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RESEARCH ARTICLE

Gastroretentive Delivery Approach to Address pH-Dependent Degradation of (+)- and (-)-Phenserine

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Abstract

(-)-Phenserine ("phenserine") and (+)-phenserine (posiphen; buntanetap) are longer-acting enantiomeric analogs of physostigmine with demonstrated promise in the treatment of Alzheimer's and Parkinson's diseases. Both enantiomers have short plasma half-lives, and their pharmacokinetics might be improved through the use of either once or twice-daily administration of an extended-release dosage form. Phenserine was observed to form a colored degradation product in near-neutral and alkaline pH environments, and at pH 7, the half-life of posiphen was determined to be ~ 9 h (40 °C). To limit luminal degradation which would reduce bioavailability, a gastroretentive tablet composed of a polyethylene oxide-xanthan gum matrix was developed. When placed in simulated gastric fluid (pH 1.2), approximately 70% of the phenserine was released over a 12 h period, and no degradants were detected in the release medium. In comparison, a traditional hydrophilic-matrix, extended-release tablet showed measurable amounts of phenserine degradation in a pH 7.2 medium over an 8 h release interval. These results confirm that a gastroretentive tablet can reduce the luminal degradation of phenserine or posiphen by limiting exposure to neutral pH conditions while providing sustained release of the drug over at least 12 h. Additional advantages of the gastroretentive tablet include reduced gastric and intestinal concentrations of the drug resulting from the slower release from the gastroretentive tablet which may also limit the occurrence of the dose-limiting GI side effects previously observed with immediate-release phenserine capsules.

Keywords Buntanetap · Extended-release · Gastroretentive tablet · Phenserine · Physostigmine · Posiphen

Introduction

The natural alkaloid product, (-)-physostigmine (physostigmine), was the first drug-like compound shown to be effective as a reversible cholinesterase inhibitor [1]. Its positive effect on memory in animals and humans suggested application as a potential treatment strategy for Alzheimer's disease (AD) [2], and it was evaluated for clinical efficacy using an extended-release tablet due to its short plasma half-life [3]. However, peripheral adverse effects due to non-selective inhibition of both acetylcholinesterase (AChE, EC 3.1.17) and butyrylcholinesterase (BChE, EC 3.1.1.8) limited its clinical development for AD [4]. To provide improved AD treatments, analogs of physostigmine with fewer peripheral adverse effects and improved actions on memory have been investigated, and (-)-phenserine [(-)-N-phenylcarbamoyl eseroline (Fig. 1)], a phenyl carbamate analog of physostigmine has been identified as a non-competitive, potent inhibitor of AChE with a reported IC_{50} of 24 nM and a 65-fold selectivity for human and rodent AChE over BChE [4]. Phenserine has preferential brain distribution, and pre-clinical studies reported a brain: plasma ratio of 10:1 compared to approximately 1:1 for physostigmine [4]. Moreover, phenserine has shown a long duration of AChE inhibitory action in rodents with a reported pharmacodynamic half-life ($t_{1/2}$) of 8.25 h. Similar to physostigmine, phenserine undergoes rapid clearance from both the plasma and brain with reported elimination half-lives of 12.6 and 8.5 min, respectively [4]. The primary metabolites of phenserine, generated through N-demethylation, also have highly potent anti-cholinesterase activities with IC_{50} values ranging from 22–40 nM, and

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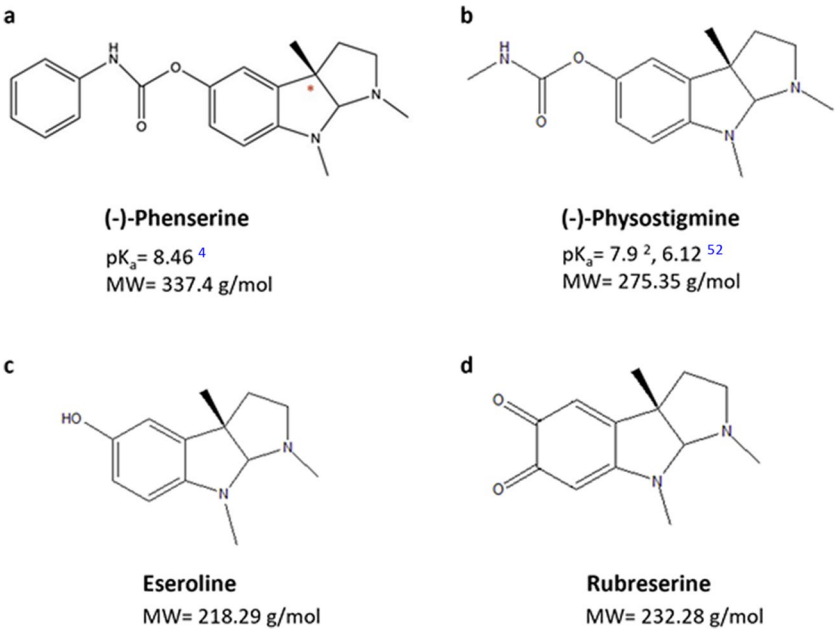

Fig. 1 Chemical structures of (-)-phenserine, (-)-physostigmine and their primary degradants, eseroline and rubreserine

these metabolites can extend the duration of phenserine's pharmacological actions [5, 6].

Beyond its effects on AChE, phenserine has demonstrated activity in lowering the generation of insoluble amyloid β peptide 42 ($A\beta_{42}$), a proteolytic fragment of β-amyloid precursor protein (APP) associated with the pathogenesis of AD [7, 8]. Phenserine also reduces the production of alpha-synuclein (αS), a neuronal protein linked to the pathogenesis of Parkinson's disease (PD); upregulates brain neuroprotective proteins, such as brain-derived neurotrophic factor (BDNF); and reduces pro-apoptotic proteins, thereby, augmenting neuronal survival [9–12].

The (+)-enantiomer of phenserine, known as posiphen and now referred to as "*buntanetap*", is devoid of inhibitory effects on AChE and BChE, but is equipotent in the suppression of the translation of APP mRNA [13]. Posiphen has also demonstrated the ability to suppress the translation of tau and αS mRNA, decreasing the CNS levels of these neurotoxic proteins and reducing their aggregation [14]. Posiphen, like phenserine, reduces αS generation, as do their primary N-demethylated metabolites [15], and both enantiomers show anti-inflammatory activities associated with significantly reduced levels of the pro-inflammatory cytokines, IL-1β and TNF-α [16, 17].

Clinical trials evaluating phenserine demonstrated that the drug was safe and well tolerated at a single oral dose of 10 mg, yet dose-limiting GI side effects (nausea and vomiting) occur at doses of 20 mg administered using immediate-release capsules [18]. A small Phase II trial showed promising results with a positive trend on cognitive measures, but a later Phase III trial failed to meet the defined cognitive milestones, and further clinical evaluations were halted [19]. A post-trial evaluation of the Phase III results indicated that subjects administered phenserine 15 mg b.i.d. and evaluated within 4 h of drug administration demonstrated cognitive improvement, and the authors attributed the clinical failure of the previous trial to the limitations in the clinical trial design, the limited dose administered, and inadequate drug exposure from the immediate release formulation [20–22] which led to drug and metabolite concentrations below therapeutic levels for 14 or more hours per day [23]. A once-or twice daily extended-release formulation was proposed as a possible alternative to improve the efficacy of phenserine by providing adequate phenserine concentrations throughout the dosing interval, thereby maintaining AChE inhibition while reducing the occurrence of adverse effects (nausea and vomiting) associated with the faster dissolution of the immediate-release doses [22–24]. During initial formulation development of a conventional hydrophilic-matrix, extended-release tablet containing phenserine, a reddish color was observed during a forced degradation study, suggesting phenserine may be chemically unstable under neutral pH conditions. Physostigmine shows similar instability at higher pH [25, 26], and based on their similar chemical structures, further investigation of the pH-dependent stability of phenserine/posiphen were undertaken in the current studies to better define the rate and mechanism of the degradation. The goal of this research was to measure the pH-dependent degradation of phenserine and propose an alternative extended-release dosage form (gastroretentive tablet) to mitigate its degradation in the GI lumen.

Gastroretentive systems are retained in the stomach allowing the drug to be slowly released into the low pH gastric fluid, maximizing absorption in the upper GI tract where the

intestinal pH remains below 7. Swelling/expandable gastroretentive tablets are retained in the stomach due to their rapid increase in size to greater than the size of pylorus (~12 mm) [27] after coming into contact with gastric fluids. Various hydrophilic swelling polymers (e.g. hydroxypropylmethyl cellulose, polyethylene oxide, Carbopol and xanthan gum) are used to achieve such systems [28–30]. Glumetza® and Gabapentin GR® are examples of swelling systems employed to achieve gastric retention [31, 32]. Gastroretentive technologies, including swellable systems, have been employed for drugs such as ciprofloxacin [33] and captopril [34] which degrade in higher pH environments, to improve their bioavailabilities.

Experimental

Chemicals

Posiphen tartrate [(+) phenserine tartrate] and phenserine tartrate [(-) phenserine tartrate] were provided by the National Institute on Aging, National Institutes of Health. Polyethylene oxide (PEO) (5,000,000 g/mol) (WSR Coagulant, NF grade) was provided by Dow Chemicals (Middlesex, NJ). Monosodium phosphate (EM Science, Gibbston, NJ); disodium phosphate heptahydrate and hydrochloric acid (Fisher Scientific, Waltham, MA), citric acid monohydrate, acetic acid and L-tartaric acid (Sigma Aldrich, St. Louis, MO); KCl (Research Products International Corp., Mt. Prospect, IL); NaOH (VWR International. Radnor, PA); hydroxypropyl methylcellulose (HPMC) (Methocel™ K100M CR) (Dow Chemical Company, Middlesex, NJ); xanthan gum NF and magnesium stearate NF (Spectrum Chemicals, New Brunswick, NJ); microcrystalline cellulose (MCC, Avicel PH 102) (FMC Corporation, Newark, DE); fumed silica (Cab-O-Sil®) (Cabot Corporation, Boston, MA); and polyvinylpyrrolidone K-30 (PVP) (Acros Organics, Geel, Belgium) were all purchased and used as received.

Preparation of pH-Controlled Media

Solutions ranging in pH between 2 and 8 were prepared using buffers (0.02M) by dissolving specific molar ratios of buffer pairs or by dissolving the appropriate acid and adjusting the pH with 10 N NaOH (Table 1) [35]. The ionic strength (0.05 M) was kept constant by the addition of potassium chloride.

pH-Dependent Phenserine Stability

Degradation of phenserine/posiphen solution was studied under ambient (21°C), physiological (37°C), and accelerated (40°C) conditions. Stock solutions of 4 mg/ml (82 mM) phenserine tartrate (MW 487.5) or posiphen tartrate were prepared in 0.01 M HCl. Buffer solutions were pre-incubated at 21°C, 37°C, and 40°C for 30 min (Precision Incubator, GCA Corporation, IL) before adding the drug to initiate the

Table 1 Influence of pH and Buffer On Degradation Rate Constant (k_{obs}) of Posiphen at 21°C, 37°C, and 40°C. Results are Reported As Mean ± Standard Deviation (n = 3)

pH	pKa	Buffer	Buffer conc. (M)	21 °C		37 °C		40 °C	
				$k_{obs} \times 10^{-5}$ (min^{-1})	$t_{1/2}$ (hr)	$k_{obs} \times 10^{-5}$ (min^{-1})	$t_{1/2}$ (hr)	$k_{obs} \times 10^{-5}$ (min^{-1})	$t_{1/2}$ (hr)
2	2.15	*Phosphate*	0.02	0*	*n/a*			0*	*n/a*
2.5	2.15	*Phosphate*	0.02	0*	*n/a*			0*	*n/a*
3	3.13	*Citrate*	0.02	0*	*n/a*			0*	*n/a*
3.5	3.13	*Citrate*	0.02	0*	*n/a*			0*	*n/a*
4	4.76	*Citrate*	0.02	0*	*n/a*			0*	*n/a*
	4.76	*Acetate* [a]	0.019						
	4.34	*Tartrate*	0.02						
5	4.76	*Citrate*	0.02	0*	*n/a*			0*	*n/a*
	4.76	*Acetate* [a]	0.019						
	4.34	*Tartrate*	0.02						
6	6.4	*Citrate* [a]	0.01	0*	*n/a*			20	57.6
6.5	6.4	*Phosphate*	0.02	0*	*n/a*			33 ± 4.7	35
7	7.2	*Phosphate*	0.02	6.33 ± 3.21	183	39 ± 6	29.3	133 ± 27	8.6
	6.4	*Citrate*						120 ± 31	
8	7.2	*Phosphate* [a]	0.017	35.6 ± 2.5	32.4	225 ± 7	5.1	356 ± 37	3.2

*Limited degradation observed during 12 h measurement interval. kobs are not statistically different from zero

[a] Buffer concentration was reduced to maintain the 0.05 M ionic strength

n/a not calculated due to negligible degradation

experiments. Phenserine/posiphen stock solution (100 µl) was added to 1.9 ml of each buffer solution, giving a final concentration of 0.2 mg/ml (0.4 mM); the solutions were stored at 21°C, 37°C, and 40°C (Precision Incubator, GCA Corporation, IL) for 10–12 h. At appropriate time intervals, 100 µl of the sample was withdrawn and added to a sample vial pre-filled with 100 µl of HPLC mobile phase (stop solution). The samples were analyzed immediately by HPLC. Care was taken to maintain pseudo-first order conditions with minimal changes in media pH during the incubation period [36]. General acid or base catalysis of degradation by the buffer components was evaluated by comparing different buffer systems (phosphate, tartrate, citrate, or acetate) at selected pH values (Table 1).

Statistical calculations (standard deviation; $f2$) were performed using Excel (Excel 365, Microsoft, WA, USA). All linear regressions were conducted using GraphPad Prism 9 (GraphPad Software Inc., CA, USA).

HPLC Method Of Analysis

An HPLC method for posiphen/phenserine using a Waters Alliance e2695 system module with a 2487 UV absorbance detector at 247 nm (Waters Corporation, Milford, MA) was used to quantify drug concentrations. The method was developed and validated during the previous manufacture of the phenserine drug products prepared for clinical investigation. The separation was carried out using a Primesep 100 column (150 mm × 4.6 mm ID, 5 µm) and a 70:30 acetonitrile: water mixture with 0.1% trifluoracetic acid as the mobile phase. The injection volume was 10 µl and the flow rate was 1 ml/min. A linear response was obtained over a working concentration range of 7.8–250 µg/ml.

Degradant Peak Identification Using Mass Spectrometry

Physostigmine degrades to form eseroline, a colorless compound, and rubreserine, a pink-colored compound. Since a pink color was also observed following the degradation of both phenserine and posiphen, a degradation scheme similar to physostigmine was proposed. To identify the primary degradants from a posiphen sample stored at pH 5 and 40°C for 2 weeks, aliquots were analyzed using an LC–MS method adapted from the HPLC method but using a mobile phase flow rate of 0.7 mL/min. An ultra-performance liquid chromatograph (Acquity UPLC, Waters, USA) coupled with a single quadruple detector (Acquity SQD, Waters, USA) was used to identify the degradant peaks resolved from a posiphen tartrate sample.

Preparation of Prototype Gastroretentive Tablets

Preliminary screening studies were conducted to develop a prototype gastroretentive tablet able to rapidly expand to a size greater than the diameter of the pyloric sphincter (≥ 12 mm). The powder blends prepared by mixing various combinations of excipients are summarized in Table 2. The excipient powders were passed through a 60-mesh (250 micron) screen before blending. Magnesium stearate (1.5–2% w/w), also pre-screened through a 60-mesh screen, was added to each powder mixture and further blended for one minute. The dry powder mixture was compressed manually using a single station Carver press (Model 3851–9, Carver Inc., IN) using 8 mm diameter, round, flat-faced punches at a compression force of ~ 200 pounds.

Swelling Study

The initial thickness and diameter of the dry gastroretentive tablets were measured using a micrometer (Mitutoyo, Japan). Due to limited supplies of phenserine and posiphen, the tablets used for swelling measurements contained 10 mg of scopolamine as a drug surrogate. Its similarity to phenserine/posiphen in molecular weight, pKa, and solubility makes it a suitable surrogate for swelling studies.

Swelling studies were performed by placing the tablets in 100 ml of either deionized (DI) water or simulated gastric

Table 2 Polymers Evaluated for Inclusion in Gastroretentive Tablets. Additional Excipients in All Tablets Included 10 mg of Tartaric Acid and 5 mg Magnesium Stearate. Based on the Composition, Tablet Weights Ranged Between 200–330 mg. Initial Tablet Diameter = 8 mm [33, 51]

HPMC K100M 30–50%	HPMC K4M 10–15%	PEO (5,000,000) 10–50%	PEO (900,000) 5–20%	Crospovidone 2–10%	PVP K30 5–15%	MCC 10–50%	Xanthan gum 20–45%	Tablet diameter (mm) after 1 h in DI water
x	x			x		x		Eroded
	x	x		x		x		Eroded
		x	x	x		x		Eroded
		x	x		x	x		11
		x			x	x	x	14

fluid in an incubator shaker (C24 Incubator Shaker, New Brunswick Scientific, NJ) at 37°C and at 50 rpm. Swelling was measured by removing the tablet at various intervals over a period of 1 h, blotting the excess water with a tissue, and measuring the diameter of the swollen tablet using a ruler with 1 mm gradations.

Preparation of Monolithic Extended-Release Tablet

Phenserine tartrate, L-tartaric acid, microcrystalline cellulose (Avicel PH102), fumed silica, HPMC K100M, and magnesium stearate were passed through a 60-mesh screen and blended manually (Table 3). The powder mass was compressed manually using a single station Carver press (Model 3851–9, Carver Inc., IN) with 8 mm diameter, round, flat-faced punches using a compression force of 380 pounds.

In-Vitro Drug Release

Drug release from the initial monolithic, extended-release tablets was evaluated in simulated gastric fluid for 12 h. In addition, drug release was further evaluated in a series of dissolution media with increasing pH to simulate the environments encountered along the GI tract. The sequence included exposure to media at pH 1.5 (~0.1 N HCl) for 1 h, pH 4.5 for 3 h, and pH 7.2 for 7 h. Medium pH change was accomplished by addition of 1.67 g of sodium acetate to the pH 1.5 medium to achieve pH 4.5 and addition of 1.66 g of tris(hydroxymethyl)aminomethane (TRIS) base to the pH 4.5 medium to achieve pH 7.2.

Phenserine drug release from the gastroretentive tablets was measured using a USP type II apparatus (Model VK 700, VanKel, Cary, NC) with 40-mesh baskets (to prevent the tablets from floating) rotating at 50 rpm in 500 ml of simulated gastric fluid (pH 1.2, 0.2% NaCl) [37, 38] de-aerated before use and maintained at 37 ± 0.5 °C. Aliquots were withdrawn over a 12 h duration, filtered (0.45 µm PES syringe filters, MDI Membrane Technologies, PA), and analyzed immediately using HPLC. Initial formulation efforts were focused on developing a gastroretentive dosage form with a similar release profile as the hydrophilic matrix, extended-release tablet and the dissolution profiles of the extended-release and gastroretentive dosage forms in 0.01M HCl were compared using the similarity factor($f2$) described in the US FDA"Dissolution Testing of Immediate Release Solid Oral Dosage Forms" Guidance[39].

Table 3 Composition of Gastroretentive and Comparator Extended-release Matrix Tablets Used to Measure Drug Release Under Varying pH Conditions

Ingredient	*Gastroretentive* (mg)	*Extended-release* (mg)
Phenserine tartrate	20.0	20.0
Tartaric acid	10.0	10.0
PEO WSR coagulant	40.0	-
HPMC K 100M	-	75.0
MCC (Avicel PH 102)	50.0	139.0
PVP K 30	50.0	-
Xanthan gum	140.0	-
Magnesium stearate	5.0	5.0
Colloidal silicon dioxide	-	1.0
Total	**315.0**	**250.0**

Results

pH-Dependent Phenserine/Posiphen Stability

The concentrations of intact phenserine/posiphen remaining in solution at pH values between 2 and 8, stored at either 21°C, 37°C, or 40°C, were measured over 10–12 h. The apparent first-order degradation rate constants (k_{obs}) were determined from the resulting first-order plots (lnC *vs* t) [36]. Posiphen tartrate showed good stability over the pH range of 2–5 (Fig. 2). Significant degradation was observed at higher pH values with only 84% and 74% of intact drug remaining at pH 6 and 6.5, respectively, after 12 h at 40°C. Posiphen tartrate also exhibited rapid degradation at pH 7 and 8 with only 44% and 12%, respectively, of the parent drug remaining after 10 h at 40°C. Stability over the entire pH range (pH 2–8) was investigated using posiphen tartrate, and the degradation of phenserine, which was expected to be similar to that of posiphen, was studied at pH 4 and pH 7. The results confirmed that the degradation rates were similar for both enantiomers (Fig. 2).

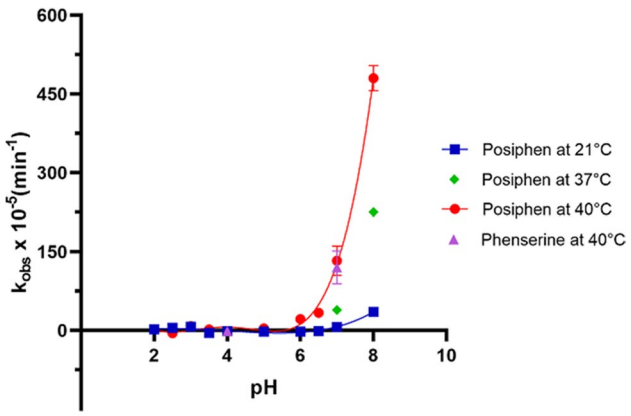

Fig. 2 pH-rate profile of phenserine/posiphen degradation. Results are presented as the mean ± standard deviation (n = 3). The curves represent the best fit to the mean values

Since the stability of a compound varies with its environment (pH, buffer, ionic strength, and temperature) potential direct effects of the buffer salts used in these studies were evaluated by comparing posiphen degradation using different buffer components (acetate, tartrate, citrate, and phosphate) while maintaining the same buffer concentration (0.02 M), pH (4, 5, or 7), ionic strength (0.05M), and temperature (40°C). At each pH value, no significant differences among the degradation rate constants were observed (Table 1), suggesting the absence of buffer salt catalysis.

Degradant Identification

Phenserine was detected as an $[M+H]^+$ ion with m/z = 338 and a product ion of m/z = 162 with a retention time of 12.7 min, similar to the results reported by Greig *et al.* [18]. Rubreserine and eseroline were detected as $[M+H]^+$ ions of m/z = 233.1 and 219.1 with retention times of 10 and 9.8 min, respectively. Several additional degradants were detected, but they represented less than 2.5% of the total mass in the sample and further evaluations of their structures were not conducted. The key steps in the degradation of phenserine are shown in Fig. 3.

Formulation of a Gastroretentive Dosage Form

Table 2 summarizes preliminary screening experiments to identify tablet compositions with desirable swelling behavior. Initial gastroretentive tablet formulations contained different combinations of hypromellose (K100M and K4M grades) and polyethylene oxide (PEO; 5,000,000 g/mol and 900,000 g/mol) as hydrophilic matrix formers, along with crospovidone as a swelling agent [40]. PEO was included based on reports of its improved swelling capacity compared to HPMC [41].

In tablets containing crospovidone, no swelling of the tablets was observed, but the tablets appeared to erode. The addition of xanthan gum to the composition significantly increased the swelling capacity [42]. Polyvinylpyrrolidone (PVP K30) was added to the formulation as a pore-forming agent, as it is soluble, and was observed to promote rapid swelling. Based on these behaviors, a final formulation able to swell to a diameter of 14 mm in one hour in deionized water and 12 mm in SGF was selected (Table 3).

In-Vitro Drug Release

Hydrophilic-Matrix Extended-Release Tablet

Drug release was evaluated from the preliminary gastroretentive tablet and from a prototype, hydrophilic-matrix extended-release tablet (Table 3). The extended-release tablet, when exposed to simulated gastric fluid, released 78% of the incorporated phenserine in 12 h. When drug release was studied using a series of increasing pH media, only 56% of the phenserine content in the tablet could be detected in the release medium after 12 h, and a pink color in the medium was observed. The release profile in the first two media at pH 1.5 and pH 4.5 (up to 4 h) was similar to the release profile in simulated gastric fluid, but the amount of intact phenserine quantified in the pH 7.2 dissolution medium was lower than the corresponding amounts measured in simulated gastric fluid. The reduced phenserine recovery, along with the appearance of a color change in the pH 7.2 dissolution

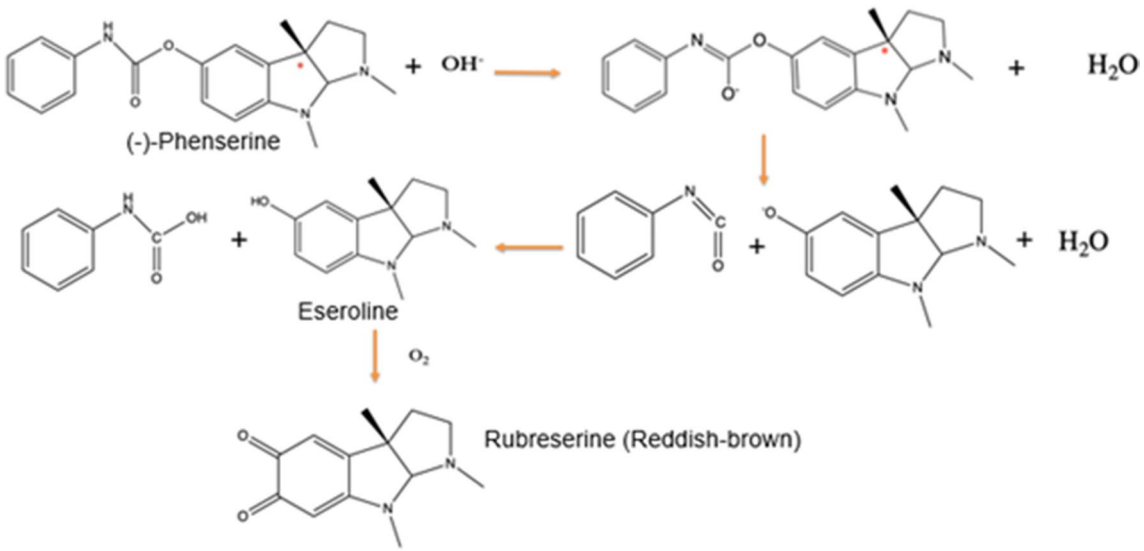

Fig. 3 Proposed chemical degradation pathway of phenserine or posiphen based on the degradation mechanism reported for aromatic carbamates [25, 26, 43]. Hydroxide-catalyzed carbamate hydrolysis forms eseroline followed by oxidation to form rubreserine

medium, suggested degradation of phenserine to rubreserine was occurring.

Gastroretentive Tablet

Since the gastroretentive tablet is intended to remain in the stomach for extended periods, release testing was performed only in simulated gastric fluid. The gastroretentive tablet released 70% of the phenserine content in 12 h (Fig. 4), demonstrating good sustained release properties for the prototype tablet.

Discussion

Due to their structural similarities, phenserine, posiphen, and physostigmine all show similar pH-dependent stabilities and undergo significant degradation in mildly alkaline environments [25, 26, 43]. The confirmed appearance of rubreserine and eseroline following phenserine degradation suggests all three compounds degrade, first through carbamate hydrolysis, followed by oxidation to form the final, red-colored rubreserine (Fig. 3). The systematic evaluation of phenserine stability across the pH range of 2–8 showed that hydroxide-catalyzed hydrolysis occurs in environments where the pH is greater than 6, a condition present in a significant portion of the gastrointestinal tract [44] where the pH in much of the small intestine and all of the large intestine is greater than 6. The short half-life of phenserine/posiphen at pH 7 suggests that a significant fraction of the drug released in the lower small intestine and large intestine may degrade in the intestinal lumen prior to absorption. The colon arrival times for most conventional monolithic extended-release dosage forms range between ~ 4–8 h, depending on dosage form type and food intake relative to dosing. Their residence times in this higher pH region can be at least an additional 16 h. *In vitro* drug release testing for the hydrophilic-matrix extended-release tablets confirmed the likelihood of phenserine/posiphen degradation within the gastrointestinal tract based on the observation of a pink coloration in the pH 7.2 dissolution media. Increasing amounts of degradants, especially eseroline and rubreserine, were also observed in the HPLC chromatograms, and the degradation of phenserine in the pH 7.2 release medium explains the lower concentrations of phenserine measured when the medium change method was employed (Fig. 4).

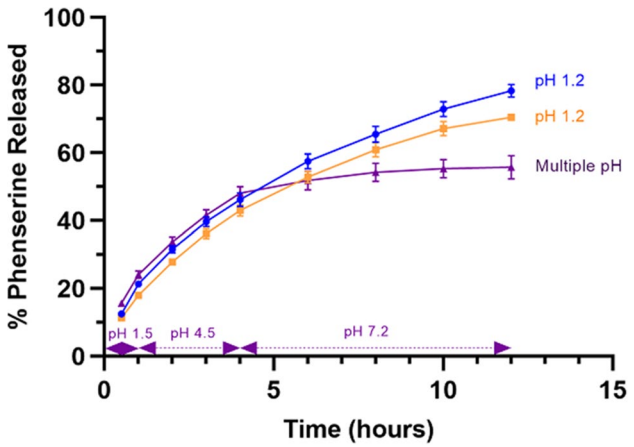

Fig. 4 Release profile of phenserine from hydrophilic-matrix extended-release tablets in simulated gastric fluid (pH 1.2) (blue-filled circles); release from gastroretentive tablets in simulated gastric fluid (pH 1.2) (orange-filled circles) and drug release from hydrophilic matrix extended-release tablets in a sequence of varying pH dissolution media (purple-filled circles) (pH 1.5 for 1 h, pH 4.5 until hr. 4 and pH 7.2 for remaining 8 h). Results are reported as mean ± standard deviation (n = 3)

The limited clinical efficacy of phenserine observed in previous phase II and phase III clinical trials was proposed, in large part, to be the result of poor pharmacokinetics with insufficient concentrations of phenserine at the target site(s) to provide adequate time-dependent maintenance of AChE inhibition in the brain [23]. Becker *et al.* proposed that the development of an extended-release formulation might resolve these pharmacokinetic/dynamic issues [22–24]. The stability results suggest that a traditional extended-release dosage form may not be the best choice for phenserine/posiphen delivery. A gastroretentive dosage form, which is likely to be retained for longer periods in the stomach (pH 1 – ~ 5), thereby minimizing the exposure of released drug to higher pH environments, may be more appropriate. The resulting improved phenserine/posiphen stability could result in improved bioavailability following drug absorption in the upper small intestine. Similar gastroretentive strategies using a variety of retention technologies have been proposed for captopril [34, 45] and clopidogrel bisulfate [46], compounds also reported to degrade in the higher pH conditions of the lower intestinal tract.

Various hydrophilic polymers with good swelling properties commonly used in gastroretentive formulations were tested to develop a prototype gastroretentive dosage form (Table 2). While HPMC exhibits good swelling behavior, it did not hydrate rapidly and primarily showed axial swelling, characteristics also observed by previous investigators [47, 48]. Other swelling agents, including PEO and crospovidone were combined with HPMC, but combinations containing crospovidone resulted in concomitant erosion likely limiting their gastric retention. Given the goal of a rapid, radially swelling tablet with reasonable mechanical integrity when hydrated, combinations of xanthan gum, a rapidly swelling polymer, and PEO, a polymer that forms a firm gel, were tested [47]. To further increase fluid uptake resulting in rapid swelling, PVP was added as a pore former [49]. The final formulation was selected based on its rapid radial

swelling to a diameter of 14 mm in deionized water in less than one hour. The final prototype gastroretentive tablet released ~ 70% of the drug over 12 h, and when compared to the control formulation (extended-release matrix), showed a similarity factor ($f2$) of 67.7 indicating good similarity in the release profiles of the two tablets [39]. While further formulation optimization could be conducted to refine drug release rates, the gastroretentive formulation approach is likely to provide a prolonged interval for drug absorption and subsequent sustained plasma and brain drug concentrations. Swelling dosage forms that increase in size to greater than pylorus, or > 12 mm during the fed state, have been shown to resist gastric emptying resulting in prolonged retention in the stomach after administration [50]. The prototype phenserine tablet expanded to ~ 12 mm in diameter in SGF in one hour, and the associated ~ 250% increase in total volume suggests that the prototype formulation would show adequate gastric retention when administered in the fed state. Further increases in the initial diameter of the tablet would likely result in a swollen tablet of sufficient diameter to be retained in the stomach for at least 12 h. The prototype gastroretentive dosage form remained intact for the duration of the drug release study (12 h), and based on its composition of hydrophilic polymers, it is expected to completely disintegrate and empty from the stomach in less than 24 h. Alternative gastroretention strategies could also be explored, including floating [34], mucoadhesive [45], or high-density formulations [46] to optimize dosage form performance.

Conclusion

Investigations of the pH-dependent degradation of phenserine/posiphen showed that only limited drug degradation occurs below pH 6. With this information, a gastroretentive extended-release approach to address the observed poor pharmacokinetics of phenserine was evaluated. Drug release from a prototype gastroretentive tablet designed using a swelling PEO-xanthan gum matrix showed that continuous release of phenserine at lower pH conditions could minimize degradation of the drug in the lower intestinal lumen, while providing for drug release and absorption over an extended time period. In comparison, a traditional hydrophilic matrix tablet showed considerable degradation of drug following release into a pH 7.2 medium representative of the conditions in the lower intestinal tract, providing evidence that decreased bioavailability of phenserine due to chemical instability in the intestinal lumen is likely to occur when using traditional extended-release tablets.

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Data Availability Data will be provided on request.

Declarations

Competing Interests The authors declare that they have no competing interests.

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