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Research Article

Nanosuspension Based *In Situ* Gelling Nasal Spray of Carvedilol: Development, *In Vitro* and *In Vivo* Characterization

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Abstract. The objective of the present investigation was to develop *in situ* gelling nasal spray formulation of carvedilol (CRV) nanosuspension to improve the bioavailability and therapeutic efficiency. Solvent precipitation–ultrasonication method was opted for the preparation of CRV nanosuspension which further incorporated into the *in situ* gelling polymer phase. Optimized formulation was extensively characterized for various physical parameters like *in situ* gelation, rheological properties and *in vitro* drug release. Formation of *in situ* gel upon contact with nasal fluid was conferred *via* the use of ion-activated gellan gum as carrier. *In vivo* studies in rabbits were performed comparing the nasal bioavailability of CRV after oral, nasal, and intravenous administration. Optimized CRV nanosuspension prepared by combination of poloxamer 407 and oleic acid showed good particle size [*d* (0.9); 0.19 μm], zeta potential (+10.2 mV) and polydispersity (span; 0.63). The formulation containing 0.5% *w/v* gellan gum demonstrated good gelation ability and desired sustained drug release over period of 12 h. *In vivo* pharmacokinetic study revealed that the absolute bioavailability of *in situ* nasal spray formulation (69.38%) was significantly increased as compared to orally administered CRV (25.96%) with mean residence time 8.65 h. Hence, such *in situ* gel system containing drug nanosuspension is a promising approach for the intranasal delivery in order to increase nasal mucosal permeability and *in vivo* residence time which altogether improves drug bioavailability.

KEY WORDS: bioavailability; Carvedilol; *in situ* gel; intranasal; nanosuspension.

INTRODUCTION

Carvedilol (CRV), (±)-1-(carbazol-4-yloxy)-3-[[2-(*o*-methoxyphenoxy)ethyl] amino]-2-propanol is an α_1, β_1, and β_2 adrenergic receptor antagonist used to treat high blood pressure and heart failure (1). CRV is practically insoluble in water and exhibits pH-dependent solubility. CRV is rapidly absorbed after oral administration from the gastrointestinal tract (80%) with low bioavailability (25–35%) due to significant first-pass hepatic metabolism by cytochrome P-450 and short plasma half-life of 6 h (2). Such low oral bioavailability and short plasma half-life may lead to poor patient compliance due to increased frequency of administration during long-term treatment of disease. Therefore, development of alternative route for its administration is the major need to overcome such obstacles. The present investigation focused on exploring nasal route for administration of CRV.

In the field of drug delivery technology, nasal route of drug administration has attracted considerable attention since the last decade because of various advantages offered by it (3). One of the main distinct advantages of nasal route is avoidance of pre-systemic metabolism, as it avoids metabolism due to hepatic first-pass effect, intestinal enzyme inactivation, and gastric acid degradation (4). The presence of rich vasculature at the nasal route as compared to oral route gives rapid drug absorption as compared to latter. Such distinct advantages of nasal route make it a potential route for administering many small molecular weight drugs, protein and peptides (5). Apart from such pharmacokinetic benefits, nasal route also offers various advantages which will improve patient compliance like self-administration, no pain at site of administration, and no trained personnel requirement (6).

However, intranasal drug delivery system has some limitations such as rapid mucociliary clearance of formulation that determines decrease in drug concentration at the site of absorption and minimum surface area of nasal mucosa as compared to oral mucosa for drug absorption (7,8). To alleviate the problem of rapid clearance, various formulation strategies were employed like mucoadhesive systems (9), *in situ* gelling formulations (10) which prolong the contact time between the nasal mucosa and drug formulation. In the present investigation, this is achieved by *in situ* gelling nasal spray formulation containing gellan gum as ion-activated carrier. The developed formulation will be administered as low viscosity solution in the form of spray into the nasal cavity. In contact with the nasal fluid, mist of solution is converted into sprayed-gel on nasal mucosa. Such technology will not only prolong the

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contact time between drug and nasal mucosa but also sustained the drug release over prolong period. In addition, such *in situ* gelling nasal spray formulation offers various advantages like combination of precise doses of medication with broader distribution throughout the nasal mucosa which gives amplified bioavailability and simple usability since medication can be taken from any position at any time without skill.

Due to low permeability and minimum surface area of nasal mucosa, low drug absorption was observed through this route. This problem was tackled by use of penetration enhancers from different classes like surfactants, bile acids, phospholipids, and cyclodextrins. But somehow they are associated with nasal mucosal toxicity when used for prolonged period during treatment, this should be considered in the chronic conditions (11). Literature reports the troubleshooting of low nasal permeability problem by different formulation strategies like microemulsion system for the drugs like clonazepam (12), insulin (13), and diazepam (14) in which there is significant improvement in absorption owing to lipophilic nature and low globule size of the microemulsion system (12). But nasal mucosal toxicity point of view, high surfactant concentration and low drug loading in such systems makes them unsuitable for administration of drugs in chronic conditions. In the present investigation, this problem of low nasal permeability was handled by developing nanoparticulate formulation of CRV. This would not only improve nasal absorption by increasing transcellular and paracellular uptake of drug through nasal mucosa but also improves its saturation solubility in nasal fluid thereby increasing passive diffusion of drug molecule. Good bio-adhesiveness to nasal mucosal membrane is another distinct advantage of the developed system which offers prolonged *in vivo* residence and contact time in nasal cavity (15). Administering nanoparticulate formulation by nasal spray will result in uniform distribution of formulation throughout nasal mucosa, making maximum drug nanoparticles come in contact with nasal mucosal membrane for longer period of time.

Therefore, the main objective of investigation was to administer CRV by nasal route in the form of nanosuspension incorporated in *in situ* gelling nasal spray formulation, in order to improve the bioavailability of drug. Thus, such drug delivery system could provide platform technology for intranasal delivery of many other drugs which suffers from low oral bioavailability and results in improved therapeutic efficacy and patient compliance.

MATERIALS AND METHODS

Materials

Carvedilol (CRV) was procured from Cipla Ltd., India. Poloxamer 407 (Lutrol F127) and poloxamer 188 (Lutrol F68) were obtained from BASF, India. Stearic acid, oleic acid, glycerol, sodium dodecyl sulfate (SDS), Tween 80, Solutol HS15, Span 40, chlorhexidine acetate, and lycerol were purchased from Merck India Ltd. Polyvinyl pyrrolidone K-30 (PVP K-30) was purchased from BASF. Trehalose and Gellan gum (Kelcogel) were procured from Hayashibara Co. Ltd., Japan, and CPKelco, USA, respectively.

Methods

Preparation of Nasal Spray of In Situ Gelling Nanosuspension of CRV

Preparation of Nanosuspension Phase. CRV nanosuspension was prepared by the modified precipitation–ultrasonication method (16; Fig. 1). Briefly, CRV and different stabilizers were dissolved in an organic solvent to form a series of organic solutions containing different concentration of stabilizers. For the development of nanosuspension of CRV, Tween 80, Solutol HS 15, Span 40, PVP K-30, poloxamer 407 and poloxamer 188, stearic acid, oleic acid, and their combinations were tried as stabilizers to get the desired particle size. Methanol, ethanol, and acetone were tried as solvent for the CRV and stabilizer. Deionized water which acts as anti-solvent system was cooled in an ice bath before injecting drug solution. Precisely, 2 ml of organic solution of CRV and stabilizer was quickly introduced into 25 ml of the pre-cooled deionized water with simultaneous ultrasonication using ultrasonic probe having a tip diameter of 8.0 mm (Dakshin Instruments, India) at ultrasonic power inputs (630–650 A). The period of ultrasound burst was set to 5 s with a pause of 5 s between two ultrasound bursts. During the process, the temperature of deionized water was controlled using an ice bath. After the precipitation, the samples were transferred to a round-bottom flask (500 ml) for the organic solvent evaporation on rotary evaporator (Rotavapor R210, Buchi, Switzerland) at 45°C for 45 min. Different formulation and process parameters were systematically investigated to clarify their effects on the particle size of drug nanocrystals. The different parameters optimized during formulation development were stabilizer or combination of stabilizers, concentration of stabilizer, volume of anti-solvent, and ultrasonication time. For solid-state characterization, the optimized CRV nanosuspension was subjected for pre-freezing at –70°C for 24 h followed by freeze-drying in freeze-drier (Labconco, USA) under vacuum (1 mbar, –30°C) till solid powder was obtained.

Preparation of In Situ Gelling Nanosuspension of CRV. An accurately weighed quantity of gellan gum (0.25%, 0.5%, 1.0% *w*/*v*) was dissolved in 25 ml of deionized water (100°C) with continuous stirring till polymer dispersion is obtained. To this polymer dispersion, other ingredients like chlorhexidine acetate (0.01% *w*/*v*) and glycerol (1.0% *w*/*v*) were added (17) and mixed well. Thereafter, nanosuspension phase was added to the *in situ* gelling phase so as to obtain final concentration of CRV and stabilizers as per Table I in the Final formulation (Fig. 1). Finally, prepared formulation was filled in nasal spray containers (Aptar Nasal Spray System, India).

Characterization of Nanosuspension

Particle Size and Zeta Potential. The particle size distribution during process optimization was measured using a Laser Diffractometer Mastersizer 2000 (Malvern Instruments,

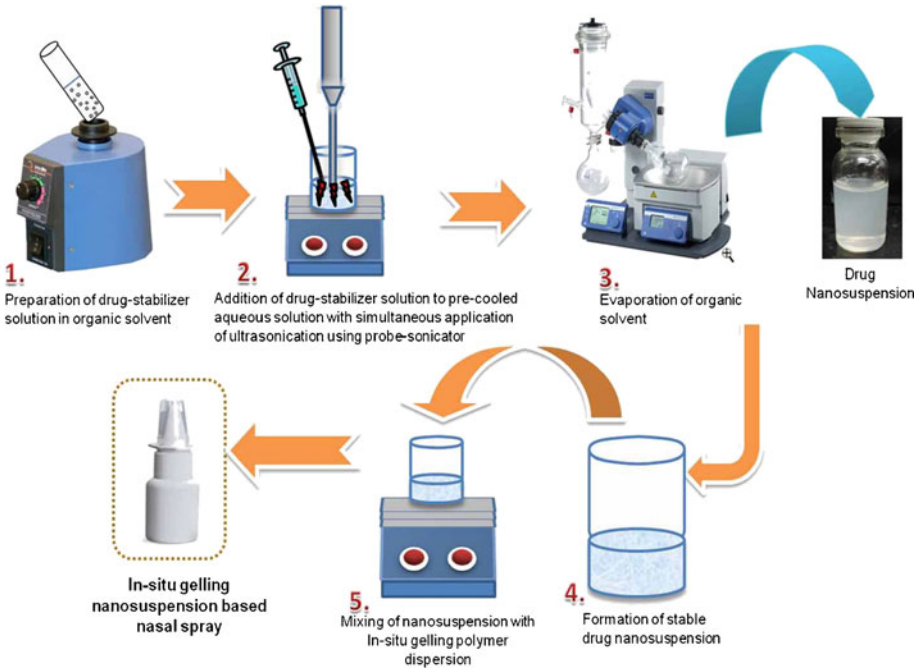

Fig. 1. Preparation of CRV nano *in situ* gelling nasal formulation

Worcestershire, UK). Particle size distribution typically includes d (*v*, 0.1), d (*v*, 0.5), and d (*v*, 0.9), which represent the percentage of particles below 10%, 50%, and 90%, respectively, of the given size. The width of particle size distribution is

Table I. Batches of CRV Nanosuspension

				Stabilizers	
Batch no.	Carvedilol (%*w*/*v*)	Anti-solvent	Ultrasonication time (min)	Type	Concentration (% *w*/*v*)
S-1	0.4	Methanol	10	Tween 80	0.2
S-2	0.4	Ethanol	10	Tween 80	0.2
S-3	0.4	Acetone	10	Tween 80	0.2
S-4	0.4	Methanol	10	Tween 80	0.1
S-5	0.4	Methanol	10	Tween 80	0.2
S-6	0.4	Methanol	10	Span 40	0.1
S-7	0.4	Methanol	10	SDS	0.2
S-8	0.4	Methanol	10	Solutol	0.1
S-9	0.4	Methanol	10	Solutol	0.2
S-10	0.4	Methanol	10	Solutol	0.4
S-11	0.4	Methanol	10	PVP K-30	0.1
S-12	0.4	Methanol	10	PVP K-30	0.5
S-13	0.4	Methanol	10	Poloxamer 188	0.20
S-14	0.4	Methanol	10	Poloxamer 407	0.20
S-15	0.4	Methanol	10	Poloxamer 188	0.40
S-16	0.4	Methanol	10	Poloxamer 188	0.60
S-17	0.4	Methanol	10	Poloxamer 407	0.40
S-18	0.4	Methanol	10	Tween	0.2
				PVP K-30	1.0
S-19	0.4	Methanol	10	Poloxamer 407	0.20
				Stearic acid	0.01
S-20	0.4	Methanol	10	Poloxamer 407	0.20
				Stearic acid	0.05
S-21	0.4	Methanol	10	Poloxamer 407	0.20
				Stearic acid	0.10
S-22	0.4	Methanol	10	Poloxamer 407	0.20
				Stearic acid	0.20
S-23	0.4	Methanol	10	Poloxamer 407	0.40
				Oleic acid	0.10

measured by parameter span which is calculated by following equation (Eq. 1)

$$Span = \frac{d(0.9) - d(0.1)}{d(0.5)} \quad (1)$$

The span is a dimensionless number which illustrates whether or not the spread of the distribution is narrow or wide (18).

Zeta potential of optimized batches was determined by Zetasizer (Malvern Instruments, Worcestershire, UK). Nanosuspension samples for analysis were prepared by diluting appropriately with help of deionized water.

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) of freeze-dried nanosuspension was performed with Perkin Elmer DSC7 instrument, USA, under a pure nitrogen flux of 20 ml/min and with a heating rate of 10°C/min in the temperature range of 40°C to 300°C. Each sample was accurately weighed (6–10 mg) in an aluminum pan, crimped and sealed. Temperature calibration was obtained using indium.

X-ray Powder Diffractometry. X-ray powder diffractometry (XRPD) of freeze-dried nanosuspension was recorded using Phillips X-ray Diffractometer, USA, using Ni-filtered, Cu Kα radiation, a voltage of 40 kV and a 25-mA current. The scanning rate employed was 1°/min over 10–40° diffraction angle (2θ).

SEM of Nanosuspension. The morphological examination of CRV nanosuspension was performed using a scanning electron microscope (SEM, Supra 35VP-24-13, Carl Zeiss, Germany) operated at an accelerating voltage of 1 kV and a secondary detector. Samples were deposited on a double-sided carbon tape (diameter 12 mm, Oxon, Oxford instruments, UK) for analysis.

Solubility Study of Nanosuspension. Solubility behavior of CRV nanosuspension was studied by a paddle method using dissolution system Electrolab TDT-08L in different media like hydrochloride acid pH1.2, acetate buffer pH 4.5, phosphate buffer pH6.8, and phosphate buffer pH7.4 at stirring speed 50 rpm at 37±0.5°C. For this study, extra amount of CRV nanosuspension was added to the medium. Samples were withdrawn and centrifuged on high-speed ultracentrifuge (Thermo WX Ultra 100, USA) at speed 40,000 rpm for 30 min and immediately analyzed after appropriate dilutions using UV-spectrophotometer (Jasco V-530 UV/VIS spectrophotometer, Japan) at λmax 240 nm and calibrated for each media. The solubility was continued till constant reading observed. For comparison, solubility of CRV was performed in the same manner in all the media.

Characterization of In Situ Gelling Ability of Formulation

In Situ Gelling Ability and Viscosity Determination. The viscosity of the CRV formulation containing different concentrations of gellan gum (0.25%, 0.5%, and 1.0% *w/v*) was determined with and without artificial nasal fluid on rotational viscometer (Brookfield Viscometers, USA). Measurements were performed using suitable spindle number at 20 rpm at 37°C. *In situ* gelling ability of CRV optimized formulation was demonstrated by mixing formulation and artificial nasal fluid (1:1 *v/v*) in a glass vial and gelation was observed by visual examination after inverting the vial (19). An *in situ* gelation of formulation was demonstrated by spraying formulation on filter paper soaked with simulated nasal fluid.

In Vitro Drug Release in Simulated Nasal Fluid. In vitro drug release study of nasal formulation was performed as per method described by Zaki *et.al.* (20). Drug release study was performed by the USP paddle method. A dialysis tube (dialysis membrane-70; molecular weight cutoff: 12,000, Himedia, India) containing 2.0-ml formulation was immersed in 500 ml of simulated nasal electrolyte solution (7.45 mg/ml NaCl, 1.29 mg/ml KCl, and 0.32 mg/ml $CaCl_2 \cdot 2H_2O$ and pH adjusted at 5.5) (21) used as a dissolution medium at 37±0.5°C and at peddle speed of 50 rpm. The amount of drug released from the formulation was measured spectrophotometrically at λmax 240 nm. The concentration of drug was determined from a previously constructed calibration curve. The drug release kinetics of three batches was performed for zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models by using Micorsoft Excel Add-Ins DD Solver.

In Vivo Evaluation of Formulation

Animal Handling and Drug Administration. Prior to initiation, study protocol was reviewed and approved by Institutional Animal Ethical Committee (protocol approval no. ICT/IAEC/2011/P 45). Five New Zealand white rabbits of either sex weighing 2.5±0.12 kg were used for this study. Animals were acclimatized to laboratory conditions for at least 7 days prior to study. They were housed individually in stainless steel cages, fed a commercial laboratory rabbit diet, and allowed water *ad libitum*. The rabbits were fasted for overnight prior to dose administration. Animals were held in rabbit restrainers during blood sampling and they were conscious throughout the duration of the experiments. In a crossover study, wash out period of 1 week given before administration of another formulation. Animals received three formulations each of dose 1.0 mg/kg, oral CRV coarse suspension in purified water by oral route, *in situ* gelling nanosuspension using nasal spray system of 100 µl (Aptar Pharma, India) by intranasal route in each nostril, CRV solution (0.1% *w/v*) prepared by using Tween 80 (1% *w/v*), and ethanol (2% *w/v*) in sterile WFI and injected into their marginal ear vein.

Sample Collection and Analysis. After administration of the different formulations, blood sample (2.0 ml) was collected at different time intervals (0.5, 1, 2, 4, 8, 12, and 24 h) from the marginal ear vein of the rabbits. Blood samples were collected in Eppendorf tube containing 10 µl of 5.0% *w/v* EDTA solution to avoid clotting and samples were centrifuged at 5,000 rpm to separate the plasma from RBC. The mean percentage recovery of CRV from spiked serum samples was 98.33±3.16% (C.V. %=2.96) and the mean correlation coefficient of the standard curve was 0.9969. At the time of analysis, to 0.5-ml plasma samples, 0.5 ml acetonitrile were added and vortexed for efficient mixing for 2 min, centrifuged at 3,000 rpm for 10 min. After centrifugation, 0.20 ml supernatant was taken and evaporated at 50°C to this solution, 0.5 ml mobile phase added. Precisely, 100 µl were injected into the HPLC column (C-18 Column, 5-µm particle size, 150.0×

6.0 mm Thermo, Scientific). The mobile phase consisted of methanol: 50 mM phosphate buffer (pH2.5; 60:40) *v/v*. The flow rate of the mobile phase was 1 ml/min (515 HPLC pump, Waters, USA) and detection wavelength was 240.0 nm (UV variable wavelength detector, 2487 dual λ absorbance detector, Waters, USA). The analysis was done on Waters HPLC system having 717 plus auto sampler and results were analyzed using Empower 2.0 software.

Data Treatment and Statistics. Standard non-compartmental pharmacokinetic parameters were calculated using the pharmacokinetic software Thermo Kinetica Version 5.0 Pk/PD analysis (Thermo Fischer Scientific, USA).

RESULTS

Screening of Solvent for Drug and Stabilizer

The choice of suitable organic solvent is a key factor to control the size and stability of the drug nanocrystal during nanoprecipitation process. In this study, three solvents were tried during preparation of nanosuspension of CRV (Table I). Sticky mass were formed in both batches of nanosuspension prepared by using acetone and ethanol (batch S-3 and S-2). But the batch prepared with methanol showed fine dispersion (batch S-1); therefore for further optimization of nanosuspension, methanol was used.

Screening of Stabilizer: Surfactants

For preparation of stable CRV nanosuspension of low particle size, various surfactants having different hydrophilic lipophilic balance (HLB) values were tried (Table I). The results of particle size reduction of CRV nanosuspension with different surfactant are shown in Fig. 2a. In the case of batches taken with Tween 80, SDS, and Span 40, no adequate reduction in particle size were observed [*d*(0.9) in S-4, S-5 S-6, and S-7 were 3 to 15 μm]. It was particularly observed that particle size results were significantly higher for batches (S-7 and S-6) with surfactants of high and low HLB values (SDS, HLB 40 and Span 40, HLB 6.7) (22) as compared to batch (S-4, 0.1% *w/v* Tween 80) with surfactant of medium HLB value (Tween 80, 15) (23).

For further improvement in particle size, higher concentration of Tween 80 (S-5 0.2% *w/v*) was tried. However, nanosuspension showed no improvement in particle size reduction. In spite, the resultant nanosuspension was with larger particles. In line to this, another surfactant Solutol HS 15 having similar HLB value (HLB 15) (24) was tried at different concentration (S-8, S-9, and S-10) but still no improvement in the particle size reduction was observed (*d*, 0.9>7.0 μm).

Screening of Stabilizer: Polymers

On the basis of steric stabilization of nanoparticles by hydrophilic polymers, PVP K-30, poloxamer 407, and poloxamer 188 were tried (Table I). In this study, poloxamer 407 showed significant reduction and mono-modal distribution of particle size [S-17, *d* (0.9) 0.24 μm, span 0.99] (Fig. 2b) as compared to poloxamer 188 (S-16, *d* (0.9) 2.52 μm, span 0.62) and PVP K-30 (S-12, *d* (0.9) 0.83 μm, span 1.97; Fig. 2). But after 2 days stability of this batch (S-17), tremendous increase in the particle size (*d* (0.9) 479 μm, span 89.2) was observed indicating inefficaciousness of poloxamer 407 alone for stabilization towards crystal growth (Fig. 3).

Screening of Stabilizer: Combination of Stabilizers

Additional stabilizers with low aqueous solubility like stearic acid and oleic acid have been tried in combination with poloxamer 407. The batches (S-19 to S-23) taken with these two stabilizers showed significant reduction in particle size (*d* (0.9)<0.36 μm, span<1.64) (Fig. 2c) but after 3 days of stability, nanosuspension prepared with stearic acid (S-19 to S-22) showed gradual increase in particle size (*d* (0.9)< 34.83 μm, span<79.34) (Fig. 3). But the nanosuspension prepared using poloxamer 407 with oleic acid (S-23) showed good stability with insignificant increment in particle size after 15 days stability (*d* (0.9)<0.23 μm, span<1.28).

The zeta potential of optimized batch (S-23) was found to be +10.2 mV indicating steric stabilization by poloxamer 407 and oleic acid.

Characterization of Nanosuspension

DSC of Nanosuspension

The thermograms of drug, trehalose, freeze-dried drug nanosuspension, and physical mixture are shown in Fig. 4. In the thermograms of plain drug, sharp melting endotherm was observed at 114°C and melting enthalpies was 175.604 J/g (Fig. 4) for CRV. Similar results were also observed in the case of physical mixture. But in the case of freeze-dried nanosuspension of CRV, complete absence of melting endotherm was observed. In

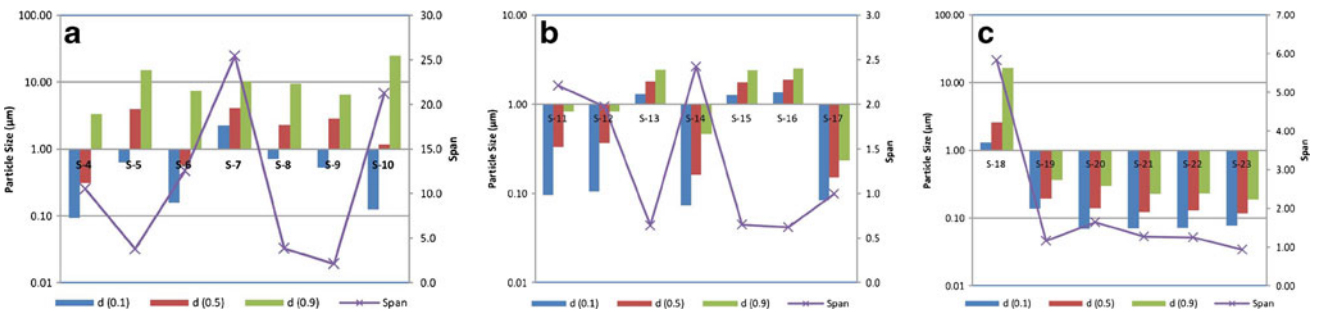

Fig. 2. Effect of different stabilizers on particle size distribution of CRV nanosuspension: **a** surfactant, **b** polymer, **c** combination of surfactant and polymer

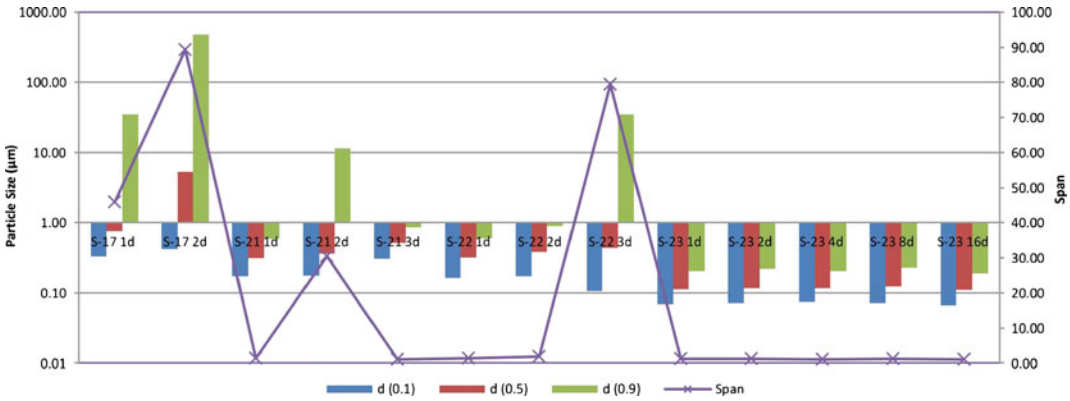

Fig. 3. Particle size distribution of CRV nanosuspension batches after stability at room temperature during different time interval (days)

addition, melting enthalpies of endotherm were at lower-energy state of 3.618 J/g as compared to crystalline form of drug.

XRPD of Nanosuspension

XRPD pattern of CRV, trehalose, and physical mixture exhibit intense peaks indicating crystalline nature of these substances, while XRPD pattern of nanosuspension showed peaks of low intensity which may be because of trehalose suggesting the amorphous nature of CRV in the nanosuspension (Fig. 5).

SEM Study

The SEM image of the drug and nanosuspension showed significant difference in the morphology of these particles. Nanosuspension sample was appeared to be spherical and amorphous with the mean particle size of 250 nm having narrow distribution while drug SEM showed coarse, irregular, more elongated, and crystal morphology (Fig. 6). Particle size of developed nanosuspension observed using SEM was in accordance with results of laser diffraction.

Solubility Study of Nanosuspension

Solubility of CRV and CRV nanosuspension was determined at different pH condition. The solubility of CRV in pH 1.2 is very low (0.33 mg/ml), while CRV nanosuspension showed 2.5 times increase in solubility as compared to plain CRV. At pH4.5, plain CRV was found to possess maximum solubility (1.6 mg/ml). On the other hand, at same pH value the drug nanosuspension showed 4.5 times (7.2 mg/ml) increase in solubility of CRV as compared to plain drug. As shown in Fig. 7, tremendous increase in solubility of CRV was observed at pH values of 6.8 and 7.5, i.e., 43 times and 71 times, respectively, as compared to plain CRV.

In Situ Gelling Ability and Viscosity Determination

Figure 8a explains the viscosity fate of developed *in situ* nasal systems in presence and absence of simulated nasal fluid. The developed *in situ* gelling system showed increase in viscosity after addition of simulated nasal fluid. The formulation containing 0.25% and 0.5% *w*/*v* gellan gum have lower initial viscosities (106.7 and 286 mPas, respectively) as compared to formulation containing 1% *w*/*v* gellan gum (3,390 mPas) (Table II). All three batches containing various concentrations of gellan gum were transformed into gel with corresponding increment in the viscosities in presence of simulated nasal fluid. Formulation containing 0.5% *w*/*v* gellan gum showed lower initial viscosity and transformation into viscous gel in presence of simulated nasal fluid (Batch S-23b). For further understanding of sol to gel conversion, *in situ* gel formation study was conducted. Figure 8b, c showed that rapid gelation was observed when the developed formulation comes in

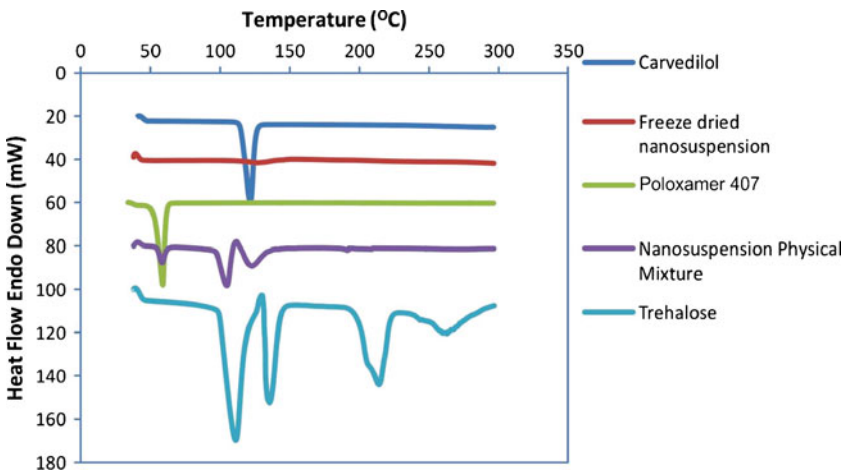

Fig. 4. Comparative DSC thermograms of powder samples

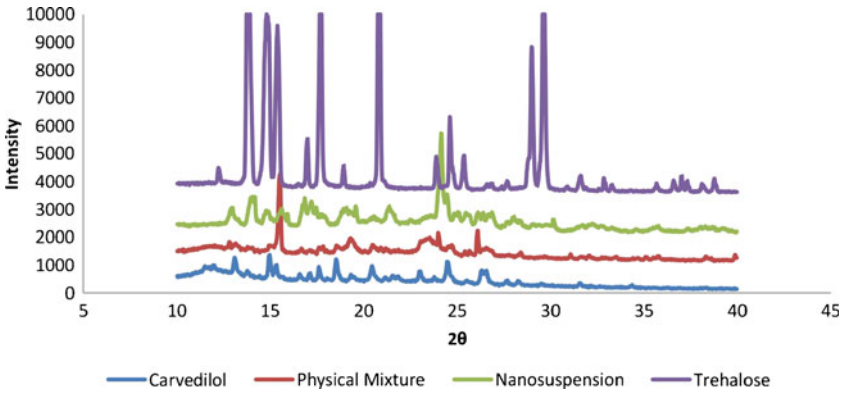

Fig. 5. Comparative XRD spectra of powder samples

contact with simulated nasal fluid as evidenced by *in situ* gel formation in inverted vial and in sprayed format, respectively. Similar kind of outcomes can be expected *in vivo* at very low ionic concentration.

In Vitro Drug Release in Simulated Nasal Fluid

Figure 9 shows the *in vitro* drug release pattern of CRV *in situ* gelling formulation in simulated nasal medium. The three batches having different concentration of gellan gum showed significant retardation in drug release pattern. With increase in the concentration of gellan gum in the formulation (batch 23a, 23b, and 23c) increase in drug release retardation was observed. As shown in Fig. 9, the batch 23a containing 0.25% *w/v* of gellan gum showed 90% of drug release in 4 h, while to achieve the 90% drug release in batches 23b and 23c containing 0.5% and 1.0% *w/v* gellan gum required approximately 8 and >12 h respectively.

In Vivo Evaluation of Formulation

In this study, the bioavailability of optimized nasal spray formulation (batch 23b) was compared to the oral CRV suspension and intravenous CRV solution. The mean plasma drug concentration–time profiles after administration of the intravenous, oral, and nasal formulations are illustrated in Fig. 10. From the study, it is clear that higher plasma levels were achieved in the case of the nasal spray formulation as compared to the oral drug administration (C_{max}; nasal 163.99 ng/ml and oral 67.34 ng/ml; Table III). Moreover, the $AUC_{(0-\infty)}$ values were 1,196.54, 830.23, and 310.695 ng/mlh^2 for the i.v. solution, the nasal spray formulation, and the oral CRV suspension, respectively. These values corresponded to absolute bioavailability values, (F_{abs}), of 69.38% and 25.96% for the nasal and the oral formulation, respectively.

DISCUSSION

For intranasal administration of CRV, a nasal spray was developed which utilizes advantages of nanosuspension and *in situ*-gelation technologies. The formulation process comprises three steps namely preparation and optimization of nanosuspension of CRV, preparation of *in situ* gelling polymer dispersion, and finally mixing of nanosuspension phase into *in situ* gelling polymer phase.

In the literature, two main methods were reported for the preparation of nanosuspension namely bottom up technology and top down technology (25); the former involves precipitation, microemulsion, the melt–emulsification methods, while in the latter, large particles are disintegrated into nanoparticles either by high-pressure homogenization or by milling methods. For CRV nanosuspension, the method employed was facile solvent anti-solvent precipitation with the aid of sonication to intensify micromixing for generation of submicron-sized particles (Fig. 1). Nanoprecipitation method was selected because of its simplicity, stable product formation, low-energy requirement, and easiness in scale-up (26).

Solvent–stabilizer pair is very crucial to obtain submicron particles and identification of an appropriate solvent–stabiliz-

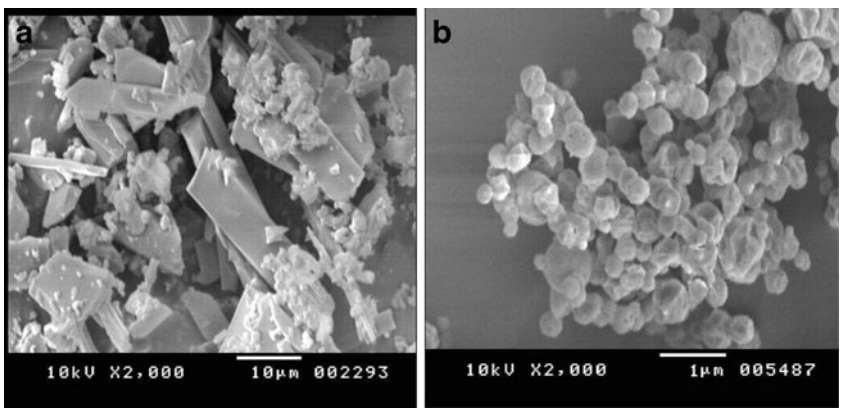

Fig. 6. SEM images of **a** carvedilol and **b** nanosuspension

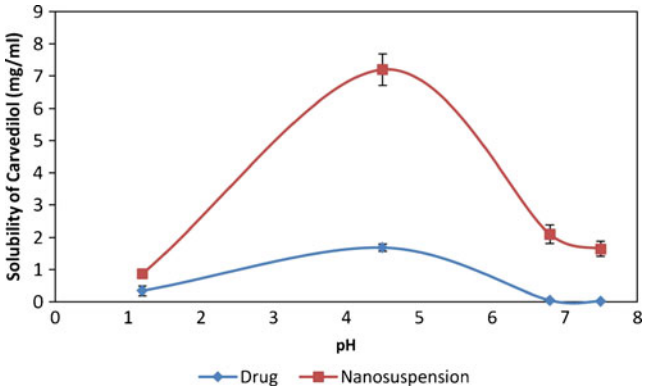

Fig. 7. The solubilty of plain CRV and CRV nanosuspension at different pH conditions

er pair is generally empirical (27). The selection of an appropriate solvent is very critical since it has to comply with numerous requirements such has high drug solubilization capacity, faster diffusions rates into the anti-solvent, ease of removal, and acceptable safety profile. As shown in Table I, three different solvents were evaluated for the nanoprecipitation of CRV. The batches (S-2 and S-3) taken with ethanol and acetone results in sticky mass formation during nanosuspension preparation which may be because of inadequate precipitation of CRV in the anti-solvent system. The batch S-1 where methanol was used as anti-solvent gave finer dispersion of CRV and hence it was selected for further optimization of nanosuspension phase.

Table II. Batches for *In Situ* Gelling Phase of Formulation

Ingredients (%*w/v*)	Batch S-23a	Batch S-23b	Batch S-23c
Nanosuspension phase			
Carvedilol	0.40	0.40	0.40
Poloxamer 407	0.40	0.40	0.40
Oleic acid	0.10	0.10	0.10
In situ gelling phase			
Gellan gum	0.25	0.50	1.00
Chlorhexidine acetate	0.01	0.01	0.01
Glycerol	1.0	1.0	1.0
Purified Water	q.s.	q.s.	q.s.

In literature, various surfactants have been successfully used in the particle size reduction and stabilization of nanosuspension formulations, due to their steric and electrostatic effect on the surface of nanoparticles (24). As shown in Fig. 2a, the batches of CRV nanosuspension taken with surfactants of high and low HLB values showed higher particle size as compared to batches taken with surfactants having medium HLB. This may be because surfactants with high and low HLB value were unable to get

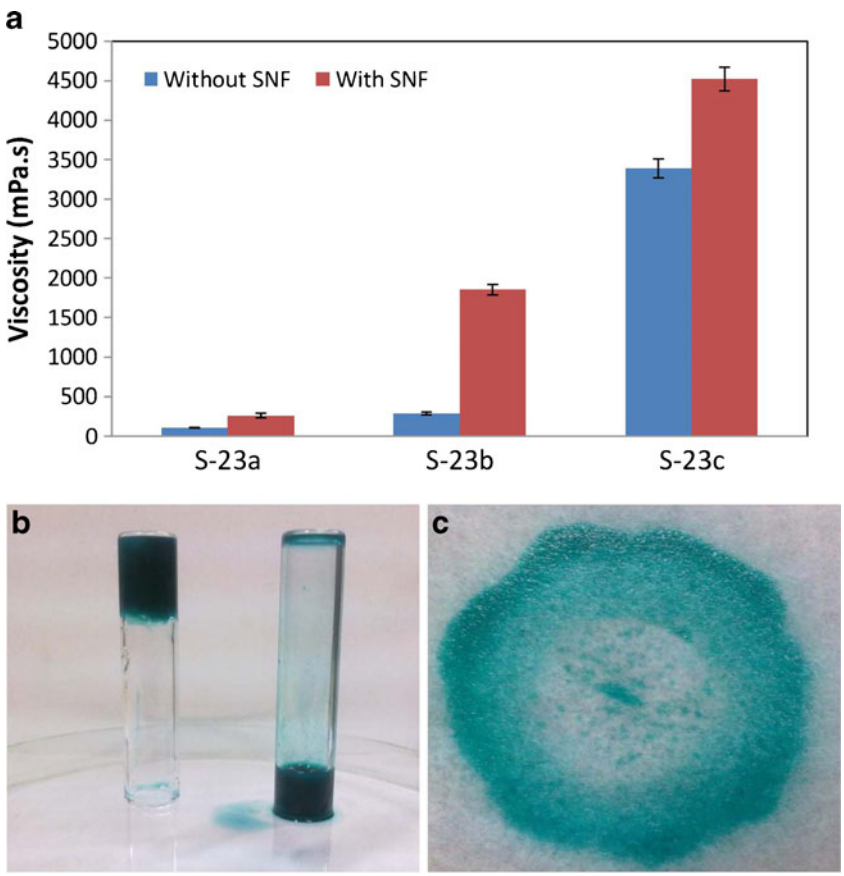

Fig. 8. *In situ* gelling study of the formulation: **a** viscosity of CRV formulations with and without SNF, **b** photograph showing *in situ* gelation of the formulation in the inverted vial (*left*) in presence of SNF (1:1 *v/v*) and solution formulation in the inverted vial (*right*) without SNF, **c** photograph showing gelation of sprayed formulation on the filter paper soaked with SNF

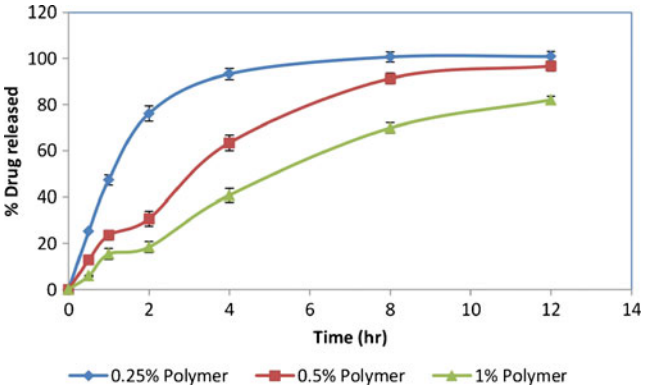

Fig. 9. *In vitro* drug release profile of CRV nasal formulations

adsorbed on hydrophobic surfaces of drug particles hence these particles were found to agglomerate rapidly.

Among the different hydrophilic polymers tried for CRV nanosuspension, the batch taken with poloxamer 407 showed desired reduction and distribution in particle size as compared to poloxamer 188 and PVP K-30. This may be because of higher molecular weight and surface active property of poloxamer 407 grades as compared to poloxamer 188 grades and PVP K-30. But tremendous increase in particle size after 2-days stability showed inefficaciousness of poloxamer F 127 alone for stabilization towards crystal growth (Fig. 3). This conversion of amorphous nanosuspension particles to crystalline particles can be explained by Ostwald ripening process. It is a process where the difference in solubility with particle size leads to a transport of material from small to larger particles, with an accompanying increase in the mean particle size with time (28,29). Ostwald ripening process was inhibited in o/w emulsions by incorporating a small amount of a second component with a very low aqueous solubility, and this has been explained theoretically by Kabalnov *et al.* (30). Briefly, the incorporation of a second component with low aqueous solubility leads to a difference in composition between large and small particles during the Ostwald ripening process. This difference may counterbalance the driving force for Ostwald ripening and eventually result in a termination.

As individual surfactant or polymer were found to be ineffective in stabilizing the CRV nanosuspension, combination of them were tried in order to get the desired particle size with long-term stability. Stearic acid and oleic acid were tried in combination with poloxamer 407. Along with low aqueous solubility of these stabilizers, the possession of amphiphilic characteristics and negative charge impartment on the surface

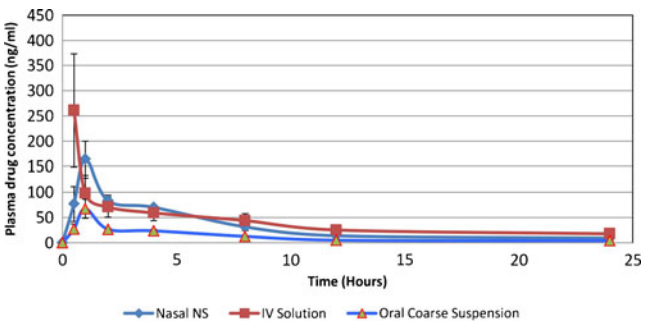

Fig. 10. Mean plasma concentration–time profiles in rabbits after oral, i.v., and intranasal administrations of CRV

of the hydrophobic particles provide synergistic effect with steric stabilizer poloxamer 407 (31). Significant reduction in particle size was observed in batches taken with these two stabilizers but only the batch taken with poloxamer 407 with oleic acid showed long-term stability (15 days) with insignificant increase in particle size.

Thermal analysis of powder sample revealed the crystalline state of the sample. The DSC thermogram of plain CRV showed characteristics of strong and sharp peak representing melting point of the drug, while the freeze-dried drug nanosuspension showed a negligible amount of less intense and broad peak. CRV physical mixture showed the characteristic of endotherm similar to that of trehalose and plain CRV. However, there was decrease in melting enthalpies were found in physical mixture. This was probably caused by the dilution effect of the drug. The enthalpy value for the drug nanosuspension was found to be very less as compared to plain CRV. This decrease in enthalpy value and low melting point indicate low lattice energy and it was very well reported that the particles with lower lattice energy are easier to dissolve (32).

The crystalline state of the freeze-dried CRV nanosuspension was evaluated by comparing its XRPD spectra with XRPD spectra of unprocessed CRV. Intense peaks were observed in the XRPD spectrum of unprocessed CRV indicating highly crystalline nature of plain drug. The peaks of lower magnitudes in the XRPD spectra of freeze-dried drug nanosuspension were consistent with the XRPD pattern of trehalose. Thus, the characteristic intense drug peaks were either reduced or absent in XRPD spectra of freeze-dried CRV nanosuspension indicating the amorphous phase formation of drug during nanoprecpitation.

The SEM study revealed that the nanoprecipitation process is very effective in converting the original CRV particles into the nanosize range. SEM micrograph of plain unprocessed CRV particles showed much larger particles of irregular shapes and missing size uniformity. On the other hand, CRV nanocrystals generated by nanoprecipitation were even, round-shaped particles. SEM micrograph of CRV nanosuspension revealed more uniform size and shape particles.

Solubility is an important parameter of drug candidate which determines the bioavailability of water-insoluble drugs. The solubility of drugs can be enhanced by reduction of particle size, this observation can be explained by Noyes and Whitney equation (Eq. 2) (33,34),

$$\frac{dm}{dt} = \frac{DA(\mathrm{Cs} - \mathrm{C})}{h} \tag{2}$$

Where dm/dt is the rate of change of mass dissolved (m) with time (t); A is the surface area of solid; D is the diffusion coefficient through a static layer of liquid of thickness h; Cs is the concentration of the solid in the diffusion layer surrounding the solid while C is the concentration of the solid in the bulk dissolution medium. Saturation solubility is chemical substance specific constant which largely depends on temperature and polymorphic state. Saturation solubility is also influenced by particle size when it is less than 1 μm. Decrease in the particle size of nanocrystal below specific limit will result in increase in saturation solubility. Thus, size reduction of drug crystal might leads to increase in the saturation solubility. Buckton and Beezer assumed that the enhancement of solubility is valid only for sparingly soluble particles having

Table III. Comparative Pharmacokinetic Parameters of Carvedilol Following Intravenous Solution, Oral Coarse Suspension and Nasal Spray of *In Situ* Gelling Nanosuspension

Parameters	Intravenous solution	Nasal in-gelling nanosuspension	Oral suspension
C_{max} (ng/ml)	260.88	163.99	67.34
T_{max} (h)	0.5	1	1
K_{elim} (h^{-1})	0.064	0.104	0.0923
$T_{1/2\ abs}$	10.8	6.63	7.45
MRT (h)	14	8.66	8.654
AUMS ng/mlh^2	17,197.8	7,196.26	2,999.72
AUC ng/mlh^2	1,196.54	830.23	310.695
F_{abs} (%)	100	69.38	25.96

particle size less than 1 μm (35). In the case of CRV, very low solubility was observed at acidic pH condition (pH1.2). This might be because at low pH, CRV was found to get protonated resulting in hydrochloride salt formation which exhibit very low solubility. But decrease in size of CRV gave the increase in solubility of the drug at same pH value. The increase in solubility of CRV at different pH conditions compared to plain drug might be attributed by increase in surface area due to significant particle size reduction.

The *in situ* gelling formulation should be of low viscosity and free flowing to allow easy reproducible administration. It must have ability of rapid transformation into gel immediately as it comes in contact with nasal fluid. It should have ability to produce strong adherence and stickiness enough to withstand external forces due to nasal ciliary movements (36). This transition from sol to gel can occur either by temperature, ionic, or pH stimulation depending on polymers used (37). In the case of gellan gum, ion-activated gelation takes place by formation of double helical junction structures to form a three-dimensional network by complexation with cations and hydrogen bonding with water (38). The batch containing 0.5% *w/v* gellan gum was found to be optimum batch as it gives the desired viscosity and gelling ability to the formulation as it come in contact with nasal fluid.

In the presence of simulated nasal fluid (SNF), formulation is rapidly converted to gel, releasing the drug in sustained manner. With increase in the concentration of gellan gum in the formulation significant retardation in drug release pattern was observed. The drug release from gel formed in presence of simulated nasal fluid was described by various mathematical model and equations. Table IV explains the release kinetics of CRV formulations containing different proportion of gellan gum (Table II). The best r^2 value of zero-order model was observed for batch 23c indicating concentration independent drug release while batches 23a and 23b showed good r^2 values for first-order model suggestive of concentration-dependent drug release (39). This is due to the batch containing high polymer concentration has formed strong diffusional gel matrix releasing drug in controlled manner independent of concentration, while low concentration of polymer matrix eroded rapidly, releasing the drug depending upon concentration. When Higuchi model was applied to drug release of these formulations, it was observed that r^2 value was not close to linearity indicating that drug is not released by matrix diffusion but by erosion of gellan gum gel. In the Hixson–Crowell model, good r^2 value was observed for batches 23b and 23c indicating change in surface area of gel matrix with progressive dissolution of matrix as function of time.

The Korsmeyer–Peppas model is fitted to all three formulations. The diffusional exponent *n* is anomalous or non-Fickian for batches 23b and 23c which indicates that the drug release rate is controlled by more than one process, i.e., erosion and diffusion, while it is Fickian for the batch 23a in which diffusional release occurs by the usual molecular diffusion of the drug due to a chemical potential gradient (40).

In vivo pharmacokinetic study in rabbits proved the bioavailability enhancement phenomenon of *in situ* gelling formulation of CRV as compared to plain CRV. The higher plasma levels of CRV after administration of *in situ* gel formulation of CRV as compared to plain drug agrees with the increase in therapeutic effectiveness of the formulation. This enhancement by nasal route can be explained by avoidance of first-pass effect and increase in permeability due to nanosuspension formulation. Concerning the rate of absorption, Tmax was similar (1 h) for nasal and oral formulation this is due to slow release of CRV from nasal formulation. Higher bioavailability of 2.7 times was achieved by *in situ* gelling nanosuspension nasal spray formulation of CRV compared to oral route (Table IV).

CONCLUSION

Novel spray formulation of CRV combining advantages of nanoparticulate and *in situ* gelling technologies was successfully developed for nasal administration. The formulation has demonstrated good stability and physical properties of nanosuspension phase, excellent sol–gel transformation ability of *in situ* gelling phase and controlled drug release profile overall. Furthermore, it has demonstrated tremendous enhancement in the bioavailability of CRV as compared to oral drug formulation. Hence, this intranasal drug delivery technology could be alternative platform for delivery of many other drugs.

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Table IV. Drug Release Kinetics of CRV Formulations

	Zero order		First order		Higuchi		Korsmeyer–Peppas			Hixson–Crowell	
Batch	r^2	K_0 (h^{-1})	r^2	K_1 (h^{-1})	r^2	K_H ($h^{-1/2}$)	r^2	K_{kp} (h^{-n})	n	r^2	K_{hc} ($h^{1/3}$)
S-23a	0.7969	11.347	0.9993	0.662	0.8137	36.131	0.9617	65.433	0.204	0.9201	0.126
S-23b	0.9463	9.729	0.9947	0.240	0.8137	28.763	0.9439	28.941	0.519	0.983	0.067
S-23c	0.9815	7.684	0.9715	0.138	0.9352	22.141	0.983	17.056	0.654	0.993	0.039

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