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Original Article

NICORANDIL MUCOADHESIVE MICROSPHERES: FORMULATION DEVELOPMENT, PHYSICO-CHEMICAL AND FUNCTIONAL CHARACTERIZATION

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ABSTRACT

Objective: The study aims to prepare and evaluate Nicorandil mucoadhesive microspheres to improve the oral physicochemical properties of nicorandil and mucoadhesion to extend the residence time at the absorption site.

Methods: Nicorandil mucoadhesive microsphere was prepared by emulsion cross-linking method using fenugreek gum, karaya gum as polymer, and glutaraldehyde as a cross-linking agent. Drug entrapment efficiency, particle size, % swelling index, mucoadhesion study, differential scanning calorimetry, powder x-ray diffraction, Fourier transform infrared spectroscopy, and *in vitro* dissolution studies were used to characterize the microspheres.

Results: The characterization studies indicated the formation of mucoadhesive microspheres. The nicorandil mucoadhesive microspheres particle size is130.83±0.48, entrapment efficiency 66.91±0.54, swelling index 82.69±0.40, % mucoadhesion 95.22±0.13 and *in vitro* drug release was found to be 89.96±0.17 % at the end of 12 h.

Conclusion: This research work successfully formed nicorandil mucoadhesive microspheres formulation using the emulsion cross-linking method. Encapsulation efficiency and other physicochemical and functional characterization of microspheres suggested the successful formation of nicorandil mucoadhesive microspheres.

Keywords: Nicorandil, Microspheres, Polymer, Glutaraldehyde

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INTRODUCTION

Nicorandil (NCR) (IUPAC: N-[2-(Nitroxy) ethyl]-3-pyridine carboxamide) is a selective ATP-dependent potassium channel opener [1]. It has been approved as long-term therapy for chronic stable angina in Japan and Europe [2]. The intrinsic mechanism of the drug (selective activation of K^+ATP channels at the sarcolemmal and mitochondrial level) allows coronary and peripheral vasodilatation with subsequent reduction of preload and afterload. Secondly, NCR has been attributed to cardioprotective effects because of the role of K^+ATP channels in ischemic preconditioning [3]. Despite its benefits, NCR has a short half-life (1 h), rapid metabolism and elimination (52 l/h), and low oral bioavailability (23-75%) [4, 5]. Considering NCR's significant pharmacological activity and its existing drawbacks, there is a strong need for unique formulations that can improve the biopharmaceutical characteristics of NCR [5].

Literature analysis demonstrated that the authors had attempted only some formulations to enhance the biopharmaceutical properties of NCR. For instance, tablets [3, 6], nanofiber [7], transdermal patch [8], proniosomes [9]. Previous reports have shown that nano-formulation improved the bioavailability of several drugs via localized and targeted delivery to the stomach, small intestine, intestinal lymphatic system, and colon. It displays various shortcomings, such as low encapsulation efficiency, an uncontrolled release profile, and poor drug absorption. This reduces the oral bioavailability of drugs. The poor bioavailability of NCR is attributed to the short retention of its dosage form at the absorption sites (in the upper gastrointestinal tract to the duodenum and jejunum).

Microspheres are multi-particulate drug delivery systems prepared to obtain prolonged or controlled drug delivery to improve bioavailability and stability and target the drug to a specific site at a predetermined rate. They are made from polymeric waxy or other protective materials such as natural, semi-synthetic, or synthetic polymers. Microspheres are characteristically free-flowing powders with particle sizes ranging from 1-1000 µm [10]. The preparation of microspheres provides multiple ways to control drug administration aspects and enhance the therapeutic efficacy of a drug. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity, improved patient compliance, and convenience. Such systems often use macromolecules as carriers for drugs [11].

In contrast, fenugreek gum and karaya gum are used as a complex of mucoadhesive polymers for synergistic effects; Span 80, castor oil, glutaraldehyde, and vanillin are used to prepare nicorandil mucoadhesive microsphere (NCR MMs). In this study, positive-charged glutaraldehyde builds up association and interaction with the negative charge of the fenugreek-karaya gum complex, leading to the production of NCR MMs via cross-linked coordinate bond interaction. This mechanism enhances further interaction with hydrophilic drugs. It increases its holding within the NCR MMs to a great extent, resulting in the enhancement of stability, encapsulation efficiency, and controlled release pattern of the drug. Subsequently, it enhances its bio-adhesion, retention, miscibility, and permeation across the biological membrane. The cross-linked coordinate bond interaction within the NCR MMs increases hydration and swelling and prevents its degradation, resulting in controlled drug release.

This research aimed to develop and evaluate nicorandil mucoadhesive microspheres to enhance encapsulation efficiency, controlled release, permeability, oral bioavailability, and improve mucoadhesive properties to extend the residence time at the site of absorption with improved patient compliance, fewer side effects, and most aspects of an ideal drug delivery system.

MATERIALS AND METHODS

Nicorandil was gifted from Supriya Life Science Pvt. Ltd., Ratnagiri, Karaya gum was purchased from Research Lab Fine Chem industries, Mumbai. Fenugreek seed was purchased from the local market of Wardha, Maharashtra. Castor oil, Glutaraldehyde, Hydrochloric acid, Span 80, Isopropyl alcohol, Acetone, and Sulphuric acid were purchased from Loba Chemic Pvt. Ltd., Mumbai, India.

Extraction and purification of fenugreek seed mucilage

The high-quality fenugreek seeds were obtained from the local market, cleaned and dried. Dried fenugreek seeds were ground using a laboratory mixture. The ground fenugreek seeds (250 g) were soaked in distilled water (500 ml) overnight to allow the seeds to swell. The swelled seeds were heated at 50 °C for 1 hour. After heating, a viscous liquid solution was obtained. The obtained solution was filtered using a multilayer muslin cloth bag to remove the marc from the viscous solution. Acetone was added to the above filtrate to precipitate the mucilage, and the mucilage was separated. Freshly obtained mucilage was purified with ethanol, followed by acetone three times. At 50 °C, dried purified mucilage was dried in an oven. Then dried gum was ground using a grinder and passed through mesh # 100 to get a fine powder. The obtained fenugreek seed gum powder was preserved in amber-colored (light-protected) glass vials, purged with N2, and stored at room temperature until further analysis [12].

Method of preparation of microspheres

An emulsion cross-linking method was used to prepare Nicorandil mucoadhesive microspheres. NCR MMs were developed in two phases. Briefly, fenugreek gum, karaya gum, and NCR were weighed on a digital balance (Model: AW-220 and BX-620SShimadzu, Japan). Both weighed gums were transferred into a 50 ml beaker and dissolved in 25 ml of distilled water. In this solution, NCR (20 mg) was dispersed (2% w/v), forming an aqueous phase. The oil phase was prepared by dispersing span 80 (2%) into castor oil as an emulsifier. The freshly prepared aqueous phase was added dropwise into the oil phase to form a w/o emulsion under vigorous stirring speed (1000 rpm) for 30 min at room temperature, forming a whitish opalescence emulsion. Then, glutaraldehyde 4% (cross-linking agent) was transferred to a 50 ml beaker and mixed with conc. H_2SO_4 (0.2 ml) for droplet cross-linking. A freshly prepared solution was dropped into the w/o emulsion and stirred continuously for 4 h to develop cross-linked microspheres in the emulsion. The cross-linked microsphere-loaded emulsion was centrifuged at 3000 rpm for 30 min. The sedimented microspheres were collected and washed with isopropyl alcohol to remove the traces of oil and untreated glutaraldehyde. The obtained mucoadhesive cross-linked microsphere was dried in an oven at 45 °C. The dried microspheres are preserved in amber-coloured (light-protected) glass vials, purged with N2, and stored at room temperature until further analysis [13].

Evaluation of nicorandil mucoadhesive microspheres

Drug entrapment efficiency

The accurately weighed sample of microspheres (50 mg) was dispersed in 20 ml of acid buffer (pH 1.2). The dispersion was sonicated for 30 min and kept overnight for the complete erosion of microspheres. The sample was centrifuged, filtered and analyzed using a UV-visible spectrometer (Model: UV 2401 PC, S.220V Shimadzu, Japan) at 260 nm. The following equation calculates the entrapment efficiency and drug content of microspheres [14]:

$$\text{Entrapment efficiency (\%)} = \frac{\text{The actual amount of drug}}{\text{Theoretical amount of drug}} \times 100$$

Particle size

Particle size is the most widely used indicator to determine the release behaviour of NCR MMs formulations in liquid media. It plays a vital role in mucoadhesive ability and the release of drugs from microspheres. Briefly, an approximate quantity of NCR microsphere was weighed and dispersed into 5 ml of distilled water. The resultant dispersion was placed into the analyzer. The dispersion was analyzed using a particle size analyzer (Model: Nanophox Sympatec, GmbH, Clausthal-Zellerfeld, Germany) within the sensitivity range of 1 nm to 1000 µm [15].

Swelling study

The water uptake of the cross-linked microspheres loaded with the NCR was determined by measuring the extent of swelling behaviour in pH 1.2 buffer solution for 12 h. Briefly, weighed Ms sample (100 mg) was immersed in an aliquot of 25 ml 1.2 buffer for 24 h at RT with gentle shaking. Samples were removed from the solution. The excess surface-adhered liquid was removed by using soft tissue paper. The swollen microspheres were weighted using an electronic microbalance. The microspheres were dried in an oven at 60 °C until there was no change in the dried mass of the samples. The swelling index was calculated by using the formula [16].

$$\text{Water uptake (\%)} = \frac{\text{Mass of swollen microspheres} - \text{Mass of dry microspheres}}{\text{Mass of dry microspheres}} \times 100$$

Mucoadhesion study

The mucoadhesion study is designed to enable prolonged retention at the application site, providing a controlled drug release rate for improved therapeutic outcomes. A 5 cm piece of freshly cut pig intestine was obtained from a local abattoir within 1 hr of the animal's killing. A newly collected piece of intestine was cleaned by washing it with an isotonic saline solution. A cleaned piece of intestine was attached to a polyethylene plate and fixed at an angle of 40° relative to the horizontal plane. An accurate no. of microspheres was placed on the mucosal surface of the intestine. Phosphate buffer (pH 6.8) warmed at 37 °C±1 °C was passed at a rate of 5 ml/min over the intestine's surface. The time required for detaching all the microspheres from the mucosal surface of the pig intestine was recorded by visual inspection. At different time intervals up to 8 h, the numbers of microspheres still adhering to the tissue were counted. The percentage of mucoadhesion was calculated by [17]:

$$\text{Mucoadhesion (\%)} = \frac{\text{No. of microspheres adhered}}{\text{No. of microspheres applied}} \times 100$$

***In vitro* drug release studies**

A dissolution method is employed to evaluate the release performance of NCR MMs. An *in vitro* dissolution study was conducted in a USP Type I (Basket Method) apparatus (Model: DA-3 Veego Scientific Devices, Mumbai). Briefly, the dissolution studies were carried out in simulated gastric fluid (pH 1.2) media. NCR MMs (~ 10 mg of NCR) were weighed, loaded into empty capsule shells, and then placed into the basket assembly. The basket containing samples was transferred into 900 ml of freshly prepared dissolution media. The dissolution flask contents were stirred at 50 rpm for the entire study and maintained at 37±0.5 °C. At the fixed intervals, 2 ml of solution was pipetted out and replaced with an equal quantity of fresh dissolution media. The removed samples were suitably diluted and analyzed for absorbance using a UV-visible spectrophotometer (Model: V-630, JASCO International Co., Ltd., Tokyo, Japan) at the maximum wavelength (λmax ~ 263 nm). The recorded absorbance values were calculated and reported in percentages of the cumulative NCR release [18].

Differential scanning calorimetry

The samples of NCR, fenugreek gum, karaya gum, physical mixture (PM), and NCR MMs were analyzed to study their thermal behaviour using a differential scanning calorimeter (Model: UCT/TEQIP/959-07/CFL/DSC, Shimadzu). Briefly, the samples (~2 mg) were weighed and filled into a previously calibrated and N_2 purged analyzing area. The added sample was heated at a rate of 10 °C/min within the heating range of 0–400 °C. Each sample heated sample-based DSC spectrum was interpreted using instrument-accompanied software (Universal Analysis 2000, V4.5A, Build 4.5.0.5) [19].

FT-IR

FTIR is an essential analytical technique used for drug-polymer interaction studies. FTIR analysis was carried out using a Fourier Transform Infrared Spectrophotometer (Model: 84005, Shimadzu Asia Pacific Pvt. Ltd., Singapore). Briefly, the samples of NCR, fenugreek gum, karaya gum, physical mixture (PM) and NCR MMSs were weighed with the FTIR grade of potassium bromide and compressed into thin transparent discs using a Mini Hand Press Machine (Model: MHP-1, P/N-200-66, 747-91, Shimadzu, Kyoto, Japan). This disc was then scanned at a wave number range of 400 to 4000 cm^{-1} under the scanning resolution of 4 cm^{1}. The scanned

image of each FT-IR sample was analyzed and interpreted using the instrument-accompanied software (IR Solution, version 1.10) [20].

Scanning electron microscopy

The NCR, fenugreek gum, karaya gum, physical mixture (PM), and NCR MMs samples were analyzed to study their surface characterization using a scanning electron microscope (Model: S-3700N, Hitachi, Japan, Hyderabad). The samples (~50 mg) were weighed and spread as a thin layer on double-faced carbon tape and then loaded into the sample chamber of the SEM. After loading, the sample was coated with gold (~400 °) via a sputter coating technique. The coated sample was scanned at an accelerating voltage of 10 kV. The scanned image of each sample at various magnifications was analyzed using the instrument attached software (Smart® SEM V05.06) [21].

RESULTS AND DISCUSSION

Preparation of NCR MMs

In this study, NCR MMs were prepared using the emulsion cross-linking method. NCR is a vasodilator. It works by reducing the workload of the heart. Nicorandil has low bioavailability and a half-life of 1 hr, requiring frequent drug administration. Therefore, the study objective was to formulate controlled-release mucoadhesive microspheres to overcome the problem with NCL. The NCR MMs were prepared by the emulsion cross-linking method using the complex of two polymers and a cross-linker. Following this, fenugreek gum was extracted from fenugreek seeds and used as a mucoadhesive polymer to help retain it at the adsorption site. Karaya gum and fenugreek gum are negatively charged polymer that allows the reaction with positively charged glutaraldehyde to form cross-linked coordinate bond interaction between them, resulting in the formation of spherical NCR MMs [22].

Estimation of encapsulation efficiency

According to results (fig. 1), the NCR MMs (B7) show higher encapsulation efficiency of around ~ 66.91±0.54 w/w. This signifies the suitability of emulsion cross-linking methodology towards enhancement of the encapsulation efficiency of NCR within NCR MMs. It suggested that the mixture of solvents, i.e., sulphuric acid and vanillin in the glutaraldehyde solution and complex of polymer, might significantly enhance the encapsulation efficiency of NCR. These results could be explained by the fact that glutaraldehyde combines with the aqueous phase of polymeric solution, forming a strong association with each other and forming an immediate gel. At the same time, sulphuric acid combined with the external phase emulsion caused a rapid hardening of the gel. This combined mechanism facilitates the encapsulation of NCR within the polymer complex and prevents its escape into the surrounding solution. This phenomenon could increase the stability of the microspheres and thereby enhance the encapsulation efficiency and the controlled release pattern of NCR [23].

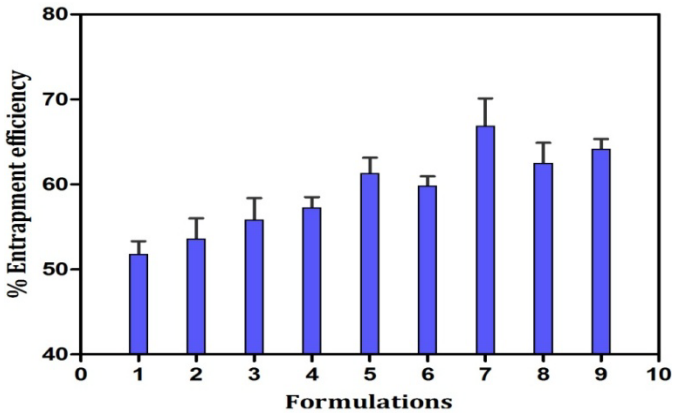


Fig. 1: % Entrapment efficiency of microsphere formulations, Each value represents mean±SD, (n=3)

Particle size

Particle size is an essential property of multi-particulate systems, indicating their physical stability, distribution, and bioavailability upon administration. The result of the particle size analysis of the prepared NCR MMs formulation is shown in fig.2. The particle size was revealed to be around ~ 130.83±0.48 µm (B7). The larger particle size may be attributed to a higher concentration of polymers, which may increase the viscosity of the solution, resulting in the formation of large droplets during emulsification and an increase in the mean particle size of microspheres [24].

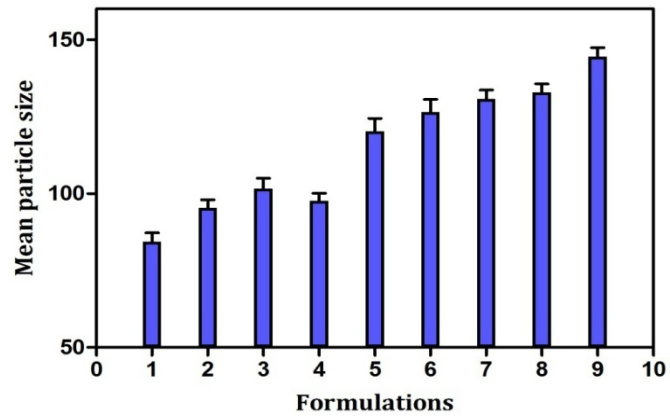


Fig. 2: Mean particle size of microsphere formulations, each value represents mean±SD, (n=3)

Swelling study

The swelling behaviour of microspheres defines the extent of mucoadhesion and drug release patterns through microspheres. The % swelling index for mucoadhesive formulation for all batches was found in the range of 55.81±0.47-89.08±0.06, respectively, as shown in fig.3. The % swelling index of optimized NCR MMs revealed around ~ 82.69±0.40. According to optimization results, NCR MMs demonstrated a high swelling index due to the excellent swelling property of fenugreek gum. It contains hydrophilic groups of the poly-branched structure, which leads to a greater extent of water absorption and holding capacity, resulting in the swelling of microspheres [25].

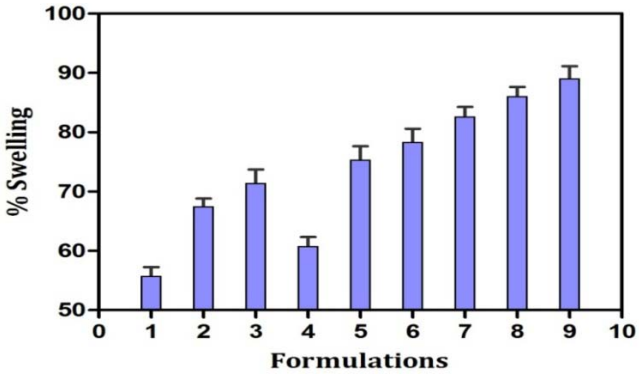


Fig. 3: % swelling of microspheres in pH 1.2, each value represents mean±SD, (n=3)

Mucoadhesion study

The mucoadhesion of microspheres at the target site increases the duration of action of a drug, thus improving its bioavailability. Polymers need to swell first for effective mucoadhesion, followed by interpenetration into biological tissue. The optimized NCR MMs exhibited higher mucoadhesion around ~ 95.22±0.13, indicating the complexity of polymer and glutaraldehyde improved the mucoadhesion of NCR to the small intestine. Fenugreek gum itself acts as a mucoadhesive polymer. Its mucoadhesive could be explained on the basis that swelled and wetted NCR MMs containing positive charge glutaraldehyde and negative charge polymer increase association and, thereby, interaction with functional groups of the biological membrane through physical entanglement and chemical forces. This interaction forms a cross-linked network with the membrane, resulting in mucoadhesion and retention in the small intestine [26].

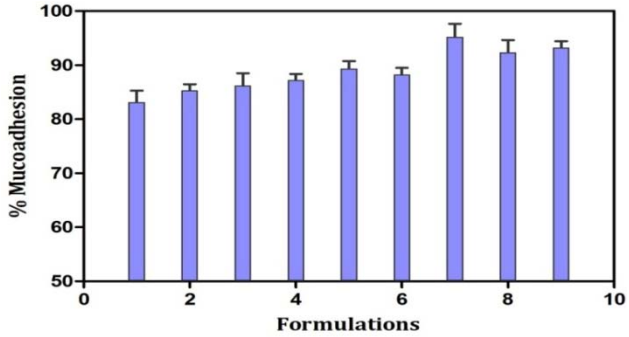


Fig. 4: Mucoadhesion study of formulations, each value represents mean±SD, (n=3)

***In vitro* drug release**

In NCR MMs, *in vitro* dissolution release was carried out. At 37±0.5 °C, the dissolution profile was performed in freshly prepared 0.1N HCl (pH 1.2). Fig. 4 depicts the release behaviour of NCR from NCR MMs for all batches, which was found in the range of 76.24±0.12-89.96±0.17% at the end of 12 h. At early 5 h, the optimized formulation showed around ~ 50% of NCR dissolution demonstrated burst release, which led to the highly aqueous solubility of the drug. The dissolution of the same formulation exhibited around ~ 90% of drug dissolution at the end of 12 h, which indicates controlled NCR release [27].

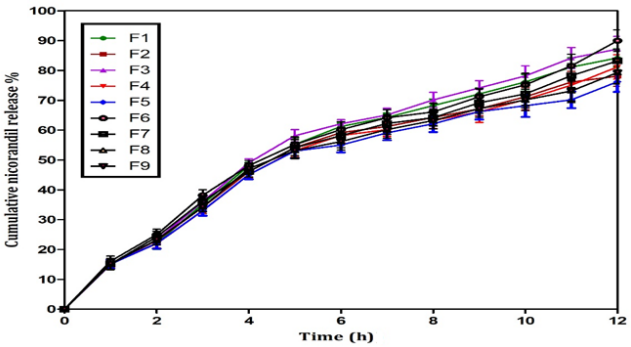
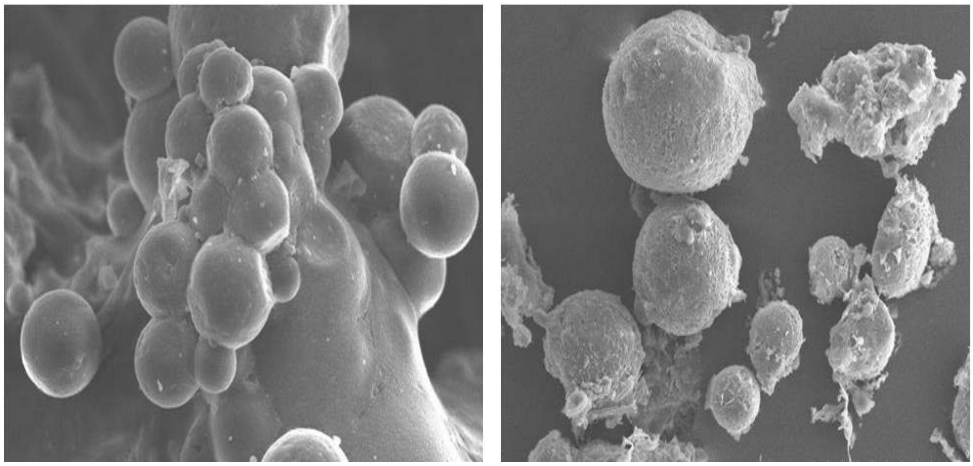


Fig. 5: %Cumulative drug release of formulations F1-F9, each value represents mean±SD, (n=3)

SEM analysis



a) **b)**

Fig. 6: Scanning electron microscopy of optimized NCR MMs

SEM photomicrographs obtained for optimized NCR MMs are shown in fig. 6. The formulation fig. a) revealed spherical particles with a smooth surface and had no rupture on the surface, indicating a positive reaction between the drug, the blend of polymers and the cross-linking agent. The external surface of the microspheres fig. b) showed a sponge-like nature. The outer layer of microspheres showed some porous structure, and it may be caused due to evaporation of solvent entrapped within microspheres after forming a smooth and dense layer which possibly imparts mucoadhesion of the microspheres [28].

DSC

The DSC thermogram of the physical mixture, nicorandil, karaya gum, fenugreek gum, and optimized NCR MMs is shown in fig. 7. Thermal analysis is employed to evaluate drug crystallinity and to determine any unexpected interactions. Nicorandil showed its sharp endothermic peak associated with a melting point of around ~ 92 °C [19]. The optimized microsphere formulation showed the broadening of the nicorandil characteristics peak. Although prepared microspheres showed less intense and broadening, reducing the intensity and early onset compared to the pure nicorandil, fenugreek gum, and karaya gum helps lower drug crystallinity. It is attributed to its amorphous form, which affects bioavailability enhancement.

FT-IR

The FTIR spectrum of the drug sample was compared with the reference spectra of NCR [23]. FTIR of NCR (fig. 8a) showed characteristic peaks at 3218.17 cm^{-1} due to N-H Stretching, 2837.09, 2883.38, 2954.74 cm^{-1} C-H Stretching, 1724.24 cm^{-1} due to C=O Stretching, 1473.51 cm^{-1} C-H bending, 1593.09 cm^{-1} due to N-H bending, 1296.86 cm^{-1} due to O-NO_2stretching, 1066.56 cm^{-1} C-O stretching, 1672.17 cm^{-1} CO-NH, 1384.79 cm^{-1} Aromatic pyridine Tertiaryamine. All these prominent peaks were identified and matched with reference spectra which confirm the authenticity of the procured NCR. The ranges of the physical mixture and optimized NCR MMs showed the absorbance pattern, indicating the compatibility of drugs and polymers. Therefore, no shifting of positions of the functional groups and no significant interaction between NCR and polymer has been observed [29].

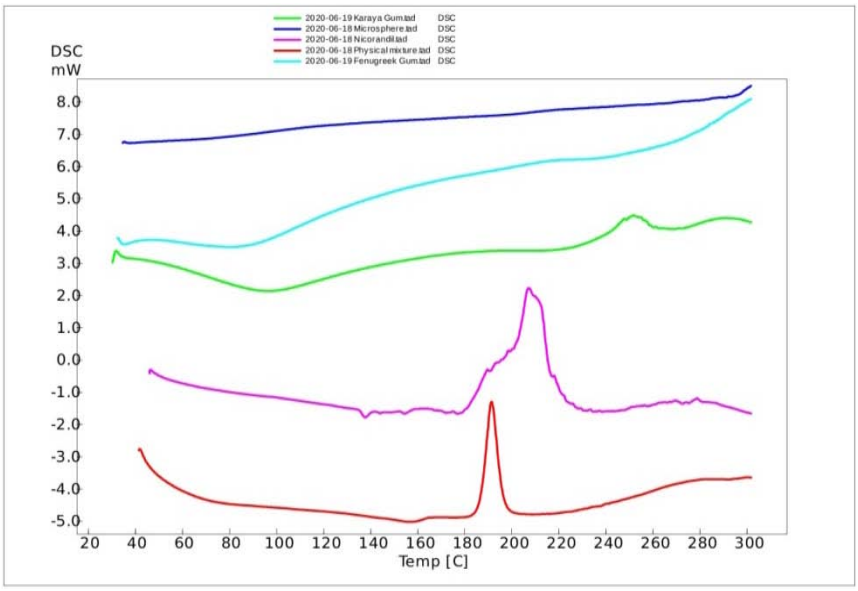
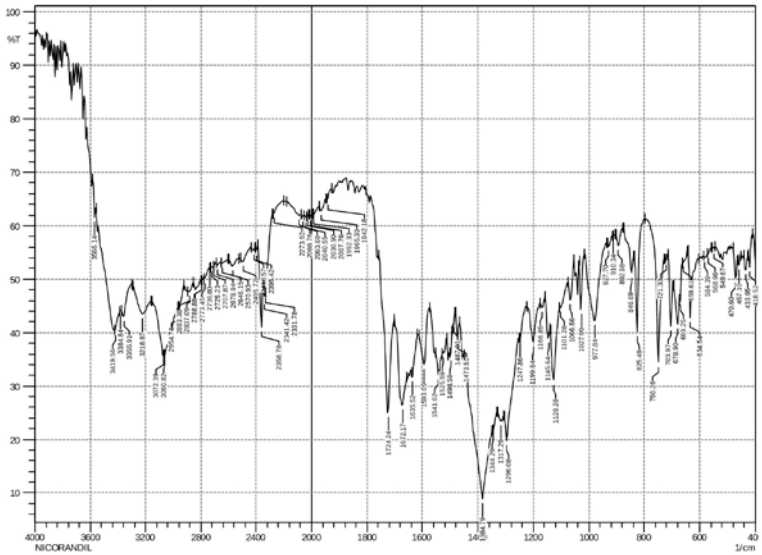
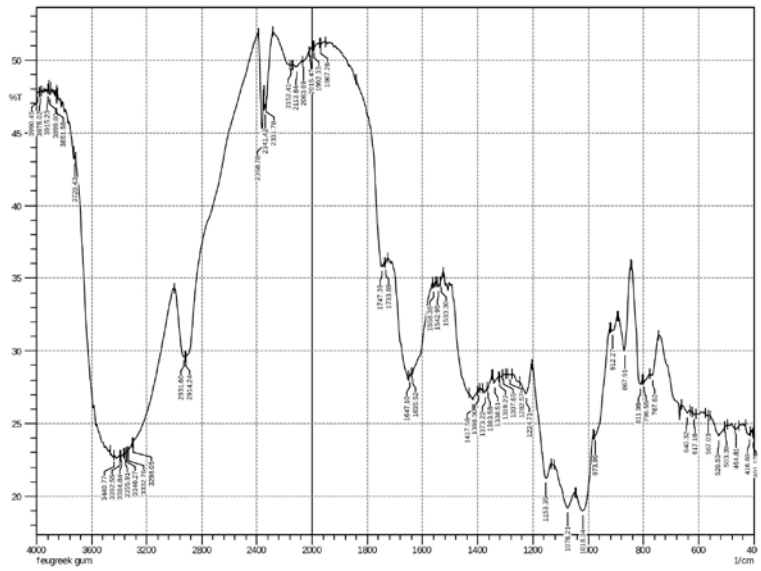


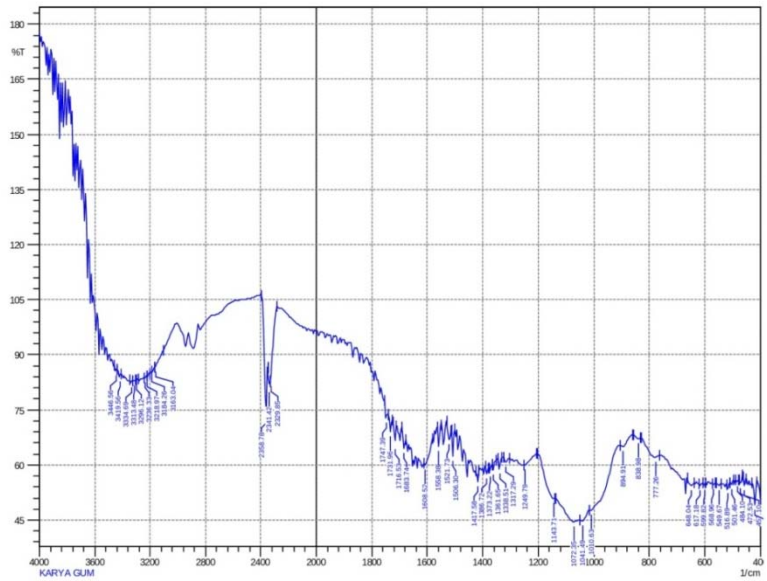
Fig. 7: Differential scanning calorimetry of the physical mixture, nicorandil, karaya gum, fenugreek gum, optimized NCR MMs



a)



b)



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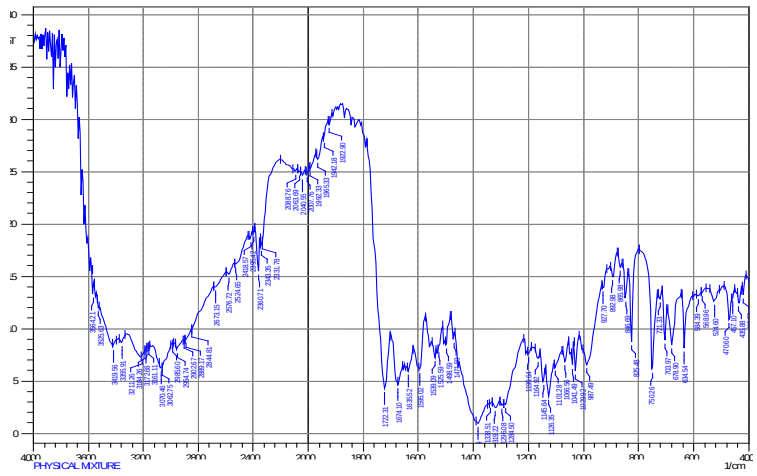
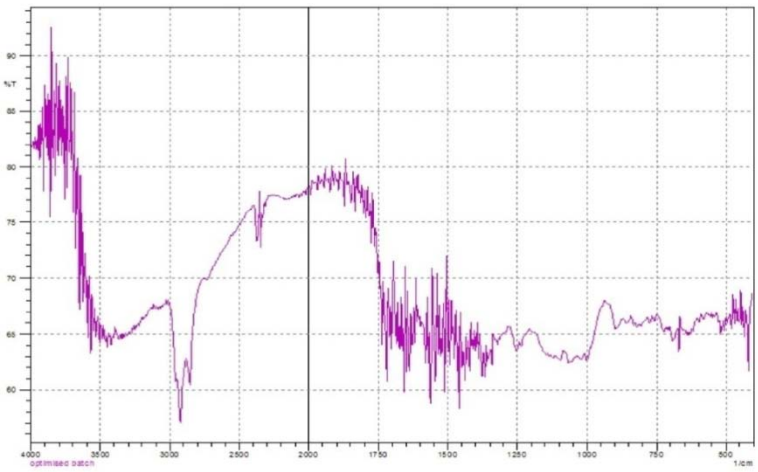


d)



e)

Fig. 8: FTIR of a) Pure Nicorandil b) Fenugreek gum c) Karaya gum d) Physical mixture e) Optimized NCR MMs

CONCLUSION

Nicorandil-loaded mucoadhesive microspheres were successfully prepared using the cross-linked emulsification method. The prepared batches of microspheres were spherical, ruptured-free, with a porous structure. The entrapment efficiency ranged from 51.83±0.50 to 66.91±0.54; mean size was in the range of 84.41±0.20 µm to 144.43±0.22µm, swelling index 82.69±0.40, % mucoadhesion 95.22±0.13. The spectra of the physical mixture and optimized mucoadhesive NCL MS indicate the drug and polymer compatibility. The release behaviour of nicorandil from prepared optimized NCR MMs was found to be 89.96±0.17 % at the end of 12 h.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

There is no conflict of interest among authors.

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