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Original Article

MUCOADHESIVE CHITOSAN MICROSPHERES OF GEFITINIB

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ABSTRACT

Objective: Gefitinib, Epidermal Growth Factor-Tyrosine Kinase Inhibitor (EGFR-TKI); has promisingly shown activity against Non-Small-Scale Lung Cancer. Currently, the formulations of this drug available are in Tablets, Capsules and liposomal suspensions taken by the oral route. These have certain disadvantages in gastrointestinal disorders like irritation of GI mucosal layer, bleeding, non-patient compliance and low bioavailability due to low aqueous solubility and thus low bioavailability. The purpose of this study was to formulate and evaluate Chitosan-based Microparticles of Gefitinib for maintaining the therapeutic index and limits its side effects.

Methods: Chitosan microspheres cross-linked with glutaraldehyde were prepared by solvent evaporation technique which is then analyzed for its particle size, encapsulation efficiency, swelling index.

Results: The release rate of the drug can be increased by using chitosan-based carrier system which will enhance its bioavailability. By this work, the anticancer activity of Gefitinib in non-small-scale lung cancer will be successfully determined.

Conclusion: It has been concluded that microspheres can be prepared by solvent evaporation technique by varying the concentration of chitosan and tween-20. Chitosan used in this work is of 85 % degree of deacetylation, 25 % solution of Gluteraldehyde suitable for the formulation of these microspheres. Optimized temperature was selected as 65 °C, and the rotation speed was taken as 1200 rpm. Finally, the objectives planned for this research work was performed and evaluated and shown promising results as the dosing frequency is reduced and maximize for 3 d rather than once in a day as per the current formulation available in the market now with a low dosage regimen of 100 mg of dosage strength, administer by pulmonary route. Microparticulate drug delivery system from microspheres is able to deliver the drug in a sustained release manner for the long period of time successfully.

Keywords: Microparticles, Gefitinib, Epidermal Growth Factor Inhibitor, Tyrosine Kinase, Lung Cancer, Bioavailability

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INTRODUCTION

Chitosan is a biodegradable, biocompatible and non–toxic natural polymer thus it has a great potential for biomedical and pharmaceutical applications. Chitosan is cationic in nature, so it has good mucoadhesive and membrane permeability enhancing properties. Chitosan has previously been shown to enhance the mucosal absorption of various compounds in a drug delivery system and have adjuvant activity in the mucosal immune response. Chitosan is a renowned rate controlling polymer for drug release which helps in prolongation of the duration of action and delivering the drug to the specific sites in the body. Also, chitosan does not cause any hypersensitivity or allergic reactions with living tissues [1]. It breaks down slowly to amino sugars which is harmless and completely absorbed by the human body. There are so many reports that demonstrated the efficacy of chitosan microspheres as a vehicle for transport of drugs in the body. Thus, this proves to be safe, widely available and cost-effective.

Microparticle, also called as *'microsphere'* or *'microcapsule'* have many applications in medicine. In most cases, microparticles are used as drug carriers to deliver the drug to the desired site and slowly release the encapsulated drug over a desired period of time to maintain an effective local drug concentration. Microparticles also have novel application in the foods, medical devices, chemical coatings, personal health testing kits, biosensors as per security systems, high throughput screening techniques, and water purification units for manned spacecraft [2]. Thus; Microparticles are that type of drug delivery systems where the particle size ranges from 1 micron (one-thousandth of a mm) to few mm. The microencapsulation technology allows protection of drug from the environment, stabilization of sensitive drug substances, elimination of incompatibilities, or masking of unpleasant taste. Hence, they play an important role as drug delivery systems aiming at improved bioavailability of conventional drugs and minimizing side effects.

Microparticles: 1 µm–1000 µm (1 mm)

MATERIALS AND METHODS

Materials

Chitosan of medium molecular weight (240 kD) having 85% degree of deacetylation obtained from Yarrow Chemicals Mumbai, Gefitinib was obtained as a gift sample from Natco Pharma Pvt. Ltd Hyderabad, Acetic acid was purchased from Merck Chemicals, S. K. Traders Indore, Glutaraldehyde (25 % aqueous solution) obtained from Loba Chemicals. All the chemicals, reagents and solvents used were of the highest analytical grades.

Equipment

Ultra–Sonicator bath–type, Shimadzu Digital Weighing Balance having 2.20 kg capacity, 2 MLH Remi Motors Magnetic Stirrer, Shimadzu–1800 UV Spectrophotometer, FTLA 2000 meter Toledo FTIR, Veego 6 Station Dissolution Apparatus, Horiba Nano Particle Sized Analyzer.

Microspheres preparation

Chitosan-based microspheres are prepared by the reported method with some modifications. A weighed quantity of Chitosan (1, 1.5, 2 gm) was dissolved in 5 % acetic acid solution and was stirred under room temperature (25 °C) on a magnetic stirrer at 700 rpm. Then the drug Gefitinib was loaded (100 mg) into this chitosan solution and stirred continuously. After obtaining a homogeneous solution, this was sonicated on ultrasonicator for 10 min to remove air bubbles during stirring. This solution was filled into 10 ml injection contains 24 gauze needle [3]. By adding this solution in injection drop by drop at a rate of 1 ml/min into the base solvent system containing 5 ml petroleum ether and 10 ml of heavy liquid paraffin containing tween 20 as an emulsifier. Then, these microspheres are obtained by solvent evaporation technique by using REMI Motors 2 MLH Magnetic Stirrer. After half an hour of continuous stirring at 60 °C, add 25 % glutaraldehyde solution and stirred this solution continuously. This was subjected to filtration, washing three times with N-hexane and then air dried. For the solvent evaporation process, the process conditions were: concentration of

chitosan, the concentration of emulsifier (Tween 20) and temperature. Gefitinib loaded chitosan microspheres were prepared by this technique.

Fig. 1: Photograph of gefitinib chitosan microspheres

Optimization

Optimization of the Gefitinib–Chitosan Microspheres by Solvent Evaporation Method was based on following parameters given by 3^2 factorial design [4].

In 3^2 factorial design

3 = Levels like **Low (-1), Medium (0), High (+1)** and

2 = Factors like **independent variations and responses**.

- The independent variations in this design are: X_1 = Concentration of chitosan, and

X_2 = Concentration of Tween-20

- The responses in this design are: Y_1 = Drug Release, and

Y_2 = Entrapment efficiency.

Batch designs in 3^2 Factorial design

Table 1: Table for batch design

Levels	-1	0	+1
Chitosan (%)	1	1.5	2
Tween–20 (ml)	0.5	1.0	1.5

Design of responses

Table 2: Table for Responses design

Chitosan concentrations X_1	Tween–20 concentrations X_2
-1	-1
-1	0
-1	+1
0	-1
0	0
0	+1
+1	-1
+1	0
+1	+1

Batch formulation according to 3^2 factorial design

Table 3: Table for the formulation of batches according to 3^2 factorial design

S. No.	Chitosan (%)	Tween–20 (ml)
1	1	0.5
2	1	1.0
3	1	1.5
4	1.5	0.5
5	1.5	1.0
6	1.5	1.5
7	2	0.5
8	2	1.0
9	2	1.5

Optimization of chitosan and tween–20 concentration

Table 4: Optimization of chitosan and tween–20 for gefitinib microspheres

S. No.	Formulation code	Chitosan concentration (mg)	Tween–20 concentration (ml)	Temperatures (°C)	Feed Rate of injection (ml/min)
1	GCM–1	1	0.5	60–70	1
2	GCM–2	1.5	0.5	60–70	1
3	GCM–3	2	0.5	60–70	1
4	GCM–4	1	1	70–80	1
5	GCM–5	1.5	1	70–80	1
6	GCM–6	2	1	70–80	1
7	GCM–7	1	1.5	80–90	1
8	GCM–8	1.5	1.5	80–90	1
9	GCM–9	2	1.5	80–90	1

☆ GCM = Gefitinib Chitosan Microspheres, The Percent Entrapment Efficiency and Particle Size Analysis will be done for selecting the best-optimized formulation.

Table 5: Percent entrapment efficiency and particle size analysis of gefitinib–chitosan microspheres

S. No.	Formulation code	Entrapment efficiency (%)	Particle size (µm)
1	GCM–1	29.45±1.2	11.236
2	GCM–2	43.87±0.87	11.698
3	GCM–3	69.69±0.64	12.765
4	GCM–4	72.66±0.10	13.863
5	GCM–5	79.87±1.2	14.442
6	GCM–6	29.38±0.69	10.720
7	GCM–7	50.38±0.87	11.687
8	GCM–8	68.29±1.0	12.754
9	GCM–9	68.29±1.0	11.798

Each data represents±SD, (n=3)

Optimization of temperature and speed

Table 6: Optimization of temperature and speed for the formulation

Formulation code	Temperature (°C)	Speed (rpm)	Chitosan Concentration (%)	Tween 20 (ml)	Entrapment efficiency (%)	Particle size (µm)
GCM–10	60	700	1.5	1	52.94±0.8	12.389
GCM–11	65	700	1.5	1	59.92±0.7	12.830
GCM–12	70	700	1.5	1	65.59±0.64	13.927
GCM–13	60	1200	1.5	1	79.93±0.58	13.934
GCM–14	65	1200	1.5	1	83.72±0.5	14.010
GCM–15	70	1200	1.5	1	80.34±0.98	13.211
GCM–16	60	1800	1.5	1	71.54±1.1	12.178
GCM–17	65	1800	1.5	1	45.76±0.58	11.892
GCM–18	70	1800	1.5	1	49.75±0.08	11.592

From the above-mentioned data, formulations of Gefitinib Chitosan Microspheres are prepared at the temperature optimized to be 65 °C and speed was optimized at 1200 rpm.

Various process parameters selection

Table 7: Various process parameters selected during optimization

S. No.	Process parameters	Optimized values
1	Chitosan concentration	1.5 gm
2	Tween–20 concentration	1
3	Temperature	65
4	Rotation Speed	1200 rpm

Table 8: Final ingredients for microsphere preparation

S. No.	Ingredients	Quantity
1	Chitosan	1.5
2	Tween–20	1 ml
3	25 % Gluteraldehyde	0.5 ml
4	5 % Acetic Acid	20 ml
5	Gefitinib	100 mg
6	Petroleum ether	5 ml
7	Heavy Liquid Paraffin	500 ml
8	N–Hexane	200 ml
9	Distilled water	30 ml

Particle size analysis was done by optical microscopy having least count

From the above-mentioned data, the Formulation Code GCM–5 shows maximum Percent Entrapment Efficiency (79.87±1.2) and Particle Size (14.442 µm) was selected to be best-optimized formulation.

Final optimized formulation table

For the preparation of Gefitinib–chitosan microspheres, the following ingredients were taken as in optimized concentrations:

Characterization of chitosan microspheres

Yields of production

The amounts of these microspheres obtained of each batch were weighed, and the percentage yield was calculated by taking into the consideration of the weight of drug and weight of polymer [5]. The Percentage Yield was calculated by using the given formula:

% Yield of Production= Practical Yield/Theoretical Yield × 100

These calculations were done in triplicate (n=3), and the mean was calculated.

Swelling index

The degree of swelling of the optimized formulation was calculated with little modifications [6]. The swelling ability of the microparticles to swell them in Phosphate Buffer pH 6.8 was determined by immersing 100 mg of microspheres in little excess of Phosphate Buffer pH 6.8 in 16 ml capacities of Franz–Diffusion Cell for 24 h and then washed. The formula used for degree of swelling:

$\alpha = W_s\text{-}W_0/W_0$

Where, α = degree of swelling, W_0 = weight of microspheres before swelling, W_s = Weight of microspheres after swelling.

Table 9: Yields of production of the optimized formulation of gefitinib chitosan microspheres

S. No.	Formulation code	Production yield (%)
1	GCM–5	48

Table 10: Degree of swelling of optimized formulations containing chitosan microspheres

S. No.	Formulation batch	Degree of swelling
1	GCM–5	45

Percent entrapment efficiency

Gefitinib Chitosan Microspheres were crushed and suspended in 10 ml methanol to extract the drug from microspheres. After 24 h, the formulation was then centrifuged to 700 rpm, and the supernatant was separated [7]. This supernatant was assayed in UV Spectro-photometer (Shimadzu 1800) at 260 nm. The blank solvent was taken as methanol.

Percent Entrapment Efficiency = Wα–Ws ×100

Wα

Wα = drug added in the formulation, Ws = drug in the supernatant.

Drug loading efficiency

Drug Loading Efficiency was also calculated by the above-mentioned process, and the supernatant was being assayed under UV Spectrophotometer under 260 nm. Blank solution was taken as methanol [8].

Drug Loading Efficiency = Wα–Ws ×100

Wα–Ws+Wp

Where; Wp = Weight of chitosan polymer

Scanning electron microscopy

Scanning Electron Photomicrographs of a formulation containing Gefitinib-Chitosan Microsphere was obtained by using Scanning Electron Microscope (Jeol, JSM 5600, Japan). Into this process, the microspheres in little quantity were spread on the aluminum stub [9]. Then this was placed under the chamber of Scanning Electron Microscope at an acceleration voltage of 15.00 kV EHT under the magnification of 88 X, and the detector SE1 has used The photomicrograph of this sample is obtained.

Table 11: Percent entrapment efficiency of optimized formulations containing gefitinib chitosan microspheres

S. No.	Formulation batch	Entrapment efficiency (%)
1	GCM–5	79.87±0.12

Table 12: Drug loading efficiency of optimized formulations containing gefitinib chitosan microspheres

S. No.	Formulation batch	Drug loading efficiency (%)
1	GCM–5	68.23±0.56

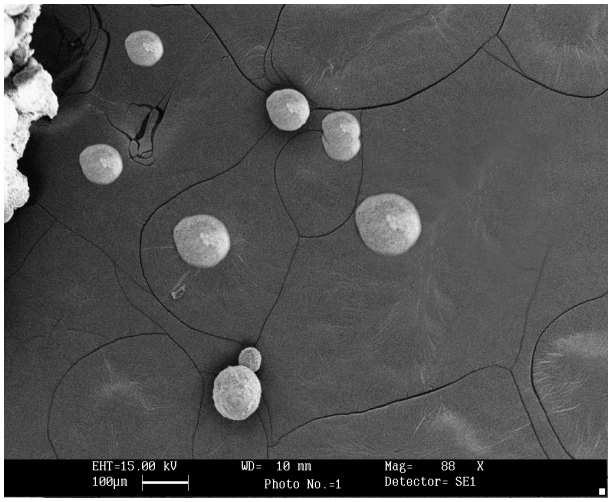

Fig. 2: SEM of gefitinib chitosan microspheres

Particle size determination

Determination of particle size of an optimized formulation containing Gefitinib–Chitosan Microspheres was obtained by appropriate hydration using pH 6.8 (5 ml) with manual shaking for 5 min through Horiba Nano Particle Analyzer at NDDS Lab, VNS Faculty of Pharmacy, Bhopal (M. P.). The result obtained was as follows:

Table 13: Particle size analysis of optimized formulation gefitinib chitosan microspheres

S. No.	Formulation batch	Particle size (µm)
1	GCM–5	14.628

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NDDS Lab
VNS Faculty of Pharmacy, Bhopal

201709191000006.nsz
Measurement Results

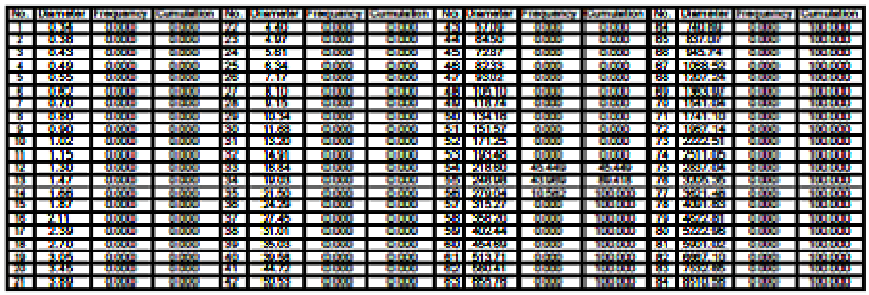
Date	:	Tuesday,September 19,2017 15:41:15 PM
Measurement Type	:	Particle Size
Sample Name	:	F Microsphere
Scattering Angle	:	90
Temperature of the holder	:	25.0 °C
T% before meas.	:	18602
Viscosity of the dispersion medium	:	0.894 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Scattering Light Intensity
Count rate	:	822 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	223.4 nm	18.4 nm	218.2 nm
2	—	— nm	— nm	— nm
3	—	— nm	— nm	— nm
Total	1.00	223.4 nm	18.4 nm	218.2 nm

Cumulant Operations

Z-Average	:	14628.8 nm
PI	:	1.658



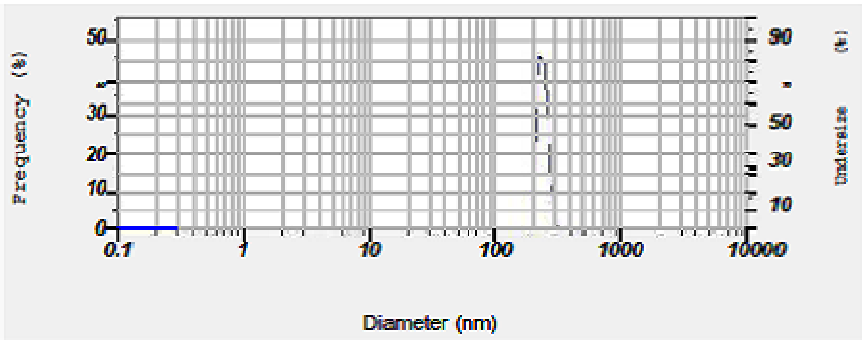


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Fig. 3: Particle size determination of gefitinib chitosan microspheres

Zeta potential measurement

Zeta Potential is the representative of positive charge. The Zeta Potential measurement of these optimized formulations was measured by Horiba Nano Particle Analyzer in NDDS Lab, VNS Faculty of Pharmacy, Bhopal (M. P.) It involves the preparation of a dispersion of microspheres in distilled water.

Afterwords, this dispersion mixture was filled in Zeta Cell and placed in an analyzer that will determine the Zeta Potential.

Table 14: Zeta potential measurement of an optimized formulation containing gefitinib chitosan microsphere

S. No.	Formulation batch	Zeta–potential (mV)
1	GCM–5	-35.2 mV

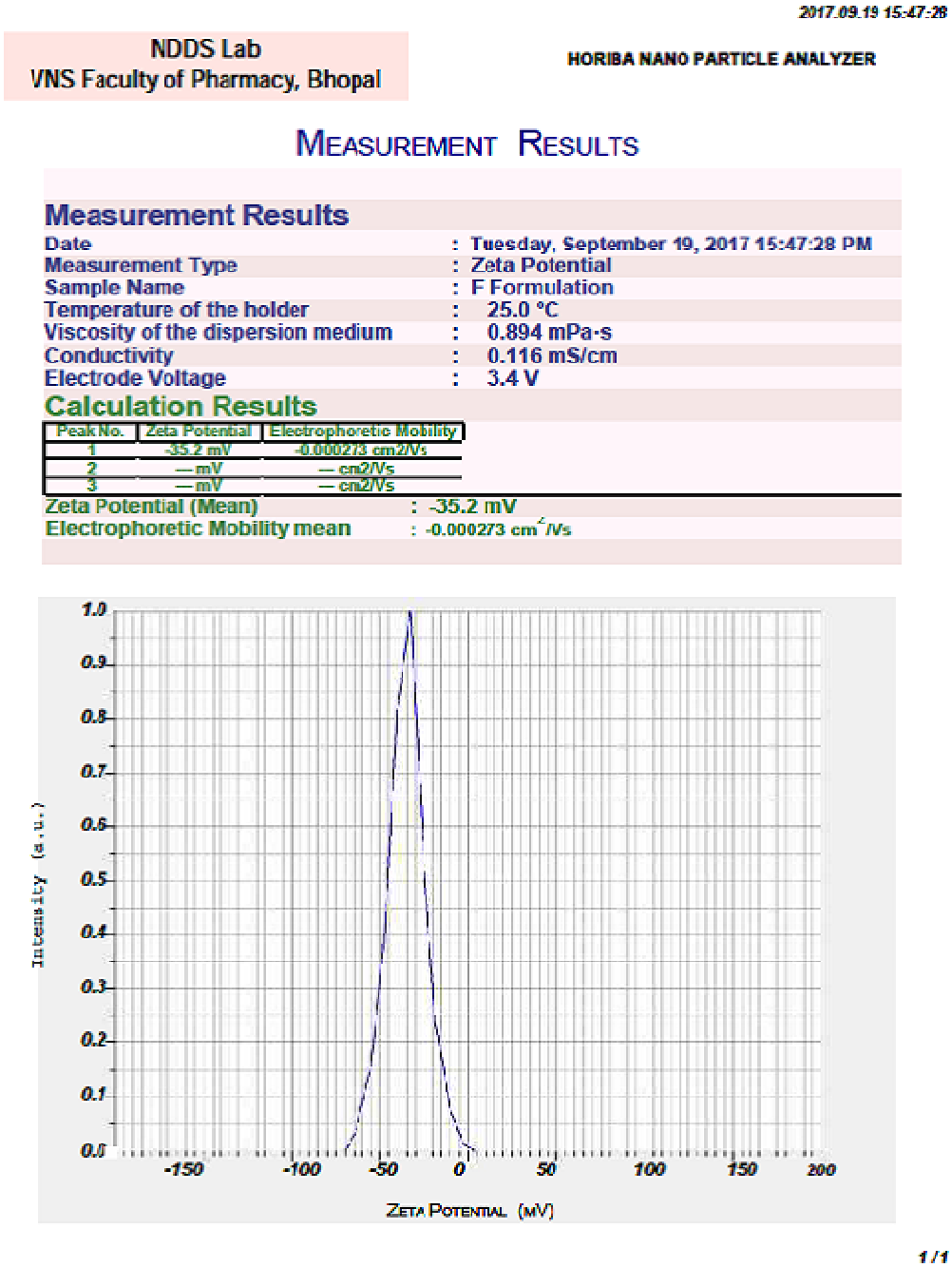

Fig. 4: Zeta potential measurement of gefitinib chitosan microspheres

***In–vitro mucoadhesion* strength measurement**

A strip of the mucoadhesive skin of Sheep or Goat was mounted on a glass slide with a fixative adhesion. Numbers of counted Gefitinib–Chitosan Microspheres were placed on the mucoadhesive membrane after washing the membrane with distilled water and then PBS pH 6.8 for 5 min continuously. The glass slide was then incubated for 15 min in a desiccator at 80 % RH to allow the polymer for interaction with the membrane that placed on the cell and was attached to the assembly inclined at 45 °C [10]. Then PBS pH 6.8 was circulated on the cell over the microspheres and skin at the rate of 2 ml/min from the burette. This was then subjected for washing and collected at different time intervals, and a number of capsules were drained off and counted by using haematocytometer chamber under an optical microscope.

Following equation gives the adhesion number as:

$$N_a = N/N_o \times 100$$

Where; N_a = Adhesion Number, N_o = Number of Microspheres present in that area, N= Number of Microspheres attached to the mucosa after washing.

Table 15: *In–vitro* mucoadhesion test of a formulation containing gefitinib chitosan microspheres

S. No.	Time (h)	*In–vitro* mucoadhesion (%)
1	1	87.82±44
2	2	79.47±83
3	4	67.81±78
4	6	56.70±84
5	7	23.56±73

In–vitro drug release studies

In–vitro release drug release studies were performed in phosphate buffer solution pH 6.8 by using 6 Station USP Dissolution Apparatus (Basket type) at 37±0.5 °C and 50 rpm (Veego, VDA–6DR, India). Formulations containing Gefitinib Chitosan Microspheres (100 mg) were tested in 900 ml of Phosphate Buffer Solution pH 6.8. 1 ml sample has been withdrawn in 10 ml volumetric flask at fixed time intervals (1, 2, 3, 4, 5, ...24, 48 h respectively), and replaced with fresh 1 ml of Phosphate Buffer pH 6 to maintain sink conditions [11]. These 1 ml withdrawn samples are then diluted with the same solvent up to 10 ml mark in a volumetric flask. Then, these samples are scanned at 254 nm using Shimadzu 1800 UV–Spectrophotometer. The drug release kinetics models are applied to determine the cumulative amount of drug release at each time and graph has been plotted.

Table 16: *In–vitro* drug release studies of optimized formulation of gefitinib chitosan microspheres

S. No.	Time (h)	Absorbance	Concentrations (µg/ml)	Amount (mg/ml)	Drug release (%)
1	0	0	0	0	0
2	6	0.056	2.05	18.45	30.75
3	12	0.08	3.25	29.25	48.75
4	24	0.122	5.35	48.15	80.25
5	36	0.138	6.15	55.35	92.25
6	48	0.143	6.4	57.6	96
7	60	0.145	6.5	58.5	97.5
8	72	0.146	6.55	58.95	98.25

Drug release kinetics

Release kinetics studies are more useful for prediction of different modified release dosage forms. These release patterns help to define the time and rate of release of drug to be followed. Different mathematical models used to predict the release patterns in a definite manner. The selection of best release model based on the highest regression value (R^2), ANOVA and MANOVA, etc. [12]. The different models are applied, and the best statistical model would be selected as Korsemeyer–Pappas Model as it has given the highest coefficient of determinants (R^2 value).

Table 17: Korsemeyer–pappas model of *In–vitro* drug release for gefitinib chitosan microspheres

Time (h) T	Log time log (t)	Concentration (µg/ml)	Amount (mg/ml)	Cumulative drug release (%)	Log of cumulative drug release (%)
0	0	0	0	0	0
6	0.7781	2.05	18.45	30.75	1.4878
12	1.0791	3.25	29.25	48.75	1.6879
24	1.3802	5.35	48.15	80.25	1.9044
36	1.5563	6.15	55.35	92.25	1.9649
48	1.6812	6.4	57.6	96	1.9822
60	1.7781	6.5	58.5	97.5	1.9890
72	1.8573	6.55	58.95	98.25	1.9923

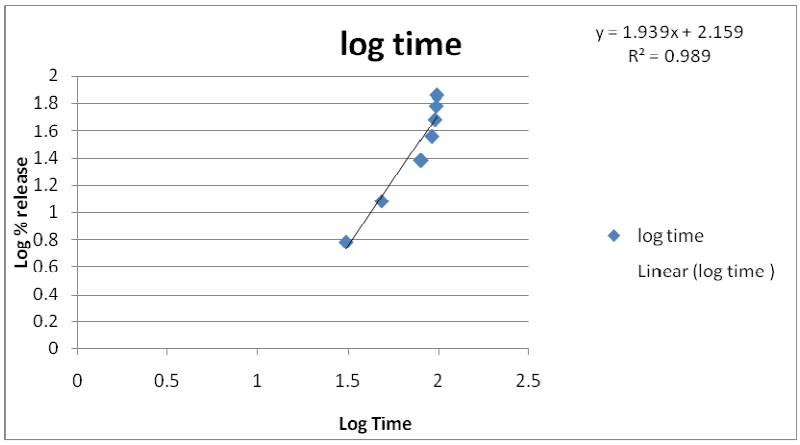

Fig. 5: Korsemeyer pappas model

Table 18: *In vitro* drug release of formulation GCM–1 to GCM-9

Time (h)	% Cumulative drug release									
	Pure drug	GCM–1	GCM–2	GCM–3	GCM–4	GCM–5	GCM–6	GCM7	GCM–8	GCM–9
0	0	0	0	0	0	0	0	0	0	0
6	34.44	24.45	34.56	44.68	30.12	30.75	22.97	24.56	23.45	20.17
12	68.67	51.45	39.87	51.67	49.81	48.75	36.89	29.94	29.55	25.50
24	96.81	60.26	55.79	60.98	61.87	80.25	45.65	32.76	32.86	39.81
36	97.45	65.58	61.64	74.58	69.43	92.25	58.11	44.98	40.58	41.86
48	97.49	79.54	72.89	82.98	72.98	96	62.64	54.81	49.94	50.67
60	97.61	80.77	88.81	88.78	79.65	97.5	69.69	59.99	50.76	61.81
72	97.44	82.84	91.92	91.98	81.23	98.25	72.47	71.76	68.98	64.62

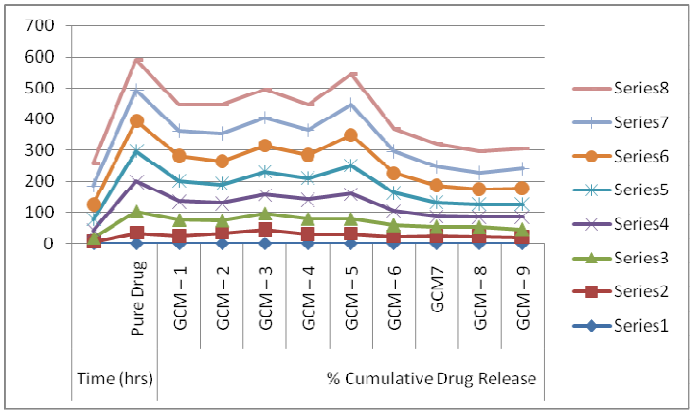

Fig. 6: Curve representing percent cumulative drug release of all formulations containing gefitinib–chitosan microspheres

Drug-excipient studies by differential scanning calorimetry

Differential Scanning Calorimetry is that thermal analysis procedure by which we can measure the interaction of the drug with the polymer before and after the formulation at high temperatures. This will also show the thermal degradation resulted if any. This analysis was done by SOPS, RGPV, Bhopal (M. P.) by the process of PerkinElmer Thermal Analysis. Standards of Indium were taken for calibration purpose of temperature and enthalpy scale. Hermetically sealed samples in aluminum pans are heated at a constant rate of temperature of 30.00 °C/min with ranges from 30.00–350.00 °C at 40.00 °C/min. Purging Nitrogen was used at a flow rate of 100 ml/ml for maintaining the inert environment.

DSC of gefitini

The heat flow during the endothermic process was observed at-34 mW. The temperature was sharply recorded at 205 °C which was approximate near to the melting point of Gefitinib itself.

DSC of chitosan

In the case of chitosan, the endothermic peak was observed at-3 mW, and the broad peak was obtained at 108 °C.

DSC of gefitinib–chitosan microsphere

The microspheres prepared by Gefitinib and Chitosan combination shows the endothermic peak in a positive direction at 2.3 mW, and the temperature was recorded at 92 °C.

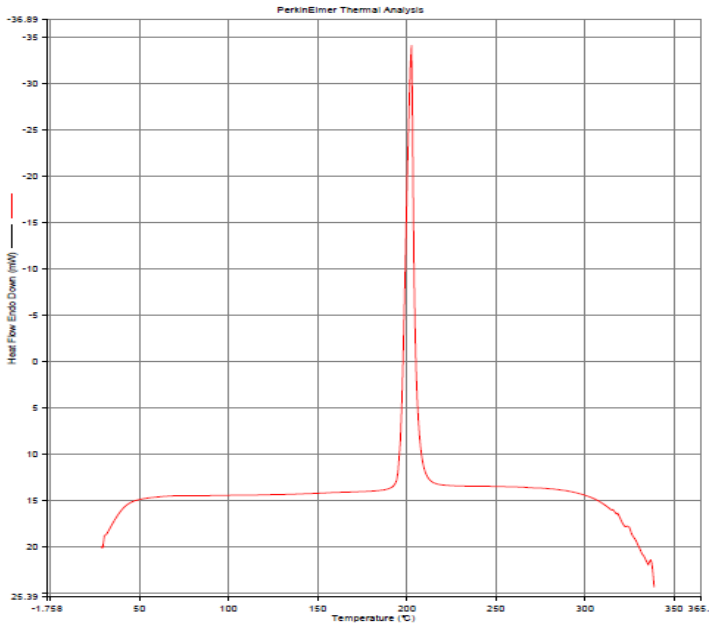

Fig. 7: DSC curve of drug (Gefitinib)

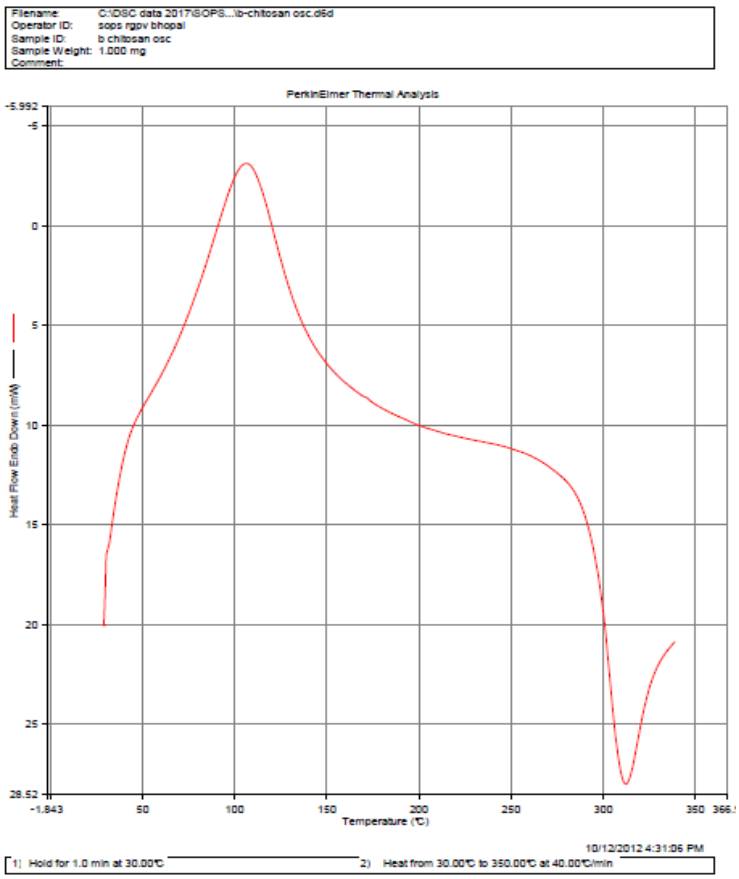

Fig. 8: DSC of chitosan

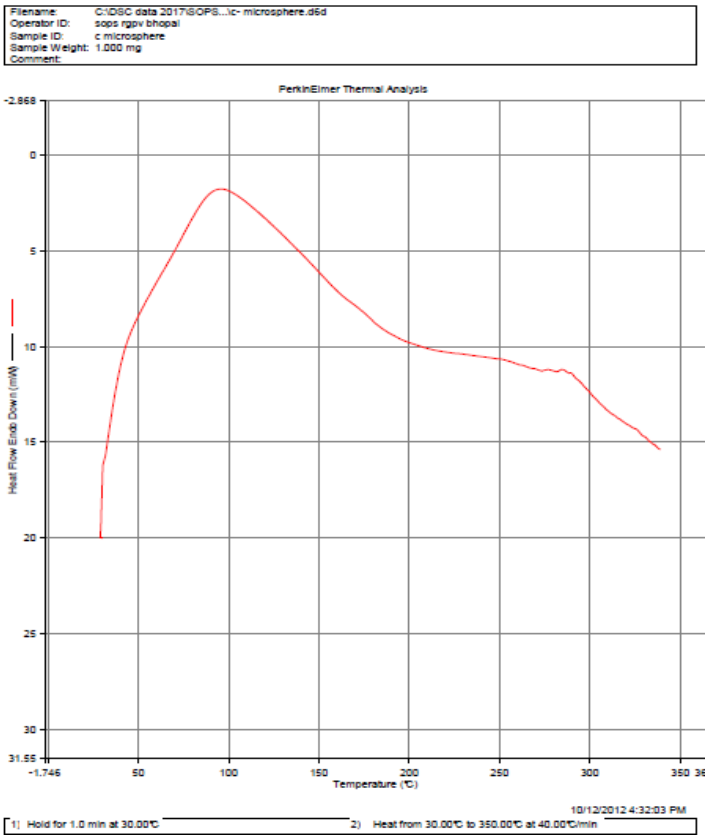

Fig. 9: DSC of gefitinib–chitosan microspheres

Table 19: Table for DSC analysis

S. No.	Endothermic peaks (mW)	Temperature (°C)	Peak types
Gefitinib	-34	205	Sharp
Chitosan	-3	108	Broad
Microspheres	2.3	92	Broad

RESULTS AND DISCUSSION

Differential scanning calorimetric studies

According to these DSC graphs of Gefitinib, Chitosan and microspheres containing a combination of these two states that, there is no deviation into the melting point of gefitinib (197 °C) from the chitosan and both the graphs shows significant peaks in this analysis. Firstly, the drug is in crystalline form, but as the microspheres are prepared by chitosan polymer, peak shifted towards the right side and slightly changes its nature to amorphous form due to chemical interactions and high temperatures. But overall, the drug doesn't have any interactions with chitosan.

Yield of production

The percentage yield of the batches prepared by Gefitinib–Chitosan Microspheres was determined, and the optimized yield of production was found to be 45 %.

Scanning electron microscopy

The morphological characteristics of chitosan-based microspheres of Gefitinib were determined by photomicrographs obtained by Scanning Electron Microscopy under 88 X. The SEM image of these microspheres also exhibits a somewhat rough texture which is due to the feeding of chitosan solution and reaction between the solvent system and chitosan solution. The SEM image also confirms that there is not any residual content of drug left on the surface and no surface was ruptured during the preparation of microspheres. All the microspheres are nearly smooth and circular in shape. The drug embedded successfully into the core content. The particle size of the optimized formulation was then further determined to confirm the size of each microsphere. Later the drug delivery system has been decided.

Particle size determination

The particle size determination of Gefitinib–chitosan microspheres was performed by Horiba Nano Particle Analyzer which has given the average particle size of 233.4 nm. The Z Average was found to be 14628.28 and PI is 1.658.

Percentage entrapment efficiency

The percentage entrapment efficiencies of Gefitinib–Chitosan Microspheres were determined under UV Spectrophotometer and later were optimized to be 79.87±0.12 %.

Drug loading efficiencies

The Drug Loading Efficiencies of Gefitinib–Chitosan Microspheres were determined by UV Spectrophotometer by using the given formula. Optimized formulation shows the Percent Drug Loading Efficiency of 68.23±0.56 %.

Degree of swelling

Chitosan having the swelling capacity will tend to swell the microsphere and rupture the cell wall that tends to show burst release effect. So to control and identify how much bursting and swelling is there, swelling parameter was studied. The degree of swelling was obtained to be 45 %.

Zeta potential measurement

Zeta Potential Measurement is an important parameter essential for the prediction of particle's stability. Higher the zeta potential value, higher will be the repulsive force between particles that resulted in less aggregation. The zeta potential measurement of the Gefitinib–Chitosan Microspheres were found to be-35.2 mW.

In–vitro mucoadhesive strength measurement

Mucoadhesive is the special characteristic property of chitosan microspheres that tend to adhere to the mucoadhesive membrane present inside the body cavities. This property decides the drug release pattern of the drug delivery system. The In–vitro mucoadhesive strength measurement was determined for Gefitinib–chitosan microspheres for 1 hour 87.82±44 % up to 7 h will be 23.56±73 % respectively.

In–vitro drug release studies

For determination of the release patterns and decide either the formulation is able to show sustained release, prolonged release, burst release, etc; the In–vitro drug release studies were performed. This is the main criteria for this whole research work to produce sustained release microspheres that reduce the dosage frequency of the drug. The percent cumulative In–vitro drug release was determined to be 98.25% for 72 h. The results indicated that Gefitinib–chitosan microspheres were prepared by solvent evaporation technique stat the promising controlled release for drug delivery system.

Drug release kinetics studies

All the formulations of GCM–1 to GCM–18 are subjected to determine the Percent Cumulative Drug Release and fitted into different kinetics release models from which, Formulation GCM–5 resulted in following Korsemeyer–Pappas Model for the drug delivery of Gefitinib–Chitosan Microspheres that have the highest regression value of $R^2 = 0.989$.

CONCLUSION

From the above thesis work, it has been concluded that microspheres can be prepared by solvent evaporation technique by varying the concentration of chitosan and tween-20. Chitosan used in this work is of 85 % degree of deacetylation. 25 % solution of Glutaraldehyde is suitable for the formulation of these microspheres. The optimized temperature was selected as 65 °C, and the rotation speed was taken as 1200 rpm.

Drug-excipient compatibility studies

This was done by Fourier transform infrared spectroscopy and Differential Scanning Calorimetry. FTIR states that there is no interaction between Gefitinib and Chitosan in the formulation. Little similar peaks are observed due to certain process parameters. DSC curves show that the drug is in crystalline form changes to amorphous form when combines with chitosan as the curve shifts towards right.

Scanning electron microscopy

This was done under 88 X magnification and confirms that there is no residual content left over the surface of the drug, and the smooth and circular shape was obtained.

Particle size determination

Horiba Nano Particle Size Analyzer was used for the particle size determination, and the size was found to be in the micro range. The Z Average particle size was found to be 14.628 μm, and PI is 1.658. This size range is more useful and suitable for the drug delivery through pulmonary route Gravitational Sedimentation.

Percent entrapment efficiency

This was obtained and optimized to be 79.87±0.12 %. That the drug loaded in a successful manner.

Drug loading efficiency

The Percent Drug Loading Efficiency of the optimized formulation (GCM–5) was determined to be 68.23±0.56 %. It concludes that 100

mg of the drug was added into the formulation and out of which 68.23±0.56 % of the drug has been loaded into the microspheres embedded by chitosan and rest of drug will be present into the supernatant.

Zeta potential measurement

The obtained value for zeta potential measurement is-35.2 mW. This concluded that there is a microsphere formed are in separate conditions and does not forms aggregates and non–sticky in nature as higher the zeta potential value, more will be the repulsive force between the particles and less aggregates will be formed.

***In–vitro* mucoadhesive test**

This was performed and evaluated and the result obtained from this that, chitosan will adhere to skin mucosal membranes for 1 hour 87.82±44 % up to 7 h 23.56±73 % respectively. This concluded as the chitosan polymer has successfully adhered to the mucoadhesive membrane and acts as a rate controlling polymer for the delivery of drug Gefitinib in a sustained release manner.

***In–vitro* drug release studies**

The release patterns obtained from this study that the formulation will release the drug in a sustained manner up to 72 h means (3 d) successfully. The cumulative percent drug release was obtained as 98.25%, and the formulation containing drug Gefitinib follows Korsemeyer–Pappas Model for the drug delivery of these Chitosan Microspheres containing Gefitinib. According to this model, the drug will release from the polymeric matrix system in the form of dissolution of drug from the core. To understand the mechanism of drug release, the 60 % of the drug release should be fitted under the equation given by Korsmeyer–Pappas Model.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Learoyd TP, Burrows JL, French E, Seville PC. Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. Eur J Pharm Biopharm 2008;68:224-34.
2. Singh MN, Hemant KSY, Ram M, Shivkumar HG. Micro-encapsulation: a promising technique for controlled drug delivery. Res Pharm Sci 2010;5:65-77.
3. Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. Int J Pharm 2004;282:1-18.
4. Bhavsar MD, Tiwari SB, Amiji MM. Formulation optimization for the nanoparticles-in-microsphere hybrid oral delivery system using factorial design. J Controlled Release 2006;110:422-30.
5. He P, Davis SS, Illum L. Chitosan microspheres prepared by spray drying. Int J Pharm 1999;187:53-65.
6. Genta I, Costantini M, Asti A, Conti B, Montanari L. Influence of glutaraldehyde on drug release and mucoadhesive properties of chitosan microspheres. Carbohydrate Polymers 1998;36:81-8.
7. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro-and nanoparticles in drug delivery. J Controlled Release 2004;100:5-28.
8. Yu X, Yang G, Shi Y, Su C, Liu M, Feng B, Zhao L. Intracellular targeted co-delivery of shMDR1 and gefitinib with chitosan nanoparticles for overcoming multidrug resistance. Int J Nanomed 2015;10:7045.
9. Zhao L, Yang G, Shi Y, Su C, Chang J. Co-delivery of gefitinib and chloroquine by chitosan nanoparticles for overcoming the drug acquired resistance. J Nanobiotechnol 2015;13:57.
10. Zhou X, Yung B, Huang Y, Li H, Hu X, Xiang G, *et al*. Novel liposomal gefitinib (L-GEF) formulations. Anticancer Res 2012;32:2919-23.
11. Abbas Z, Swamy NGN. Mucoadhesive microspheres as intranasal drug delivery systems: a review. Indian Drugs 2012;49:5-23.
12. Gavini E, Hegge AB, Rassu G, Sanna V, Testa C, Pirisino G, et al. Nasal administration of carbamazepine using chitosan microspheres: in vitro/in vivo studies. Int J Pharm 2006;307:9-15.