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Bioavailability of Oral Colon-Specific Ketoprofen Microcapsules

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ABSTRACT

Oral, colon-specific drug delivery has attracted much interest recently. It is of considerable advantage to have an oral drug delivery system that could be targeted to the colon. This could be either for the local treatment of diseases such ulcerative colitis, irritable bowel syndrome and tumors, or to exploit the colon as a preferred region for systemic drug absorption, possibly as a site for polypeptides absorption. Delivery systems that rely on pH and/or time dependent mechanisms for drug release are less reliable in achieving consistent site-specific drug delivery to the colon. The successful delivery of prodrugs of 5-aminosalicylic acid is an evident of the potential of the bacterial flora. However, it has been less successful in the development of polysaccharide – based dosage forms or synthetic polymer coatings. This work was aimed at the development of ketoprofen microcapsules based on polysaccharide coating. The *in-vitro* release studies in simulated colonic media showed sensitivity of such dosage form to bacterial enzymes with subsequent increase in dissolution rate. The *in -vivo* profiles in dogs showed that the tested product exhibited a lag-time prior to commencement of drug absorption. The plasma profiles were not accompanied by a high initial plasma concentration associated with conventional dosage forms.

Key Words: colonic delivery, cell-free extract (CFE), ketoprofen, moment analysis parameters.

1. INTRODUCTION

The benefits of developing a universal colon-specific system for the oral delivery of drugs are large both in terms of local or systemic treatment [1]. The colon demonstrates a longer residence time, reduced enzymatic activities and greater responsiveness to agents that enhance the absorption of poorly absorbed drugs, when compared to the upper gastrointestinal tract (GIT). Oral delivery of both labile and conventional drugs directly to the colon dramatically decreases their chances of degradation by presenting them to a site low in host enzymatic activity, permitting lower dosing and resulting in fewer side-effects. Also, site-specific local delivery may permit the utilization of some drugs which are currently precluded from routine use due to their systemic toxicity [2, 3]. Many formulations have been proposed for achieving such goals based on pH and/or time dependent delivery and systems utilizing colonic bacteria [4-7]. Delivery systems that rely on pH and/or time dependent mechanisms for drug release are less reliable in achieving consistent site-specific delivery in the colon [8, 9]. More reproducible delivery systems are described in the literature exploiting environmental differences in the GIT [10, 11]. The colon has a greater concentration of bacteria than the rest of the GIT, and many of these bacterial species produce glycosidic enzymes which are capable of many metabolic reactions, including fermentation of dietary fibers. Formulations based on colonic microbial enzymes as a trigger mechanism appeared more promising [12-14]. The exploitation of the properties of the colonic bacteria has been extremely successful in the development of prodrugs of 5-aminosalicylic acid [15, 16]. Hence, the aim of this work is to design a multi-particulate system in the form of microcapsules containing the model drug ketoprofen for possible targeting to the colon. The trigger mechanism will be based on bacterial colonic enzymes, and both *in-vitro* and *in-vivo* studies will be performed for the evaluation for this novel dosage form.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this work were of analytical grade, while the high quality grades were reserved for HPLC analysis. The following materials were obtained from the indicated sources: Avicel PH101 (FMC, Belgium), Eudragit® NE 30D (Rohm Gmbh, Germany), gellan gum (Gellrite, Scott Lab., USA), Gamanase™ (Novo Nordisk, Denmark), guar gum (Sigma, USA) and ketoprofen (S.I.M.S, Italy).

2.2. Preparation of colon-specific ketoprofen microcapsules

Ketoprofen pellets of 1-mm in diameter were prepared by the method of extrusion-spheronization, and film coated using pan technology [17, 18]. The coating dispersions were based on a combination of natural polysaccharide such as guar gum and/or gellan gum, in combination with Eudragit NE 30D. Details of the nature of core and coat are given in Table 1.

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Table 1. Percentage composition of ketoprofen products examined in the dog trials.

Product	Core composition			Coat composition			
	Drug	Avicel	End . NE	Guar	Gellan	End . NE	FeO
Control	60%	40%	0 %	---	---	---	---
P2	60%	40%	0 %	9%	9%	72%	10%
P4	60%	35%	5 %	18%	0%	72%	10%

NB: Control = uncoated pellets , Drug = Ketoprofen , End . NE =Eudragit NE , FeO=Iron Oxide.

2.3. *In-vitro* dissolution experiments at variable pH

The rotating basket method was used with a six-station dissolution apparatus (Erweka DT6, Germany) at pH's 1.2, 5.4 and 6.8. To simulate passage to the colon 0.015 g/ml Gamanase™ was added to the media after 4 hr of dissolution. A weigh of pellets equivalent to 100-mg ketoprofen was added to each basket and immersed in the dissolution medium. Samples of 5-mL were withdrawn and replaced by equivalent volumes of pre-heated dissolution media at specific time intervals. The samples were assayed spectrophotometrically at 260 nm (Shimadzu UV-160, Japan), and the data was plotted as time *vs*. percentage drug released.

2.4. Release studies in presence of Cell-Free Extracts

Cell-Free Extract (CFE) was prepared from rat caecal contents according to the method described in the literature [19, 20]. The activity of CFE was compared to Gamanase™ by evaluating their ability to reduce the viscosity of a standard solution of guar gum. Dissolution experiments were performed in 100 mL of phosphate buffer saline (PBS pH 7), with or without CFE (25%) at 37°C in sealed glass bottles which were shaken at 80 rpm. Samples of 1 mL were withdrawn in duplicate and drug concentration was determined by spectrophotometric analysis after dilution. To maintain statistical significance, the mean of 6-experiments was reported.

2.5 *In-Vivo* Study in dogs

Six male Labrador dogs weighing 20.2 to 25.4 kg were fasted from solids 18 hr before dosage administration. Doses of microcapsules equivalent to 3 mg/kg ketoprofen were packed in hard gelatin capsules and were administered to the dogs orally. Blood sampling was carried-out hourly during the first 12 hr and after 24, 27 and 30 hr. A cross-over design was used with a washout period of 7 days.

2.6 Analytical procedure

Plasma was separated by centrifuging blood samples at 5000 rpm for 15 min. Aqueous solution of the internal standard oxyphenbutazone (50 mg/mL) and 1 mL of 1 M HCl were added to the plasma and agitated in a test tube. Ketoprofen and internal standard were then extracted by adding 3 mL of diethyl ether, vortexed and centrifuged for 5 min at 3500 rpm. The clear organic phase was withdrawn and evaporated using nitrogen. The samples were reconstituted using 200 mL of the mobile phase [acetonitrile-phosphate buffer pH 6.8 (9:1 v/v)]. At 1.2 mL/min flow rate of mobile phase, 20 µL samples were injected through a 40 µL injection loop of Shimadzu chromatographic system (LC-5A) equipped with a SPD-2A variable wavelength detector.

2.7 Statistical analysis

Where applicable, student\s t-test was used to evaluate the significance of the generated data.

3. Results and Discussion

3.1 *In-vitro* Results

The dissolution profiles for the two tested products at pH 5.4 are shown in Figure 1. The addition of Gamanase™ enzyme resulted in significant increase in drug release. The increase in dissolution rate was 12% and 11% for P2 and P4 respectively. It should be noted that the pH of 5.4 was selected because it enhances the activity of the external enzyme system Gamanase™.

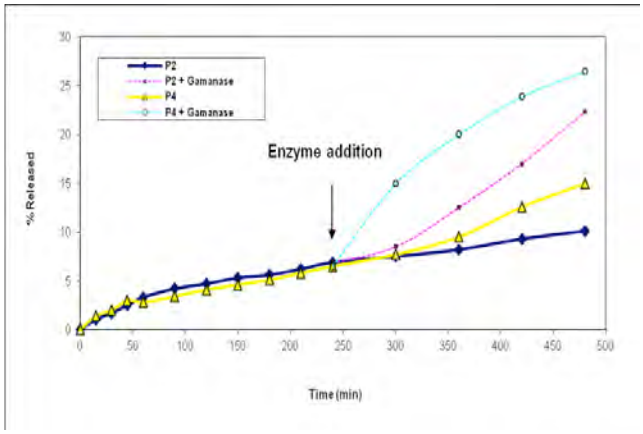

Figure 1. Release of ketoprofen from P2 & P4 at pH 5.4 with or without the addition of 0.015 g/mL Gamanase™ after 4 hr of dissolution.

Based on the positive results from dissolution testing, further experiments to evaluate the release profiles were conducted using extracts from rat-caecal contents. Dissolution testing in presence of CFE was conducted in media of phosphate buffer saline containing 25% rat caecal extract (equivalent to 2.5% caecal content). The two tested products (P2 & P4) showed affinity to enhance ketoprofen release upon the addition of CFE to the medium, while the drug levels in the control experiments were maintained to acceptable level which did not exceeds 25% (Figure 2).

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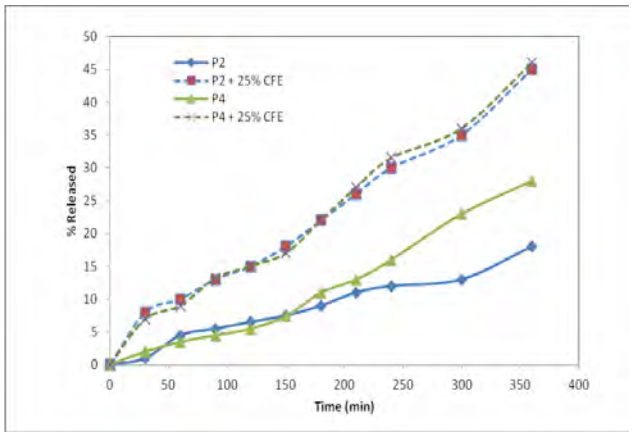

Figure 2. Ketoprofen release from P2 & P4 in PBS pH 7 with or without 25% of cell free extract (CFE).

3.2 *In-Vivo* Results

The mean plasma profiles for the three formulations are shown in Figure 3. The standard deviation for the data obtained at various time points from the dogs ranged between 0.2 and 2.7 ¥ g/mL. The variability of the data could be attributed to the large intra-subject variation in the gastric emptying of dogs. Dogs have been reported to have higher gastric pH values and longer gastric emptying times, compared to humans [21, 22]. Drug-plasma concentrations attained after the administration of uncoated pellets (control) were significantly above the therapeutic levels of ketoprofen for a considerable period of time. Ketoprofen blood levels after the administration of P2 and P4 were mostly within the recommended therapeutic limits for humans of 0.4-6.0 ¥ g/mL. Ketoprofen was detected in plasma after 1 hr following the administration of uncoated pellets. When administering P2 and P4, ketoprofen blood levels indicated a lag period before the start of drug appearing in plasma. The length of such lag time was variable (3-6 hr for P2 and 2-8 hr for P4). With reference to the plasma profiles for each formulation, uncoated pellets data showed a trend of a sharp peak after 3-5 hr, which was followed by steady state period, while variable observations were associated with P2 and P4. Such variability could be attributed to the two main physiological barriers in the GIT: emptying from the stomach and stasis at the ileo-caecal junction (ICJ) [2, 23]. Gastric emptying might be less significant with P2 and P4 for the following reasons: the dogs were in the fasted state which probably implies a shorter residence time in the stomach, the two tested products administered has a very low release rate at gastric pH, residence in the stomach allows the film coating applied to the formulation to reach equilibrium prior to passage to the small intestine, and the small size of the pellets (1-1.3 mm) would allow the preparations to pass through the pyloric sphincter during normal gastric emptying. The more unpredictable barrier is the ICJ. The residence time at the ICJ is both variable and independent of whether the dosage form is a non-disintegrating tablet or a multiparticulate [24, 25]. Stasis at the ICJ allows the regrouping of multiparticulates prior to entry to the colon, which allows the pellets to enter the colon as a bolus [26, 27]. Such event may give rise to a sharp peak or a C_{max} from a slow – release multiparticulate delivery system activated by colonic bacterial anzymes.

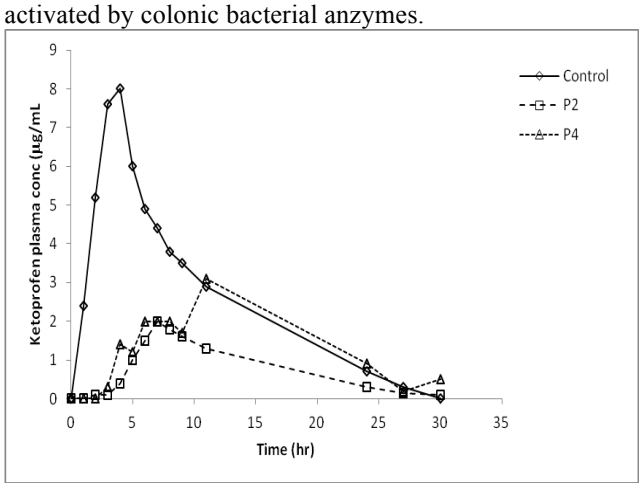

Figure 3. Mean plasma profile of formulations used in the *in- vivo* dog study.

The mean pharmacokinetic parameters for the three preparations, are presented in Table 3. The mean peak concentration of ketoprofen was 8.7 µg/mL for the uncoated pellets observed at 3.5 hr, 2.51 µg/mL for the P2 preparation observed at 7.8 hr, and 4.55 µg/mL for P4 observed at 8.5 hr. Both P2 and P4 exhibited a lag time during which no drug can be detected in samples. The *in-vitro* dissolution profiles of ketoprofen for P2 and P4 in simulated gastric juices showed negligible drug release over extended periods of time, which may imply that the lag time observed may be related to dog gastric emptying and dosage form transport along the upper GIT. Once the drug starts to appear in the plasma, it increases in a regular manner. It is clearly that extended ketoprofen release was achieved from P2 and P4 for over 20 hr. The values of T_{max} for P2 and P4 of 7.8 and 8.5 hr respectively, may imply that drug absorption was boosted in a certain region of the GIT, most likely the colon. Ketoprofen was reported to show appreciable colonic absorption if extended release form was achieved [28].

The statistical analysis of the obtained AUC_{0-t} values using uncoated pellets as a reference, revealed that P2 bioavailability was significantly lower than that of control ($P < 0.05$), while P4 was similar ($P > 0.05$). This would imply that despite the lag – time observed from P4, the product maintained the bioavailability of ketoprofen to an acceptable level. The low bioavailability of P2 could have been caused by loss of unabsorbed drug in feces. The degree of fluctuation at steady state parameter (Fl) was used to compare the sustained – release properties of the three formulations. The Fl of a controlled – release preparation should be similar to or less than that for the immediate release form. This Fl index is analogous to the coefficient of variation; small values are desired for the prolonged – release preparations. The index was 0.091 and 0.087 for P2 and P4 respectively, and 0.114 for uncoated pellets. The Fl index suggests that the uncoated

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pellets produces about 25 and 31 % more fluctuation in ketoprofen serum levels than P2 and P4 respectively.

The statistical moment theory offers an attractive alternative for the evaluation of absorption data. Non-compartmental methods for estimating the rate of absorption of a drug after administration are based on differences in mean residence time (MRT) after different modes of administration. The MRT concept may also be useful for comparing the absorption characteristics of a drug from different formulations [29]. The parameters given in Table 3 indicate that the MRT of P2 and P4 are significantly higher than that of uncoated pellets. The difference between the MRT values of the control and products, MAT or MDT, is a representation to the mean absorption time of ketoprofen from each formulation. MDT for P4 was significantly higher than that of P2, which implies that the absorption of ketoprofen from P4 continued over a longer period of time. The $t_{1/2}$ of the two tested products was significantly longer than the control. The half – life of ketoprofen was increased from 5.1 hr (control) to 11.6 and 15.1 hr from P2 and P4 respectively.

Table 3. Mean pharmacokinetic parameters of ketoprofen following oral administration of uncoated pellets, P2 and P4 to dogs.

Parameter	Uncoated pellets	P2	P4
L_t (hr)	1	3	2
C_{max} (µg / mL)	8.7	2.51	4.55
C_{min} (µg / mL)	0.65	0.5	0.74
T_{max} (hr)	3.5	7.8	8.5
T_e (hr)	22.33	14.2	17
AUC_{o-t} (µg / hr)	70.63	22.11	43.60
F_{rel}	1	0.31	0.62
Fl	0.114	0.091	0.087
$t_{1/2}$ (hr)	5.1	11.6	15.1
$AUMC_{o-t}$ (µg / hr)	536.2	244.35	559.2
MRT (hr)	7.6	11.1	12.83
MDT (hr)	---	3.5	5.23
K_a (µg / hr)	---	0.29	0.20

NB: L_t = lag-time before drug appearance in the plasma, C_{max} = maximum plasma concentration, C_{min} = minimum plasma concentration, T_{max} = time to maximum plasma concentration, T_e = length of plasma levels exceed the minimum therapeutic concentration, AUC_{o-t} = area under plasma concentration-time curve, F_{rel} = relative bioavailability compared to uncoated pellets, Fl = degree of fluctuation at steady state $[(C_{max} - C_{min})/ C_{av}]$, where C_{av} is estimated from the ratio of AUC_{o-t} to dosing interval, $AUMC_{o-t}$ = area under the first moment curve (area under the drug concentration-time versus time plot), MRT = mean residence time ($AUMC_{o-t}$ / AUC_{o-t}), MDT = mean dissolution time ($MRT_{test} - MRT_{control}$), K_a = first order absorption rate constant (1/MDT), $t_{1/2}$ = absorption half-life (0.693*MDT).

4. CONCLUSIONS

The ketoprofen formulations prepared in this work may be useful in the treatment of inflammatory bowel diseases which are normally treated with NSAIDs, or in the control of arthritic conditions which are susceptible to diurnal rhythms. A dose given at bed time might provide the required delay to allow systemic absorption at a time commensurate with the morning stiffness in arthritic patients. Irrespective of any colonic specificity, the newly developed products have potential for one-a-day delivery, maintaining therapeutic levels of ketoprofen over extended periods with minimal side-effects associated with reduced plasma spikes. The technique used in the preparation of such dosage forms can be considered "universal" and be applied for the delivery of other classes of drugs such as steroidal or non-steroidal anti-inflammatory drugs, and anticancer agents which are used in the treatment of local colonic tumors. Although the tested products showed promising *in-vitro / in-vivo* result , confirmation by the use of radiographic images to determine the various retention times of the dosage from across the GIT and by testing in humans is required preferably with diseased states amenable to such therapy [30].

REFERENCES

1. Thakur B, Pandit V, Ashawat MS, Kumar P. Natural and Synthetic Polymers for Colon Targeted Drug Delivery. Asian Journal of Pharmacy and Technology. 2016;6(1):35-44.
2. Ashford M, Fell J. Targeting drugs to the colon: delivery systems for oral administration. journal of drug targeting. 1994 Jan 1;2(3):241-57.
3. Chourasia MK, Jain SK. Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharm Sci. 2003 Jan 1;6(1):33-66.
4. Pozzi F, Furlani P, Gazzaniga A, Davis SS, Wilding IR. The time clock system: a new oral dosage form for fast and complete release of drug after a predetermined lag time. Journal of controlled release. 1994 Aug 1;31(1):99-108.
5. Jain A, Gupta Y, Jain SK. Perspectives of biodegradable natural polysaccharides for site-specific drug delivery to the colon. J Pharm Pharm Sci. 2007 Apr;10(1):86-128.
6. Gidwani B, Vyas A, Ahirwar K, Shukla SS, Pandey R, Kaur CD. Factorial Design and a Practical Approach for Gastro-Retentive Drug Delivery System. Research Journal of Pharmacy and Technology. 2016 Jun 20;9(6).
7. Oliveira GF, Ferrari PC, Carvalho LQ, Evangelista RC. Chitosan–pectin multiparticulate systems associated with enteric polymers for colonic drug delivery. Carbohydrate polymers. 2010 Oct 15;82(3):1004-9.
8. Watts PJ, Lllum L. Colonic drug delivery. Drug development and industrial pharmacy. 1997 Jan 1;23(9):893-913.
9. Akhgari A, Heshmati Z, Garekani HA, Sadeghi F, Sabbagh A, Makhmalzadeh BS, Nokhodchi A. Indomethacin Electrospun Nanofibers for Colonic Drug Delivery: In vitro dissolution studies. Colloids and Surfaces B: Biointerfaces. 2016 Dec 23.

Corresponding author: idrispharmacy@yahoo.com, P,O.Box 5341, Tel: 00218913826640.

10. Krishnaiah YS, Satyanarayana S, Prasad YR, Rao SN. Gamma scintigraphic studies on guar gum matrix tablets for colonic drug delivery in healthy human volunteers. Journal of controlled release. 1998 Nov 13;55(2):245-52.
11. Maestrelli F, Cirri M, Corti G, Mennini N, Mura P. Development of enteric-coated calcium pectinate microspheres intended for colonic drug delivery. European journal of pharmaceutics and biopharmaceutics. 2008 Jun 30;69(2):508-18.
12. Sinha VR, Kumria R. Polysaccharides in colon-specific drug delivery. International journal of pharmaceutics. 2001 Aug 14;224(1):19-38.
13. Liu L, Fishman ML, Kost J, Hicks KB. Pectin-based systems for colon-specific drug delivery via oral route. Biomaterials. 2003 Aug 31;24(19):3333-43.
14. Shah N, Shah T, Amin A. Polysaccharides: a targeting strategy for colonic drug delivery. Expert opinion on drug delivery. 2011 Jun 1;8(6):779-96.
15. Klotz U. Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-aminosalicylic acid. Clinical pharmacokinetics. 1985 Apr 1;10(4):285-302.
16. Sinha VR, Kumria R. Binders for colon specific drug delivery: an in vitro evaluation. International journal of pharmaceutics. 2002 Dec 5;249(1):23-31.
17. El-Mahdi IM, Deasy PB. Tableting of coated ketoprofen pellets. Journal of microencapsulation. 2000 Jan 1;17(2):133-44.
18. El-Mahdi IM, El-Shehebia TA. Three-Dimensional Presentations of Factorial Experimentation Data of Extrusion–Spheronization. Lebda Medical Journal. 2016 Oct 26;2(1).
19. Brøndsted H, Kopeček J. Hydrogels for site-specific drug delivery to the colon: in vitro and in vivo degradation. Pharmaceutical research. 1992 Dec 1;9(12):1540-5.
20. Sinha VR, Mittal BR, Bhutani KK, Kumria R. Colonic drug delivery of 5-fluorouracil: an in vitro evaluation. International Journal of Pharmaceutics. 2004 Jan 9;269(1):101-8.
21. Akimoto M, Furuya A, Nakamura M, Maki T, Yamada K, Suwa T, Ogata H. Release and absorption characteristics of chlorphenesin carbamate sustained-release formulations: In vitro-in vivo and in vivo dog-human correlations. International journal of pharmaceutics. 1995 Apr 4;117(1):31-9.
22. Lui CY, Amidon GL, Berardi RR, Fleisher D, Youngberg C, Dressman JB. Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans. Journal of pharmaceutical sciences. 1986 Mar 1;75(3):271-4.
23. Dar MJ, Ali H, Khan A, Khan GM. Polymer based drug delivery: The quest for local targeting of inflamed intestinal mucosa. Journal of Drug Targeting. 2017 Feb 23(just-accepted):1-44.
24. Sugito K, Ogata H, Goto H, Noguchi M, Kogure T, Takano M, Maruyama Y, Sasaki Y. Gastrointestinal transit of non-disintegrating solid formulations in humans. International Journal of Pharmaceutics. 1990 Apr 30;60(2):89-97.
25. Yang L, Chu JS, Fix JA. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. International Journal of Pharmaceutics. 2002 Mar 20;235(1):1-5.
26. Khosla R, Davis SS. Gastric emptying and small and large bowel transit of non-disintegrating tablets in fasted subjects. International journal of pharmaceutics. 1989 May 15;52(1):1-0.
27. Asghar LF, Chandran S. Multiparticulate formulation approach to colon specific drug delivery: current perspectives. J Pharm Pharm Sci. 2006 Sep 1;9(3):327-38.
28. Deasy PB. Microencapsulation of drugs by pan and air suspension techniques. Critical reviews in therapeutic drug carrier systems. 1990 Dec;8(1):39-89.
29. Gibaldi, M., (1991). Biopharmaceutics and Clinical pharmacokinetics, 4 th Ed ., Lea & Febiger , USA
30. Ibekwe VC, Liu F, Fadda HM, Khela MK, Evans DF, Parsons GE, Basit AW. An investigation into the in vivo performance variability of pH responsive polymers for ileo- colonic drug delivery using gamma scintigraphy in humans. Journal of pharmaceutical sciences. 2006 Dec 1;95(12):2760-6.

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