ORIGINAL ARTICLE

Study of mucoadhesive microsphere of pirfenidone for nasal drug delivery

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The present research work involves formulation development and evaluation of nasal mucoadhesive microsphere in view to, improve bioavailability and reduce dosing regimen. Microspheres were prepared by spray drying and cross-linking method using chitosan and HPMC K4M, using 32 central composite design. Microspheres were evaluated for particle size, drug content, swelling ability, and percentage yield. Compatibility was checked by doing Fourier transform infrared spectroscopy and Differential scanning calorimetry study. The polymorphism and particle shape were studied by X-ray diffraction and scanning electron microscopy. The average particle size of spray-dried and cross-linked formulations were found in the range between 20-50 μm and 30-60 μm with percent mucoadhesion in the range of 80%-90% and 60-70%, respectively. *In vitro* drug release was found to be proportional to drug to polymer ratio. *In vitro* drug release for optimized formulation, that is, (F1), for spray-drying method and cross-linking method was found to be 88.73% and 70.93% at the end of 6 h, respectively. Release of drug from microspheres followed non-Fickian diffusion kinetics. *Ex vivo* studies were performed with sheep nasal mucosa for mucoadhesion, histopathological study, and drug permeation. The histopathological study indicates nonirritant nature of microsphere. The microspheres were found to be stable at accelerated storage conditions for 1 month, as per International Conference of Harmonisation guidelines.

Key words: *Cross-linking method, nasal mucoadhesive microspheres, pirfenidone, spray drying*

INTRODUCTION

Drug action can be improved by developing new drug delivery system, such as the mucoadhesive microsphere. These systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the site of action leading to improved bioavailability.[1] Pirfenidone is widely used in the treatment of mild-to-moderate idiopathic pulmonary fibrosis. Presently, pirfenidone is available as tablets dosage form. Pirfenidone in conventional dosage form possesses low bioavailability, requires large amount of dose, and has to pass first pass metabolism. Oral routes may become inconvenient or impractical depending on the patient's condition. Hence, there arises a need for an alternative route of administration. The nasal route provides a good solution to the aforementioned problem.[2-5] Microspheres swell in contact with nasal mucosa and form a gel, which controls the rate of clearance from the nasal cavity. In the presence of microspheres, the nasal mucosa is dehydrated due to moisture uptake by the microspheres. The result is reversible shrinkage of the cells, providing a temporary physical separation of the tight (intercellular) junction, which increase the absorption of the drug.[6] Considering the usefulness of mucoadhesive microspheres in enhancing the solubility and bioavailability with added advantage of avoidance of the first pass hepatic metabolism, mucoadhesive microspheres can be explored as drug delivery carriers for pirfenidone via nasal route. Recently, microsphere approach has been used in designing formulations for nasal drug delivery. The primary intention behind selection of microspheres is to serve a better chance for the drug to be absorbed by allowing a large surface area and prolonged contact between the drug and the mucosal membrane.[7]

MATERIALS AND METHODS

Pirfenidone was supplied as a gift from Cipla Limited (Mumbai, India). Chitosan was purchased from India Sea

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Foods (Kochi, India). Hydroxy propyl methyl cellulose K4M was supplied as a gift sample from Colorcon Limited (Goa, India). All other chemicals were of analytical grade. A freshly cut piece of sheep nasal mucosa was obtained from a local abattoir.

Preparation of microspheres

Experimental design

In the present study, formulations development is based on 3^2 central composite design containing two factors. A total of nine batches were developed [Table 1].

Factor A: Chitosan (X1) and Factor B: HPMC K4M (X2).

Drug-polymer dispersion

Chitosan (0.5%w/v) for spray drying and (2.0%w/v) for cross-linking was dissolved, respectively, in acetic acid (2%v/v). Hydroxy propyl methyl cellulose K4M (0.5%w/v) for spray drying and (2.0%w/v) for cross-linking, respectively, were soaked in water. HPMC K4M was mixed with chitosan solution. Pirfenidone (400 mg) was dissolved in sufficient amount of methanol (96%v/v). Drug solution was mixed with the above-mentioned polymers solution. Hydroxy propyl methyl cellulose K4M is used as a mucoadhesive polymer along with chitosan.

Table 1: Composition of mucoadhesive microspheres

Formulation code	Pirfenidone (mg)	Variable level in coded form (mg)	
		X1	X2
F1	400	−1	−1
F2	400	0	−1
F3	400	+1	−1
F4	400	+1	0
F5	400	0	0
F6	400	−1	0
F7	400	−1	+1
F8	400	0	+1
F9	400	+1	+1

Note: (−1): 200, (0): 300 and (+1): 400

Table 2: Optimized parameters for spray drying

Parameter	Optimized values for processing of dispersion containing drug and polymer
Input temperature	90-110°C
Output temperature	70-90°C
Aspirator speed	40-45%
Feed rate speed	2-3 mL/min
Compressed air pressure	3.5 Barr

Preparation of microsphere by spray drying method

Drug-polymer dispersion subjected to spray drying under optimized process parameters [Table 2]. Microspheres were collected from drying chamber and cyclone separator and stored in dry atmosphere.[8]

Preparation of microsphere by emulsion cross-linking method

Light liquid paraffin (100 mL) containing span 80 (0.75% v/v) was allowed to stir for 30 min. Then drug-polymer dispersion was incorporated dropwise into the above solution with constant stirring at 1000 rpm. Three milliliters of glutaraldehyde was added after 5 min and stirring was continued for 3 h. Dispersion was allowed to stand for 30 min. Microspheres were allowed to settle down in dispersion. The obtained microspheres were collected by filtration and washed with petroleum ether several times to minimize irritating effect of glutaraldehyde, dried at room temperature, and stored in desiccators.[9]

Evaluation of developed microspheres

Placebo microspheres were evaluated for particle size, flow characteristics, degree of swelling, mucoadhesion etc. Based on the results obtained, further loaded microspheres were evaluated and do not show much variation due to drug loading in primary characteristics of microspheres.

Percentage yield

The percentage yields [Table 3] for all batches were calculated as follows:

$$\% \text{ Yield} = \text{weight of microspheres/theoretical weight of drug and polymer} \times 100.$$

Table 3: Percentage yield and particle size

Formulation	Percentage yield	Particle size (μm)		
	Spray drying	Spray drying	Cross-linking	Cross-linking
F1	41.73±1.22	25.07±1.87	30.41±0.73	81.27±0.92
F2	43.85±1.96	29.85±2.14	35.27±1.64	84.55±1.86
F3	47.32±0.87	37.60±0.79	55.54±0.79	86.42±0.62
F4	55.27±0.79	41.27±1.63	57.61±1.83	90.68±1.24
F5	49.80±1.66	38.44±1.73	54.94±0.91	85.14±0.78
F6	42.97±1.43	28.78±2.10	48.37±2.32	82.76±1.34
F7	46.64±0.61	35.59±0.35	56.19±0.54	84.69±1.71
F8	54.73±1.50	40.74±1.92	58.53±1.75	88.90±0.53
F9	59.91±1.56	46.97±2.86	60.17±0.37	92.03±1.20

n=3, Mean±SD

Particle size analysis

Particle size analysis [Table 3] is carried out by using a Motic optical microscope.

Micromeritic properties

Mucoadhesive microspheres were studied for compressibility index, Hausner's ratio, and angle of repose [Table 4].

Encapsulation efficiency

Twenty-five milligrams of microspheres were dissolved in 50 mL phosphate buffer (pH 6.6) and sonicated for 15 min and kept overnight for 24 h to extract the drug from microspheres [Table 5]. Resulting solution is filtered through Whatmann filter paper. One milliliter solution was withdrawn and diluted to 10 mL with phosphate buffer (pH 6.6). Absorbance was measured at 221 nm using UV-Vis spectrophotometer (UV- 1800 Shimadzu, Japan) against blank. Encapsulation efficiency was calculated as:

EE (%) = actual drug content/theoretical drug content × 100.

Swelling index

Fifty milligrams of microspheres were immersed in phosphate buffer (pH 6.6) [Table 5]. The degree of swelling was calculated using the following formula:

$$\text{Swelling ratio (SR)} = \frac{We - W_0}{W_0}$$

(W_0 = weight of microspheres before swelling and We = weight of microspheres after swelling).

In vitro drug release study

The *in vitro* drug release was performed using Franz diffusion cell with dialysis membrane. The dialysis membrane was soaked in phosphate buffer (pH 6.6) for 24 h. The temperature was maintained at 37°C ± 2°C. One milliliter sample was withdrawn from receptor compartment at hourly intervals. The samples were analyzed by UV spectrophotometer (UV-1800 Shimadzu, Japan) at 221 nm.

Drug release kinetics

The release data were fitted to the PCP Disso software version 2.08. The statistical treatment of data was done by Design Expert software version 8.0.7.1. Based on the data supported, optimized formulation was selected for further evaluation study.

Mucoadhesion studies

The mucoadhesive property was evaluated by *in vitro* adhesion testing method known as wash-off method. One centimeter piece of goat nasal mucosa was tied on glass slide using thread. Microspheres were spread on tissue specimen and prepared slide was hung onto one of the groves of USP tablet disintegrating test apparatus. The apparatus was operated such that, tissue specimen was given regular up and down

Table 4: Compressibility index and hausner's ratio

Formulation	Compressibility index (%)		Hausner's ratio		Angle of repose (θ)	
	Spray drying	Cross-linking	Spray drying	Cross-linking	Spray drying	Cross-linking
F1	9.09±0.01	10.8±0.75	1.10±0.03	1.12±0.89	25.1±1.51	27.4±0.25
F2	11.2±0.85	11.2±0.85	1.12±0.90	1.12±0.90	26.5±0.13	28.7±0.33
F3	12.3±1.04	14.0±0.45	1.14±0.02	1.16±0.34	28.3±0.10	30.1±0.02
F4	15.4±0.84	15.0±0.03	1.18±0.02	1.17±0.05	31.7±0.11	31.4±0.01
F5	13.6±1.21	13.1±0.07	1.15±0.02	1.15±0.03	27.9±0.17	29.8±0.01
F6	10.5±0.78	8.8±0.55	1.11±0.32	1.09±0.55	26.9±0.07	28.6±0.31
F7	13.5±1.02	12.4±0.04	1.15±0.08	1.14±0.03	29.7±0.75	29.5±0.25
F8	14.2±1.32	15.5±1.03	1.16±0.05	1.18±0.02	30.3±0.08	32.8±0.09
F9	16.1±1.27	16.3±0.05	1.19±0.01	1.19±0.85	32.2±0.15	34.2±0.11

n=3, Mean±SD

Table 5: Entrapment efficiency and degree of swelling (%)

Formulation	Entrapment efficiency (%)		Degree of swelling (%)		Mucoadhesion (%)	
	Spray drying	Cross-linking	Spray drying	Cross-linking	Spray drying	Cross-linking
F1	73.85±0.27	41.34±0.35	60.87±0.57	50.92±0.62	80.73±0.98	59.92±2.36
F2	74.13±1.83	46.77±1.64	62.55±1.86	53.60±1.98	83.64±1.86	62.25±1.45
F3	76.56±0.75	49.51±1.87	65.25±1.74	55.48±0.88	85.49±0.73	65.43±1.93
F4	80.46±1.46	55.56±0.58	68.99±0.98	58.37±1.34	87.55±0.82	68.80±1.48
F5	76.98±1.50	50.84±2.93	64.48±1.87	54.88±1.68	85.03±1.79	62.73±0.57
F6	72.59±0.69	43.26±1.83	61.75±2.78	51.82±2.45	82.90±0.58	60.84±2.34
F7	75.72±0.67	52.76±2.23	63.80±1.42	53.64±1.69	84.76±1.63	64.78±1.76
F8	78.81±1.97	56.94±1.96	67.62±1.62	56.50±0.53	86.82±2.43	67.85±0.67
F9	82.04±0.31	60.60±0.28	70.33±0.21	59.73±2.12	90.73±0.17	70.13±0.31

n=3, Mean±SD

movements in jar containing phosphate buffer (pH 6.6). At hourly intervals up to 6 h, number of microspheres remained adhere to the tissue was counted.

$$\% \text{ Mucoadhesion} = (Wa - W_1) \times 100/Wa$$

Where, Wa = weight of microspheres applied; W_1 = weight of microspheres leached out.

Compatibility studies

Compatibility studies were carried out by performing infrared spectroscopy and differential scanning calorimetry. Infrared spectroscopy spectral analysis of pure drug alone and physical mixture of drug-polymer was studied. Differential scanning calorimetry was performed for pure drug, blank, and drug-loaded microsphere.

Surface morphology

Shape and surface morphology was carried out by X-ray diffraction (XRD) and scanning electron microscopy (SEM). XRD (Brucker AXS D8 Advance) and SEM (Juol-Jsm 6360A) was performed for pure drug, blank, and drug-loaded microsphere.

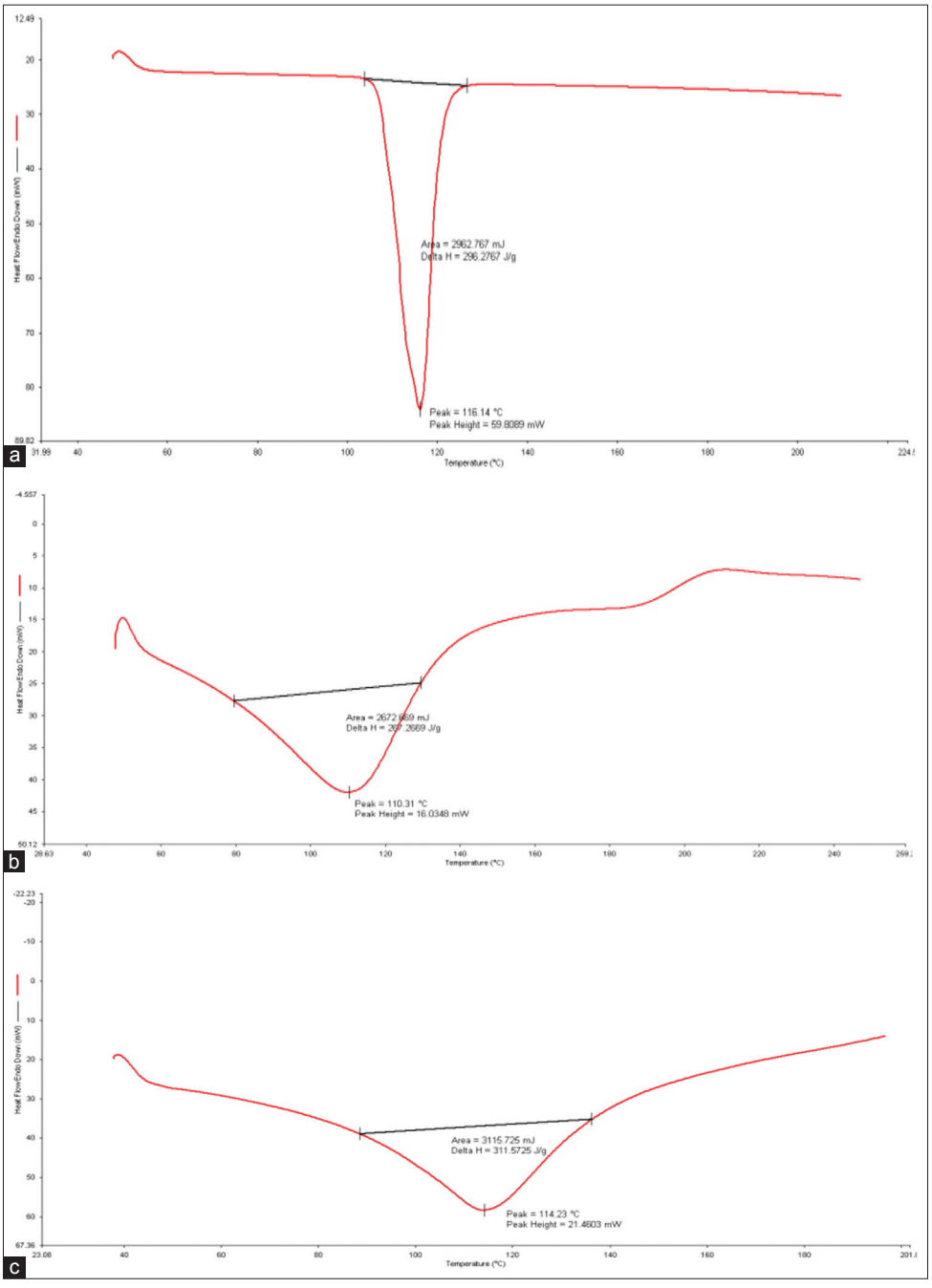

Figure 1: Differential scanning calorimetry of (a) pure drug (b) drug-loaded microsphere for spray drying (c) drug-loaded microsphere for cross-linking

Ex vivo drug permeation study

The permeation study was performed using sheep's nasal mucosa. The procedure was remaining same as that of *in vitro* drug release study.

Histological study

The nasal mucosa was fixed with 10% neutral carbonate-buffered formalin solution and later embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin. After 6 h of application of drug-loaded microspheres, sections were examined under microscope to detect any damage to tissue.

Stability studies

The batches of optimized microsphere formulation were stored at ambient accelerated conditions of temperature and humidity as per ICH guidelines.[10-12]

RESULTS AND DISCUSSION

The percentage yield of spray-dried and cross-linked formulations was found in the range of 40%-60% and 80%-90%, respectively [Table 3].

Percentage yield of formulation prepared by spray drying method was less as compared with cross-linking method. Increase in drug-to-polymer ratio slightly increases the size of microspheres. The average particle size of spray-dried and cross-linked formulations was found in the range of 20%-50% and 30%-60%, respectively [Table 3]. Average particle sizes of spray-dried microspheres were small as compared with cross-linked microspheres-such particles are considered to be suitable for nasal administration.

Spray-dried and cross-linked formulations were evaluated for micromeritic parameters, which showed good flow properties [Table 4].

Entrapment efficiency of spray-dried microsphere was found to be high as compared with that of cross-linked microspheres. Also entrapment efficiency was found proportional to drug-to-polymer ratio. Similar results were observed for swelling capacity [Table 5].

No interaction was absorbed as no addition peaks were reported in the IR spectra of physical mixture of drug and polymers. Sharp melting endotherm was found for pure drug Pirfenidone at 116°C, which is very similar to the reported melting point. In DSC of drug-loaded microsphere for spray drying and cross-linking method, endotherm was comparatively broad than pure drug and shows little shift in the melting point. This suggests encapsulation of drug in microsphere [Figure 1].

All the developed microsphere batches showed good release up to or more than 6 h, at varying compositions of chitosan and HPMC K4M. Drug release was found to be inversely proportional to polymer concentration used. At low levels of polymer, rapid release was found [Figures 2 and 3].

In vitro drug release data were fitted to different diffusion models, using PCP Disso software version 2.08. For optimized formulation for both the methods, that is, F1, respectively, *n* value was found to be 0.6, suggesting non-Fickian diffusion mechanism.

Further optimized formulation F1, was subjected to surface morphology. In SEM study, microsphere developed by spray drying process shows smooth surface and spherical shape, whereas microsphere of cross-linking methods were showing rough surface. Microsphere of spray drying was found to be small in size than that of cross-linking [Figure 4].

XRD of pure drug and drug-loaded microsphere by both methods were obtained. XRD pattern suggest changes in physical state of drug, from crystalline nature to amorphous form in that of drug-loaded microsphere. These changes were more prominent in the spray drying than cross-linking [Figure 5].

Statistical analysis of *in vitro* drug release data and *in vitro* mucoadhesion data illustrate model significance when done on Design Expert software version 8.0.7.1. 3D Surface plot

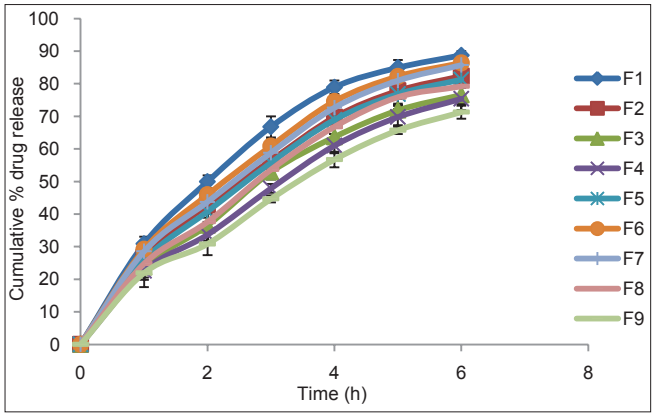

Figure 2: *In vitro* dissolution profile of F1–F9 (spray dried)

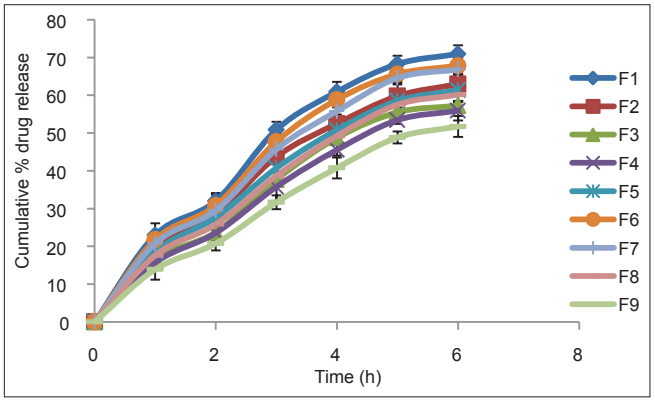

Figure 3: *In vitro* dissolution profile of F1–F9 (cross-linking)

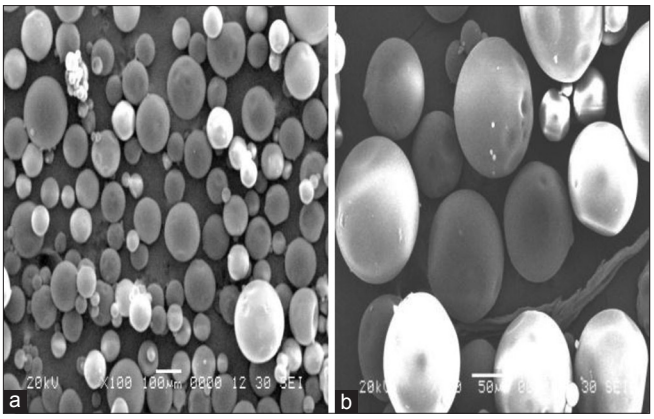

Figure 4: Scanning electron microscope photographs (a) spray drying method (b) cross-linking method

and counter plotted were obtained for microsphere prepared by spray drying method [Figures 6 and 7].

Ex vivo study using goat nasal mucosa was performed. The results obtained revealed more tissue permeation of microsphere prepared by spray drying method. More permeation may be because of small size of microsphere [Figure 8].

Histological study revealed [Figure 9] mucomimitic nature of developed microsphere.

From stability study of optimized batch, it was found that microspheres remained stable even after exposing to stress conditions of temperature [Table 6].

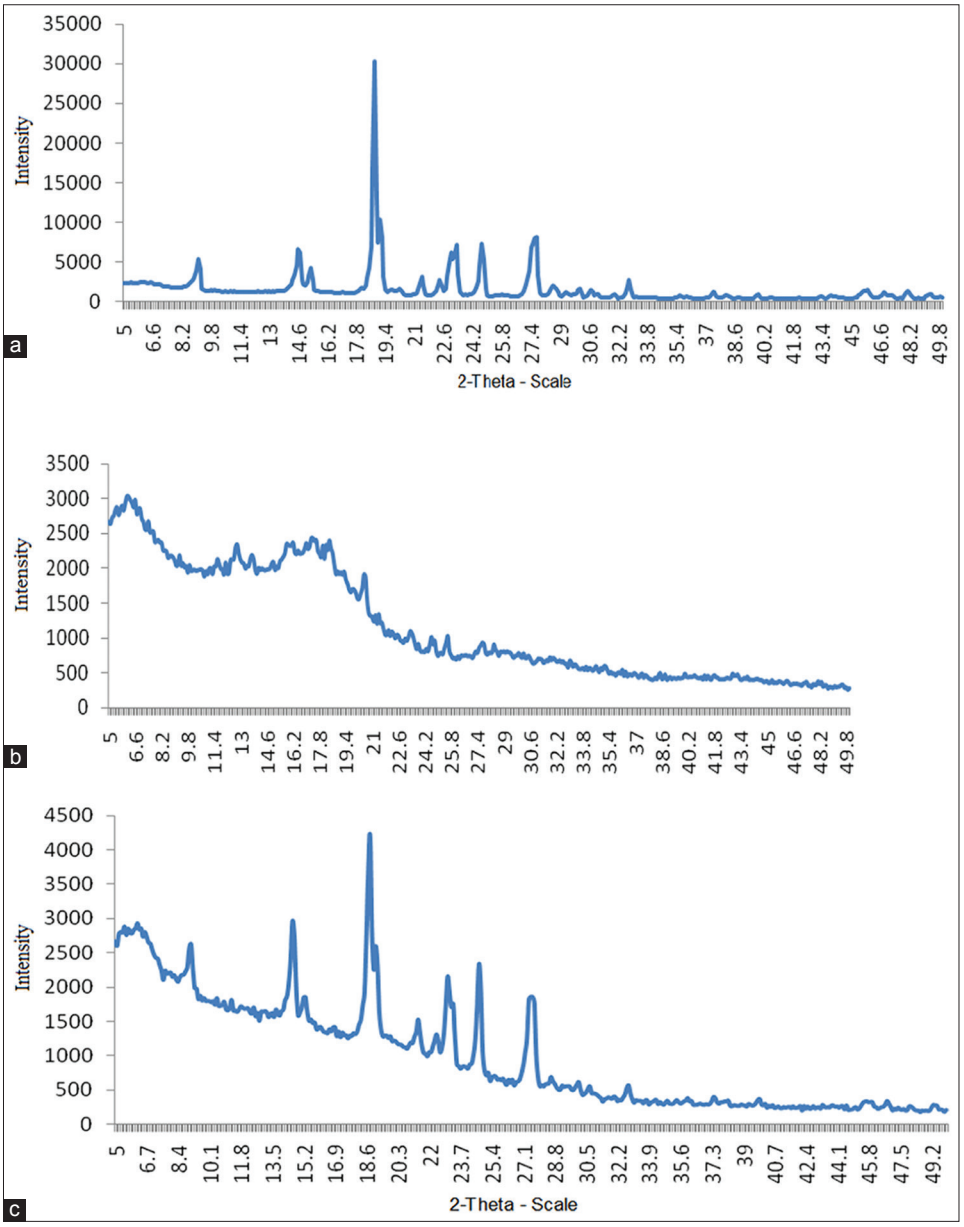

Figure 5: X-ray diffraction of pure drug pirfenidone; (a) drug-loaded microsphere (spray drying), (b) drug-loaded microsphere (cross-linking), (c) spray drying microsphere, shows more mucoadhesion than cross-linking on goat nasal mucosa [Table 5]

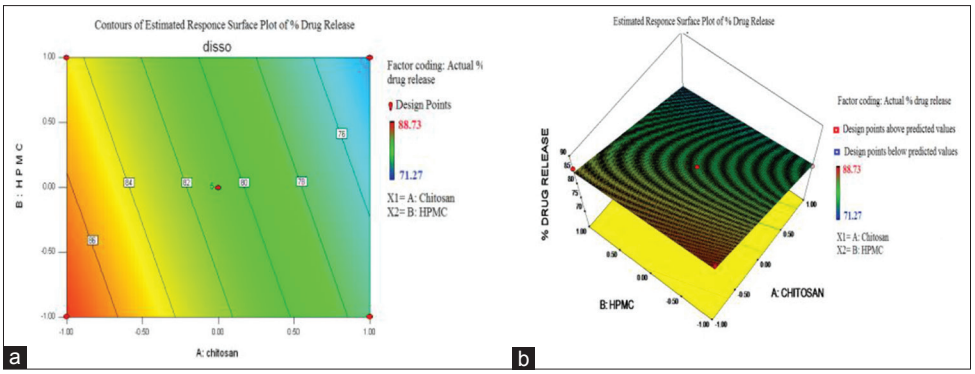

Figure 6: (a) Contour plots and (b) 3-D surface plot of spray-dried formulation for percentage drug release

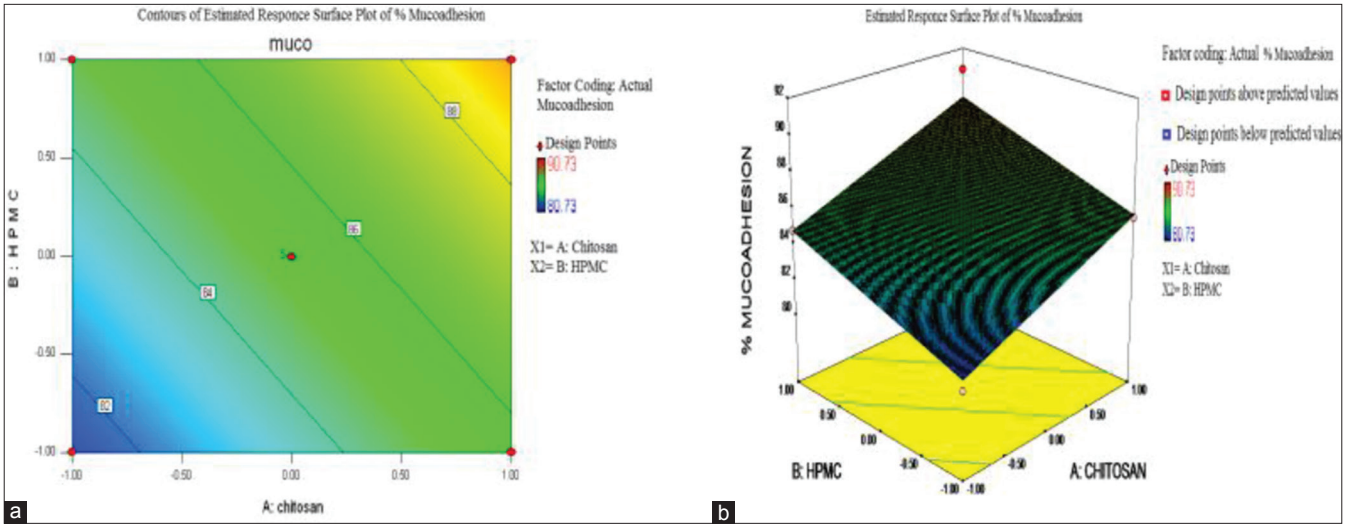

Figure 7: (a) Contour plots and (b) 3-D surface plot of spray-dried formulation for percentage mucoadhesion

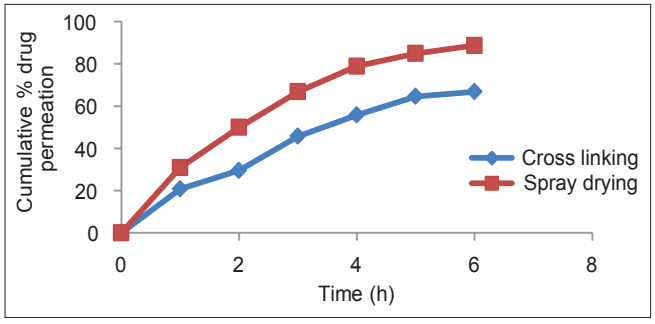

Figure 8: *Ex vivo* permeation profile of optimized formulation (F1)

CONCLUSION

Stable, effective nasal mucoadhesive microspheres of pirfenidone can be successfully developed by optimizing the concentrations of chitosan and HPMC K4M in order to achieve the desired controlled release characteristics for the treatment of idiopathic pulmonary fibrosis. The present study indicates optimized F1 spray-dried formulation as best as compared with optimized F1 cross-linked formulation.

Table 6: Stability data

Evaluation parameters	Storage time (30 days) (F1 batch)					
	Spray drying method			Cross-linking method		
	0	15	30	0	15	30
Mucoadhesion (%)	80.73	80.02	79.83	59.92	58.93	58.87
Drug release (%w/w)	88.73	87.37	85.23	70.93	68.71	65.43

REFERENCES

1. Kaurav H, Harikumar SL, Kaur A. Mucoadhesive microspheres as carriers in drug delivery: A review. Int J Drug Develop Res 2012;4:21-34.
2. Schaefer CJ, Ruhrmund DW, Pan L, Seiwert SD, Kossen K. Antifibrotic activities of pirfenidone in animal models. Eur Respir Rev 2011;20:85-97.
3. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, *et al*. CAPACITY Study Group. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): Two randomised trials. Lancet 2011;377:1760-9.
4. Macías-Barragán J, Sandoval-Rodríguez A, Navarro-Partida J, Armendáriz-Borunda J. The multifaceted role of pirfenidone and its novel targets. Fibrogenesis Tissue Repair 2010;3:16.
5. Raghu G, Johnson WC, Lockhart D, Mageto Y. Treatment of idiopathic pulmonary fibrosis with a new antifibrotic agent, pirfenidone: Results of a prospective, open-label phase II study. Am J Respir Crit Care Med 1999;159:1061-9.

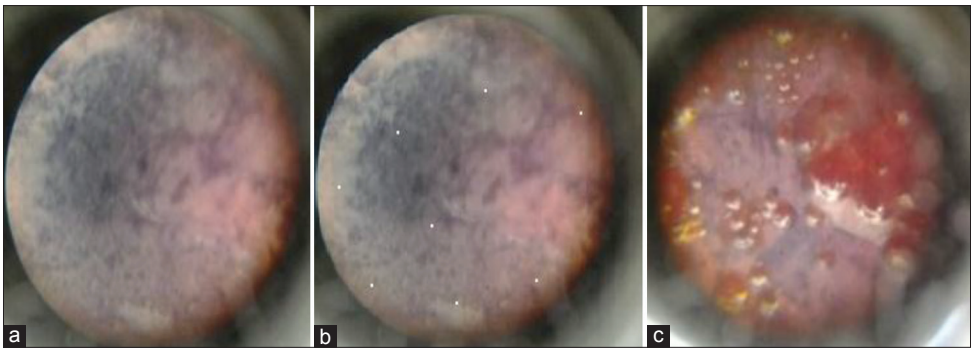

Figure 9: Microscopic images of nasal mucosa. (a) Control (normal). (b) F1 formulation treated for spray drying method. (c) F1 formulation treated for cross-linking method

6. Rathananand M, Kumar DS, Shirwaikar A, Kumar R, Kumar DS, Prasad RS. Preparation of mucoadhesive microspheres for nasal delivery by spray drying. Indian J Pharm Sci 2007;69:651-7.
7. Tadwee Imran AK, Shahi SR, Thube MW, Shaikh AM, Tribhuwan SD, Mahajan HS. Spray dried nasal mucoadhesive microspheres of carbamazepine: Preparation and *In vitro/Ex vivo* evaluation. Res Pharmaceutica 2011;2:23-32.
8. Shahi SR, Tribhuwan SD, Tadwee Imran AK, Gupta SK, Zadbuke NS, Shivanikar SS. Formulation of atenolol mucoadhesive microspheres for nasal delivery by spray drying technique: *In vitro/Ex vivo* evaluation. Der Pharmacia Sinica 2011;2:54-63.
9. Das MK, Senapati PC. Furosemide-loaded alginate microspheres prepared by ionic cross-linking technique: Morphology and release characteristics. Indian J Pharm Sci 2008;70:77-84.
10. Patel VR, Patel S, Tank HM, Jobanputra C, Brahma D. Formulate and evaluate the mucoadhesive microsphere of HMG co-A reductase inhibitor for the treatment of hyperlipidemia. Int J Pharm Res Bio Sci 2012;1:283-308.
11. Jain SK, Jain NK, Gupta Y, Jain A, Jain D, Chaurasia M. Mucoadhesive chitosan microspheres for non-invasive and improved nasal delivery of insulin. Indian J Pharm Sci 2007;69:498-504.
12. Yadav AV, Mote HH. Development of biodegradable starch microspheres for intranasal delivery. Indian J Pharm Sci 2008;70:170-4.

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