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FORMULATION & EVALUATION OF SUSTAINED RELEASE MUCOADHESIVE MICROSPHERES

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ABSTRACT
Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity, urinary and gastrointestinal tract, thus offering the possibilities of localized as well as systemic controlled release of drugs.microspheres used for Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, birth control and also used for Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intra-arterial/intravenous application. In the present work, an organoleptic characteristic of drug is determined and it seems to be yellow crystalline powder, tasteless and odourless. Its melting point is 332^0C and solubility was determined in different solvents and found freely soluble in DMSO and other solvents. The standard curve of Meletine in hydroalcholic solution was drawn at 370nm by taking different aliquots, a line is obtained with the regression value r^2=0.981. Then the interaction study between the drug and polymer done and considered as compatible i.e. there is no interaction between drug and polymer mixture. The In-vitro release study of different batches were done at different time intervals in which % cumulative release is determined by considering drug release at 210 min as 100% drug release by following first order kinetics. The graph is plotted between % cumulative release v/s time and a semi log graph is plotted between log % cumulative release v/s time. Finally Mucoadhesive strength of different batches were determined and found that the batch F6 showed maximum mucoadhesion. Hence Meletine can be formulated as Mucoadhesive microsphere and in this Meletine can be utilized for its protective efficacy as antidote to mustard agents particularly for sustained release, protection of mucosal membrane and in case of accidental ingestion.

KEYWORD: Microspheres, Mcoadhesive, Sustain Released, Meletin.

INTRODUCTION

Mucoadhesive Microspheres

Mucoadhesive microspheres which are of 1—1000m m in diameter and consisting either entirely of a mucoadhesive polymer or having an outer coating of it[1], respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of mucoadhesive properties to microspheres has additional advantages[2], *e.g.* efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio[3], a much more intimate contact with the mucus layer, specific targeting of drug to the absorption site achieved by anchoring plant lectins, bacterial adhesions and antibodies, *etc.* on the surface of the microspheres.[4]

Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity[5], urinary and gastrointestinal tract, thus offering the possibilities of localized as well as systemic controlled release of drugs.[6]

Medical Application

- Release of proteins, hormones and peptides over extended period of time[7]
- Gene therapy with DNA plasmids and also delivery of insulin[8]
- Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, birth control.[9]
- Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intra-arterial/intravenous application.[10]
- Tumour targeting with doxorubicin and also treatments of leishmaniasis.[11]
- Magnetic microspheres can be used for stem cell extraction and bone marrow purging.[12]

- Used in isolation of antibodies, cell separation and toxin extraction by affinity chromatography.[13]

MATERIALS AND METHODS

Method of Formulation

For the formulation of Carbopol based Meletine microsphere we use the Double Solvent Evaporation Method.[14]

Process

Bioadhesive microspheres were prepared by an oil-in water-in-oil (O/W/O) double-emulsion method.[15] Aqueous polymer solution was prepared and subsequently stored in sealed containers at 48 ºC for 24 h prior to use. Carbophil (0.50 g) was dispersed in 50.0 g of deionized water and mixed by rapid vortexing; the pH was adjusted to 7 using dilute aqueous sodium hydroxide.[16] Meletine was dissolved in dichloromethane. For the first emulsion, Meletine dissolved in dichloromethane was emulsified into 50.0 g of aqueous polymer solution.[17] The addition of 0.15 ml of Tween 80 aided the emulsification process.[18] A Silverson homogenizer was used for rapid mixing of the emulsions for 15 min.[19] The first emulsion (25 ml) was added drop wise to 250 ml light liquid paraffin containing 1% Span 80.[20] The resultant double emulsion was stirred at 800 rpm.[21] The samples were heated to 60-70 ºC to promote evaporation of water.[22] Solid polymer microspheres were subsequently separated from the oil by centrifugation, washed in hexane and dried in a vacuum oven at 40 ºC for 24 h.[23] For each polymer to drug ratio, six batches of microspheres were prepared in order to assess the reproducibility of drug loading by this method.[24]

Evaluation Parameters

1. **Bulk Density[25]:** Bulk density is defined as the mass of many particles of the material divided by the total volume they occupy. The total volume includes particle volume, inter-particle void volume and internal pore volume.

2. **Tapped Density[26]:** The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

3. **Angle of Repose[27]:** The angle of repose or, more precisely, the critical angle of repose, of a granular material is the steepest angle of descent or dip of the slope relative to the horizontal plane when material on the slope face is on the verge of sliding. This angle is in the range 0°–90°.

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]**

It indicates the flow property of the powder and calculated by the following equation.

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

6. **Drug Entrapment Efficiency[30]:** Microspheres (50 mg) were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 10mL of phosphate buffer (pH 7.4). After 24 hours, the solution was filtered and the filtrate was analyzed for the drug content.

7. **In Vitro Release Studies[31]:** To carry out In Vitro release, accurately weighed 50 mg of loaded microspheres were dispersed in dissolution fluid in a beaker and maintained at 37± 2^0C under continuous stirring at 100 rpm. At selected time intervals 5 ml samples were withdrawn through a hypodermic syringe fitted with a 0.4µm Millipore filter and replaced with the same volume of pre-warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analysed Spectrophotometrically.

8. **Mucoadhesion Study[32]:** The in vitro mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segment of jejunum were everted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the everted sac from the position of 2 cm above. Then the sac was suspended in a 10ml tube containing 8 ml of saline by the wire, to immerse in the saline completely. The sac were incubated at 37^{0}C and agitated horizontally. The sac were taken out of the medium after immersion for 0.5, 1, 1.5, 2, 2.5 and 3 hrs, immediately repositioned as before in a similar tube containing 8ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation.

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

RESULT And DISCUSSION

1. Organoleptic characteristic

Table No. 1.

Property	Meletine	Carbopol
Colour	Yellowish	White
Nature	Crystalline	Fluffy
Odour	Odourless	Odourless
Taste	Tasteless	Tasteless

2. MELTING POINT

Table No. 2,

Compound	Melting point ^{0}C (Literature)	Melting point ^{0}C (Practical)
Meletine	316	332
Carbopol	106	110

3. SOLUBILITY STUDY

Table No. 3 Solubility Studies of Meletine.

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

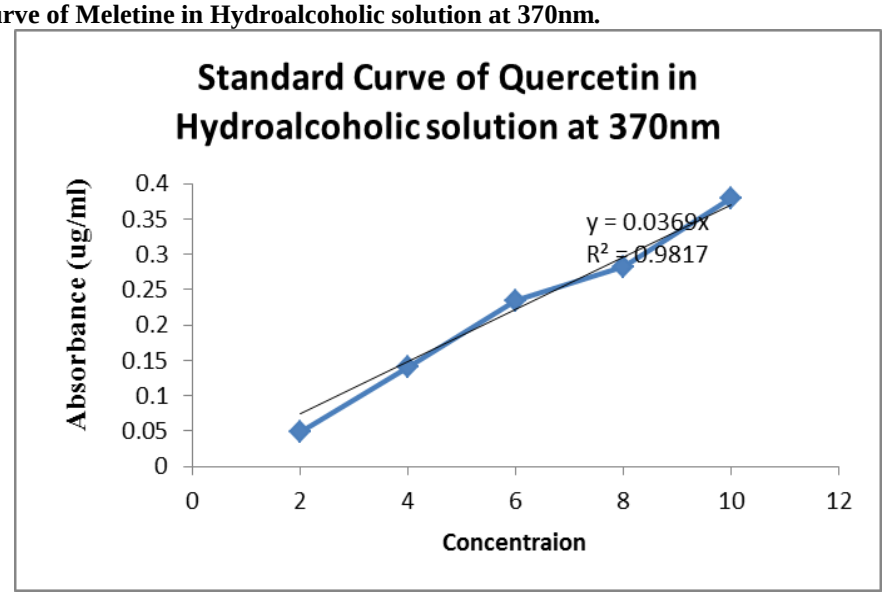
+++ Freely soluble ++ slightly soluble + partly soluble

4. CALIBRATION CURVE OF MELETINE IN HYDROALCHOLIC SOLUTION

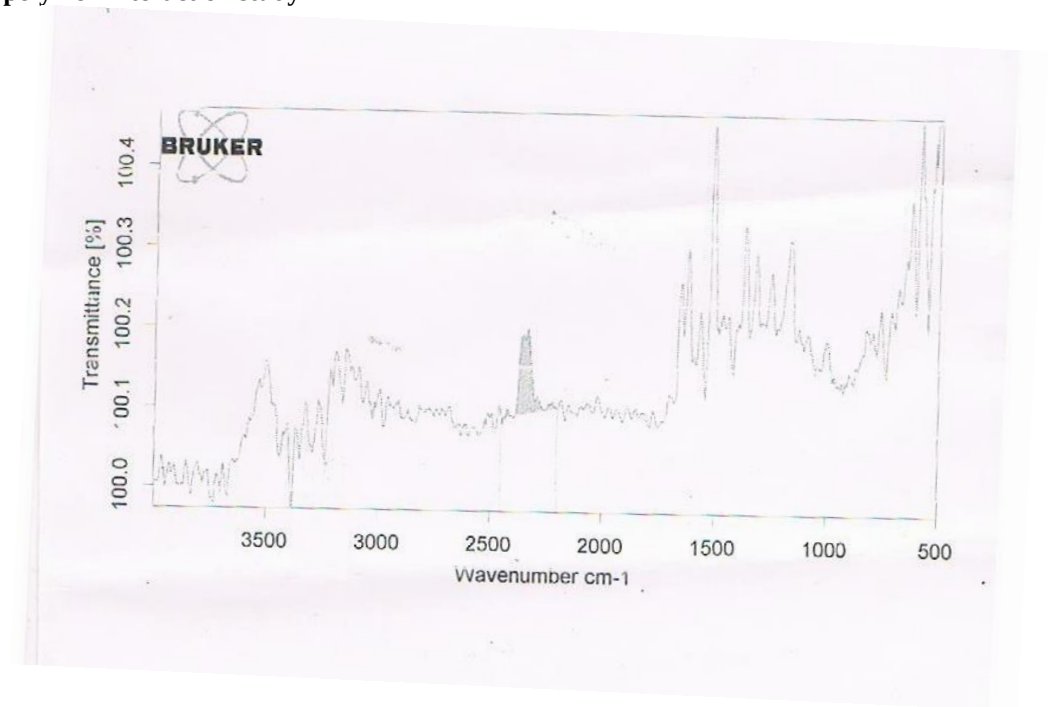
Table No. 4.

S.No.	Concentration (mcg/ml)	Absorbance (370 nm)
1.	2	0.048
2.	4	0.14
3.	6	0.235
4.	8	0.282
5.	10	0.38

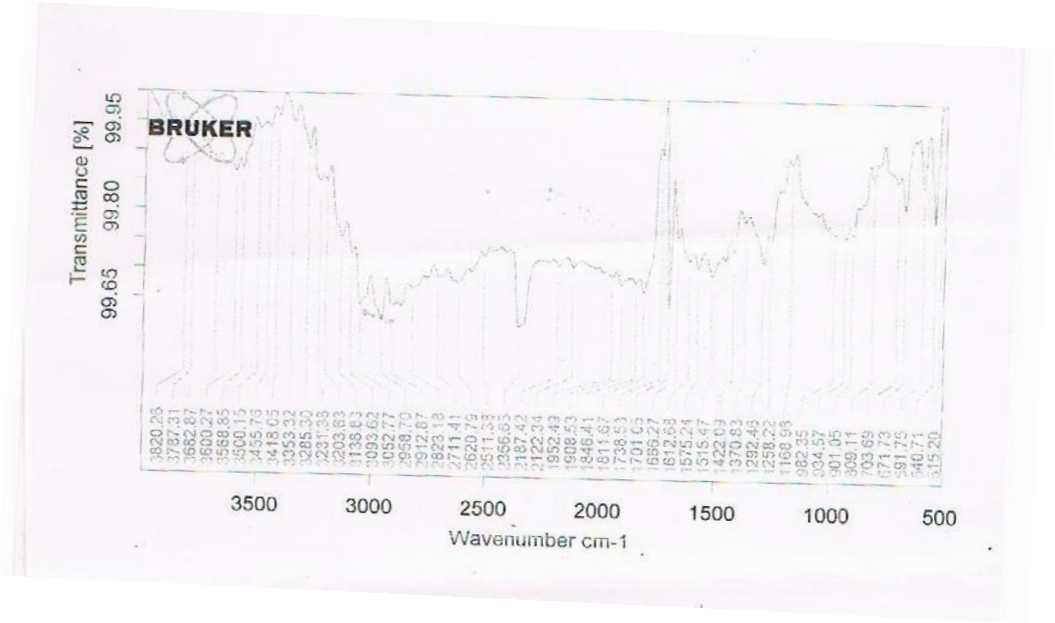
5. Standard Curve of Meletine in Hydroalcoholic solution at 370nm.



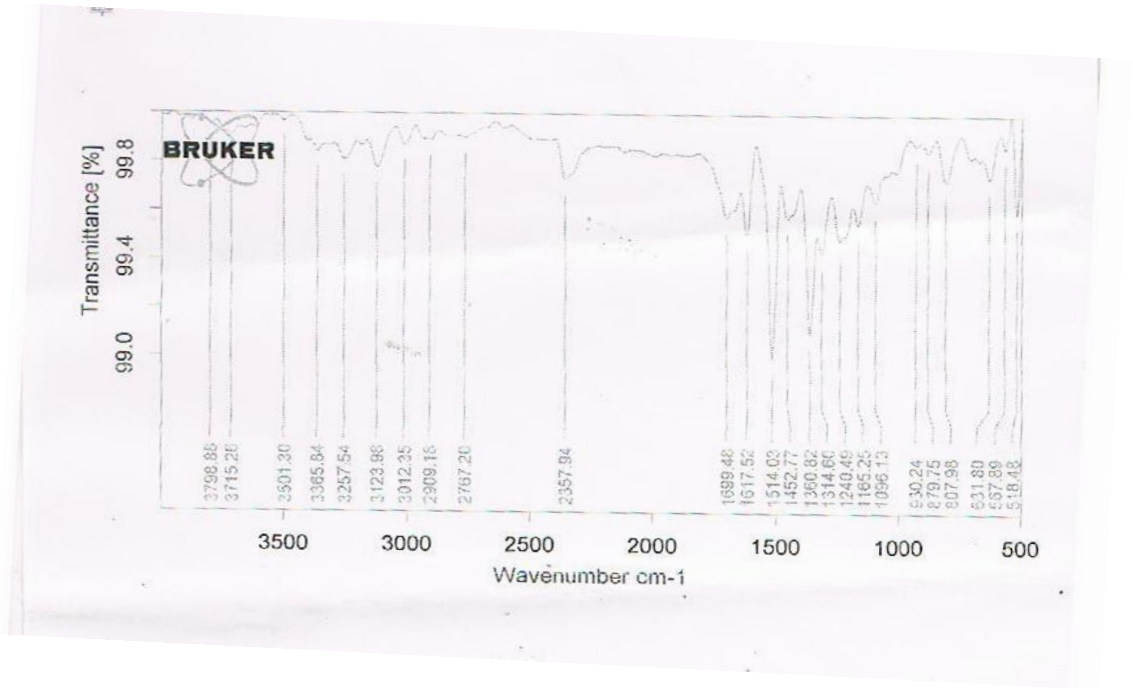
6. Drug polymer Interaction study



Sample 1: IR graph of Drug sample.



Sample 2: IR graph of Polymer sample.



Sample 3: IR graph of Drug Polymer Mixture.

7. FORMULATION

Table No. 5.

S.No.	Formulation code	Drug (g)	Polymer (g)	Dichloro Methane (ml)	Span-80(%)	Liquid paraffin light (ml)	n-Hexane (ml)
1.	F1	0.500	0.500	10	1.0	250	50
2.	F2	0.500	1.00	10	1.0	250	50
3.	F3	0.500	1.50	10	1.0	250	50
4.	F4	0.500	2.00	10	1.0	250	50
5.	F5	0.500	2.50	10	1.0	250	50
6.	F6	0.500	3.00	10	1.0	250	50

8. EVALUATION PARAMETERS

Table No. 6.

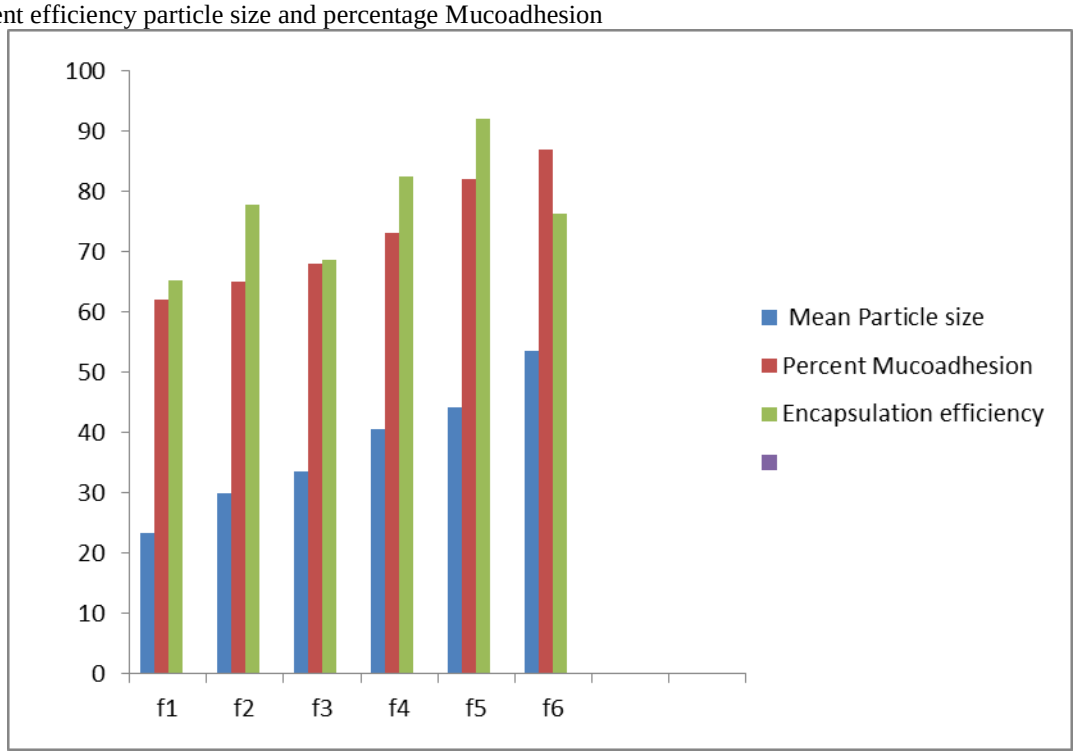
Batch	Bulk density (gm/ml)	Tapped density (gm/ml)	Angle of Repose	Carr's Index	Hausner's Ratio
F1	0.54±.02	0.98±.02	30.2±.02	44.89±.002	1.81±.01
F2	0.86±.01	1.09±.07	27.9±.05	23.21±.1	1.30±.02
F3	0.91±.03	1.01±.02	30.8±.11	8.91±.05	1.09±.12
F4	1.3±0.1	0.86±.03	25.3±.02	- 39.53±.02	0.71±.002
F5	0.8±0.3	1.06±.12	33.1±.001	20±.003	1.25±.1
F6	0.92±0.02	0.83±.01	20.3±.03	- 8.69±.01	0.91±.03

9. Particle Size, Encapsulation Efficiency and Percent Mucoadhesion

Table No. 7: % Drug Content and Drug Entrapment Efficiency of various formulations.

Batch	Encapsulation Efficiency* (%)	Mean Particle size* (µm)	Percent Mucoadhesion
F1	65.12	23.40±1.10	62±1.81
F2	77.79	29.82±2.54	65±1.90
F3	68.64	33.53±1.76	68±1.51
F4	82.5	40.55±1.87	73±1.31
F5	92.04	44.23±0.85	82±1.20
F6	76.19	53.60± 1.92	87±1.30

Entrapment efficiency particle size and percentage Mucoadhesion

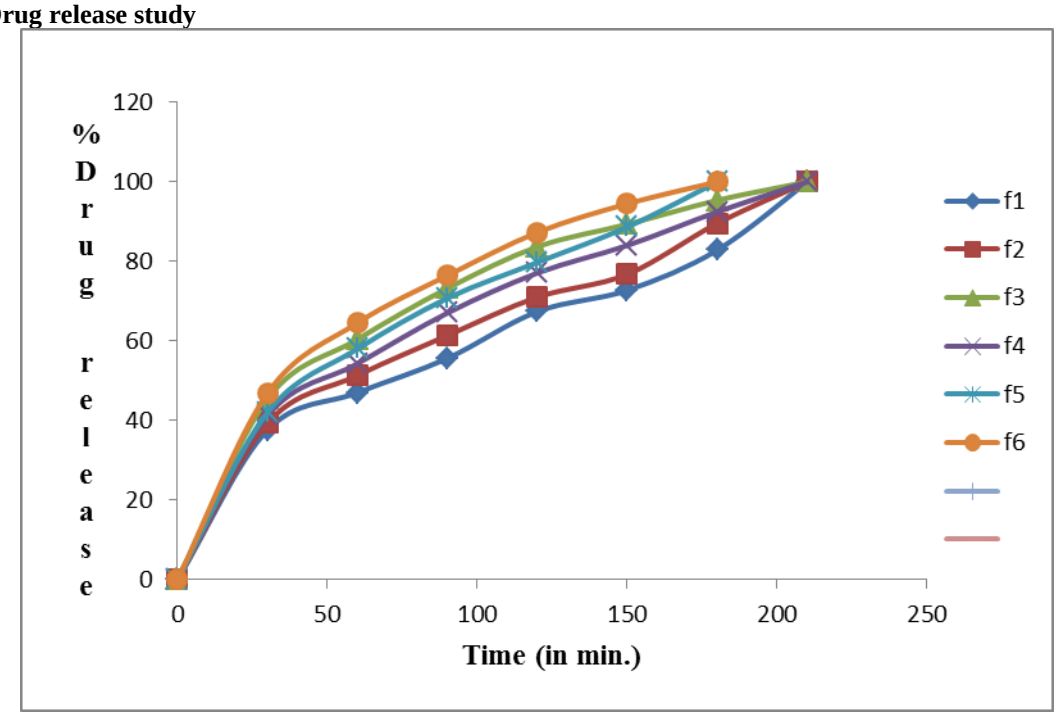


10. In-vitro drug release study

Table No. 5.7.3.

Time	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
30	37.61	39.32	45.41	41.23	42	46.83
60	47.01	51.33	60.4	54.28	58.01	64.54
90	55.63	61.37	73.23	67.04	70.75	76.45
120	67.4	71.02	83.69	77.09	79.76	87.29
150	72.74	76.77	89.33	84.04	88.67	94.51
180	80.85	89.58	95.37	92.46	100	100
210	100	100	100	100		

Invitro Drug release study



DISCUSSION

In the present work, an organoleptic characteristic of drug is determined and it seems to be yellow crystalline powder, tasteless and odourless. Its melting point is 332^0C and solubility was determined in different solvents and found freely soluble in DMSO and other solvents.

The standard curve of Meletine in hydroalcholic solution was drawn at 370nm by taking different aliquots, a line is obtained with the regression value r^2=0.981. Then the interaction study between the drug and polymer done and considered as compatible i.e. there is no interaction between drug and polymer mixture.

After that six batches of microspheres were formulated by varying the drug: polymer ratio by double emulsion solvent evaporation method and further evaluated its physicochemical characteristics. The bulk density and the tapped density of six batches were determined and found that the formulated batch F4 has good bulk density which is 1.3±0.1 and the batch F2 has the tapped density 1.09±0.07.

The flow property of various formulated batches determined and found that the batch F6 has excellent flow property with angle of repose 20.3±0.03 and the batch F3 has excellent Carr's index with the value of 8.91±0.05.

After that Particle Size of various batches was determined by means of sieving method, by passing the powder through various sieves and agitated by placing it on mechanical shaker.

The particle size of batch F1 is 23.40±1.10 and considered as good among all other batches. Then the encapsulation efficiency of different batches was determined of varying drug: polymer ratio and was found that the batch F5 shows maximum encapsulation efficiency of 92.04.

The In-vitro release study of different batches were done at different time intervals in which % cumulative release is determined by considering drug release at 210 min as 100% drug release by following first order kinetics. The graph is plotted between % cumulative release v/s time and a semi log graph is plotted between log % cumulative release v/s time. Finally Mucoadhesive strength of different batches were determined and found that the batch F6 showed maximum mucoadhesion.

Hence Meletine can be formulated as Mucoadhesive microsphere and in this Meletine can be utilized for its protective efficacy as antidote to mustard agents particularly for sustained release, protection of mucosal membrane and in case of accidental ingestion.

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