Assistant Professor, Sardar Patel College of Pharmacy, Anand, Gujarat

Keyword:

Novel API delivery system, Micropaticles, Carbopol, Invitro release

Abstract

An organoleptic property of the API is identified in this study, and it appears to be a yellow, tasteless, and odorless powder. It was found to be freely soluble in DMSO and other solvents, and its melting point was determined to be 333.20 C. Meletine in hydroalcholic solution's standard curve was drawn at 370 nm using various aliquots; the regression coefficient was r2=0.981. The API and polymer mixture was then subjected to an interaction study, and it was determined to be compatible, indicating that there was no interaction. Following first-order kinetics, the release study of various batches was conducted at various time intervals. The percentage of cumm. release was calculated by release of API at two hundred ten minutes to be hundred percent API release. The chart is plotted between percent total delivery verses time and a semi log diagram is plotted between log percentage combined discharge verses time. Finally, the mucoadhesive strength of the various batches was measured, and batch F6 had the highest mucoadhesion. As a result, Meletine can be made into a mucoadhesive microsphere that can be used as an antidote to Toxic gases for sustained release, defense of the mucosa lining, and in the event of accident.

1. Introduction

Microspheres, that are either entirely made of a mucoadhesive polymer or have an outer coating of it and have a diameter between one and one thousand millimeters.[1] Targeted and controlled API delivery can be accomplished with microspheres in general; However, there are additional advantages to coupling mucoadhesive properties to microspheres[2], such as a high surface/volume ratio, a much closer contact with the mucos lining, precise API target to the assimilation site.[4]

The ability to tailor mucoadhesive microspheres to adhere to any mucosal tissue, including those in the eye, nasal cavity[5], urinary tract, and gastrointestinal tract, makes it possible to control the localized as well as the systemic release of APIs[6].

Application of Micro Drug Delivery System

- Long-term release of proteins, hormones, and peptides[7].
- Gene therapy using DNA plasmids and insulin delivery[8].
- Vaccine delivery for the treatment of diseases such as hepatitis, influenza, pertussis, ricin toxoid, diphtheria, and birth control.[9]
- Doxorubicin targeting of tumors as well as treatments for leishmaniasis.[10]
- Active targeting of tumor cells and antigens by intra-arterial or intravenous application.[11]
- Stem cell and bone marrow purging can be accomplished with the help of magnetic microspheres.[12]
- Used in affinity chromatography to extract toxins, separate cells, and isolate antibodies.[13]

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2. Materials & Methods

Description of the Formulation Process:

A double-emulsion technique known as O/W/O was used to make bioadhesive microspheres.[15]. Before use, the polymer solution in water was prepared and kept in preserved containers at 48 degree celcius for twenty four hours. Carbopol (0.50 g) was rapidly vortex-mixed into 50.0 g of deionized water; Aqueous sodium hydroxide in dilute form was used to bring the pH down to 7.[16]. Meletine was disintegrated in dichloromethane. The API (Meletin) dissolved in dichloromethane was emulsified into 50.0 g of solution pf polymer in water for the first emulsion.[17] The emulsification process was aided by the addition of 0.15 milliliters of Tween 80.[18] The emulsions were quickly mixed for 15 minutes in a Silverson homogenizer.[19] The two fifty ml of light liquid paraffin contain one percent Span 80[20], the first blend (25 ml) was added drop by drop. At 800 rpm[21], the resulting double emulsion was stirred. To encourage the water to evaporate, the samples were heated to 60-70 °C.[22] After centrifugation separated the solid polymer microspheres, they were cleaned in hexane, washed, and dehydrated for 24 hours in a vacuity oven at forty degrees Celsius.[23] Six batches of microspheres were made for each polymer to API ratio to check the reproducibility of API loading using this method.[24]

Testing Parameters-

1. **Bulk Density[25]:** The quantity of material's mass divided by the volume it occupies is known as its bulk density. The complete volume incorporates molecule volume, between molecule void volume and inside pore volume..
2. **Tapped Density[26]:** After the powder sample has been mechanically tapped into a container, the result is the tapped density, which is an increase in bulk density.

3. **Angle of Repose[27]:** The point of rest or, all the more definitively, the basic point of rest, of a grainy material is the steepest point of drop or plunge of the slant comparative with the level plane when material on the slant face is nearly sliding. The range of this angle is 0° to 90°S

4. **Carr's Index[28]:**

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density} - \text{Poured density}}{\text{Tapped density}} \times 100$$

5. **Hausner Ratio[29]:**

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

6. **API Entrapment Efficiency[30]:** A glass mortar and pestle was used to crush 50 mg of microspheres. The powdered microspheres were then suspended in 10 mL of phosphate buffer with a pH of 7.4. The solution was filtered after 24 hours, and the API content in the filtrate was checked.

7. **In Vitro Release Studies[31]:** A beaker containing precisely weighed 50 mg of loaded microspheres was used for In Vitro release. The microspheres were immersed in dissolution fluid, kept at 37 20 C, and continuously stirred at 100 rpm. In order to maintain a constant volume of the receptor compartment, 5 ml samples were withdrawn through a hypodermic syringe equipped with a 0.4 m Millipore filter at predetermined intervals and replaced with the same volume of freshly prepared buffer solution. Spectrophotometry was used to examine the samples.

8. **Mucoadhesion Study[32]:** Chicken small intestine was used for the in vitro mucoadhesive test. The tissue of the small intestine was removed and flushed with saline. Using a glass rod, five centimeter-long segments of the jejunum were everted. The segment was joined at both ends with a ligament. From a height of 2 centimeters, 100 microspheres were distributed uniformly across the everted sac. Then the sac was suspended in a 10ml cylinder containing 8 ml of saline by the wire, to totally drench in the saline. The sac were brooded at 370C and fomented on a level plane. After immersion for 0.5, 1, 1.5, 2, 2.5, and 3 hours, the sac was taken out of the medium, immediately repositioned in a similar tube containing 8 ml of fresh saline, and the number of unbound microspheres was counted. The equation that followed showed the adhering percent.

$$\text{Mucoadhesion} = \frac{\text{No.of Microspheres adhered}}{\text{No. of Microspheres applied}} \times 100$$

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3. Result & Discussion:

1. Organoleptic characteristic:

Table No. 1

Property	Meletine	Carbopol
Colour	Yellow	Whitish
Nature	Crystal	Fluffish
Odour	Without odour	No odour
Taste	No Taste	No Taste

2. MELTING POINT:

Table No. 2

Compound	Melting point ^{0}C (Literature)	Melting point ^{0}C (Practical)
Meletine	315.5	333.4
Carbopol	105.7	109.8

3. Testing of Solubilization:

Table No. 3 Testing of Solubilization

S.No.	Solvent	Meletine
1.	Ethanol	++++
2.	Methanol	++++
3.	Water	+++
4.	n-hexane	+++
5.	DMSO	++++
6.	PBS (7.2)	+++

+++ Freely soluble ++ slightly soluble + partly soluble

4. Ultraviolet Spectrophotometer Studies (Calibration Curve)-

Table No. 4

S.No.	Conc (microgram per ml)	Absorbance (370 nm)
1.	2.00	0.049
2.	4.00	0.13
3.	6.00	0.234
4.	8.00	0.281
5.	10.00	0.37

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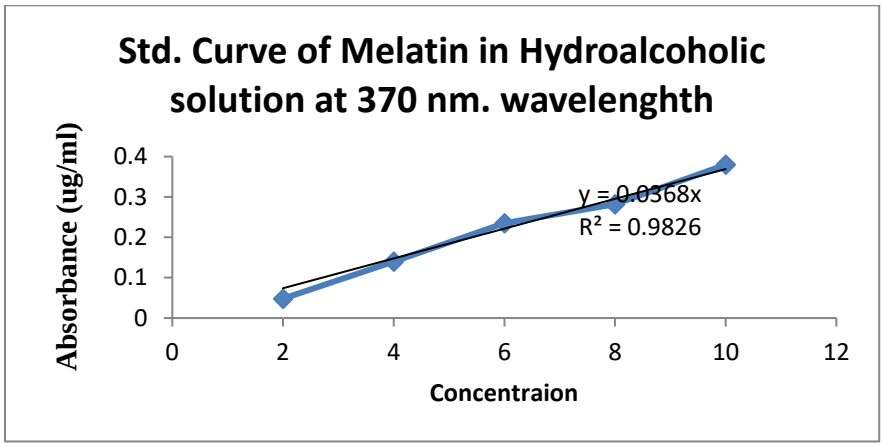
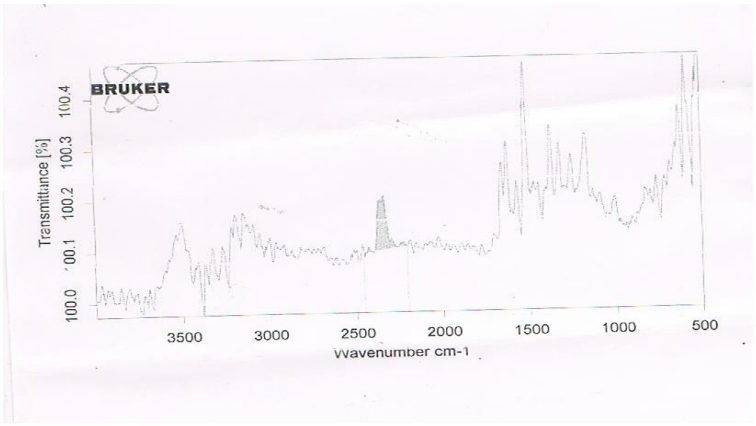
5. Standard Curve of API -

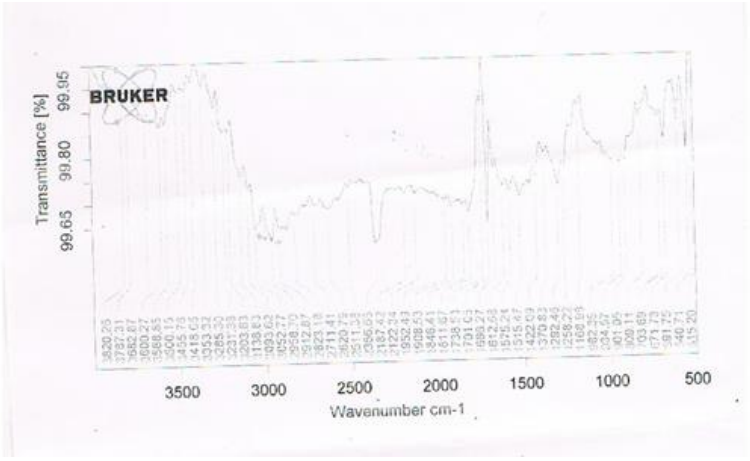


6. Interaction Testing:

Sample 1: FT-IR Results of Sample compound (API)



Sample 2: FT-IR Results of Carbopolr Sample



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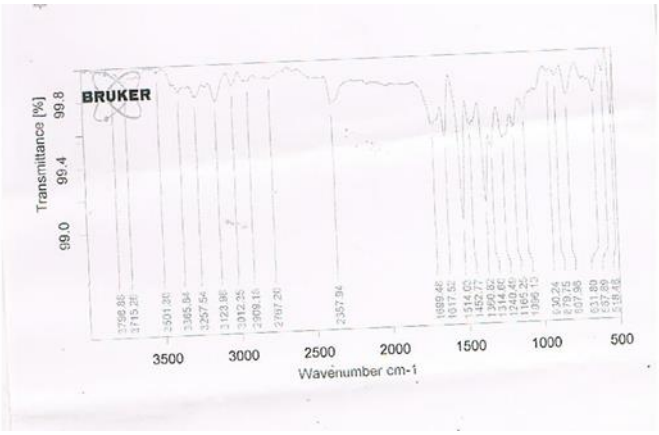
Sample 3: FT- IR Results of API & Carbopol



7. Formulation Table:

Table No.05

S.No.	Formulation code	Drug (in g.)	Polymer (In g.)	Dichloro Methane (ml)	Span-80(%)	Liquid paraffin light (In.ml)	n-Hexane (In ml)
1.	F1	0.5000	0.5000	10.00	1.00	250.00	50.00
2.	F2	0.5000	1.000	10.00	1.00	250.00	50.00
3.	F3	0.5000	1.500	10.00	1.00	250.00	50.00
4.	F4	0.5000	2.000	10.00	1.00	250.00	50.00
5.	F5	0.5000	2.500	10.00	1.00	250.00	50.00
6.	F6	0.5000	3.000	10.00	1.00	250.00	50.00

8. Testing Parameters I:-

Table No. 6

Batch	Bulk density (gm/ml)	Tapped density (gm/ml)	Angle of Repose	Carr's Index	Hausner's Ratio
F1	0.53±.010	0.97±.010	31.2±.02	43.89±.002	1.71±.01
F2	0.85±.010	1.07±.060	28.9±.05	22.21±.1	1.20±.02
F3	0.92±.0.20	1.02±.030	31.8±.11	8.71±.05	1.08±.12
F4	1.2±0.30	0.84±.020	24.3±.02	38.53±.02	0.72±.002
F5	0.7±0.10	1.07±.130	32.1±.001	22±.003	1.23±.1
F6	0.91±0.22	0.81±.0160	21.3±.03	8.59±.01	0.94±.03

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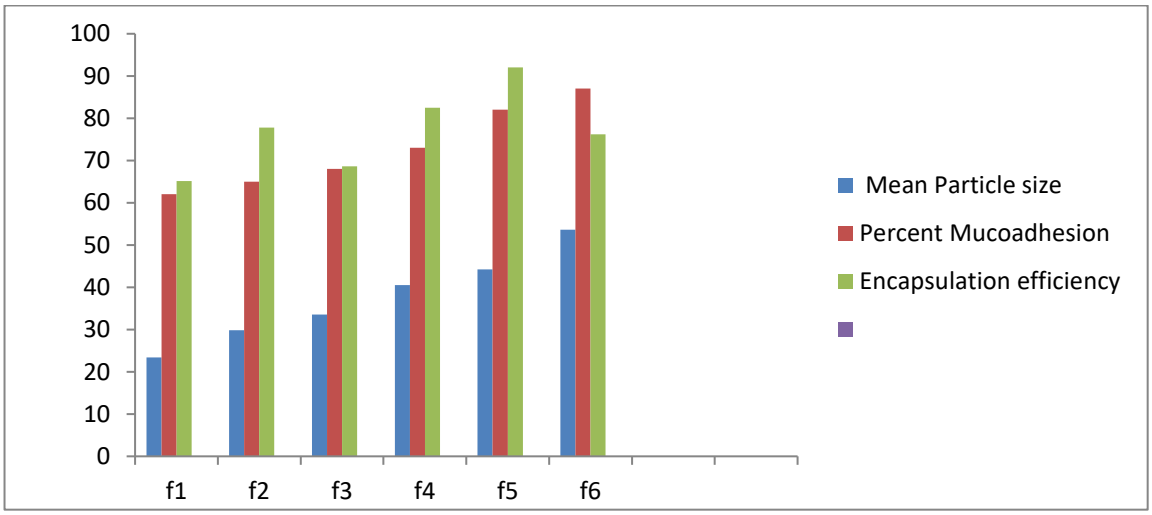
9. **Testing Parameters II-**

Table No.07

Batch	Encapsulation Efficiency* (%)	Mean Particle size* (µm)	Percent Mucoadhesion
F1	64.12	22.40±1.10	61±1.81
F2	76.79	28.82±2.54	64±1.90
F3	66.64	32.53±1.76	67±1.51
F4	81.5	41.55±1.87	74±1.31
F5	91.04	42.23±0.85	83±1.20
F6	77.19	51.60± 1.92	88±1.30

Entrapment efficiency particle size and percentage Mucoadhesion of Preparation



10. In-vitro API release study of preparation:

Table No. 8

Time	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
30.0	37.57	39.42	45.51	41.11	42.10	46.53
60.0	47.23	51.23	60.38	54.25	58.04	64.22
90.0	55.52	61.13	73.65	67.72	70.65	76.47
120.0	67.65	71.09	83.48	77.17	79.53	87.25
150.0	72.23	76.66	89.88	84.53	88.23	94.57
180.0	80.61	89.43	95.91	92.49	100.00	100.00

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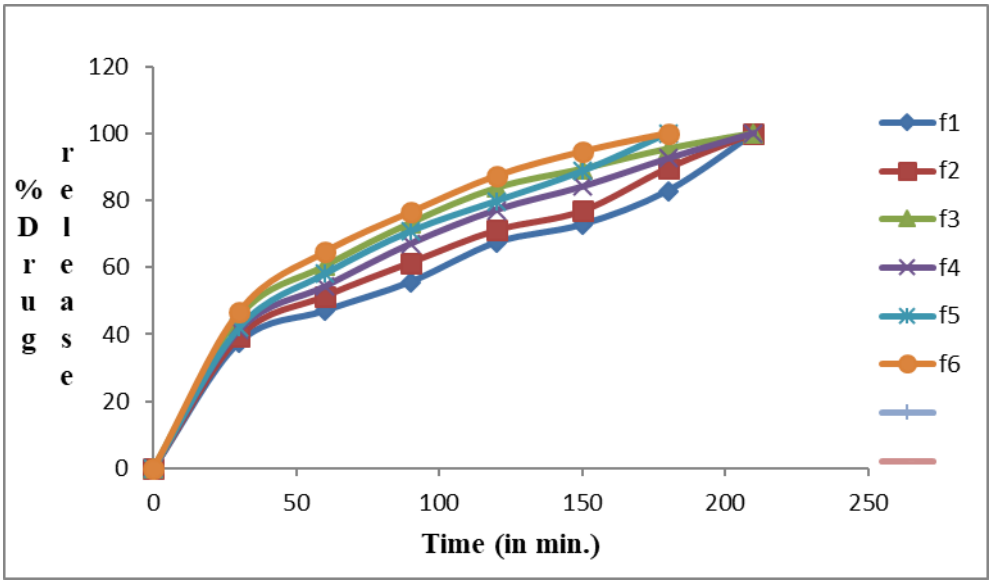

Invitro API release study of Preparation

4. Discussion:

An organoleptic property of the API is identified in this study, and it appears to be a tasteless and odorless yellow crystal powder. It was found to be freely soluble in DMSO and other solvents, and its melting point was determined to be 333.40 C.

Meletine in hydroalcholic solution's standard curve was drawn at 370 nm using various aliquots, and the regression coefficient was set at r2=0.9826. The API and polymer mixture was then subjected to an interaction study, and it was determined to be compatible, indicating that there was no interaction.

Total Six No. of batches of formulations were created using the double emulsion solvent evaporation technique, and further examined its physicochemical properties. Six batches' bulk and tapped densities were examined, and it was discovered that the formulated batch F4 has a superior bulk density of 1.20±30 and the tapped density of 1.070±060.

After determining the flow properties of various formulated batches, the particle sizes of those batches were determined by sieving, in which the powder was agitated by placing it on a mechanical shaker and passed through a variety of sieves. B. No. F6 had brilliant flow property with an angle of repose of 21.3 Degree and B.No. F3 had admirable Carr's index with a value of 8.71±5.

Batch F1's 22.40±1.10 particle size is regarded as the best of all the batches. Then, the encapsulation effectiveness of various API batches were evaluated: polymer ratio, and a maximum encapsulation efficiency of 91.04 percent was found for batch F5.

Following first-order kinetics, the study of in-vitro release, various batches were conducted at various time duration. The percentage of collective release was calculated by considering API release at two hundred ten minutes to be hundred percent API release. The chart is plotted between % total delivery verses time and a semi log diagram is plotted between log % combined discharge verses time. Finally, the Adhesion strength of the various batches was measured, and batch F6 had highest mucoadhesion.

As a result, Meletine can be made into a mucoadhesive microsphere that can be used as an antidote to toxic agents, defense of the mucos membrane.

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