

Research Article**DESIGN AND IN VITRO EVALUATION OF PREDNISOLONE TABLETS AS A POTENTIAL COLON DELIVERY SYSTEM**

*MOHANAD NAJI SAHIB Department of Pharmaceutical Technology; School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia, E-mail: mohanad_pharm@yahoo.com
SHAYMAA ABDALWAHED ABDULAMEER Department of Clinical pharmacy, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia
ALAA A. ABDUL RASOOL Dean of the College of Pharmacy, University of Baghdad, Iraq

ABSTRACT

Prednisolone is a corticosteroid drug that is used to treat many diseases. The present investigation was concerned with the formulation of prednisolone as an oral modified release tablet for colonic targeting. Many trials were performed to prepare a satisfactory formula using the wet granulation method with various additives and coatings. We found that lactose as a diluent provided the most reasonable release for prednisolone among other diluents. In addition, the formula containing 1% Eudragit RS PM was the best with regard to 100% release of drug in comparison with other concentrations and other retardant types. Avicel was used as a canalising agent, and the results showed that the formula containing 30% Avicel PH 302 demonstrated faster release. Eudragit S 100 provided the best release of drug in phosphate buffer, pH 7.4. The effect of the percent of binding agent polyvinylpyrrolidone (PVP) (5%, 10%, and 15%) was studied, and the best results were obtained with a concentration of 10%. The trials in this study successfully formulated prednisolone-modified release tablets (coated matrix) using a wet granulation method as a potential colon delivery system.

Key words: Prednisolone; Colon Delivery System; Eudragit

INTRODUCTION

The oral route is considered the most suitable for administration of drugs to patients. The orally administered dose normally dissolves in the stomach fluid and/or in intestinal fluid, and it absorbs from these regions of the gastrointestinal tract (GIT) depending on the physicochemical properties of the active constituent¹. In the colon, both local and systemic drug delivery can take place². As a result, oral drug delivery systems have considerable advantages that could be targeted to the colon; for example, such a delivery system would permit topical treatment of inflammatory bowel disease, e.g., ulcerative colitis or Crohn's disease³. Target-specific means of drug delivery could allow oral administration of peptide and protein drugs, which normally become inactive in the upper parts of the gastrointestinal tract^{4, 5}. When diurnal rhythm is evident, colon-specific systems

could be used, e.g., for asthma, rheumatic disease, ulcer disease, and ischemic heart disease⁶. The colon forms the lower region of the GIT and extends from the ileocaecal junction to the anus⁷. Many drugs are absorbed from the colon by passive diffusion via the paracellular route. The surface area of the colon for absorption is smaller than that of the small intestine, and this is compensated by the slow transit time⁸.

An oral colonic delivery system should delay the release of drug in the stomach and small intestine but permit complete release in the colon. Many approaches and systems have been used to manufacture such colonic targeting systems. The major approaches used for targeting mechanisms are as follows^{9, 10}:

1. pH-dependent delivery: The pH-dependent systems take advantage of the pH changes in the human GIT, which gradually increase from the stomach (pH 1-2, which increases to 4 during digestion)

^{11, 12} to the small intestine (pH 6-7) at the site of digestion, and finally to pH 7-8 in the distal ileum ¹³.

The polymers used for colon targeting should be able to resist the lower pH values of the stomach and proximal portion of the small intestine; moreover, the polymers should be able to disintegrate or dissolve at the neutral or slightly alkaline pH of the terminal ileum, preferably at the ileocaecal junction ^{14, 15}.

2. Time-dependent delivery: The principle of designing timed-released systems is used to counteract the low pH of the stomach and establish a lag time of a predetermined time span, after which the release and absorption of drug take place. The lag time in this situation is the time needed to transit from the mouth to colon ¹⁶.

3. Pressure-dependent delivery: As a consequence of peristalsis, higher pressures are found in the colon than in the small intestine; to take advantage of this increased pressure, Takaya and co-workers developed pressure-dependent colon-delivery capsules prepared from ethyl cellulose, which is insoluble in water ¹⁷. In these systems, drug release occurs after disintegration of a water-insoluble polymer capsule due to pressure in the lumen of the colon. The thickness of the ethyl cellulose membrane is a crucial factor for disintegration of the formulation ¹⁸.

4. Bacteria-dependent delivery: These systems clearly appear in the prodrug sulphasalazine, which is composed of two separate moieties, sulphapyridine and 5-aminosalicylic acid, linked by an azo-bond. This system remains intact across the upper portion of the gut; once in the colon, the azo-bond is cleaved by the bacteria present in the colon, resulting in liberation of the carrier molecule sulphapyridine and the pharmacologically active agent 5-aminosalicylic acid ¹⁹. This idea has led to the development of other novel azo-bond-based polymers (azo-polymers) for this purpose; such potential materials include inulin ²⁰ and dextran ²¹.

Prednisolone is also known as 1, 2-Dehydrohydrocortisone, Deltahydrocortisone, metacortandralone, and 11, 17, 21-Trihydroxy pregna-1, 4-diene-3, 20-dione; its chemical formula is $C_{21}H_{28}O_5$ and its molecular weight is 360.4 KD ²². Prednisolone is completely absorbed from the gastrointestinal tract. When administered orally, around 80% of the prednisolone is absorbed. The bioavailability of prednisolone depends on the dissolution rate of the dosage form. Peak plasma concentrations are obtained 1 or 2 hours after oral administration, and it has a typical plasma half-life of 2 to 4 hours. Its initial absorption, but not its overall bioavailability, is affected by food ²³.

MATERIAL AND METHODS

Chemicals and reagents

The following materials of the highest available purity were used in this study:

Acetone (BDH chemicals, Ltd, Liverpool, England), Dibutylphthalat (USB, B. Brussels, Belgium), Ethanol 99% (BDH chemicals, Ltd, Liverpool, England), Eudragit L 100, S100, RSPM, RL-copolymers of methacrylic acid and methyl acrylate (Rhom Pharma GmbH Weiterstadt, Germany), Glucose, Mannitol, Starch (Merck, Germany), Lactose, Hydrochloric acid (Riedel De Haen Ag Seelze Hanover), Ethyl cellulose (BDH chemicals, Ltd, Liverpool, England), Methanol (BDH chemicals, Ltd, Liverpool, England), Microcrystalline cellulose-Avicel -PH 101, PH 102, PH 302 (FMC Cooperation, Pennsylvania, USA), Polyvinylpyrrolidone (PVP K 30) (BDH chemicals, Ltd, Liverpool, England), Potassium dihydrogen phosphate (BDH chemicals, Ltd, Liverpool, England), Sodium hydroxide (Sodium hydroxide), Zinc stearate (Barlocher, GmbH, Germany), and Prednisolone (gift from AL-Furat Drug industry, Iraq, sigma origin).

Method

Formulation of prednisolone tablets

Different formulas (Table 1) were prepared using the wet granulation method. After appropriate dry blending of the previously sieved ingredient, the powder was granulated by adding an appropriate amount of binding solution. It was subsequently kneaded to the proper

²⁴, and the absorbance was then recorded and plotted versus the concentration.

RESULTS AND DISCUSSION

Different formulas were prepared as shown in Table 1; an acceptable formula could resist the 0.1 N HCl, not release the active ingredient in phosphate buffer at pH 6.8, and release the active ingredient at pH 7.4 within an acceptable period of time.

Table - 1 Constituents of Different Formulas of Prednisolone-Modified Release Tablets.

Formula No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Prednisolone	14.28	14.28	14.28	14.28	14.28	14.28	14.28	14.28	14.28	14.28	14.28	14.28	14.28
PVP K 30	10	5	15	10	10	10	10	10	10	10	10	10	
Eudragit RS PM	1	1	1			1	1	1	1	1		2	3
Eudragit RL				1									
EC					1								
Mannitol						44.22							
Glucose							44.22						
α -Lactose	44.22	49.22	39.22	44.22	44.22				44.22	44.22	45.22	43.22	42.22
Starch								44.22					
Avicel 302	30	30	30	30	30	30	30	30			30	30	30
Avicel 102									30				
Avicel 101										30			
Zinc Stearate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

consistency. The wet mass was passed through a 1-mm sieve (18 mesh) and dried in a pre-warmed tray dryer. The dry granules were then reduced in size by passing them through a 0.8-mm sieve (20 mesh). A known weight of granules was mixed with a specified amount of disintegrant in a closed container; the samples were then mixed with zinc stearate (previously sieved and dried) for 1 minute and compressed into tablets using 6-mm biconcave punches at 6 ton/inch². Three coating formulas were used: Eudragit S 100, Eudragit L 100, and cellulose acetate phthalate (CAP). Calibration curves of the drug in phosphate buffer at pH 6.8 and 7.4 and in 0.1 N HCl were constructed by preparing various concentrations of the drug (5, 10, 15, 20 μ g/ml); the samples were analysed spectrophotometrically at a λ_{max} of 248 nm

Effect of the diluent Types

Although diluents are normally considered inert ingredients, they may affect the biopharmaceutical, chemical, and physical properties of the final tablet²⁵. Generally, the more soluble the carrier (diluent), the faster the dissolution of the drug; correspondingly, when carrier materials with low solubility are used, the drug particles are less rapidly exposed to the dissolution medium as primary drug particles²⁶.

FIG 1 shows the drug release for formulas 1, 6, 7, and 8, which contain lactose, mannitol, glucose, and starch, respectively. In formulas 6 and 7, in which mannitol and glucose were used, the dissolution rate was relatively equivalent ($T_{50\%}$ was 98.9 and 97.8 min, respectively) and higher than the others ($T_{50\%}$ for formulas 1 and 8 was 130 and 162 min, respectively) because of their

water solubility properties. However, both formulas were excluded because of the ability of glucose to absorb moisture from the atmosphere, as well as the high cost of mannitol²⁷.

On the other hand, the slow dissolution rate of formula 8 ($T_{50\%}$ was 162 min) was due to the presence of starch, which is an insoluble filler and reaches a moisture plateau of 11 to 14%. As a result, in steroid tablet form, this localisation of moisture may result in a decrease in the dissolution rate. Finally, formula 1 with lactose gave a reasonable dissolution rate ($T_{50\%}$ was 130 min) due to its low water solubility compared to other types of sugars; in addition, it is widely used in tablets employing a small amount of active ingredients (e.g., steroids), and its cost is relatively low in comparison to many other diluents²⁵. The $T_{50\%}$ release shows non-identical superscripts that represent a significant difference among the release rate of these formulas ($P < 0.01$).

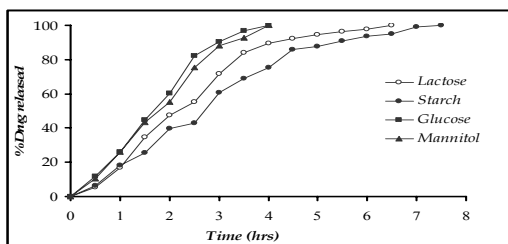


FIG.1 Effect of diluent types on prednisolone release from modified release tablets in phosphate buffer, pH 7.4 at 37° c.

Effect of the Eudragit RS PM concentration

Eudragit RS PM acrylic resin is a copolymer of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups. The ammonium groups are present as salts and are responsible for the permeability of the polymer. The amount of Eudragit RS PM added is dependent on the solubility characteristics of the drugs and the release rate needed; in general, 10 to 30 parts of Eudragit RS PM

by weight is used to provide suitable granules and matrix tablets²⁸.

Formulas 1, 12, and 13 contain Eudragit RS PM at concentrations of 1%, 2%, and 3%, respectively. Figure 2 shows the 100% release of the drug for 1% Eudragit RS PM over about 6.5 hrs; in comparison, 100% release of the drug using 2% and 3% Eudragit RS PM occurred in about 10 hrs and 12 hrs, respectively. These results are consistent with those obtained by Savarna Somade et al.²⁹, who found that as the polymer concentration was increased, drug release was reduced. In addition, the $T_{50\%}$ release showed a significant difference ($p < 0.01$) for the release of drug among these formulas and non-identical superscripts [$T_{50\%}$ for 1%, 2%, and 3 % was 130, 292.5, and 365 min, respectively]. The same result was also obtained by Gareeb M. M. (Formulation and *in vitro/ in vivo* evaluation of sustained release tablets of orphenadrine citrate. M.Sc. Thesis, College of Pharmacy, University of Baghdad, Baghdad/Iraq, 1998; 46-57), who reported a similar retardation effect for a sustained release tablet of orphenadrine citrate with a dissolution time of 5-8 hrs.

In this study, the effect of the retardant appeared to be reduced. This retardation in drug release might be due to the low solubility of prednisolone, in addition to inclusion of the drug within the inert insoluble polymer matrix. AL-Hurre, M.Y. (Formulation and clinical study of diclofenac sodium and indomethacine as oral modified release tablets. M.Sc. Thesis., College of Pharmacy, University of Baghdad, Baghdad/Iraq, 2003) reported a similar effect for the retardant amount between indomethacine and diclofenac sodium, where indomethacine has a lower solubility than diclofenac sodium.

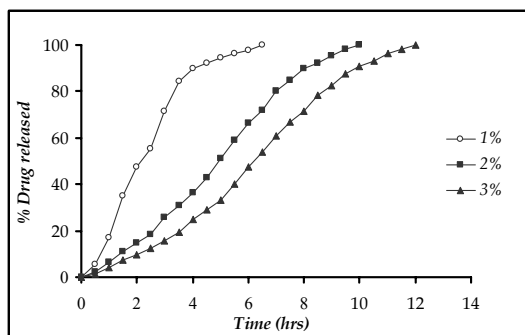


FIG. 2 Effect of Eudragit RS PM Concentration on The Release of Prednisolone From Modified Release Tablets in Phosphate Buffer, pH 7.4 at 37° C.

Effect of changing the grade of Avicel

Avicel is microcrystalline cellulose $[(C_6H_{10}O_5)_n]$ with $n \sim 220$, a molecular weight of ~ 36000 Daltons] that can be used as a canalising agent³⁰. The results shown (Figure 3) a fast release profile for formula 1, in which Avicel PH 302 was used, since it gave 100% release within 6.5 hrs compared to formulas 9 and 10 (80% within the same time period), which contained Avicel PH 102 and Avicel PH 101, respectively. On the other hand, the $T_{50\%}$ release showed non-identical superscripts [$T_{50\%}$ for Avicel PH 302, 102, and 101 were 130, 210 and 244 min, respectively]; this represents a significant difference among these formulas ($P < 0.01$). The above result may be due to differences in the porosity, surface area, particle size, and/or density of Avicel pH 302, as previously stated^{31, 32}.

These results were consistent with the data obtained by Landin M. et al.³³, who found that Avicel PH 302 shows the highest drug release rate compared to Avicel PH 101, which exhibits the lowest.

In addition, Avicel offers the possibility to controlling drug release rates when it is combined with lactose in water-insoluble drugs²⁵.

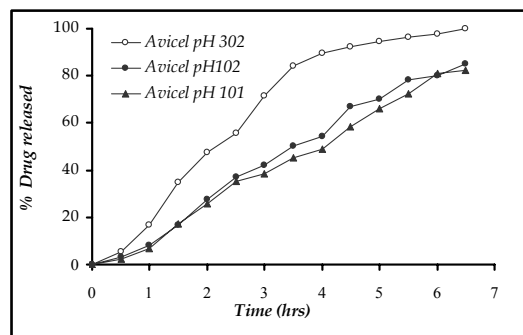


FIG. 3 Effect of Changing The Grade of Avicel on The Release of Prednisolone From Modified Release Tablets in Phosphate Buffer, pH 7.4 at 37° C.

Effect of changing the type of coating formulas

The effect of changing the type of coating formulas were investigated for matrix tablets (formula 1) and plain tablets (formula 11) using Eudragit S 100, L100, and cellulose acetate phthalate (CAP). The three dissolution media were 0.1 N HCl, and phosphate buffers at pH 6.8 and 7.4.

Figure 4 shows the release profile of the coated plain tablets. The results indicate that less than 5% of the prednisolone was released in 0.1 N HCl within two hrs, compared to 100% in phosphate buffer at pH 6.8 within 1 hr for plain tablets coated with cellulose acetate phthalate (CAP) and Eudragit L 100. While no release was obtained in 0.1 N HCl and 100% release was obtained in phosphate buffer at pH 6.8. 100% release was obtained within 2 hrs in phosphate buffer at pH 7.4 for plain tablets coated with Eudragit S 100.

Ashford et al. and Fukui et al.³⁴ have shown that pH-sensitive polymers are not suitable for colon-targeted drug delivery systems due to their poor site specificity and because these polymers may start to dissolve even in the lower small intestine. However, two controlled release mechanisms (time- and pH-dependent) could achieve colonic delivery following oral administration³⁵.

FIG 5 shows that 100% release of the prednisolone was obtained from matrix tablets coated with cellulose acetate

phthalate (CAP) and Eudragit L100 in phosphate buffer at pH 6.8 within 4 and 6 hrs, respectively; meanwhile, the matrix tablets coated with Eudragit S 100 provided 100% release in phosphate buffer at pH 7.4 within 6.5 hrs. This is because cellulose acetate phthalate (CAP) and Eudragit L 100 films dissolve above pH 5 and 6, respectively, while the film of Eudragit S 100 dissolves above pH 7³⁶. Therefore, Eudragit S 100 is more suitable for colonic targeting because the tablet will reach a farther distance in the intestine with the sustained effect of the matrix tablets.

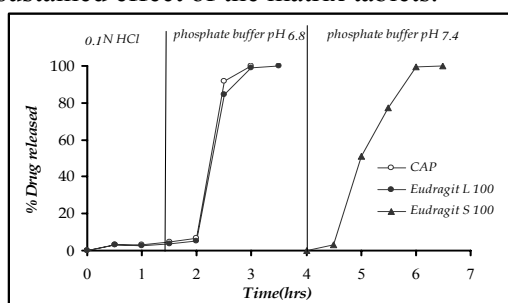


FIG. 4 Effect of changing the coating formulas on the release of Prednisolone plain tablets. (Dissolution Media, 0.1 N HCL, Phosphate Buffer at pH 6.8 and 7.4, 37° C.)

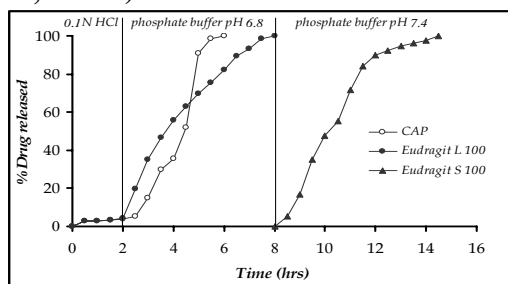


FIG. 5 Effect of changing the coating formulas on the release of Prednisolone from modified release tablets. (Dissolution Media: 0.1 N HCL, Phosphate Buffer at pH 6.8 and 7.4, 37° C.)

Effect of changing the type of polymer matrix tablet

Eudragit RS, Eudragit RL, and ethyl cellulose (EC) provide pH-independent sustained release properties^{35, 36}. Figure 6 shows the release profiles of formulas 1, 4, and 5, which contain Eudragit RS PM,

Eudragit RL, and EC, respectively. These profiles were used to study the relationship between the permeability of the polymer and the retardation of drug release.

The results (Figure 6) show that the release of drug from formulas 4 and 5 reached 100% within 4 and 3.5 hrs, respectively, while 100% drug release occurred in about 6.5 hrs for formula 1. Therefore, it appeared that Eudragit RS PM possesses a better dissolution rate than Eudragit RL and EC at the concentration used (1%). In addition, the $T_{50\%}$ release shows non-identical superscripts [$T_{50\%}$ for EC, Eudragit RL, and Eudragit RS PM were 68, 92 and 130 min, respectively], which represents a significant difference ($P < 0.01$) in the release rate for these formulas.

These results were consistent with those obtained by Kaul-D et al.³⁷, who found that both types of Eudragit exhibit significant release retardation, but Eudragit RS PM is more suitable than RL in decreasing drug release at all concentrations.

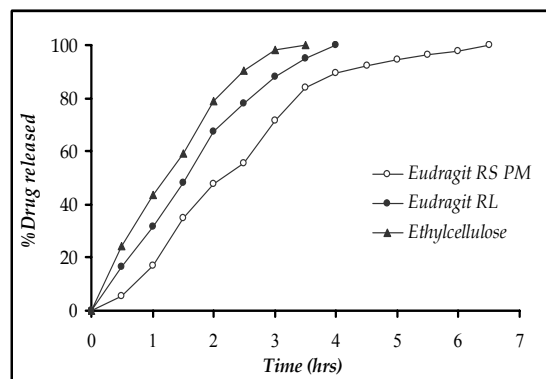


FIG. 6 effect of changing the type of polymer matrix on the release of prednisolone from modified release tablets in phosphate buffer, ph 7.4 at 37° C.

Effect of changing the binder concentration

Polyvinylpyrrolidone (PVP) is an alcohol-soluble material³⁸ that can be used to make an alcoholic binder solution for hygroscopic material. Figure 7 shows the release profiles for formulas 1, 2, and 3, which contain PVP at concentrations of

10%, 5%, and 15%, respectively. The results show that the release of drug from formulas 2 and 3 reached 100% within 4 and 8 hrs, respectively, while drug release for formula 1 was about 6-7 hrs (100% release). Additionally, the $T_{50\%}$ release showed non-identical superscripts [$T_{50\%}$ for formulas 1, 2, and 3 were 130, 85 and 159 min, respectively]. This represents a significant difference ($p < 0.01$) in the release of drug, which was delayed from 85 min for formula 2 to up to more than 130 to 159 min for formulas 1 and 3, respectively. These results were attributed to the increase in concentration of alcoholic PVP in the presence of Eudragit RS polymer, which decreases the rate of tablet dissolution³⁹.

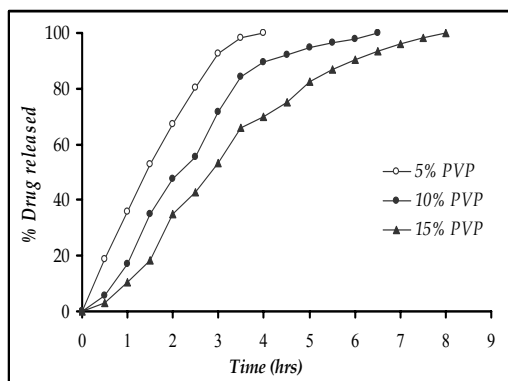


FIG.7 Effect of Changing The Concentration of Binder (Polyvinylpyrrolidone on The Release of Prednisolone From Modified Release Tablets in Phosphate Buffer, pH 7.4 at 37° C.

Mechanism of prednisolone release from different matrices

In order to characterise the mechanism of drug release from the matrices, the data were fit to the following mathematical model⁴⁰:

$$F = \frac{M_t}{M_\infty} = K t^n$$

where, M_t/M_∞ is the fraction of drug released up to time t (F), K is the rate constant, and n is the release exponent indicative of the release mechanism.

The results (Table 2) showed high n values (> 0.5), which indicate a coupling of diffusion and erosion mechanisms, a so-called anomalous diffusion due to the presence of swelling polymers within the matrix structure during dissolution. Similar results have been observed by other investigators⁴¹. Tablets containing 1% ethyl cellulose (formula 5) remained intact during the release studies; therefore, erosion can not consider as one of the release mechanisms. The n value obtained was 0.5, indicating that the mechanism was Fickian diffusion.

CONCLUSION

Prednisolone is a corticosteroid drug that is used to treat many diseases, such as ulcerative colitis or Crohn's disease. The trials investigated in this study resulted in successful formulation of prednisolone-modified release tablets (coated matrix) using the wet granulation method. Based on the results obtained, the following may be concluded:

Lactose can be selected as a diluent due to its reasonable dissolution release profile compared to other diluents. Eudragit RS PM at a concentration of 1% w/w of the total tablet weight was utilised for prepared tablets, and the formula provided acceptable retardation of drug release with a dissolution time of about 6.5 hrs. Avicel PH 302 was the best type of Avicel for use, as it provided a reasonable dissolution rate compared to other types. Eudragit S 100 is more suitable for colonic targeting in comparison to other types of coating formulas (Eudragit L100 and cellulose acetate phthalate). Polyvinylpyrrolidone (PVP) at a concentration of 10% was an excellent binder. Further investigations will be carried out (*in vivo* evaluation) to evaluate the efficiency of prednisolone-modified release tablets for the treatment of ulcerative colitis or Crohn's disease.

Table - 2 Values of The Kinetic Parameters For the Release Mechanism of Prednisolone From Different Prepared Formulas.

Formulation Variable	Formula no.	n value	Type of release
Effect of Eudragit RS PM concentration	1	1.4	anomalous
	12	1.3	anomalous
	13	1.2	anomalous
Effect of changing the grade of Avicel	1	1.4	anomalous
	9	1.17	anomalous
	10	1.12	anomalous
Effect of changing the type of polymer matrix tablet	1	1.4	anomalous
	4	0.66	anomalous
	5	0.5	Fikian diffusion
Effect of changing the concentration of binder	1	1.4	anomalous
	2	0.86	anomalous
	3	1.28	anomalous

ACKNOWLEDGEMENTS

Corresponding author would like to thank Universiti Sains Malaysia in offering electronic data base services which have helped in writing this article and for its Fellowship program. This work was funded by College of Pharmacy, University of Baghdad, Iraq.

REFERENCES

- Sarasija S, Hota A. Colon-specific drug delivery systems. *Ind. J. Pharm. Sci.* 2000; 62: 1-8.
- Bussemer T, Otto I, Bodmeier R. Pulsatile drug-delivery systems. *Crit. Rev. Ther. Drug Carrier Syst.* 2001; 18: 433-458.
- Boot C, Rudolph MW. In vivo evaluation of a novel pH-and time- based multiunit colonic drug delivery system. *Alimentary pharmacology and therapeutics* 2004; 3: 347-353.
- Yang L, Chu J, Fix J. Colon-specific drug delivery: new approaches and *in vitro/in vivo* evaluation. *Int. J. Pharm.* 2002: 235: 1-15.
- Mackay M, Phillips J, Hastewell J. Peptide drug delivery: colonic and rectal absorption. *Adv. drug Del. Rev.* 1997: 28(2): 253-273.
- Shareef MA, Khar RK, Ahuja A, Ahmad FJ, Raghav S. Colonic drug delivery: An update Review. *AAPS Pharmscitech* 2003: 5(2): E 17.
- Watts PJ, Illum, L. Colonic drug delivery. *Drug Dev Ind Pharm* 1997: 23: 893-913
- Edwards CA. Anatomical and physiological basis. In *Physiological factors influencing drug absorption. Colonic drug absorption and metabolism.* Bieck PR (ed). Marcel Dekker Inc., New York, 1993; pp. 1-28.
- Ibekwe VC, Kendall RA, Basit AW. Drug delivery to the colon. *The Drug Delivery Companies Report*(Spring/ Summer), PharmaVentures Ltd, Oxford, UK 2004: 27-30.
- AL-Taani BM, Tashtoush BM. Effect of microenvironment pH of swellable and erodable buffered matrices on the release characteristics of diclofenac sodium. *AAPS Pharmasci Tech.* 2003; 4(3) : Article :43
- Rubinstein A.: Approaches and opportunities in colon-specific drug delivery. *Crit. Rev Ther. Drug Carrier Syst* 1995: 12: 101-149.
- Wilson CG, Washington N. The stomach: Its role in oral drug delivery. In *Physiological Pharmaceutics:Biological Barriers to Drug Absorption*, Rubintein MH(ed). Chichester, UK: Ellis Horwood Limited, , 1989; 47-70.
- Evans DF, Pye G, Bramley R, Clark AG, Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut* 1988; 29: 1035-1041.

14. Dew MJ, Hughes PJ, Lee MG, Evans BK, Rhodes J. An oral preparation to release drugs in the human colon. *Br. J. Clin. Pharmacol.* 1982; 14: 405-408.
15. Tuleu C, Andrieux C, Cherbuy C, Darcy-Vrillon B, Duee PH, Chaumeil JC: Colonic delivery of sodium butyrate via oral route: Acrylic coating design of pellets and in vivo evaluation in rats. *Methods Find Exp Clin. Pharmacol* 2001; 23(5): 245.
16. Li J, Yang L, Ferguson SM, Hudson TJ, Watanabe S, Katsuma M, Fix JA. In vitro evaluation of dissolution behavior for a colon-specific drug delivery system (CODES™) in multi-pH media using United States Pharmacopeia Apparatus II and III. *AAPS PharmSciTech.* 2002; 3(4): article 33.
17. Takaya T, Ikeda C, Imagawa N, Niwa K, Takada K.: Development of a colon delivery capsule and the pharmacological activity of recombinant human granulocyte colony-stimulating factor in beagle dogs. *J. Pharm. Pharmacol.*, 1995; 47(6): 474-478.
18. Lieberman HA, Lachman L. Tablets. In *Pharmaceutical dosage forms*, Marcel Dekker, Inc. New York and Basel, 1980; Vol. 1: pp. 214.
19. Peppercorn MA, Goldman P. The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. *J. Pharmacol. Exp. Ther* 1972; 181(3): 555-562.
20. Fish NW, Bloor JR. Drug delivery to the colon. *Expert Opinion on Ther Patents* 1999; 9: 1515-1521.
21. Pang N-Y, Zhang Y, Zhang, Z-R. Synthesis of an enzyme-dependent prodrug and evaluation of its potential colon targeting. *World J Gastroenterol* 2002; 8(5): 913-917.
22. The pharmaceutical Codex (12thed). *Principle and practice of pharmaceuticals*, Walter Lund (ed). The Pharmaceutical Press, London, 1994; pp. 1048.
23. Mycek MJ, Harvey RA, Champe PC. Androgens. In: *Lippincotts Illustrated Reviews: Pharmacology* (3rd ed.). Alwolters Kluwer Company 2004: 271-277.
24. Konishi H, Kanemoto K, Ikuno Y, Minouchi T, Inoue T, Hodohara K, Fujiyama Y, Yamaji A. Fluctuation in Therapeutic Control Associated with Interchange of Prednisolone Tablet Formulations: Assessment of Bioequivalence by Dissolution Test. *Yakugaku Zasshi* 2002; 122(10): 813-817.
25. Banker G, Peck G, Baley G. Tablet formulation and design. In *Pharmaceutical dosage forms tablets*. Liberman and Lachman (ed). Marcel Dekker, New York 1982: 61-140.
26. Westerberg M, Nystrom C. Physiochemical aspect of drug release XII. The effect of some carrier particle properties and lubricant admixture on the drug dissolution from tableted ordered mixtures. *Int J Pharm* 1991; 69: 129-141.
27. Laako R, Erikainen S. Effect of core components on indomethacin release from film-coated granules. *Int J Pharm* 1991 67(1): 79-88.
28. Lachman L, Lieberman HA, Kanig JA. *Theory and practice of industrial pharmacy* (3rd ed). Lea and Febiger 1986: 372.
29. Somade S, Singh K K. Comparative Evaluation of Wet Granulation and Direct Compression Methods for Preparation of Controlled Release Ranitidine HCL Tablets. *Indian J Pharma Sci* 2002; 9(11): 110-112.
30. Handbook of pharmaceutical excipients (3rd ed.), Washington, DC, American pharmaceutical association, 1988.
31. Podczek F, Revesz P. Evaluation of the properties of microcrystalline and microfine cellulose powder. *Int J Pharm* 1993; 91:183-193.
32. Pesonen T, Paronen P. Evaluation of a new cellulose material as a binding agent for the direct compression of tablets. *Drug Dev Ind Pharm* 1986;12: 2091-2111..
33. Landin M, Gonzaloz MP, Soute C, Concheiro A, Martinez-Pacheco R *et al*: Comparison of two varieties of microcrystalline cellulose as filler-binder part 2 hydrochlorothiazide tablets. *Drug Dev Ind Pharm* 2003; 19(10): 1211-1210.
34. Ashford M, Fukui JT, Attwood D, Sharma H, Woodhead PJ. An in vivo investigation into the suitability of pH-dependent polymers for colonic targeting. *Int J Pharm*; 1993; 95: 193-199.
35. Cheng G, An F, Zou M-J, Sun J, Hao X-H, He Y-X. Time- and pH-dependent colon-specific drug delivery for orally administered diclofenac sodium and 5-aminosalicylic acid. *China World J Gastroenterol* 2004;10(12):1769-1774.
36. Fatima L, Asghar A, Chandran S. Multiparticulate Formulation Approach to Colon Specific Drug Delivery: Current Perspectives. *J Pharm Pharmaceut Sci* 2006; 9(3):327-338.

37. Kaul D, Venkataram S. Sustained release tablet formulation for a new iron chelator. *Drug Dev Ind Pharm* 1992; 18(9): 1023-1035.
38. Shivakumar HN, Sarasija S, Desai BG. Design and evaluation of ph sensitive minitabets for chronotherapeutic delivery of theophylline. *Indian J Pharm Sci* 2007; 69(1): 73-79.
39. Abbaspour MR, Sadeghi F, Garekani HA. Preparation and characterization of ibuprofen pellets based on Eudragit RS PO and RL PO or their combination. *Int J Pharma* 2005; 303(1-2): 88-94.
40. Martin A. Physical pharmacy (4thed). Lea and Febiger, Philadelphia, 1993: 497, 515-517,595.
41. Masuda K, Ashraful SM et al. Controlled release of naproxen sodium from Eudragit RS 100 transdermal film. Dhaka University J Pharm Scie 2002; 59(3): 193-198.
42. Anjali M, Steven H. Wet granulation fine particle ethylcellulose tablets; AAPS Pharm. Sci. . 2003; 5(2), article 13