The spray-dried mucoadhesive microparticles of rizatriptan with chitosan and carbopol in migraine

Sachin Jadhav, Sagar Mishra

UDPS, Utkal University, Bhubaneshwar, 751004.

Correspondence to Sachin Jadhav, M.Pharm, UDPS, Utkal University, Bhubaneshwar, 751004. Tel: +91 7020885919; e-mail: srksmj@gmail.com

Received: 31 March 2022
Accepted: 2 May 2022
Published: 19 July 2022

Egyptian Pharmaceutical Journal 2022, 21:293–301

Background
The traditional oral formulation for migraine treatment has the drawbacks of first-pass metabolism, plasma-protein binding, and poor blood–brain-barrier penetration. This study was conducted to establish the nasal route of administration for rizatriptan formulations in migraine.
Materials and methods
Rizatriptan mucoadhesive microparticles were synthesized by spray-drying and evaluated for infrared spectroscopy, differential scanning calorimetry, and scanning electron microscopy. The *ex vivo* study was done with Franz's diffusion cell using goat nasal mucosa. The *in vivo* study was performed on the Albino rat's nasal route for determining drug concentration by high-performance liquid chromatography analysis in brain tissue at single-point evaluation.
Result
The microparticles were of optimum size with no drug–polymer interaction in infrared spectroscopy and differential scanning calorimetry. Scanning electron microscopy exhibited the morphology of spherical or ellipsoid microparticles with efficient drug entrapment. The percentage of drug permeability for chitosan microparticles was 76.53–91.09 and for carbopol microparticles was 78.49–92.25 in the ex vivo permeability study. In vivo studies showed that drug concentrations of 126.46–148.50% for chitosan batches and 152.83–165.04% for carbopol batches were superior to controls.
Conclusion
Ex vivo permeability study revealed drug-permeation patterns as high as 91.09 ±0.03% for RCH3 formulation and 92.25±0.2% for RC3 formulation. In *in vivo* study formulation, RCH3 displayed a drug concentration of 132.22±8.32% and RC3 showed 159.46±4.05% over the control batch, which is conclusive for improved drug delivery of rizatriptan through mucoadhesive microparticles for the nose-to-brain targeting in migraine.

Keywords:
microparticles, nose-to-brain drug delivery, spray-drying

Egypt Pharmaceut J 21:293–301

1687-4315

Introduction

Migraine is the third leading cause of disability in people under the age of 50 around the world [1]. Its been a quarter-century since the discovery of sumatriptan, the parent of triptans, in the last spring of migraine acute treatment. After that, the triptan family grew to include six more members, signaling the end of ergot derivatives. This triptan event marked a watershed moment for all primary headaches, which received unprecedented cultural and scientific attention. Following that, researchers focused on assessing the efficacy of the seven triptan brothers, either alone or in conjunction with NSAIDs [2]. The conventional oral formulation has the disadvantage of first-pass metabolism, plasma-protein binding, and limited penetration through the blood–brain barrier (BBB) (Fig. 1).

Various routes of administration have been tried for central nervous system disorders, but the major hurdle is the passing BBB. We have selected the nasal route of administration through the olfactory pathway as it can penetrate drugs in the brain as no BBB is involved [3].

Rizatriptan is a triptan medication that is used to treat migraine headaches. It is a 5-hydroxytryptamine1 receptor-subtype agonist that is selective. Rizatriptan stimulates 5-HT1 receptors on peripheral terminals of the trigeminal nerve that innervate cranial blood vessels, which may contribute to the antimigraine effect of rizatriptan in humans, in addition to inducing vasoconstriction. Following oral tablet administration, rizatriptan is swiftly and thoroughly 90% absorbed from the gastrointestinal system, with an

absolute bioavailability of 47% due to modest first-pass metabolism [4].

The advantage of establishing a nasal route over other routes is to avoid first-pass metabolism and also it is noninvasive compared with oral and intravenous routes, respectively [5]. In this research work, we have formulated spray-dried microparticles of rizatriptan using mucoadhesive polymers chitosan and carbopol 934 [6]. The prepared formulations were evaluated for preliminary properties, as well as some specific parameters to establish the nasal route of administration and the advantage of the formulation over other conventional formulations.

In comparison with conventional oral and other formulations, rizatriptan nasal mucoadhesive microparticles will be a promising dosage form because it provides advantages such as avoiding first-pass metabolism, no issue with plasma-protein binding, and importantly, penetration into the brain via the olfactory route, which avoids the BBB.

Materials and methods

Chemicals and reagents: rizatriptan was purchased from Lab India Ltd, Mumbai, chitosan was procured from India Sea Foods, Cochin, carbopol 934 was obtained from Lab India Ltd, ethanol was purchased from Loba Chemie Pvt Ltd, Mumbai, and high-performance liquid chromatography (HPLC)-grade methanol purchased from Merck Pvt Ltd, Mumbai, India. The remaining reagents used were of analytical grade.

Formulations: formations were created using the following ratios of rizatriptan to the polymers chitosan and carbolpol 934, with a unique formulation code assigned to each batch (Table 1).

Preparation of spray-dried microparticles: the spray-drying process was used to create the formulations [7]. To produce batches with varying drug–polymer ratios, a spray dryer from Techno Search Instruments, model SPD-D-111, was used [8]. For all batches, the flow rate was kept constant at 1 ml/min, and the temperature range was kept between 70 and 100°C. The suspensions of drug and polymer were prepared in a 1 : 1 ratio of solvent water and ethanol with nozzle adjustment to get a particle-size range between 5 and 50 μm.

Characterization of microparticles

Appearance: all the batches of formulation were characterized for some primary parameter like color and the results were recorded for different batches of formulation.

Particle-size analysis: all the batches of formulation were characterized for particle size using optical microscopy and average particle size was determined for all the batches [9].

Percentage yield: all the formulations were used to find out the percentage yield after spray-drying using the formula:

$$\text{Percentage Yield} = \frac{\text{Actualyield}}{\text{theoreticalyield}} \times 100\%.$$

Drug-content determination: drug-content determination was done using ultraviolet (UV) spectroscopy on Shimadzu UV–vis spectrophotometer model UV-3600i [10]. The formulations of rizatriptan were dissolved in methanol and dilutions were made using distilled water. The readings for formulation were taken at λ_{max} 228 nm [11]. The drug content was calculated using a calibration curve of rizatriptan in the lambert bear range of 1–10 μg/ml concentration.

Infrared spectroscopy: infrared spectroscopy was carried out on the Bruker alpha ATR instrument. About 1 mg for sample from the formulation batches was taken for analysis. The selected formulations RCH4 and RC3 were used to study IR spectroscopy [12].

Differential scanning calorimetry: for differential scanning calorimetry, the Mettler Toledo instrument was utilized. Analysis was performed using an aluminum sample holder with a temperature range of 25–300°C. The selected formulations RCH2 and RC3 were analyzed for differential scanning calorimetry [13].

Field-emission scanning electron microscopy: particle size and morphology of microparticles were analyzed by field-emission scanning electron microscopy that was performed on the instrument FEI Nova NanoSEM 450. The instrument was having an ultrahigh resolution of 1.0 nm at 15 kV, 1.4 nm at 1 kV, and 1.8 nm at 3 kV and 30 Pa. The 1-mg sample was first plated with gold particles and then the sample was mounted on carbon-conductive adhesive tape on the sample holder. The analysis was carried out at various resolutions to observe microparticle size and morphology [14].

Ex vivo permeability study: permeability study is an important evaluation to justify drug absorption through nasal membrane, hence using Franz's diffusion-cell study was carried out. The Franz's diffusion cell with a capacity of 50 ml was utilized, and for the membrane, the fresh nasal mucosa of a goat was obtained from the wet market. The optimized two formulations from each polymer chitosan and carbopol 934 were taken for further study of diffusion [15].

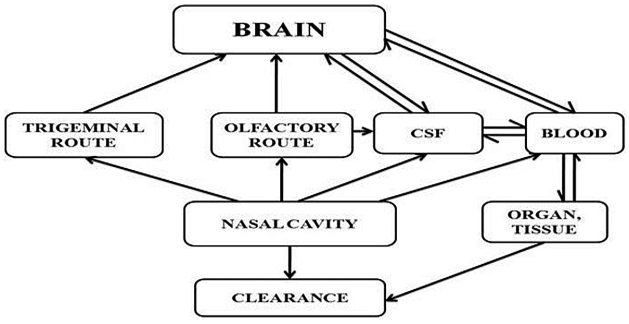
Phosphate buffer with pH 6.5 was prepared as a solvent in the receptor compartment using potassium dihydrogen phosphate and dipotassium hydrogen phosphate [16]. A sample equivalent to 4 mg of drug rizatriptan was taken from each formulation and used in the donor compartment placed on the nasal mucosal membrane of the goat [17]. After placing the sample, the diffusion study was initiated and 1-ml aliquots were taken from each sample at time intervals of 5, 10, 15, 30, 45, 60, and 90 min. The diluted samples were analyzed with a UV spectrophotometer using 228 nm as λmax [18].

In vivo studies

Ethical treatment: the in vivo studies were performed as per the CPCSEA guidelines.

The animal study protocol was duly approved by the CPCSEA Approved Drug Testing Laboratory with registration 1410/c/11/CPCSEA. The CPCSEA/IAEC number given for the *in vivo* study is CPCSEA/IAEC/0220/156.

Figure 1



Drug distribution through various routes in the brain.

Animal model method: in-bred Albino rats were used for the experiment. The animals were housed in individually ventilated cage systems in noise-controlled and temperature-controlled rooms. Animals were fed on a standard pellet diet and water ad libitum [19].

Test samples were diluted in PBS at a concentration of 50 mg/ml. About 20-µl solutions were instilled in each nostril of the animal and allowed to stand for 15 min. The animals were sacrificed 15 min postadministration and brains were dissected and kept on ice. The brains were homogenized (Remi) in PBS at a concentration of 100 mg/ml [20]. Homogenates were centrifuged at 5000 rpm (Remi) and the supernatant was collected. The supernatants were precipitated by methanol (500-µl homogenate +50-µl methanol). The samples were centrifuged and the supernatant was used for HPLC analysis.

HPLC analysis: the HPLC analysis was carried out on Shimadzu HPLC model LC-2050. The column utilized was a C-18 column of 250-mm length, 4.6-mm internal diameter, and 5-µm particle sizes were used in the analysis [21,22]. For rizatriptan isocratic mode with sodium dihydrogen orthophosphate as a buffer (pH 3.5): acetonitrile (80 : 20), a flow rate of 1.0 ml/min at ambient temperature. Quantification was attained with UV detection at a wavelength of 228 nm [23].

Results

Appearance, particle size, % yield, and drug content

The rizatriptan and chitosan batches were observed to be yellowish in appearance, while rizatriptan and carbopol 934 appeared to be white. The particle-size range of chitosan formulations was found between 17 and 48 µm, while carbopol 394 batches were between 20 and 49 µm. The mean particle size for chitosan formulations was observed between 19.4 and 29.1 µm and for carbopol 29.7 and 35.8 µm. The percentage yield for chitosan formulations was ranging between 63.57–73.26% and 52.94–69.40% for carbopol 934. The drug content was also calculated using UV spectrophotometry and it was observed between

Table 1 Formulation design

Polymer	Chitosan				Carbopol 934			
Drug	Rizatriptan				Rizatriptan			
Formulation code	RCH1	RCH2	RCH3	RCH4	RC1	RC2	RC3	RC4
Drug–polymer ratio	0.5 : 1	1 : 1	1 : 1.5	1 : 0.5	0.5 : 1	1 : 1	1 : 1.5	1 : 0.5
Drug (mg)	500	1000	1000	1000	500	1000	1000	1000
Polymer (mg)	1000	1000	1500	500	1000	1000	1500	500

89.65 and 98.20% for chitosan formulations and 91.83 and 96.67% for carbopol 934 formulations (Table 2).

Infrared spectroscopy

The formulations RCH4 and RC3 were used to study IR spectroscopy. After coinciding both spectra, it was observed that the most of characteristic peaks of the drug rizatriptan functional group were intact. In RCH4 formulation, the peaks were observed at 3254 cm^{-1} (N–H primary), 3201 cm^{-1} (N–R tertiary), 1704 cm^{-1} (C=O), 1692 cm^{-1} (C=N), 1639 cm^{-1} (C=C), and 1612 cm^{-1} (C=C). In RC3 formulations, peaks were observed at 3297 cm^{-1} (N–H primary), 3281 cm^{-1} (N–H primary), 3261 cm^{-1} (N–R tertiary), 1696 cm^{-1} (C=O), 1638 cm^{-1} (C=C), and 1625 cm^{-1} (C=C).

Differential scanning calorimetry

Differential scanning calorimetry was performed on RCH2 and RC3 formulations. The curves obtained exhibit characteristic endothermic peaks of rizatriptan in both formulations RCH2 and RC3. The initial broad peak was first observed at 60°C in both the formulations RCH2 and RC3, this peak was more prominent in chitosan formulation RCH3. The characteristic peak of the melting point of rizatriptan was observed as a broad peak at 180°C in RC3, that is, carbopol 934 formulations. The melting-point peak of rizatriptan was not found prominently seen in chitosan formulation RCH3.

Field-emission scanning electron microscopy

The analysis was carried out at various resolutions to observe microparticle size and external morphology. It was observed that the particle size was in the range of 5–50 μm, which was needful for the nasal drug delivery of microparticles as more than 50-μm size will not allow the particle to adhere to the mucosal surface, while less than 5-μm size may take microparticles to the lung. The morphology was also observed as spherical or ellipsoid particles. With higher resolutions, we can observe the drug entrapped within the polymers.

Ex vivo permeability study: permeability study is an important evaluation to justify drug absorption through nasal membrane, hence, using Franz's diffusion-cell study was carried out. The formulations from each polymer chitosan and carbopol 934 were taken for this study.

The absorbance using a UV spectrophotometer was taken to find out the percentage of drug permeability from each formulation. Permeability kinetic equations were studied to find out possible drug-release models. The % drug permeability for chitosan batches was found to be between 76.53 and 91.09 and the RCH3 formulation was with the highest 91.09 ±0.25% of drug permeability. The % drug permeability for carbopol 934 batches was found to be between 78.49 and 92.25 and the RC3 formulation was with the highest 92.25±0.25% of drug permeability.

The drug-permeability kinetics was studied and applied with the Korsmeyer–Peppas model, and it was observed that the regression value was less than 1 in all the formulations of chitosan and carbopol 934 [24] (Fig. 2 and Table 3).

In vivo studies

Inbred Albino rats were utilized for the study. The homogenized brains in PBS were centrifuged and the supernatant was collected. The supernatants were precipitated by centrifugation and the supernatant was used for HPLC analysis.

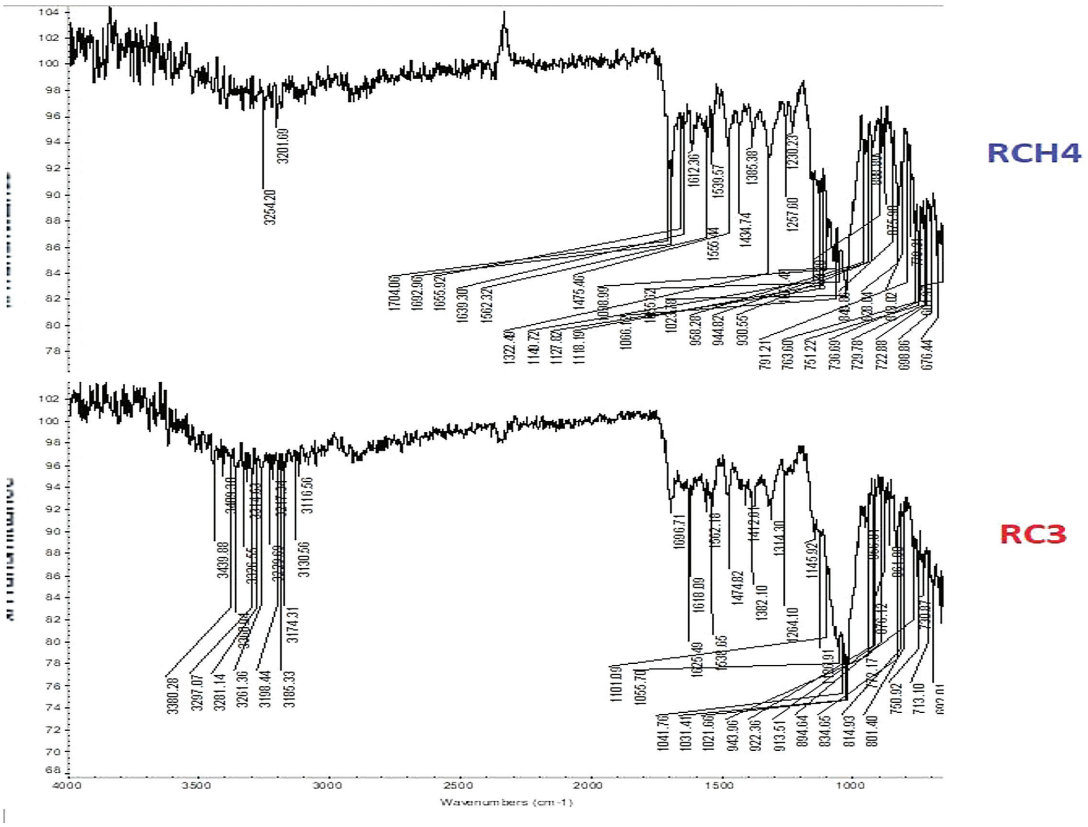
The calibration of rizatriptan was done on HPLC using sodium dihydrogen orthophosphate as a buffer (pH 3.5): acetonitrile (80 : 20), a flow rate of 1.0 ml/min at ambient temperature, and area under curve of 35698 was obtained for 0.01 μg/ml concentrations, which were utilized as a standard for further calculations. The area under curve observed for optimized formulations RCH3 and RC3 was obtained by the HPLC method.

The homogenized supernatants obtained after centrifugation were analyzed on HPLC to find out concentrations of drug in brain homogenate. The concentrations of drug in brain-tissue homogenate after 15 min were determined, and single-point

Table 2 Preliminary characterization of formulations

Batch Color	RCH1 Yellowish	RCH2 Yellowish	RCH3 Yellowish	RCH4 Yellowish	RC1 White	RC2 White	RC3 White	RC4 White
Particle-size range (μm)	17–42	25–48	23–43	28–46	25–45	23–38	25–49	20–39
Mean size (μm)	23.4	29.1	27.6	19.4	30.2	35.6	29.7	35.8
% yield	73.26	65.89	63.57	68.49	53.79	69.40	56.94	52.94
Drug content	89.65	98.20	96.84	91.59	96.67	95.82	96.51	91.83

Figure 2



Drug-permeability pattern of chitosan formulations and carbopol 934 formulations.

Table 3 Permeability kinetics for all formulations

Formulation	Release-curve equation	R^2
RCH1	y=0.7222x+19.622	0.9692
RCH2	y=0.6628x+28.934	0.9055
RCH3	y=0.7672x+28.056	0.9599
RCH4	y=0.6786x+18.614	0.9733
RC1	y=0.7037x+20.527	0.9832
RC2	y=0.732x+26.398	0.943
RC3	y=0.7795x+27.31	0.9457
RC4	y=0.7971x+13.441	0.9593

detection at 15 min was carried out as we need to study the faster onset of action through this route. Also, the mucociliary clearance time was generally observed for up to 20 min, the estimations must be done within that time window (Table 4).

The concentration of rizatriptan for a pure drug as control and selected formulations RCH3 and RC3 was obtained from the in vivo study. The chitosan formulation RCH3 was having 132.22±8.32% more drug concentration in brain tissue than that of pure drug. Also, the carbopol 934 formulation RC3 exhibited as high as 159.46±4.05% more drug concentration compared with pure drug rizatriptan (Fig. 3).

Discussion

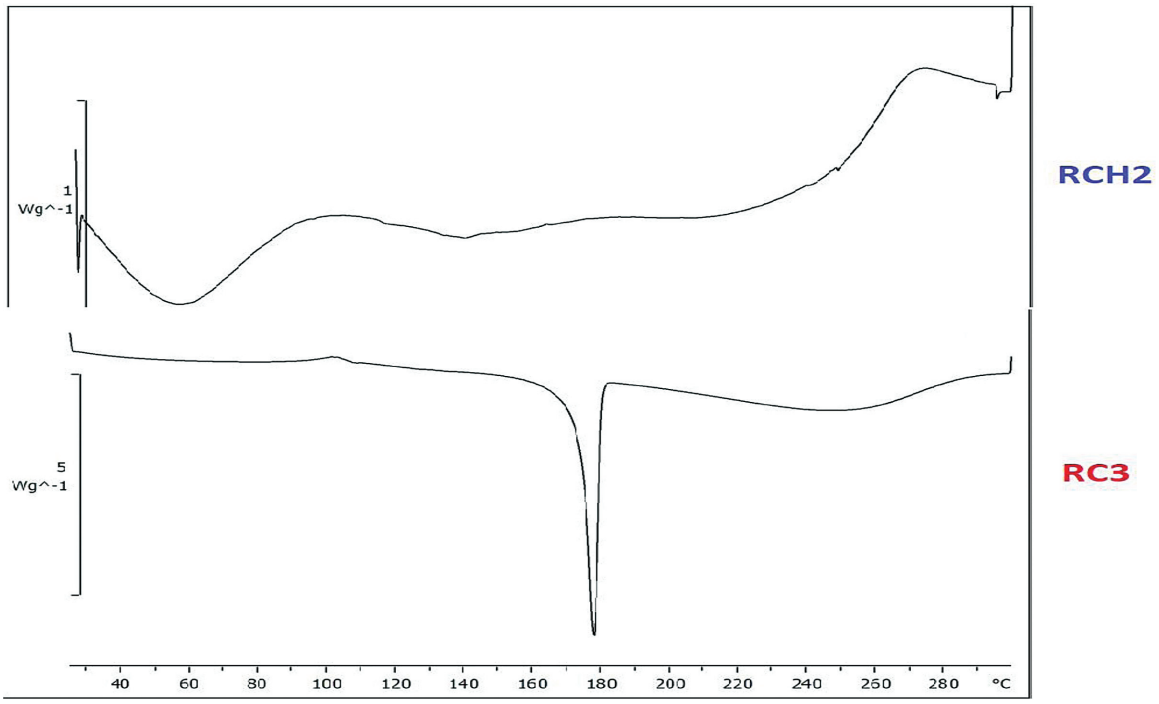
Central nervous system illnesses such as migraine, Alzheimer's disease, epilepsy, bipolar disorders, and a few others are on the rise as a result of stress and a variety of other variables. The majority of these illnesses' therapies rely on traditional dose forms such as tablets, capsules, and, in certain cases, parenteral. Because these traditional dosage forms have drawbacks such as first-pass metabolism, protein binding, and limited penetration into the

Table 4 High-performance liquid chromatography analysis for selected formulations

Formulation code	1	2	3	4	5	Average	SD
R	0.01000	0.01021	0.00967	0.00999	0.01024	0.01002	0.00023
RCH3	0.01271	0.01303	0.01436	0.01313	0.01295	0.01324	0.00065
RC3	0.01595	0.01652	0.01596	0.01580	0.01565	0.01598	0.00033

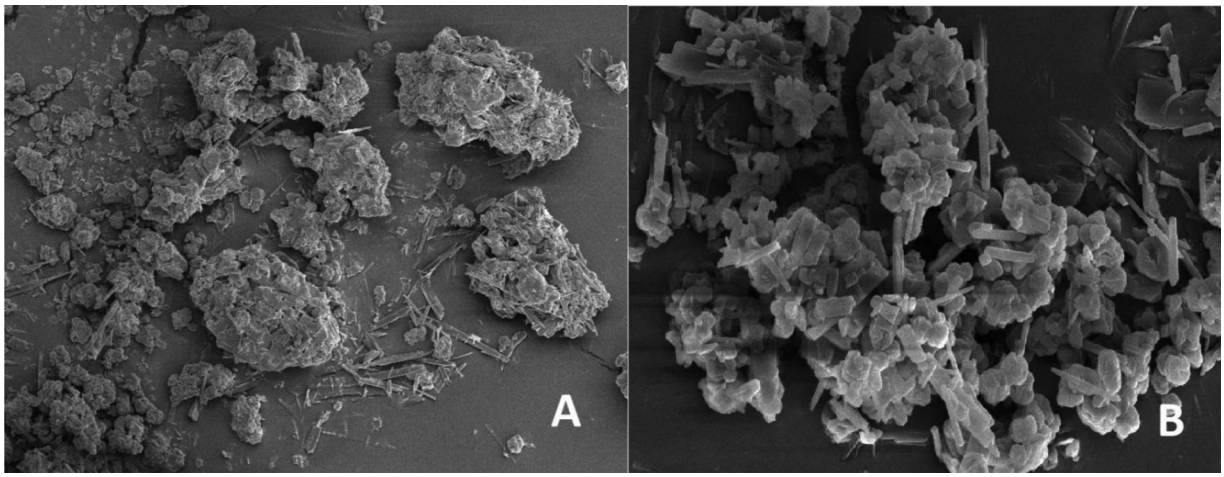
All concentrations are in μg/ml.

Figure 3



Concentrations of rizatriptan in brain tissue.

Figure 4



Infrared spectroscopy of RCH4 and RC3 formulations.

brain, they must be replaced with enhanced dosage forms utilizing novel routes of administration. This study was focused on establishing nose-to-brain route of administration, especially through the olfactory area as it is not having a BBB. The nasal route has the advantage of faster penetration of drugs in brain tissue even to the parenteral route, which was concluded at the end of this study.

The spray-dried microparticles developed had particle sizes ranging from 5 to 50 μm, which were suitable for administration to the nasal cavity [25]. Most formulations had promising percentage yields and drug content, and the formulations with the best findings were picked for further testing.

Following infrared spectroscopy as shown in Fig. 4, it was discovered that the rizatriptan peaks were either intact or slightly shifted by polymers, with no significant change in the drug's characteristics, indicating that there is no probable drug–polymer interaction in the formulations [26].

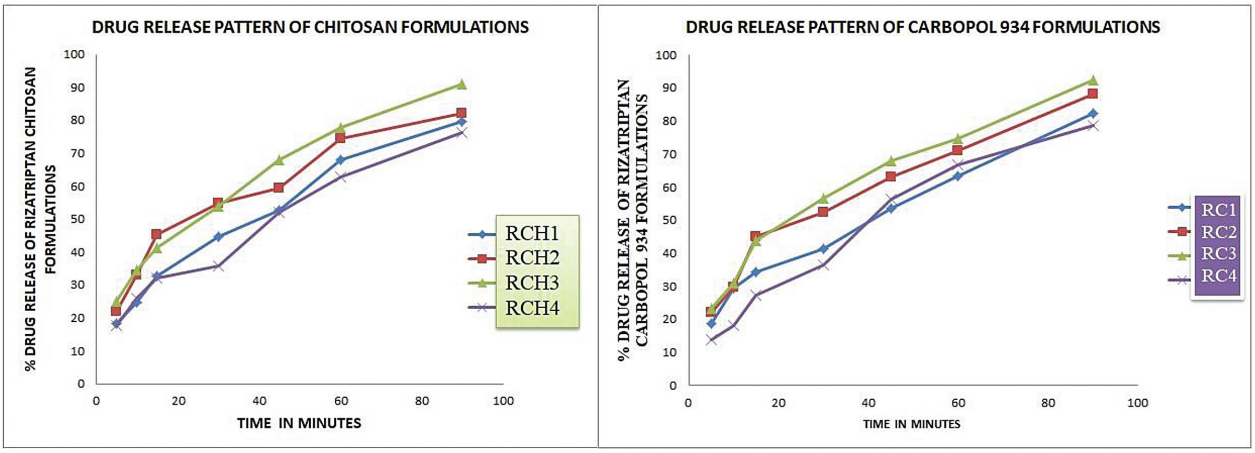
RCH2 and RC3 were analyzed using DSC to determine the physical condition of the drug in microparticles. The results are displayed in Fig. 5. The DSC thermogram of RC3 showed a sharp endothermic peak at 180°C due to the melting of the drug. The drug peak for rizatriptan-loaded microparticles was not visible in the thermogram of RCH2, indicating that rizatriptan was molecularly distributed inside the microparticles [27].

Field-emission scanning electron microscopy photographs as shown in Fig. 6 exhibited regular spherical or ellipsoid morphology for RCH3 formulation and no free drug was present. At higher resolution, the drug was found to be entrapped inside the polymer network with a very little exception for needle-like structures [28].

When the drug-release kinetics were analyzed and used with the Korsmeyer–Peppas model, it was discovered that the regression value was smaller than 1 in all chitosan and carbopol 934 formulations. The *n* value for formulations was recorded as more than 0.5, which represents the fickian type of release pattern. The polymer relaxation time and solvent diffusion time were observed to be closer, hence fickian release pattern was followed [29].

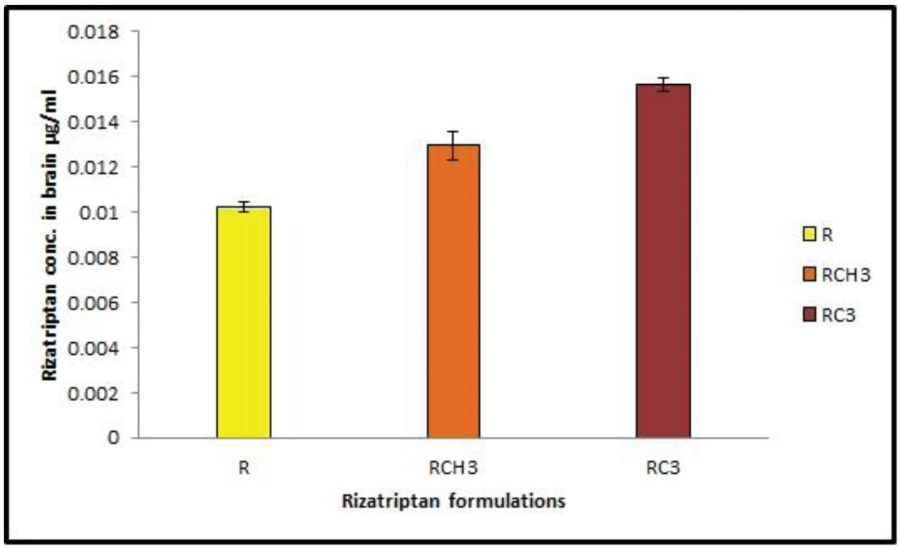
The purpose of the *in vivo* study was to establish the nose-to-brain route of administration for rizatriptan in

Figure 5



Differential scanning calorimetry of RCH2 and RC3 formulations.

Figure 6



The field-emission scanning electron microscopy of RCH3 formulation. (a) Particles with size range 5–50 µm. (b) Drug found to be embedded in the polymer after spray-drying.

migraine. *In vivo* study was also performed to observe the absorption of spray-dried formulations of rizatriptan in comparison with pure drug rizatriptan as a control [30].

The chitosan formulation RCH3 was having 132.22 ±8.32% more drug concentration in brain tissue than that of pure drug. Also, the carbopol 934 formulation RC3 exhibited as high as 159.46±4.05% more drug concentration compared with pure drug rizatriptan. This represents the efficaciousness of spray-dried formulations and we can utilize these formulations for enhanced drug delivery of rizatriptan from nose-to-brain route of administration in migraine.

The study establishes the usefulness of nose-to-brain microparticles, which is a powder formulation for patients with migraine [31]. Because few devices can give such a dosage form at the moment, device development for such formulations is the future focus of the research. Moreover, due to the small surface area of the nasal cavity, drugs with small doses can only be utilized for this kind of formulation.

Patient compliance will be crucial for nasal formulations in the future since they are easier to administer than other dosage forms and do not require specific medical help. They are considered superior to parenteral dosage forms in terms of patient compliance [32].

Conclusion

The morphology and drug entrapment of the formulation with the polymer was confirmed with field-emission scanning electron microscopy. The particle size was found to be between 5 and 50 μm, which is a necessary parameter for successful nasal drug delivery.

Ex vivo permeability study showed excellent drug-permeation patterns with as high as 91.09±0.25% for RCH3 formulation, and 92.25±0.25% for RC3 formulation, which concludes the efficacy of the formulations in terms of drug permeation. The permeation kinetic was observed to be following quasi-fickian diffusion model with an n value less than 0.5.

In vivo animal study exhibited encouraging results as it showed a higher concentration of drug rizatriptan in brain tissue for the formulations in comparison with pure drug as control after homogenization and HPLC analysis. The formulation RCH3 exhibited a drug concentration of 132.22±8.32% and RC3 showed 159.46±4.05% over the control batch, which is conclusive for enhanced drug delivery of drugs using these prepared formulations for the nose–brain drug delivery in migraine.

Thus, these formulations of rizatriptan synthesized by spray-drying using chitosan and carbopol were found to be efficacious for the nose-to-brain drug delivery and can prove beneficial for migraine patients in an emergency due to ease of administration and faster onset of action compared with oral formulations available in the market.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Martelletti P. Acute treatment of migraine: quo vadis?. Expert Opin Pharmacother 2017; 18:1035–1037.

2 Jeremic D, Jiménez-Díaz L, Navarro-López JD., Past, present and future of therapeutic strategies against amyloid-β peptides in Alzheimer's disease: a systematic review. Ageing Res Rev 2021; 72:101496.

3 Pires PC, Santos AO. Nanosystems in nose-to-brain drug delivery: a review of non-clinical brain targeting studies. J Control Release 2018; 270:89–100.

4 Rajendra V, Khedkar A, Kulkarni A, Dehghan MH, Saifee M, Lahoti S. Spectrophotometric estimation of rizatriptan benzoate. Asian J Res Chem 2010; 3:175–177.

5 Mignani S, Shi X, Karpus A, Majoral JP. Non-invasive intranasal administration route directly to the brain using dendrimer nanoplatforms: an opportunity to develop new CNS drugs. Eur J Med Chem 2021; 209:112905.

6 Porfiryeva NN, Nasibullin SF, Abdullina SG, Tukhbatullina IK, Moustafine RI, Khutoryanskiy VV. Acrylated Eudragit® E PO as a novel polymeric excipient with enhanced mucoadhesive properties for application in nasal drug delivery. Int J Pharm 2019; 562:241–248.

7 Kulkarni AD, Bari DB, Surana SJ, Pardeshi CV. In vitro, ex vivo, and in vivo performance of chitosan-based spray-dried nasal mucoadhesive microspheres of diltiazem hydrochloride. J Drug Deliv Sci Technol 2016; 31:108–117.

8 Díaz AG, Quinteros DA, Llabot JM, Palma SD, Allemandi DA, Ghersi G, *et al.* Spray-dried microspheres based on chitosan: a promising new carrier for intranasal administration of polymeric antigen BLSOmp31 for prevention of ovine brucellosis. Mat Sci Eng 2016; 62:489–496.

9 Barth T, Lecrivain G, Hampel U. Particle deposition study in a horizontal turbulent duct flow using optical microscopy and particle size spectrometry. J Aerosol Sci 2013; 60:47–54.

10 Tfelt-Hansen P, De Vries P, Saxena PR. Triptans in migraine. Drugs 2000; 60:1259–1287.

11 Asiri YA, Mostafa GA.. Donepezil Prof Drug Sub Excipients Relat Methodol 2010; 35:117–150.

12 Yuksel N, Baykara M, Shirinzade H, Suzen S. Investigation of triacetin effect on indomethacin release from poly (methyl methacrylate) microspheres: evaluation of interactions using FT-IR and NMR spectroscopies. Int J Pharm 2011; 404:102–109.

13 Sipos P, Szabó A, Er☒s I, Szabó-Révész P. A DSC and Raman spectroscopic study of microspheres prepared with polar cosolvents by different techniques. J Thermal Anal Calorimetry 2008; 94:109–118.

14 Kong M, Chen XG, Liu CS, Liu CG, Meng XH. Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli*. Coll Surf B Biointerf 2008; 65:197–202.

15 Ng SF, Rouse JJ, Sanderson FD, Meidan V, Eccleston GM. Validation of a static Franz diffusion cell system for in vitro permeation studies. AAPS PharmSciTech 2010; 11:1432–1441.

16 Washington N, Steele RJ, Jackson SJ, Bush D, Mason J, Gill DA, *et al.* Determination of baseline human nasal pH and the effect of intranasally administered buffers. Int J Pharm 2000; 198:139–146.

17 Praveen A, Aqil M, Imam SS, Ahad A, Moolakkadath T, Ahmad FJ. Lamotrigine encapsulated intra-nasal nanoliposome formulation for epilepsy treatment: formulation design, characterization, and nasal toxicity study. Coll Surf B Biointerf 2019; 174:553–562.

18 Salehi S, Boddohi S. New formulation and approach for a mucoadhesive buccal film of rizatriptan benzoate. Prog Biomater 2017; 6:175–187.

19 Illum L. Nasal delivery. The use of animal models to predict performance in man. J Drug Target 1996; 3:427–442.

20 Li W, Zhou Y, Zhao N, Hao B, Wang X, Kong P. Pharmacokinetic behavior and efficiency of acetylcholinesterase inhibition in rat brain after intranasal administration of galanthamine hydrobromide loaded flexible liposomes. Environ Toxicol Pharmacol 2012; 34:272–279.

21 Łabuzek K, Suchy D, Gabryel B, Bielecka A, Liber S, Okopień B. Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide. Pharmacol Rep 2010; 62:956–965.

22 Mercolini L, Mandrioli R, Iannello C, Matrisciano F, Nicoletti F, Raggi MA. Simultaneous analysis of diazepam and its metabolites in rat plasma and brain tissue by HPLC-UV and SPE. Talanta 2009; 80:279–285.

23 Pappa H, Farru R, Vilanova PO, Palacios M, Pizzorno MT. A new HPLC method to determine Donepezil hydrochloride in tablets. J Pharm Biomed Anal 2002; 27:177–182.

24 Costa P, Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001; 13:123–133.

25 Pereswetoff-Morath L. Microspheres as nasal drug delivery systems. Adv Drug Deliv Rev 1998; 29:185–194.

26 Jose S, Ansa CR, Cinu TA, Chacko AJ, Aleykutty NA, Ferreira SV, Souto EB. Thermo-sensitive gels containing lorazepam microspheres for intranasal brain targeting. Int J Pharm 2013; 441:516–526.

27 Mahajan HS, Gattani SG. Nasal administration of ondansetron using a novel microspheres delivery system. Pharm Dev Technol 2009; 14:226–232.

28 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.

29 Jain SA, Chauk DS, Mahajan HS, Tekade AR, Gattani SG. Formulation and evaluation of nasal mucoadhesive microspheres of Sumatriptan succinate. J Microencapsul 2009; 26:711–721.

30 Gao Y, Almalki WH, Afzal O, Panda SK, Kazmi I, Alrobaian M, *et al.* Systematic development of lectin conjugated microspheres for nose-to-brain delivery of rivastigmine for the treatment of Alzheimer's disease. Biomed Pharmacother 2021; 141:111829.

31 Salade L, Wauthoz N, Vermeersch M, Amighi K, Goole J. Chitosan-coated liposome dry-powder formulations loaded with ghrelin for nose-to-brain delivery. Eur J Pharm Biopharm 2018; 129:257–266.

32 Ruby JJ, Pandey VP. Antiemetic drugs as a nasal drug delivery – a review. Int J Pharm Sci Res 2014; 5:1624.