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Research Article

Preparation and *In Vitro/Ex Vivo* Evaluation of Buccoadhesive Discs of an Anti-Parkinson Drug: Relationship between Mucoadhesivity, Drug Release and Permeability

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ABSTRACT

Background: Selegiline hydrochloride (Sel) is a drug applied for the therapy of early- step Parkinson's disease. In usual clinical doses, it is an elective irreversible MAO-B inhibitor**.** This study intended to formulate mucoadhesive microspheres of selegiline with the objective of improving the therapeutic efficacy, patient compliance and bioavailability.
Methods: The microspheres were prepared by emulsion solvent evaporation method (O1/O2) using hydroxypropyl methylcellulose (HPMC). In the current study, bucco- adhesive microspheres were prepared with different drug to polymer ratios and characterized by encapsulation efficiency, particle size, Differential Scanning Calorimetry (DSC), FTIR Fourier Transform Infrared Spectroscopy (FTIR), flowability, the degree of swelling and surface pH, mucoadhesive character, retentive time, and drug release studies.
***Results*:** The best drug to polymer ratio in microspheres was 1:2 (as F1). The production yield microspheres F1 showed production yield of 84.79%, mean particle size of 744.73 µm and loading efficiency of 53.33%. The DSC exhibited the property of selegiline loaded microspheres changed to amorphous form. The FTIR spectrum proposed that the drug kept its chemical stability during the emulsification process. The results showed that the microspheres of F1 had faster release than the microspheres of F2 (1:4), F3 (1:6), and commercial tablet (p<0.05). The microspheres did not exhibit good retention time properties (276.66- 329.66 min). The results of mucoadhesion strength (8.3- 18.3 g/cm2) and surface pH of discs (6.44- 6.97) showed the better characterization of microspheres in buccal.
Conclusion: The formulations were found to be appropriate candidates for the improvement of microspheres for the remedial objects.

Introduction

Selegiline hydrochloride (Sel) is a drug utilized for the therapy of early- stage Parkinson's disease, depression, and dementia. In usual clinical doses, it is an elective invariable MAO- B inhibitor.

Nasal and subcutaneous routes exert some limitations such as lower retention time for nasal solution and difficulty in self-administration for injectable ones. These forms have certain disadvantages. Film and disc of bucco-mucoadhesive drugs are of delivery profit, as the film solves and the oral drug is administered through absorption in the mouth (buccally or sublingually) and/or in small gut (intestinally).[1]

Hence the aims of present project were; to extend a novel drug delivery system for Sel which might be carried via the buccal route (discs created with microspheres), to exclusively target the mucous area in order to attain a quick relief by rapid beginning of operation; to enhance bioavailability; and to elude the secondary dose administration. The buccal discs can be better alternates that may decrease the side effects related to oral and parenteral remedies.

In treating the Parkinson's, constant levels of the drug are required in the blood for an extended period. This can be settled by designing a buccal drug delivery system that could deliver the drug via oral buccal mucosa.[2]

The hydrophilic Sel may show low bioavailability when administered through the buccal route. And once in the systemic circulation, hydrophilicity is required for effective distribution. Therefore, the use of buccoadhesive Sel was proposed. However, in order to achieve the result, the modification of its absorption was

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proposed by preparing microspheres using suitable polymer.

Preparing the microspheres/discs was based on literature reports that believed the transport of small particles was better across the mucosa and also there was a buccal –to –blood transport via the mucous region. The microparticulate systems offer versatility in terms of size, mucoadhesiveness, and variety of matrix materials and these features confer flexibility to the formulator to select the dosage form in a way which could satisfy the ultimate requirements. The quantity of drug achieved the location of action is mostly only a little fraction of the utilized dose. Moreover, accumulation of administered dose at non-target sites can result in harmful reactions and unpleasant side effects. A route suggested for improving the principal biodistribution of materials is to trap them in submicroscopic drug vehicles. Amongst so many particles, polymeric microspheres caught undivided attention in the present research scenario. Attendance of free carboxyl group importantly increases the destruction of the polymer. These hydrophilic groups cause superior penetration of water molecules within the polymer matrix allowing the quicker release of the loaded drug. It was reported that this polymer possesses a biphasic release pattern. The pattern showed an initial burst release followed by slow release. It was also reported that this release has the maximum entrapment for hydrophilic drugs.

Hydroxypropyl methylcellulose (HPMC) is the combinatorial modification of the natural polymer, cellulose. The reasons for its wide acceptation are: (1) solubility properties of the polymer in gastrointestinal fluid, and inorganic and aqueous solvent systems, (2) non-intervention with tablet decomposition and drug accessibility, (3) elasticity, chip-resisting and lack of flavor and smell, (4) consistency in the attendance of heat, light, air or sensible levels of humidity.

Oral transmucosal sorption usually occurs rapidly because of the rich vascular provision to the mucosa and the absence of a stratum corneum epidermidis. This small barrier to drug transport results in a quick increase in blood concentration level. The drug reaches the blood in 1 min, while peak blood levels of remedies are reached generally within 10 to 15 min. This time span is considerably quicker than the time when the drug is administered through the orogastric tube. Oral transmucosal usage held the benefit of eluding the enterohepatic excursion and immediate degradation by stomach acid or little first-pass effects of hepatic metabolism. The drug ought to have a prolonged access to the mucosal area, as it results in significant absorption of the drug across the oral mucosa[3] Buccal drugs are made either in the form of little, fast solving tablets, in the form of inhalers, torches, or in liquid dosage forms. These drugs are administered by locating the medication in the buccal or between the gum and the cheek (mouth). This form of drug administration suffices as it bypasses the gastrointestinal system and is adsorbed in the blood stream in minutes.[4]

Having created an intense drug condensation in the mouth area, the buccal system is systemically absorbed through the mucosa. The oral mucosa is attended as a further prominent way for systemic transmucosal drug delivery.[5] At the beginning, the appropriateness of buccal transmucosal route is linked to the permeability of the field, where the buccal mucosa is not much penetrable and is therefore not capable to signal a quick beginning of absorption (i.e., more competent for an extended release formulation). In the second stage, the buccal mucosa has a stretch of soft muscle and is approximately motionless or not washed which makes it a further favorite area for retentive systems like mucosal drug delivery systems applied for oral transport.[6]

Hence this study intended to prepare and evaluate the buccal discs of Sel through the application of mucoadhesive polymer, develop the therapeutic efficacy of these medications, and decrease their dose-dependent side effects and times of administration in Parkinson's therapy.

Materials and Methods

Materials

Selegiline hydrochloride (Di-pharma, Italy), HPMC (E-15) (Sigma-Aldrich, USA), ethanol, dichloromethane, buffer phosphate (pH 6.8), sodium chloride, potassium chloride, sodium sulfate, ammonium acetate, urea, lactic acid, liquid paraffin and span 80 were provided from Merck Chemical Company (Darmstadt, Germany). All solvents and reagents were of analytical grade.

Preparation of Selegiline microspheres

Microspheres of Sel were prepared using HPMC with three different Sel/HPMC ratios (1:2, 1:4 and 1:6 W/W). Briefly, 100 mg of Sel was dissolved separately in 5 mL of ethanol (O_1) for 30 min through shaking at 500 rpm using magnetic stirrer. HPMC (200, 400 and 600 mg) was then solved in 5 mL of ethanol (O_1) and stirred until complete dissolution. Then, 5 ml HPMC solution was mixed thoroughly with 5 ml ethanol solution containing 100 mg Sel under magnetic stirring. Next, the resultant drug-polymer suspension (O1) was poured through a No. 20 needle into 100 mL of light liquid paraffin containing 3% v/v span 80 (O1/O2). Two hours later, 25 mL n-hexane was added while shaking for complete reaction. Stirring was continued for an additional 2 h at 600 rpm till perfect solvent evaporation and microspheres preparation at 70˚C. The hardened microspheres were accumulated by filtration and eluted with three parts of 25 mL n-hexane and air dried at room temperature for 24 h (Table 1).

Buccal mucoadhesive microspheres characteristics

Measurement of loading efficiency and production yield

The loading efficiency was computed through the following equation:

$$Loading\ efficiency\ (\%) = (Actual\ drug\ content\ in\ microspheres / theoretical\ drug\ content) \times 100 \quad \text{Eq. (1)}$$

Table 1. Selegiline hydrochloride microspheres prepared by emulsion solvent evaporation (O_1/O_2).

Formulation code	Drug: Polymer ratio	Emulsion O_1/O_2				
		Organic phase (O_1)			Oil phase (O_2)	
		Selegiline (mg)	HPMC (mg)	Ethanol (ml)	Liquid Paraffin (ml)	Span80 (%w/w)
F_1	1:2	100	200	10	120	2.5
F_2	1:4	100	400	10	120	2.5
F_3	1:6	100	600	10	120	2.5

The production yield of the microspheres was measured by dividing the ultimate weight of the polymeric particles to the primary weight of the solid substances. Each measurement was carried out in triplicate.[7]

Particle size analysis

Formulations of microspheres were measured for frequency distribution with an adjusted optical microscope, equipped with a stage and a visual micrometer.[8] Small amounts of microspheres were expended on a clean glass slide and the average particle size of 100 numbers, frequency distribution and mean particle size was analyzed in each sample applying scion image and sigma plot software packages.

Flowability characterization of microspheres

The angle of repose for various samples was determined according to fixed funnel standing method. When microspheres were added onto a horizontal area, a conical pile was made.[9] According to Eq. 2, the internal angle of the surface of the pile and the straight surface was measured as the angle of repose.

$$\theta = tan - 1\ h / r \qquad \text{Eq. (2)}$$

Where θ is the angle of repose, *r* is the radius, and *h* is the height of the pile.

Bulk and tapped densities were also determined through a 10 mL graduated cylinder. The sample was added to the cylinder and compacted mechanically for 200 times, then tapped volume was recorded and consequently the tapped and bulk densities were computed.[10] Each test was carried out in triplicate.

Carr's index value of microspheres was calculated according to the following formula (A-3):

$$Carr's\ index\ (\%) = (Tapped\ density - bulk\ density)\ x\ 100\ /\ tapped\ density \qquad \text{Eq. (3)}$$

Hausner's ratio of microspheres was measured by dividing the tapped density to the bulk density using the formula (A-4):[11]

$$Hausner's\ ratio = Tapped\ density\ /\ bulk\ density \qquad \text{Eq. (4)}$$

Differential Scanning Calorimetry (DSC)

The physical situation of the drug in the microspheres was determined by Differential Scanning Calorimetry (DSC) (Shimadzu, Japan). The thermograms were taken at a scanning speed of 10°C/min operated upon a temperature limit of 25-300°C.

Fourier-transform infrared spectroscopy

Fourier Transform Infrared spectroscopy (FTIR) was recorded on physical mixtures and prepared formulations as well as pure substances using FTIR spectrophotometer (Bomem, MB-100 series, Quebec, Canada) in the limit of 400–4000 cm^{-1} through potassium bromide discs. The spectrum was an average of ten sequential scans on the similar sample. Processing of the FTIR data was carried out applying GRAMS/32 version 3.04 (Galactic Industries Corporation, Salem, NH).

Disc production method and physicochemical characterization

Every disc included 100 mg of microspheres (with a various drug to polymer ratios of 1:2, 1:4 and 1:6). The discs were compacted with a single punch (diameter 6±0.1 mm) and fixed compaction force (2.5 tones). The hardness of the discs was measured for six discs applying Erweka hardness tester (Erweka, Germany), and the friability of the provided discs was evaluated utilizing friability tester (Erweka, Germany). Determination of the surface pH of the discs, swelling properties, and permeation and release behavior were performed as follows;

Evaluation of surface pH

The surface pH of the discs was measured using a composed glass electrode in order to study their facile side effects *in vivo*.[12] An acidic or alkaline sample causes the inflammation of mucosal membrane and therefore this is the main parameter in extending a mucoadhesive dosage form.[13] The discs were primarily authorized to inflate exposing to 5 mL of phosphate buffer pH 6.8 for 2 h in 50 mL beakers which simulated the saliva. pH was then noted by bringing the electrode near the surface of the formulation and allowing equilibration for 1 min. Surface pH was determined at predetermined time intervals (15, 30, 60, 90 and 120 min). The tests were performed in triplicate.[14]

Disc swelling analysis

Mucoadhesive formulations may swell in the presence of saliva. The swelling speed of mucoadhesive samples was determined by locating the exactly weighed discs (W_1) in 50 mL of phosphate buffer solution (pH 6.8) at 37°C.[15] Swelling was measured at predesigned time intervals. Then, the discs were eliminated from the beaker after attentively omitting the extra surface water using the filter paper. The swollen disc was weighed anew (W_2) and the swelling index was computed consequently (Eq. 5):

$$Swelling\ index = (W2 - W1)/W1\ x\ 100 \qquad \text{Eq. (5)}$$

Ex-vivo Permeation test

The *in vitro* permeation study via the buccal mucosa was carried out by a Franz diffusion cell at 37 ± 0.2°C. The mucosa was acquired from buccal of sheep. Freshly obtained sheep mucosa was located among the donor and receptor portions such that the smooth surface of the tissue faced the donor section.[16] The discs were located on the mucosa and the sections were clipped with one another. The donor section was filled with 3 mL simulated saliva solution (sodium chloride 4.50 g, potassium chloride 0.30 g, sodium sulfate 0.30 g, ammonium acetate 0.40 g, urea 0.20 g, lactic acid 3 g, and purified water up to 1000 mL) and pH of the solution was regulated to 6.8 by 1 M NaOH solution. The receiver section was filled with 22-25 mL isotonic phosphate buffer, pH 7.4, and shaken with a magnetic bead at 700 rpm. Three milliliters of samples (receptor portion) was removed at predestined time intervals and measured for drug content using spectrophotometer at 258 nm.

In vitro release studies

In order to perform *in vitro* release investigations, dissolution test apparatus type II (USP) was employed through a rotating paddle method. The studies were conducted for all formulations in triplicate, using 500 ml (37 °C, 100 rpm) of phosphate buffer (pH 6.8) as the dissolution environment. An aliquot of 5 mL sample was taken at 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h intervals and a similar volume was displaced with new phosphate buffer (pH 6.8) retained at the similar temperature. Samples were afterward measured at 258 nm with spectrophotometer UV-160 (Shimadzu, Japan).

Adhesion studies

Adhesion studies were performed in 3 different steps:

Ex vivo mucoadhesion time

This investigation was carried out according to the guidelines for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran (National Institutes of Health Publication No 85-23, revised 1985). The chosen sample was exposed to *ex vivo* bioadhesion trial. The disintegration medium was subjected to 900 mL phosphate buffer pH 6.8 retained at 37°C. A buccal section of sheep, 3 cm length, was attached to an area of the glass slide, vertically linked to the disintegration device (Erweka, Germany).[17] The mucoadhesive discs were moisturized from one area and next was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the disc was perfectly soaked in the buffer solution at the lowest spot and was brought out of the solution at the highest point. The time essential for complete erosion or separation of the discs from the mucosal area was indicated. The test was performed 3 times.

Determination of bioadhesive strength

Bioadhesive performance of the prepared discs was evaluated by "tensile strength". This is the power per unit area that is needed to separate the mucoadhesive preparation from the tissue area.[18] As the results in power per unit area be too small, it was normalized to the specific mass of the unit area. The bioadhesive forces of discs were determined using a biooadhesive force-measuring apparatus, by mucosa cut from a buccal mucosal area of sheep. The equipment was locally collected. The apparatus mostly consisted of a two-armed balance (Figure 1).

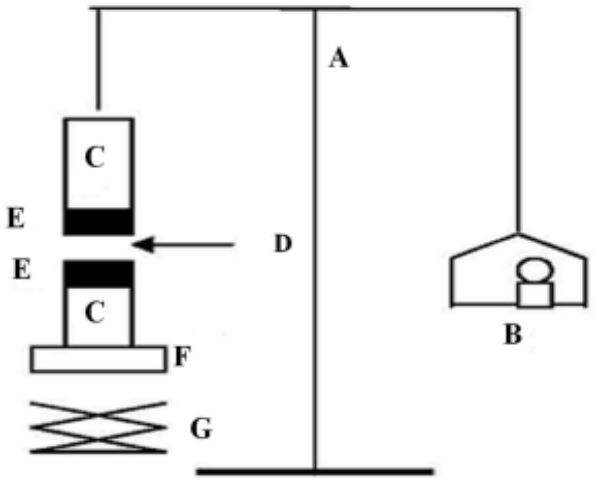

Figure 1. Mucoadhesive strength measuring instrument: (A) changed balance; (B) weight, E) glass vial; (C) selegiline discs; (D) buccal of sheep; (F) Weights; (G) height-modifiable pan.

The segments of mucosa were supplied frozen in phosphate buffer and pH of 7.4, and thawed to the room temperature prior application.[19] At the time of the experiment, a segment of tissue was fixed on the superior glass vial (C) by a cyanoacrylate adhesive (E). The diameter of the mucosal membrane was 2 cm. The vials were balanced and retained at 37°C for 10 min. Then, one vial with the segment of mucosa (E) was linked to the balance (A) and another vial was stabilized on a height-modifiable pan (F). To expose tissue to this vial, a constant amount of discs (D) was applied. The height of the vial was regulated to such a degree that the discs might stick to the mucosal tissues of both vials. Instantly, a steady force of 0.5 N was used for 2 min to assure a close contact between the mucosa and the discs. The vial was then moved upwards at a constant speed and connected to the balance. Weights were attached at a constant speed to the pan on the other side of the adjusted balance of the applied apparatus till two vials were unconnected. Within determination procedure, 150 µL of phosphate buffer (pH 6.8) was equally expanded upon the area of the trial membrane. The mucoadhesive force, indicated as the detachment stress in g/cm^2, was measured from the minimal weights that separated the mucosa from the area of each sample by the following formula:[19]

$$Detachment\ stress\left(\frac{g}{cm^2}\right) = \frac{m}{A} \qquad \text{Eq. (6)}$$

Where *m* is the weight put on the balance in grams and *A* is the area of mucosa displayed. Determinations were

replicated three times for each of the discs. All the above tests were carried out thrice.

Histopathological studies

Histopathological results of HPMC discs on the exposed mucosa were also examined. The mucosa was stabilized with 10% formalin, usually performed, and fixed in paraffin.[20] Paraffin segments were cut on glass plates and colored with hematoxylin and eosin. Any injury to mucosa was shown by a light microscope.

Results and Discussion

Drug-loaded mucoadhesive microspheres were prepared by emulsion solvent evaporation/extraction (O1/O2) technique. Sel, a hydrophilic drug, cannot distribute out in the oily (O_2) processing stage within the preparation of microspheres using emulsification procedure and thus was selected as an ideal precursor form of the drug molecule. The physicochemical properties (i.e. molecular weight, polarity, etc.) of a drug are important for its inactive carry through the mucosa of the mouth cavity. For drug absorption to be accomplished by the buccal tissue of the oral cavity, the dosage form must be solved in saliva to release the Sel into a medium.[21] Then, the drug is distributed into the mucus coating the buccal tissue at the time which is accessible for permeation. For substances transferred by the paracellular route, tortuosity and intercellular space are the major impediments to permeability. Hence, because the intercellular spaces and cytoplasm are hydrophilic in property, this path is preferred by hydrophilic composites.[22]

Physicochemical characterization of microspheres

The physical characteristics of prepared Sel buccoadhesive microspheres are shown in Table 2.

The measurement of the mean weight of six discs from each formulation exhibited that all the discs were in the weight limit of 177.57±7.56-187.60±5.45 mg. The hardness and friability were between 10.5-16.33 N, and 0.85- 0.95%, respectively. In all the formulations, friability fell below 1-2%, which is an exhibition of the well mechanical persistence of discs.

Figures S1A and S1B in Supplementary Information display that yield follows any particular pattern of change with drug loading and loading efficiency. The production yield was known to be at a maximum level for sample F1 (84.79%) containing high viscosity of HPMC (15.99 % drug loading) and the smallest amount was obtained to be 73.79 % for F3 (6.79% drug loading). The entrapment efficiency was found to be the lowest for sample F3 (47.50 %) and the highest for formulation F1 53.33 %. According to the results (Table 2), increasing the HPMC polymer amount (F3, 600 mg HPMC) increased the production yield (84.79%). The reason for this might be a decrease in the distribution rate of solvent from concentrated solutions (organic phase) within the outer phase of the emulsion.

The reason for decreased entrapment (loading efficiency < 100%) may be the difficulty in the formation of microspheres (solidification). This result was in accordance with the results previously reported on Neostigmine bromide microspheres.[23]

An outer oil phase (O2) was applied as the harvesting medium with the supposition that it would be unsuitable for the drug, to spread out of the microspheres and thus it is possible to form rigid and discrete particles containing drug molecules.

Thus higher entrapment efficiency of F_1 can be explained by low viscosity of the internal phase (O_1, with 100 mg HPMC) and subsequent precipitation of the drug.[24]

The reason for the reduced drug entrapment seen (F_1 to F_3) may be explained by the difficulty in the production of microspheres due to the maximum concentration of a hydrophilic polymer such as HPMC and consequently viscosity increase.[25]

Table 2. Effect of drug to polymer ratio on the loading efficiency, production yield, particle size and flowability characteristics of microspheres and physicochemical characteristics of disc formulations.

Variables	Formulation code		
	F_1	F_2	F_3
Drug : Polymer ratio	1:2	1:4	1:6
Production yield(%±SD)	84.79±3.95	77.42±8.13	73.79±11.65
Theoretical drug loading(%±SD)	33.33	20	14.29
Mean drug entrapped(%±SD)	15.99±0.15	8.97±0.05	6.79±1.00
Drug loading efficiency(%±SD)	53.33±0.46	44.86±0.28	47.50±7.01
Mean particle size(µm±SD)	744.73±10.72	758.58±35.48	1009.02±10.96
Bulk density(g/cm^3± SD)	0.48±0.01	0.47±0.05	0.46 ±0.03
Tapped density(g/cm^3± SD)	0.61±0.0	0.56 ±0.08	0.55 ±0.00
Carr's index(%±SD)	21.15±0.9	16.79±1.01	16.43±0.87
Hausner ratio(±SD)	1.27±0.04	1.20±0.003	1.20±0.06
Angle of repose(°θ ±SD)	14.03±0.07	12.95±0.02	10.20±0.10
Weight variation(mg ± SD)	177.57±7.56	182.35±5.6	187.6±5.45
Hardness (N ± SD)	10.5	12.83	16.33
Friability(%±SD)	0.85±0.02	0.87±0.04	0.95±0.05
Drug content (%±SD)	30.01±5.46	18.95±5.52	11.23±7.75
pH surface(±SD)	6.44±0.030	6.53±0.057	6.97±0.076
Swelling Index(%±SD)	13.58±0.63	13.04±0.57	16.76±0.39
Mucoadhesive strength (g/cm^2±SD)	8.30±0.29	13.30±0.18	18.30±0.28
Residence time(min±SD)	276.66±3.51	297.66±5.86	329.66±6.66

Incorporation of HPMC increases the viscosity of the microsphere base and offers an effective mucoadhesive base for the topical delivery of the drug as a buccal disc.[26] This can cause the enhancement of the viscosity, which in turn increases the droplet size during the polymer mixture poured to the harvesting medium.[26] The mean particle size or average diameter of the microspheres (from F1 to F3) significantly increased with increasing the HPMC concentration ($P<0.05$) and was in the range of 744.73±10.72 to 1009.02±10.96 µm (Table 2). Testing the data exhibited that all prepared microspheres followed a log-probability distribution. Microspheres of F1 (1:2 drug to polymer ratio) were smaller in size and had a smoother surface than microspheres of F3 (1:6 drug to polymer ratio).

Micromeritics of prepared microspheres

The microspheres showed good to excellent flow characteristics (Table 2). The bulk density and tapped density quantities were in the limit of 0.46–0.48 and 0.55–0.61 g/cm^3, respectively. The highest suitable flowability was exhibited by F2 and F3 and the worst flow characteristics were shown by untreated Sel powder. Using these values, Carr's index and Hausner ratio were obtained to be in the range of 16.43-21.15% and 1.20-1.27, respectively. The outcome represented that the produced microspheres have well to fair passable flow specifications.

Low angle of repose indicates less cohesive powder and more free-flowing. Powder flow characteristics were examined based on Carr's index and angle of repose.[27] Carr's index is determined by particle size and its distribution. Smaller particles tend to stick together. Thus, when measuring the angle of repose, small particles as F1 (744.73 µm) are able to form a denser packing, therefore increasing the angle of repose (14.03˚). Large particles as F3 (1009.02 µm) tend to push other particles, thereby resulting in a lower angle of repose (10.20˚).

Differential Scanning Colorimetry

Differential Scanning Calorimetry (DSC) experiments were carried out for pure Sel, HPMC, physical mixture and microspheres prepared (Figure S2 in Supplementary Information). Pure Sel exhibited a sharp melting peak around 150.58°C. HPMC demonstrated a very wide endothermic peak at 65.84°C which may be related to the initial water content of the powder, such that a mass loss resulted in the baseline change as well. The physical mixture F1 exhibited an endothermic peak around 146.05°C which is missed in the prepared microspheres. The melting peak of the drug in physical mixture slightly shifted; it may be related to the drug dissolution in molten polymer before reaching the drug melting point. The absence of melting endotherm peak of the pure drug in the microspheres was obvious.[28,29] These are sufficient to conclude the amorphous structure of the drug in the microspheres because the melting peak of the drug is appeared in the physical mixture (146.05 ˚C).

Fourier-transform infrared spectroscopy

FTIR spectra of pure Sel, physical mixture of F1, HPMC, and microspheres prepared are displayed in Figure S3 in Supplementary Information. The FTIR spectrum of pure Sel exhibited property absorption peaks at 1456, 1093, 1464 and 858 cm^{-1} which show the attendance of wide, -N- bending fluctuation, C-N bending, and -N- wagging vibration, respectively. Additionally, C-H stretching vibrations at the end of the aliphatic chain, CΞC stretching vibrations and bending vibrations bonds were exhibited at 2942, 2120 and 698 cm^{-1}, respectively. According to the FTIR spectra of Sel-loaded microsphere indicated no change of functional groups but a new peak exhibited at 1735 cm^{-1}, which is related to span 80 (as an emulsifier) that influences the microspheres. This peak was not seen in the physical mixture (absence of emulsification process).

Measurement of surface pH

The similarity of outside pH of the discs to buccal pH is necessary to avoid each possible inflammation to the buccal tissue after consecutive usage of buccoadhesive discs. All samples, as F1, F2, and F3, displayed surface pH values near the physiological pH (Table 2). These consequences manifest that the prepared formulations produce a suitable pH in the area of salivary pH (5.5-7.0) offering no hazard of mucosal harm or irritation on utilization.[30,31]

Swelling determination

Swelling status of a mucoadhesive system is serious for effective adhesion and alike for sustained release of the drug.[32] Swelling index for every sample was estimated with regard to the time, and the quantities found at 15 to 480 min are displayed in Table 2. The outcomes offer that concentration of the inserted buccoadhesive polymer can have a considerable role in attaining the appropriate mucoadhesion and drug release profiles.

Although the mucoadhesive polymer applied in the investigation was hydrophile and held big quantities of water, discs including HPMC exhibited low concentration-related swelling characteristics.[33] Thus, the results of the swelling research displayed that samples F1, F2 and F3 were able to support their entirety and decomposition after 8 h may be due to an increase in surface humidity and water permeation into the matrix.[34] Mucoadhesive polymers are water-soluble and have swellable networks. The acceptable polarity of this polymer permits enough moistness by the mucus and sufficient fluidity of it allows the reciprocal adsorption and interpermeation between polymer and mucus.[31,32]

Drug release from buccoadhesive disc

According to Figure 2, the release of 14.13-46.52% of the drug after 0.5 h assures that there is a burst of effect from the disc. Moreover, samples with a proper sustained drug release profile of at least 72.16-84.90% over a period of 8 h were preferred for the aim of present research.

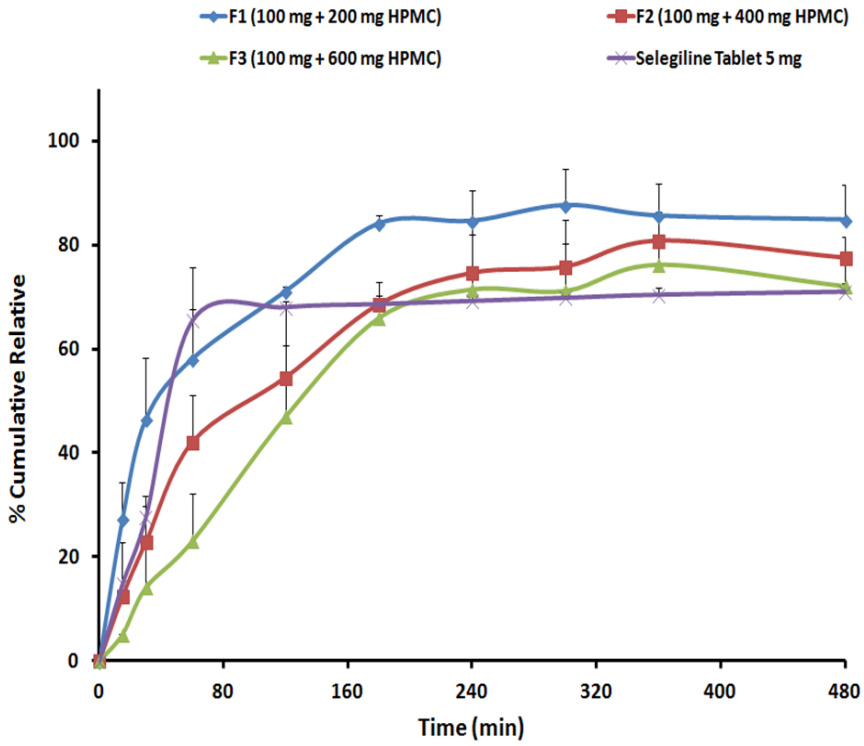

Figure 2. Accumulative percentage of selegiline release from discs made with various drug to polymer ratios, and selegiline tablet (commercial)®.

The accumulative percentage of drug release profiles of Sel from mucoadhesive discs displayed that the drug release was effected by the concentration of bioadhesive polymer used or the polymer to drug ratio in each formula (Figure 2).

In all formulations, a burst release was reported in half an hour, including a too slow increase in the accumulative percentage of drug delivered up to 8 h. The drug release was considerably quicker from commercial Sel tablet which can be due to the dissimilarity in the hydration, gelling, and abrasion status of the polymer matrix. Theoretically, the higher the absorption of water through the polymer, the more the quantity of drug distributed from the polymer matrix.[35] Therefore, HPMC as a hydrophile polymer has the capability to hydrate, hold water in its structure, and automatically form a gel which is expandable, erodible and quicker in drug release.

In addition, the hydrophile buccoadhesive polymers would solve and create more pores and ducts for the drug to distribute from the disc.[36,37] Hence, these outcomes offer that the permeation rate of the release environment within the discs and therefore the rate of release of the solved drug are actions of the quantity of the hydrophile polymer distributed over the matrix.

The time necessary for 50% (MDT) of Sel to be delivered from various buccoadhesive discs (F1 to F3) is exhibited in Table 3, as another determination of the sustained release of the drug from the made mucoadhesive discs (49.69-92.13 min, respectively). Formulae showing small MDT values (F1 with 49.69 min) should not be applied for controlled drug delivery of Sel as the drug would be gradually delivered from the buccal discs over an extended time to increase the perfect absorption of the drug from the oral mucosa and decrease the washing out by human saliva.

Table 3. Flux or amount of drug release per unit surface area after 8 h, permeability coefficient for various formulations and comparison of different release properties of selegiline from various formulations and Selegiline commercial Tab® (*P<0.05), (**p>0.05).

Formulation code	[a]$Rel_{0.5}$ (%±SD)	[b]Rel_8 (%±SD)	[c]DE (±SD)	[d]MDT (min±SD)	[e]f_1 (±SD)	[f]Flux [(mg/cm^2/min*10-4±SD]	[g]Kp [(cm/min)*10^4±SD]
F_1	*46.52±12.94	*84.90±3.15	*76.11±4.23	*49.69±5.52	22.61±2.13	38±0.000	8.70±0.02
F_2	**22.89±11.67	**77.55±6.77	**64.46±5.11	**81±6.45	13.74±4.23	26±0.000	7.96±0.01
F_3	*14.13±8.92	**72.16±4.10	*58.31±6.72	*92.13±4.34	18.97±3.45	18±0.000	7.12±0.01
Selegiline commercial Tab®	27.71±2.06	71.15±1.51	64.56±5.64	44.43±3.27	0	-	-

[a] $Rel_{0.5}$ = Percent of drug release after 0.5 h; [b] Rel_8 = Percent of drug release after 8 h; [c]DE = dissolution efficiency; [d]MDT = Mean dissolution time for 50% fractions; [e] f_1 = Differential factor, [f]Flux was provided from regression analysis among the amount of drug release per unit surface area and time; [g] permeability coefficient.

Regarding the concentration of bioadhesive polymer, discs displayed a proportional considerable decrease in MDT with reducing HPMC buccoadhesive polymer ratio. As discussed earlier, this decrease in MDT is probably due to the low concentration of HPMC to retain large amounts of water leading to a higher rate and extent of swelling. On the other hand, commercial tablet® exhibited low and slow drug release all over the investigation time without attaining 100% drug release (Figure 3). In the discs, as the amount of HPMC increased from F1 to F3 (1:2, 1:4 and 1:6 ratios, respectively), both the viscosity and strength of gel formed enhanced which reduced the water distribution into the disc and as a result reduced the percentage of drug release (F3, 72.16%) and increased the MDT (F3, 92.13 min), respectively.

This behavior is according to the outcomes acquired within the swelling study displaying an increase in swelling index upon increasing the HPMC content and suggests that higher concentration of polymer is suitable for delaying the release of Sel from the mucoadhesive discs.

The observed effects of polymer to drug ratio on the accumulative release of Sel from the discs corresponds with formerly showed release investigation of the similar drug from the buccal film.[38]

The addition of HPMC decreases the drug release which may be due to the enhancement in the swelling of the polymer and subsequently, opening the pores (into the network of polymer) on the surface of microparticles, which in turn increases the barrier effect and decreases the drug release.

It should be noted that HPMC is a hydrophilic polymer and thus the barrier of the microparticles. When it comes to contact with medium, the liquid initially enters the microspheres through the pores and the particles simultaneously start to hydrate, swell and form a gel layer, showing that the formed gel blocked these liquid pores almost directly after medium exposure and that further liquid transport through the pores was stopped after a while (barrier effect). The accumulative release of famotidine remarkably reduced with increasing the polymer concentration.[39]

Study of mucoadhesive force

The mucoadhesive force (g) of the produced discs was determined using sheep buccal as a model mucosa. The outcomes displayed that the mucoadhesive strength observed in all samples is acceptable for holding them at the buccal place (Table 2). However, the mucoadhesive properties were influenced by the concentration of matrix polymer applied, which upon hydration, stuck to the mucosal surface.

The mucoadhesive force and time were deviously proportionate to the drug/mucoadhesive polymer ratio. When the mucoadhesive polymer concentration was at maximum level, the amount of penetrating polymeric chains per unit volume of the mucus was high, resulting in larger interaction and vice versa. The highest bioadhesive force was shown in F3 formulation containing 1:6 drug to polymer ratio with 18.33 g/cm^2 (Table 2). This can be related to the capacity of HPMC to form exterior bioadhesive links via mucin.[40]

This maximum bioadhesion of F3 disc may be ascribed to quicker swelling and higher flexibility of polymeric bands of HPMC pointing a better interplay with mucin.[41]

Bioadhesive microspheres hold advantages like efficient absorption, and improved bioavailability of the drugs is related to a maximum surface to volume ratio. A very closer exposure to the mucus membrane and drug targeting to absorption place extending the retentive time of the dosage form at the place of absorption or function and focusing on drug function of the delivery system at a designated target place.[42] The mechanisms liable in the organization of buccoadhesive links include a three-stage process: spreading, moistening, and swelling of the mucoadhesive dosage form at the mucus surface. Surface initiates intimate contact between mucoadhesive polymer and biological mucosa. Internal distribution and interpenetration of the buccoadhesive polymer bands within the mucosa or surface of the tissue membrane produce a higher region of contact. The force of this mucoadhesive link relates to the degree of permeation between the polymer band and glycoprotein. To make powerful adhesive links, one polymer category ought to be soluble in another and polymer grade should be of similar chemical structure. At the present stage, there were entanglement and covalent links as well as van der Waals interactions and hydrogen bonds among the polymer bands and mucin molecule.

Ex vivo permeation investigation

According to the results, the superior samples were chosen based on the foundation of surface pH, *in vitro* release research, and mucoadhesive profile for more *ex vivo* permeation investigation. Discs formulae that showed 6.44-6.97 surface pH values, more than 80% drug release after 8 h, loss of fragments in release media, in bioadhesion time less than 6 h for the buccal mucosa were considered acceptable and were excluded from further permeation. Formulae F3 containing 100 mg Sel and 600 mg HPMC showed suitable mucoadhesion properties and no irritation during the study. They also showed MDT in the range of 92.13 min and a percentage of cumulative drug release, more than 90% after 8 h. In addition, their pH values (F1 to F3) were in the acceptable range (6.53-6.97). Therefore, they were chosen for *ex vivo* permeability and *in vivo* study.

The permeation parameters of Sel through sheep buccal membrane for tested formulations are shown in Table 3. The outcomes showed that the drug can penetrate quickly through the sheep buccal membrane and therefore might penetrate through the buccal membrane, too. F1 which included Sel and HPMC in the ratio of 1:2 displayed the maximum penetration parameters with steady-state flux (J_{ss}) of 3.8 (mg/cm^2/min × 10^{-4}), followed by F2 and F3 with J_{ss} equal to 26 and 18 (mg/cm^2/min × 10^{-4}), respectively. P>0.05 shows considerable variation among F1, F2 and F3 for the flux or permeability coefficient.

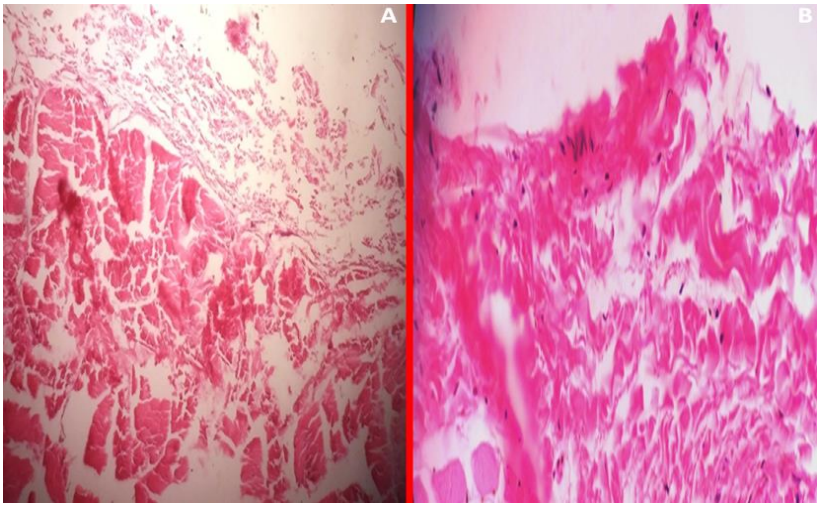

Figure 3. Histopathological evaluation of segments of buccal mucosa of sheep (A) untreated (B) treated with microspheres of discs containing selegiline (magnitude X).

Hydrophilic substances may have a tendency to apply the paracellular path and penetrate the intercellular spaces, which show a lower surface area.[43-45]

The flux of drug permeation through this pathway may be explained as Eq. 7:

$$JH = \frac{D_H \varepsilon}{h_H} CD \qquad \text{Eq. (7)}$$

Where D_H is the diffusion coefficient, h_H is the length of the tortuous path followed by the paracellular route, C_D is the concentration of the drug on the giver side, and ε is the aliquot of the surface region of the paracellular route.[46]

Although the drug in its non-ionized form can be well absorbed by the surface of the membrane, the pH in the profound layers of the membrane can convert the ionization and so the absorption. In addition, the extent of ionization of a drug reflects the partitioning into the membrane, but may not reflect the permeation through the lipid layers of the mucosa.

In addition, the effects of lipophilicity, pH, and pK_a will depend on the transport pathway used by the drug Studies conducted using buspirone displayed that the unionized form of the drug applied more lipophilic passageway, the transcellular route, but a rise in the pH raised the ionization of the drug and later the absorption.[47]

It was indicated that this transfer of ionized form of the drug was via the further hydrophilic paracellular passageway. Hence, at neutral pH, the passageway was obtained to be rather transcellular, but at acidic pH, the ionized types of the drug were also supplied to the absorption through the membrane.

The mean residence times (MRT) following F1 to F3 discs were 276.66 min to 329.66 min ($p<0.05$), respectively which is another implication on the *in vivo* performance of the buccal mucoadhesive disc in supplying a sustained drug delivery.

The microscopic studies showed that none of the discs had visible damage to the microscopic structure of the buccal tissue. As shown in Figure 3, no cell necrosis was exhibited.

Conclusion

This investigation introduced a novel effort for the Sel controlled release buccal discs. A mucoadhesive dosage form suggests a prolonged contact at the site of administration, low enzymatic activity, and high patient compliance. A buccoadhesive drug delivery system for Sel was developed as an alternate to elude the first-pass effect associated with oral administration, prepare a sustained release and optimize drug bioavailability. New mucoadhesive formulation for controlled release of Sel was prosperously produced in which release patterns and mucoadhesion characteristics may be controlled by varying concentrations of bioadhesive polymer and their ratios. They were easy to use and take from the buccal mucosa and did not show to harm the underlain mucosa. Then, they might be helpful for buccal administration of the drug.

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Conflict of Interests

The authors claim that there is no conflict of interest.

Supplementary Materials

Supplementary file contains Figure S1-S3 is available on the journal's web site along with the published article.

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