Development of Modified Release Nicotine Tablet Formulation for Treatment of Ulcerative Colitis

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Abstract

One of the therapeutic effects of nicotine is used as a protective against developing ulcerative colitis . ulcerative colitis is an inflammatory disease of the bowel affecting the superficial lining mucosa in the rectum and large intestine. In this study nicotine tablets were formulated as modified release tablets targeted to the colon. All formulas were studied for drug release , effect of diluent, retardant concentration, avicel grade,and compression force, the selected formula was then further studied for drug release in 3 different pH (coated tablets) .The kinetic study revealed acceptable shelf life . Finally the selected formula was given to 6 patients in a pre-liminary clinical study which showed that nicotine can stabilize mild to moderate ulcerative colitis attacks.

Key words: Ulcerative colitis, Nicotine, Modified release , Colon delivery.

الخلاصة

التهاب القولون التقرحي احد التهابات الجهاز الهضمي التي تصيب الطبقة المخاطية السطحية للمستقيم و الامعاء الغليظه ويعتبر النيكوتين عامل حماية ضد تطور هذا المرض. في هذا البحث تم تحضير مضغوطات النيكوتين محورة التحرر موجه الى القولون. وتم دراسة تحررالدواء و تأثير نوع المواد المضافة غير الفعالة و تركيز المواد المثبطه للتحرر للصيغ المحضرة, وتأثير قوة الكبس على تحرر النكوتين. وتم أجراء دراسة اوسع للصيغه المختارة من حيث تحرر النيكوتين في اوساط مختلفة الاس الهيدروجيني و من حيث ثباتها في درجات حرارة مختلفة. وتم ايضا اجراء دراسة سريريه اولية على 6 مرضى للصيغة المختارة حيث أظهرت الدراسة ان النيكوتين يمنع تطور المرض و يزيد من استقرارية هذا المرض في الراحل البسيطة و المعتدلة الشدة.

Introduction

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local disease associated with the colon like Crohns disease , ulcerative colitis and irritable bowel syndrome..etc., but also for the potential systemic delivery of proteins and therapeutic peptides. The large intestine, though difficult to reach by preoral delivery is still deemed to the delivery of agents to cure the local disease of the colon [(1,2)]. Colonic delivery formulations are in general may be designed to provide either the burst release or for sustained/ prolonged release once reaching the colon [(3)]. The proper selection of a formulation approach depends upon several important factors [(4)] which are : the pathology and pattern of the disease , the physicochemical and biopharmatical properties of the drug and finally the desired release profile of the active ingredient. The most common physiological factors considered in the design of delayed release colonic formulations is pH gradient of the gastrointestinal tract[(5,6)].delayed release formulations such as single unit or multiparticulate system for colon targeting , nanoparticulate system, microspheres, pelletsand beads, coating with pH sensitive polymers, embedding in matrices and bioadhesive systems[(7)] can be considered for the design of colon deliveryformulations. A wide array of polymers has been employed as drug release retarding agents each of which presents a different approach to matrix concept. Plastic matrix system , due to their chemical inertness and drug embedding ability , have been widely used for sustaining the release of drugs. Plastic polymers e.g. ethylcellulose and acrylates, which are capable of forming insoluble or skeleton matrices, have been widely used for controlled release of drugs due to their inertness and drug embedding ability . Acrylate polymers are widely used as tabletcoating and as retardents for drug release in sustained release formulations[(8)]. Ulcerative colitis (U.C) is an inflammatory disease of the bowel affecting the superficial lining mucosa in rectum and large intestine. The disease typically starts from the rectum and continues through the large bowel sparing the deeper layers of the intestinal wall [(9)]. A variety of anti-inflammatory, immunosuppressive, and biological agents have been used to induce and / or maintain remission in UC . Sulfasalazin, olsalazine, balsalazide, oral and rectal mesalamine and topical corticosteroids are the standard first line therapies for UC.Patients who fail to respond to these agents are usually treated with oral corticosteroids.

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There is a need for additional first line treatments in patients with UC and for alternatives to corticosteroid therapy in refractory patients. Nicotine may be such an agent for which epidemiologic studies have shown that smoking protects against the development of UC and controlled clinical trials have demonstrated that transdermal nicotine is efficacious for active UC[10-15]. Nicotine is a drug obtained from the plant *Nicotina tabacum*. It's a weak base. Its available as a colorless to pale yellow oily liquid with an unpleasant tobacco-like odour and burning taste [16]. Its most preferred that absorption of nicotine occurs predominantly in the colon. So post-gastric delayed release composition, the composition will pass through the small intestine in about 4-8hr and will resides in the colon for about 48hr. further more nicotine is absorbed more slowly in the colon than in the small intestine, therefore, nicotine delivered for absorption predominantly in the colon will be absorbed more slowly over a sustained period and will give rise to more uniform plasma concentration. By predominant absorption from the colon we mean to include preferably 80-90% of the total dose of nicotine[17].The present study is to develop modified release of 20mg nicotine tablets as a targeted delivery system in the colon with pre-liminary clinical study. Eudragit RS PM as a retardant polymer which is responsible for the sustained release behavior of the drug was used for preparation of the core matrix and the selected formula was targeted to release in the colon by using enteric coating (a mixture of Eudragit L and S).

Materials and Method

Nicotine (Sigma) gifted from Pharmacognosy Department. College of Pharmacy/ University of Baghdad, Sodium hydroxide (Fluka chemie AG. Buchs/Scheiz), Dibutylphthalate (USB, B. Brussels,Belgium), Hydrochloric acid , Iospropanol, and Orthophosphoric acid (Riedal De Haen Ag Seelze Hanover), Polyvinylpyrrolidone (PVP K30) , Acetone, Potassium dihydrogen phosphate, Ethanol 99% (BDH chemicals, Ltd, Liverpool, England)Microcrystalline cellulose- Avicel® - PH101, PH302, PH200 (FMC Corperation, Pennsylvania, USA), Eudragit® L100, S100, RS PM – Rhöm Pharma GmbH Weiterstadt, Germany),Trisodium phosphate, Talc (Hopkins and Williams Ltd. England), coloring agent (deep orange lakes) Zinc stearate (Barlocher, GmbH, Germany), Disodium hydrogen phosphate, Mannitol, Starch (Merk, Germany). Table (1) summarizes 8 formulas to prepare modified release nicotine tablets by wet granulation method with alcohol. A known weight of the granules were mixed with a speicified amount of Zn stearate (1%) in a well closed container and compressed into tablets using 9mm punches (tablet machine single punch – Korch, type EKO, Erweka GmbH, Kr Offenbanch/ Germany).

Table 1 : Different formulas of nicotine prepared as modified release tablets.

Constituents	Formulas (mg)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Nicotine	20	20	20	20	20	20	20	20
PVP (10%)	20	20	20	20	20	20	20	20
Avicel® PH302	40	40	40	40			40	40
Eudragit® RS PM	20	20	40	60	20	20	20	20
Starch	100		100	100	100	100	100	100
Zinc stearate	1%	1%	1%	1%	1%	1%	1%	1%
Mannitol		100						
Avicel® PH101					40			
Avicel® PH200						40		
Compression force	4Kg	4Kg	4Kg	4Kg	4Kg	4Kg	6Kg	8Kg
Total weight of final tablet	200	200	200	200	200	200	200	200

Evaluation of the prepared tablets

The following parameters were used to compare the prepared formulas to obtain the final selected formula.

1. **Effect of diluents type on the percent released of nicotine**.
   Formula 1 and 2 were used to study the effect of two different diluents (starch andmannitol) on drug release.
2. **Effect of Eudragit RS PM concentration**. Formula 1,3 and 4 were utilized to study the effect of different concentrations (10% , 20% and 30% respectively) of Eudragit RS PM on the drug release.

3. **Effect of Avicel grade**.
   Formulas 1,5 and6 which contain Avecil PH 302, PH101,and PH200 respectively were used to study the effect of different grades of Avecil as channeling agent on the drug release.
4. **Effect of compression force on nicotine release**.
   Formulas 1, 7and 8 were used to study the effect of changing the compression force 4Kg, 6Kg and 8Kg respectively on the drug release.

Drug release : **(** USP dissolutuion apparatus type II**,** Coply scientific Ltd, England)

The Medium used **:** pH 6.8 phosphate buffer 750ml, Apparatus II , rotation 75 rpm, with a Sampling time**:** 1,2,3,4 and 6hr. The amount of nicotine dissolved was determined spectrophotometecally at λ_{max} 260nm of filtered samples.(UV visible spectrophotometer, Carrywin UV. Varian, Australia). The samples were diluted with dissolution medium if necessary and compare with a standard solution having a known concentration of nicotine in the same medium.

Assay: HPLC analytical method

The chromatographic separation was achieved on a C-18 colum with UV detection at 260nm the HPLC system comprised a (Waters 1500 series HPLC pump(USA) , waters 2487 dual λ absorbance detecter, water breeze soft ware.) was operated at ambient temperature and used citrate buffer: methanol (85: 15 % v/v) with an appearent pH 2.4 as the mobile phase. The flow rate was maintained at 0.7 ml / min. and the retention time 6.94 min.[18].

Preparation of coating formula

The coating solution was prepared according to the Rhom pharma recommendations (the manufacturer) as follows:

Formula :

Eudragit *	6gm
isoprpanol	115.7gm
acetone	77.1gm
Dibutylphthalate	1.2gm
talc	3.25gm
Magnesium stearate	0.25gm
Color	0.25gm
Titanium oxide	1.55gm
Semithicone	Q.S.

*mixture of Eudragit L 100 and S 100 in a ratio 1:2

The final coating solution formula prepared was 205.3gm.

Procedure

The formula was prepared by mixing the solvents together with the plasticizer (dibutylphthalate) in a high shear mixer MLW type LR10 (VEB ML W Prufgerate-werk, Medingen/ Stizfrtal/ Germany). Eudragit mixture was added slowly at room temperature , the powder was thoroughly wetted and care was taken to ensure that nothing settled at the bottom or formed lumps . mixing lasted for at least 30mins, until the solution was clear, the fillers were added step by step .

Calculation of the amount of lacquer required [19]

A specific thickness of coating is required based on the purpose of the coating and the amount needed depending on the surface area of the cores which may be calculated from the following equation assuming that the tablets are cylindrical in shape:

$$S.A = \pi (d.h + \tfrac{1}{2} d^2)$$

Where d is the diameter (mm)
h is the height (mm)
S.A is the surface area (mm^2)
The nicotine tablets had a diameter of 9mm and a surface area approximately equals to $240 mm^2$.
Since 3-5mg lacquer / cm^2 of tablet cores required to produce a core resistant to acidic environment (enteric coated tablets). So multiplying the surface area of the tablet core by the amount required and dividing it by the weight of tablet, the quantity of the lacquer to be applied as a percentage will be obtained.
The amount to be applied
(% dry lacquer substace)= S.A(mm^2) / w (mg) x (mg/ mm^2)
= 240 / 200 x 5
= 6%

Tablet coating

The selected Formula was coated by dipping method . each tablet was held by forcipes and dipped in the coating lacquer in and out 15-20 times , the coat was dried by a stream of warm air between each dip.[20]

Dissolution study of the coated tablets [21]

The dissolution rate of the selected formula for nicotine (coated tablets) was determined using USP apparatus at $37 \pm 0.5^{o}C$ with paddle and the rotation speed was set at 75rpm in order to simulate the pH change of

the GIT , pH change dissolution procedure was applied as follows:
2hr. testing in 0.1N HCl solution followed by testing for one hour in phosphate buffer pH 4 obtained by adding 195ml 0.2M tribasic sodium phosphate solution during which samples were withdrawn at specified times and replaced immediately by fresh medium. Then the medium was changed to pH 6.8 by adding 55ml 0.2 M tribasic sodium phosphate adjusted by 2N NaOH or 2N HCl if required . samples were withdrawn at different time intervals and analyzed spectrophotometrically at 260nm.

Kinetic study

Effect of temperature:

The effect of temperature on the degradation of the selected formula of nicotine modified release tablets was studied . The study was done by storing 90 tablets in ovens (Mermert UL 80 (Rostfrei, Schwach, Germany))at different temperatures 50°C, 60°C and 70°C.
Samples were taken at specified time intervals and analyzed for nicotine. Since the degradation of the drug follows 1st order kinetics, the expiration date $t_{10\%}$ at 25°C could be calculated using the following equation :

$$T_{10\%} = 0.105/k_{25^oC}$$

Pre-liminary clinical study

Before giving the preparation we obtained a written consent of the patients who were included in this study. The modified release nicotine tablets of the selected formula was given to 6 patients suffering from mild to moderate ulcerative colitis (high mucus secretions, irritable bowl syndrome, mild to moderate bleeding and gases). All patients were put on 20mg single dose of nicotine for 2 weeks. The patients were evaluated clinically(physical examination and endoscopy) before and during treatment (physical examination) under the supervion of Dr. Mumtaz k. Hanna at his private clinic.

Results and discussion

Effect of diluent's type on nicotine release

Although diluents are normally thought to be inert ingredients they can significantly affect the biopharmaceutical, chemical and physical properties of the final tablets.[22] Formula 1and 2 which contain maize starch and mannitol as diluents, it was seen that starch gave the best drug release compared with mannitolas shown in fig. 1 .This behavior may be attributed to the swellability property of starch when compared with mannitol which the release is due to water solubility. [23]

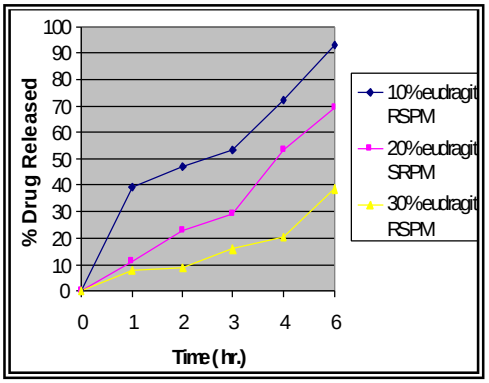

Figure 1: Effect of diluent type on the release of nicotine at pH 6.8 and 37°C

Effect of Eudragit concentration:

Eudragit RS PM can be in corperated in a percent of 10-30% (w/w) by weight to provide suitable granules and matrix tablets. The amout of Eudragit RS PM to be added , depends upon the solubility characteristics of the drugs and the rate required [20]Formulas 1 , 3 and 4 which contain 10% , 20% and 30 % w/w of Eudragit (as a retardant) respectively were evaluated . formula 1 gave the best modified release of nicotine when compared with the requirements of drug release to the colon [17]. The results from dissolution profiles of formulas 1 ,3 and 4 indicates that the retardant content affects the release of nicotine from the tablet, this result is in a consistent with the results obtained when Eudragit RSPM polymer was used as a retardant material for diclofenac sodium and indomethacin tablets.[24] It appears that the amount of retardant needed is 10 % as shown in fig. 2 .This is in agreement with the reported data which indicated that the retardation effect on the release of drug is dependent on the amount of Eudragit included [25]

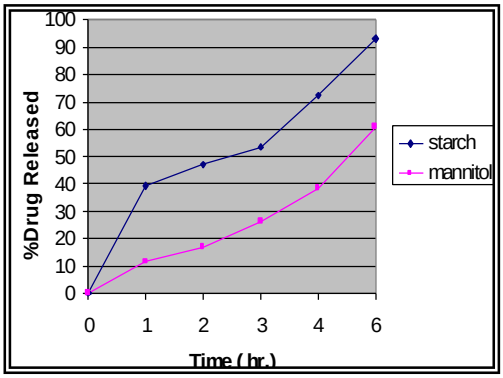

Figure 2 : Effect of Eudragit RS PM concentration on the percent released of nicotine at pH 6.8 and 37°C.

Effect of Avicel grade

Avicel is microcrystalline cellulose , it is partially depolymerized cellulose prepared by treating alpha- cellulose obtained as a pulp fibrous plant material with acids. The grade of Avicel depends on its normal loss on drying , bulk density and degree of polymerization values. [26] The results showed that formula 1 in which Avicel PH 302 present, gave the best dissolution profiles while the difference in the release that occurred in the other formulas 5 and 6 which contain Avicel PH 101 and PH 200, respectively. is due to the difference in the porosity , surface area, particle size and density of Avicel as stated [27] as shown in fig. 3

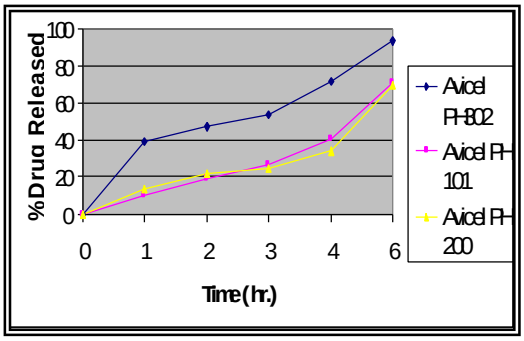

Figure 3 : Effect of Avicel grade on the percent released of nicotine at pH6.8 and 37°C.

Effect of compression force

Compression forces affect the hardness of a tablet and its thickness at a constant die fill as additional compression force is applied , the hardness values increase and the thickness decrease and the decrease as shown in fig.4 [19] , thereby the dissolution of the drug decrease in surface area and increase in hardness. This may be related to the low porosity resulted from the increase in compression force.[8] Therefore, formula 1 was selected because it gave the best drug release profile which complies with absorption properties of nicotine from the colon and it was further investigated for enteric coating, kinetic study and pre-liminary clinical study [17].

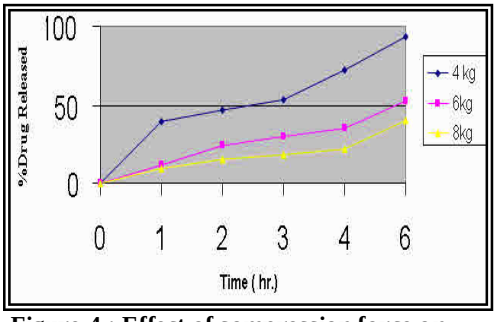

Figure 4 : Effect of compression force on percent released of nicotine at pH 6.8 and 37°C.

Coating formula

The tablets showed good appearance with no signs of craking or splitting or peeling. Induction of hydrophobic materials and inert fillers (Mg stearate , talc , titanium oxide , aluma lakes with an orange color) these fillers facilitate processing of the lacquer by reducing its stickness, help to smooth the permeability to water and decrease the tackiness of the drying lacquer. In addition they reduce the permeability of the film as long as the mechanical strength is maintained thereby enhancing the enteric properties of the film [28,29]. Formula 1 was coated to target the drug to the colon. The coated tablets showed no drug release in 0.1 N HCl for 2hr. period of the test and the release of the drug increased rapidly when the pH changed to 6.8 as shown in fig 5.

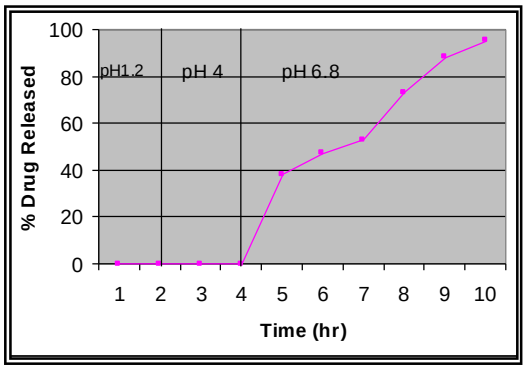

Figure 5 : The cumulative percent of enteric coated nicotine tablets release at different pH-media at 37°C.

Kinetic study

Effect of temperature

The stability of the coated modified release tablets were studied at different exaggerated temperatures (50° C , 60 °C and 70°C) for 3 months. Fig 6 shows the change in the log percentage remaining of nicotine versus time at different temperatures. The obtained profiles were linear , indicating that nicotine degradation follows 1st order kinetics. The slopes of these lines were determined and the calculated rate constants (k) are summarized in fig (6). Arrhenius plot was then constructed as shown in fig 7. the linearty of the curve indicates its utility in predicting the rate of degradation at lower temperatures. since the degradation of the drug followed 1st order kinetics the expiration date can be calculated at 25 ° C for nicotine coated matrix tablets and it was 52 month.

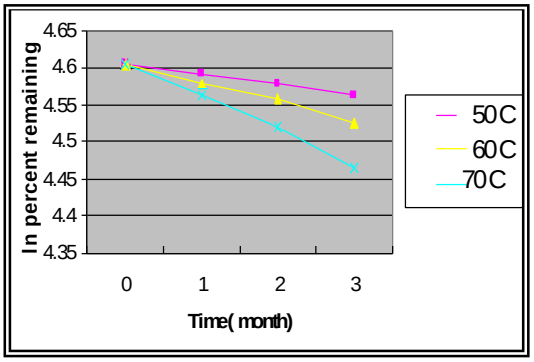

Figure 6 : Percent remaining of drug versus time.

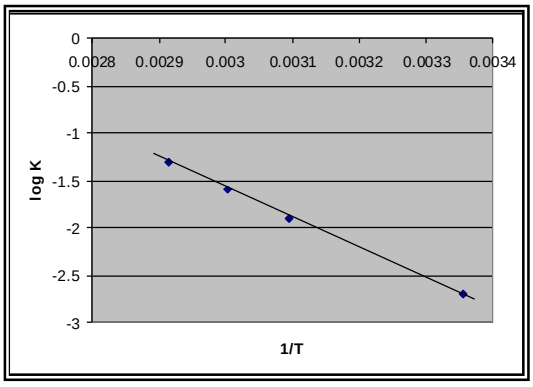

Fig7: Arrhinous plot for expiration date estimation of nicotine tablet (Formula 1).

Clinical study

Nicotine was given to 6 non smoking patients (5 males and 1 female) with an age range of 27- 65 yr. The patients took 20mg once a day for 10 days suffering from mild to moderate ulcerative colitis. The out come of this preliminary study indicates that 67% (4 out of 6 patient) were responsive to nicotine therapy (relief of bleeding and most sign and symptoms) although all patients suffered from adverse effect reaction towards nicotine therapy because the patient were non smokers (lightheadedness or dizziness, nausea, headaches, central nervous system stimulation and tachycardia).[30] further studies in the future should be done including in vivo nicotine blood concentration to optimize the dose.

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