How to Cite:
Avhad, P. S., Gupta, R. A., Firke, S. N., Roge, A. B., Sarje, S. K., Raut, A. S., & Devaliya, R. (2022). Formulation and evaluation of chitosan based polyelectrolyte complex of levodopa for nasal drug delivery. *International Journal of Health Sciences*, *6*(S3), 1316–1327. https://doi.org/10.53730/ijhs.v6nS3.5555

Formulation and Evaluation of Chitosan Based Polyelectrolyte Complex of Levodopa for Nasal Drug Delivery

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***Abstract*---**Because chitosan is biodegradable, biocompatible, non-toxic, and mucoadhesive, it is commonly used in the formulation of nasal drug delivery nanoparticles employing polyelectrolyte complexes. However, chitosan's lower solubility in aqueous and alkaline conditions limits its use in the pharmaceutical and biomedical fields. This needs the development of improved chemically altered chitosan mimics that can overcome the solubility barrier. Although Levodopa is an alternative in the treatment of Parkinson's disease, it has a low oral bioavailability and very low brain absorption due to its extensive degradation by aromatic amino acid decarboxylase in the peripheral

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
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Manuscript submitted: 18 Nov 2021, Manuscript revised: 09 Feb 2022, Accepted for publication: 27 March 2022

circulation. As a result, levodopa and carbidopa, a peripheral amino acid decarboxylase inhibitor, are given together. The nose to brain medication delivery of levodopa desolate via the olfactory pathway and the trigeminal neurons has been investigated in an effort to improve brain uptake and avoid degradation of levodopa in peripheral circulation and the use of carbidopa in combination. Ionic interactions mediate charged functional groups of former polysaccharides at dissimilar pH situations (pH 5, 8, 10, and 12) and identify polyelectrolyte complexes based on chitosan and pectin during physicochemical (particle size and zeta potential) and solid-state characterizations.

***Keywords*---**chitosan, nasal delivery, polyelectrolyte complex, levodopa, carbidopa.

Introduction

Nasal route has captivated wide recognition of researchers as drug delivery route because of the benefits delivered like bypassing of blood brain barrier, circumvention of hepatic first-pass metabolism, feasibility, security, benefit of administration and intraoperative [1-3]. Intranasal administration provides numerous advantages in the therapy of neurodegenerative disorders (NDs) between blood brain barrier avoid drug delivery. Nose to brain delivery is highest probable conciliate across the olfactory and trigeminal nerve passage. It is dominant to commendation here especially similar a passage has been demonstrating to subsist in animal imitation, yet it is quiet unknown regardless near transport takes place in humans [4]. The several nasal passages, crucial anatomical brain composition and nerves included in blood–brain and naso-brain drug delivery are attractively assessed by Djupesland, Messina and Mahmoud [5]. NDs, the ultimately usual of that are Alzheimer's disease (AD) and Parkinson's disease (PD), encompass a clump of manifold situation especially are distinguish by the tenacious deprivation of neuronal subtypes and are across the preeminent destructive situation of recent century [6,7].

Chitosan (CS) is a type of linear polysaccharide made up of glucosamine and N-acetylglucosamine units joined by (1,4) glycosidic linkages. Chitosan is made by partially deacetylating chitin, a naturally occurring carbohydrate that is basically polysaccharide (N-acetylglucosamine). The degree of acetylation (DA) and molecular weight of chitosan will rely on the reaction parameters that are involved, as well as the natural source and conditions employed to extract and deacetylate chitin [8]. Chitosan is a non-toxic, biocompatible, and biodegradable substance. Other biological features include wound healing ability, antibacterial activity, and hemostatic activity. It can be processed into fibres, gels, microspheres-microcapsules, and micro/nanoparticles and is a great film forming [9]. For many of these uses, chitosan is preferred over other cationic polymers such as polylysine, polyarginine, or polyethyleneimine because of its lower toxicity [10].

Pectin is a polysaccharide that is mostly made up of esterified D-galacturonic acid and has an alpha chain. Pectin has been extensively researched in a variety of disciplines, including food, agriculture, and medicine. In the pharmaceutical sector, pectin has been widely explored for medication administration, wound dressing, and tissue engineering. Pectin has a number of benefits in formulations, including the ability to easily adapt to hydrogels, films, scaffolds, and nanoparticles [11].

When the solutions of two polyelectrolytes with opposite charges (polycation or polyanion) are mixed together, polyelectrolyte complexes develop. When oppositely charged polyelectrolytes are mixed in solution, strong but reversible electrostatic connections emerge, resulting in self-assembly or spontaneous association. Without the use of covalent cross-linkers, these direct interactions between polymeric chains result in the formation of polyelectrolyte complex networks with non-permanent structures. These polymeric networks or hydrogels are often well tolerated, biocompatible, and more sensitive to environmental changes. The degree of ionization of each of the oppositely charged polyelectrolytes, the density of the charge on the polyelectrolytes, the charge distribution over the polymeric chains, the concentration of the polyelectrolytes, their mixing ratio, the mixing order, the duration of the interaction, the nature of the ionic groups, the position of the ionic groups on the polymeric chains, the nature of the ionic groups on the polymeric chains, the nature of the ionic [12,13].

A non-stoichiometric complex is created when polyelectrolytes are combined in such a ratio that there is an excess of charge. These complexes are usually soluble. A stoichiometric polyelectrolyte complex, on the other hand, has an equal amount of each opposite charge and has a net charge of zero. When formulated, stoichiometric polyelectrolyte complexes are usually insoluble and precipitate out of solution [14]. Levodopa is the predominant drug utilized in the therapy of Parkinson's disease. Levodopa is basically administered orally or intravenously in combination with a decarboxylase inhibitor [15]. Orally administered levodopa bring about inconsistent and dishonest clinical reactions due to its conflicting oral absorption and first-pass metabolism. The alternative of the drug during assembling polyelectrolyte complex was based upon the phenomenon that it is a confined absorption window (NAW) drug possessing moderately small disposal plasma half-time (t1/2 = 1 h) and oral bioavailability of levodopa desolate is assessed at around 5%, and below 1% of the orally administrated dose extent the brain [16], consequently evolution of a moderate free implementation could diminished variation in the therapeutic results and thus ameliorate its clinical effectiveness. Carbidopa was utilized in coalescence among levodopa as a peripheral decarboxylase inhibitor, that consequences in productive amount of dopamine and depletion of adverse effects [17].

Material and Method

Materials

Levodopa and Carbidopa (10 g) was purchased from Sigma Aldrich. Pectin, Chitosan, Mannitol, Glacial acetic acid was purchased from Loba chem Pvt. Ltd, Mumbai. All other reagents were of analytical reagent grade.

Method

Preparation of Chitosan-Pectin PECs [18]

The ionic gelation process was used to create chitosan-based polyelectrolyte complexes. Chitosan aqueous solution (0.4 g chitosan in 40 ml 2% acetic acid) produced at room temperature with magnetic stirring. The chitosan solution was then split into four aliquots and adjusted with aqueous sodium hydroxide and hydrochloric acid to a desired value (5,8,10, and 12). After that, 12 mL of 1% w/v pectin solution was added to 10 mL of chitosan solution of 5, 8, 10, and 12 pH at room temperature with magnetic stirring. Following creation, polyelectrolyte samples were lyophilized at -70°C for 48 hours at a working pressure of 0.23 mbar using mannitol as a cryoprotectant. Before adding the chitosan solution, 40 mg of Levodopa and Carbidopa were scattered in it.

Characterization of PECs
FTIR spectroscopy

The goal of the study was to see if natural polymers like chitosan and pectin were compatible with Levodopa and Carbidopa. It also aids in determining the appropriateness of polymers for nanoparticle production. A Shimadzu FTIR spectrometer was used to investigate the FTIR spectra. The samples of pure drug and physical mixtures, such as Levodopa with carbidopa, chitosan, and pectin, were made separately with KBr after drying in a hot air oven for about 1 hour and then kept in desiccators before scanning the spectra between the ranges of 4000 and 500 cm-1.

Measurement of hydrodynamic diameter and zeta potential

PEC particle hydrodynamic diameter, polydispersity index, and zeta potential were measured using the DLS technique and the Zetasizer (Malvern ZS90). To achieve the best particle count, samples were dilute properly with bidistilled water. The measurements were made at room temperature, with a scattering angle of 90o and an electric field strength of 25 Vm-1.

Drug loading

Each formulation batch's weighed amount of nanoparticle was dissolved in methanol and left overnight to extract levodopa and carbidopa, after which the solution was filtered using Whatmann filter paper. 1 mL of this solution was taken out and diluted in methanol to make 10 mL. The % drug loading of this solution was estimated using the formula below using a UV spectrophotometer at specific wavelength and used methanol as a blank.

$$\% \text{ Drug loading} = \frac{\text{Practical mass (final product)}}{\text{Theorotical mass}} \times 100$$

Entrapment efficiency

Separation of nanoparticles from aqueous medium containing free drug by centrifugation at 14000 rpm for 30 minutes was used to measure entrapment efficiency. The amount of entrapped drug was estimated by measuring the absorbance of free drug in the supernatant using UV at specific wavelength. The following equation was used to calculate it.

$$\% \text{ Entrapment Efficiency} = \frac{\text{W total} - \text{W}}{\text{W total}} \times 100$$

In-vitro drug diffusion study
Preparation of sheep nasal mucosa tissue

Biological membranes can be used to determine drug penetration characteristics. As a result, the current permeation tests were performed on the excised sheep nasal mucosae, covering the ventral nasal conchae. The tissue is very simple to use, and there are a variety of colours to choose from. There are increased odds of getting undamaged, unstrained nasal mucosa.

Experimental setup and permeation studies

Ex-vivo drug permeation investigations were conducted on lyophilized TMC-DS PEC10 utilizing a modified Franz's diffusion cell as a model permeation membrane across an excised sheep nasal mucosa (barrier). In the receptor compartment, permeation medium (PBS, pH 6.6) was added and stirred constantly. The mucosa of an excised sheep nasal mucosa was acquired from a local farm butchery, which was positioned on the diffusion chamber with mucosal and serosal surfaces that were kept clean respectively, towards the donor and receptor compartments Formulation of PEC that has been improved was applied to the mucosal membrane in the donor compartment after it had been soaked in 3.5 mL of SNEF. At certain time points, 300 L of media from the receptor compartment was taken and the sampled volume was replaced with the same volume of fresh PBS and the results were analyzed using a UV-visible spectrophotometer (1700, Shimadzu®, Tokyo, Japan).

Ex vivo permeation study

A modified Franz diffusion cell was used to conduct an ex-vivo permeation investigation on lyophilized polyelectrolyte complex pH 5,8,10, and 12 over an excised sheep nasal mucosa as a model permeation medium (PBS pH 6.6) was filled in the receptor compartment and kept under constant stirring. The mucosal and serosal surfaces of excised sheep nasal mucosa were placed in the diffusion chamber with mucosal and serosal surfaces towards the donor and receptor compartments, respectively. In the donor compartment, an optimized polyelectrolyte complex formulation was distributed over the mucosal membrane. After that, 1 ml of sample was collected and replaced with fresh buffers at each

time interval. Following the proper dilution, the obtained samples were diluted with phosphate buffer solution up to 10 ml and spectrophotometrically examined.

Ex – vivo mucoadhesive study

The ex-vivo mucoadhesion potential of lyophilized polyelectrolyte complex pH 5,8,10, and 12 was determined using a modified Rao & Buri falling liquid film technique. From a local slaughterhouse, a fresh slice of sheep nasal mucosa was procured. Nasal mucosa was cleaned in saline and glued to a polyethylene plate set at a 45o angle to the horizontal plane. On the mucosa, an appropriately weighed amount of optimal polyelectrolyte complex was applied. On the formulation, 100 litters of SNEF were used. PBS (pH 6.6) was peristaltically pumped over the mucosal tissue at a rate of 5 mL min. The concentration of levodopa plus carbidopa in the perfusate was measured spectrophotometrically at specific wavelength after 1 hour. The following equation was used to calculate the mucoadhesion potential.

$$\text{Mucoadhesion potential (\%)} = \frac{[Wt - Wp]}{[Wt]} \times 10$$

Result and Discussion

Physiochemical characterization

The hydrodynamic radii of PECs were measured using the DLS approach to estimate their size. The PEC hydrodynamic radii were determined to be in the nanoscale (colloidal) size range (187.3-210.4 nm), with a size distribution that was acceptable (PDI: 0.365-0.890). The particle size of the PEC was shown to be pH dependent. In the beginning (up to pH 10), increasing pH reduced particle size; after that, particle size increased. Using the DLS approach, the influence of pH on the zeta potential of PECs was examined. The existence of an extra layer of pectin chains on the surface of the produced nanocomplex was ensured by the negative charge (-2.90 to -38.1 mV) in all of the prepared PECs. The DL of Levodopa plus carbidopa in PEC particles was found to be between 78 and 87 percent, while the EE was found to be between 86.09 and 89.78 percent, as determined immediately after PEC manufacturing. Because of the high loading and encapsulation, the manufactured PEC system appears to be appropriate for loading hydrophilic medicinal drugs into biopolymer-based PEC particles.

Drug–excipient interactions study
Fourier transform infrared spectroscopy

The following FTIR spectrum of Levodopa exhibited characteristic peaks indicating the presence of functional groups as claimed by its chemical structure. As a result, we may conclude that the Levodopa was of excellent quality. Following the assessment of the drug's FT-IR spectrum, it was determined that all of the typical peaks belonging to the functional group contained in Levodopa's molecular structure were discovered within the reference range, confirming its identity. The

characteristic stretching vibrations of C-H and O-H bonds in PECs were linked to a broad band in the FTIR spectra of all PECs in the range of 1234.65 – 3023.87 cm-1. Following the analysis of the polymer's FT-IR spectrum, it was determined that all of the typical peaks related to the functional group contained in the molecular structure of chitosan were located within the reference range, confirming its identity. Chitosan has an FTIR spectrum that ranges from 1208.45 to 2823.95. After interpreting the FT-IR spectrum of Chitosan and its physical mixture with the drug, it was determined that all of the characteristic peaks corresponding to the functional group present in the molecular structure of Levodopa were not found intact within the reference range, confirming its chitosan reactivity. The choice of polymer is aided by this interaction. The FTIR spectrum of a physical mixture ranged from 1278.87 to 3534.86. The positions of typical absorption bands and linkages of various functional groups contained in the medication did not vary significantly. This observation clearly indicates that the Levodopa's properties do not alter much in its physical combination. The interaction between the medication and the polymer was revealed by the FTIR spectra. It was discovered that Levodopa and chitosan were compatible. The optimized formulation's FTIR spectrum was discovered in the region of 1076.09 - 2965.12.

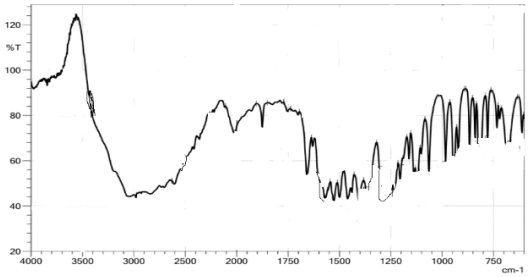

Fig. FTIR spectra of Levodopa

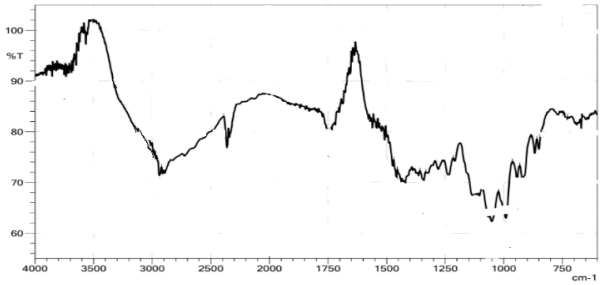

Fig. FTIR Spectra of Pectin

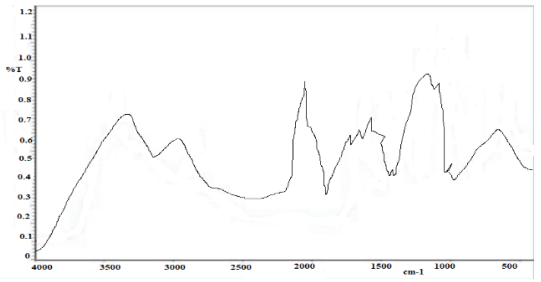

Fig. FTIR spectra of Chitosan

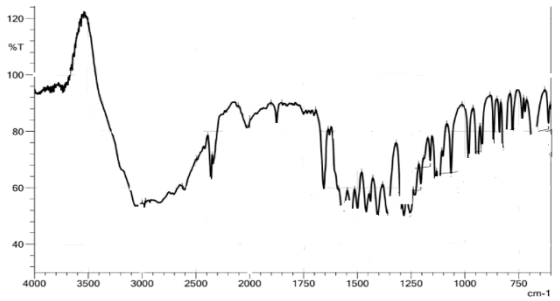

Fig. FTIR spectra of Physical mixture

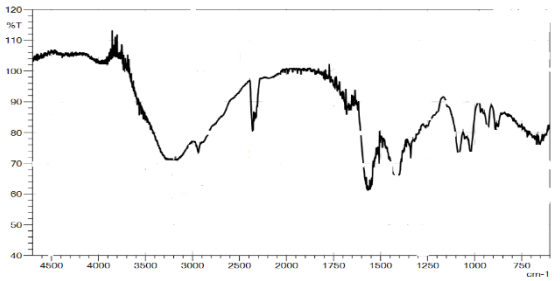

Fig. FTIR spectra of Formulation pH 10

Differential scanning colorimetry

The thermal analysis of pure drugs levodopa and carbidopa, chitosan, pectin and pH 10 formulation were studied by using Differential Scanning Calorimetry (DSC) as shown in fig. respectively. The Levodopa manifest endothermic peak at approximately 278℃ and carbidopa at 207℃. Chitosan shows a sharp endothermic peak at 89.96℃ corresponding to its melting point. DSC thermogram of PEC of formulation pH10 shows the endothermic peaks at 75.29℃, 204℃ and 271℃ respectively.

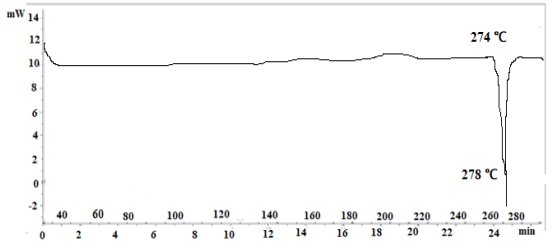

Fig. DSC thermogram of Levodopa

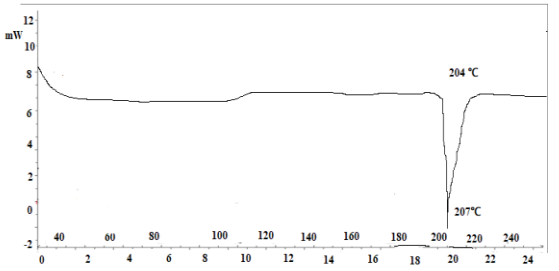

Fig. DSC thermogram of Carbidopa

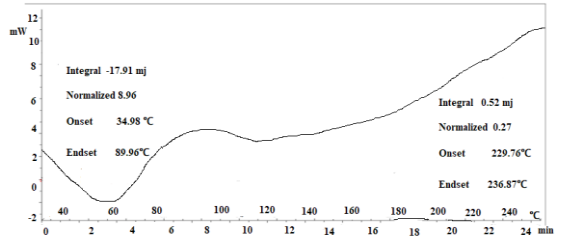

Fig. DSC thermogram of Chitosan

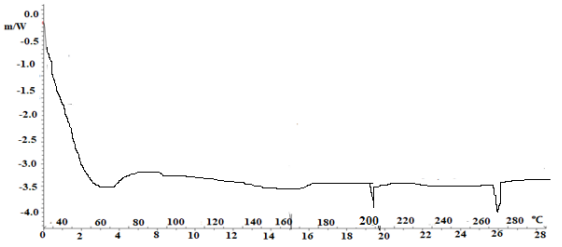

Fig. DSC thermogram of formulation pH 10

Drug content

The amount of medication in chitosan-based polyelectrolyte complex nanoparticles ranged from 45.23 mg to 65.12 mg. The medication content of the pH-based PEC nanoparticle varies. The maximal drug content of chitosan-based PEC was reported to be 65.12 mg.

Drug loading

The drug loading of levodopa in conjunction with carbidopa in chitosan-based polyelectrolyte complexes was reported to be between 78 and 85 percent. The highest drug loading in chitosan-based PEC was reported to be 85 percent.

Entrapment efficiency

The entrapment efficiency of chitosan-based polyelectrolyte complex of different pH varied. The entrapment efficiency was found to be minimum and maximum of 81.93 % to 89.67 %. But the maximum entrapment efficiency was not considered as optimum. The optimum percentage efficiency was based on the drug content and polymer usage. The optimum entrapment efficiency was found to be 85.26% from drug content and entrapment efficiency results chitosan-based PEC pH 10 batch were consider as optimum.

***In vitro* diffusion study**

In-vitro diffusion investigations of chitosan-based PECs revealed that drug release is influenced by the pH nature of the various PECs. Increases in pH concentration resulted in significantly longer drug release durations for all formulations. In comparison to pH 5 and 8, the higher the pH (pH 10 and pH 12, i.e., alkaline pH), the greater the drug diffusion. Less pH of formulation (pH 5 and pH 8) results in decreased drug diffusion, since the complex between polymer and drug is greater in alkaline pH than in acidic pH. As a result, the pH 10 and pH 12 PECs were deemed adequate for the regulated delivery of the combination medication levodopa and carbidopa.

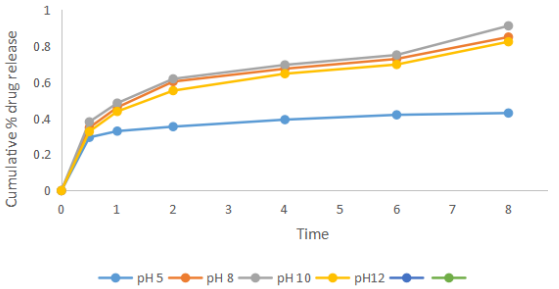

Fig. In vitro diffusion study

***Ex vivo* permeation study**

Similarly, in ex-vivo diffusion tests of chitosan-based PECs, it was discovered that drug release is dependent on the pH of the various PECs. Increases in pH concentration resulted in significantly longer drug release durations for all formulations. In comparison to pH 5 and 8, the higher the pH (pH 10 and pH 12, i.e., alkaline pH), the greater the drug diffusion. Less pH of formulation (pH 5 and pH 8) results in decreased drug diffusion, since the complex between polymer and drug is greater in alkaline pH than in acidic pH. As a result, the pH 10 and pH 12

PECs were deemed adequate for the regulated distribution of levodopa and carbidopa.

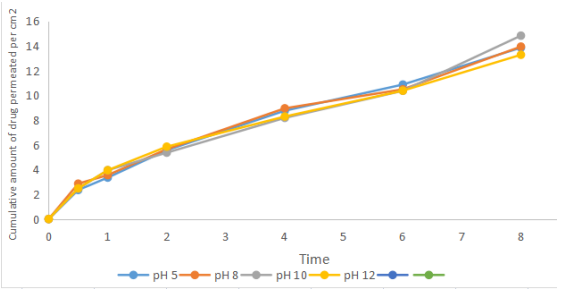

Fig. Ex – vivo permeation study

Ex-vivo bioadhesion study

The falling film approach was used to assess the ex-vivo bioadhesion potential of PECs at pH 5, 8, 10, and 12. For pH 10 and 12, the larger bioadhesion was determined to be 78.430.45% and 88.340.98%, respectively. The strong electrostatic interaction between cationic chitosan oriented outside of the PEC particles and the anionic pectin moieties found in the mucin may account for the PEC high bioadhesion.

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