Formulation Development & Pharmacokinetic Laboratory, Pharmacy Group, Birla Institute of Technology & Science, Pilani, India

Design and evaluation of pH modulated controlled release matrix systems for colon specific delivery of indomethacin

L. F. A. Asghar, S. Chandran

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Dr. Sajeev Chandran, Formulation Development &Pharmacokinetic Laboratory, Pharmacy Group, Birla Institute of Technology & Science, Pilani- 333 031, Rajasthan, India
sajeev@bits-pilani.ac.in

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Indomethacin, a potent non steroidal anti-inflammatory drug (NSAID), is indicated for the local treatment of colorectal carcinoma. The aim of the present study was to design and investigate various matrix systems for controlled and site specific delivery of indomethacin to the colon. Various pH sensitive and hydrophobic polymers were investigated for their effect on drug release and site specificity. Effect of proportion of Eudragit L100 and Eudragit S100 in matrix either alone or in combination was evaluated. Effect of hydrophobic non-swellable polymer ethyl cellulose on the release pattern of drug from the Eudragit bases was also investigated. Matrix tablets prepared with Eudragit showed pH dependent release profile with the formulations of Eudragit L100 showing faster rate of drug release than Eudragit S100 in alkaline pH. The release profile from matrix tablets containing Eudragit L100 and Eudragit S100 in combination or with ethyl cellulose correlated well with the relative proportion of the two polymer types in the matrix base. Selected formulations when evaluated in simulated gastric fluid pH without enzymes showed negligible to low drug release (less than 10%) in the first 4–6 h followed with controlled release for 14–16 h. It was concluded that pH sensitive matrix bases in combination with a hydrophobic polymer like ethyl cellulose can be ideal for site specific delivery of drugs to colon with controlled release profile.

1. Introduction

Colon targeted drug delivery systems for local action have been attempted in case of inflammatory bowel diseases (ulcerative colitis and Crohn's disease) and colorectal cancer (Patel et al. 2007). Site specific delivery of drugs to the colon is also advantageous for the systemic absorption of protein and peptide drugs that are susceptible to degradation in the upper portion of gastrointestinal (GI) tract (Sinha et al. 2007). The pH of GI tract gradually increases from the esophageal end to the rectal end. It varies from around 1.5–3.0 in the stomach to around 7.0–8.0 in the terminal ileum. It has been reported that the early colonic region has a pH range of 5.6–7.0 (Evans et al. 1988). As the pH in the terminal ileum and colon is higher than in any other earlier region of the GI tract, the dosage forms which preferentially release the drug at pH 6.0–7.5 have the potential for site-specific delivery of drugs into the colon (Rodriguez et al. 1998).

Several approaches have been reported for achieving site specific drug release in colon like prodrugs, pH and enzyme controlled systems (Chourasia and Jain 2003). One of the most employed approach for colonic delivery is coating the drug delivery system with pH sensitive polymers (Ashford et al. 1993). The pH sensitive polymers for colonic delivery are designed to be solubilized at a pH around 7.0 so as to exploit the increase of pH in the large intestine. However, as the pH of the colon drops from 7.0 in the terminal ileum to 6.5 in the ascending colon (due to the fermentation of undigested food by colonic bacteria leading to the formation of organic acids which lower the pH), it is possible that coatings which dissolve at pH 7.0 would release the active agent in the ileum rather than the colon. On the other hand, if the coating is too thick or non-uniform, there is a possibility that no drug will be released in the colon (Leopold 1999). Hence, matrix embedding approach where the formulation core (drug embedded in a polymeric matrix) controls the release behavior wherein negligible to low amount of drug is released up to proximal colon ($pH < 7.0$) offers far better advantage in terms of colon specific action than coated systems. The drug is then released in a controlled fashion during its passage though the distal ileum and the colon.

Amongst all the NSAIDs indomethacin is reported to have the most potent antineoplastic activity against *in vitro* models of colorectal cancer and is also reported to prevent colon cancer (Hull et al. 2003). Therefore, a colon targeted formulation of indomethacin would release the drug in the colon for local action and reduce the incidence of adverse effects due to its systemic absorption. A literature survey has revealed a few colon specific formulations of indomethacin. These formulation approaches include: use of pH sensitive polymers (Eudragit L100 and Eudragit

S100) for coating drug loaded pellets (Akhgari et al. 2005); compression coating of tablets using either guar gum (Krishnaiah et al. 1998); or pectin and chitosan mixtures (Fernandez and Fell 1998); guar gum based matrix tablets (Sinha et al. 2002) and more recently drug embedded in HPMC/pectin/calcium chloride matrix tablets (Wu et al. 2007).

pH sensitive polymers like Eudragit L100 (EL100) and Eudragit S100 (ES100) have been conventionally employed for coating tablet and other formulations intended for colonic delivery (Khan et al. 1999; Ibekwe 2004). EL100 dissolves at pH 6.0 while ES100 dissolves at pH 7.0. The high pH solubility of these polymers is the basis of drug release in the relatively alkaline environment of the distal ileum and the colon. Ethyl cellulose (EC) is an inert, hydrophobic polymer and has been extensively used as a retardant polymer for controlled release of a variety of drugs (Rekhi et al. 1995; Saha et al. 2000; Sajeev and Saha 2001; Saha et al. 2004).

Sustained release tablets of 5-amino salicylic acid prepared with Eudragit S100 using hot melt extrusion technique has been reported for colonic delivery (Diane et al. 2005). However, the potential of utilizing pH sensitive polymers in matrix embedded formulations for colonic delivery has not been explored yet. In the present study, various pH responsive matrix systems were designed and evaluated for colon specific controlled delivery of indomethacin. The primary objective of this study was to investigate the effect of polymer type in the matrix base on drug release behavior and colon specificity. It was also envisaged to study the effect of pH sensitive polymers in combination on controlled release pattern. Effect of hydrophobic non-swellable polymer EC on the release pattern of drug from pH responsive matrix bases was also investigated. Effect of simulated GI fluid pH without enzymes (0–2 h pH 1.2; 2–4 h pH 4.5; 4–14 h pH 7.4) on drug release from designed formulations was also evaluated as were batch reproducibility and effect of storage on drug stability and release kinetics.

2. Investigations, results and discussion

The given drug sample of indomethacin was found to comply with the various official tests and specifications for standard as per Indian Pharmacopoeia 1996. The formulation additives (in the concentrations used) did not affect the stability and UV absorbency profile of the drug. Composition of designed indomethacin formulations containing varying proportions (at 25% w/w and 50% w/w of drug) of EL 100 and ES 100 either alone and in combination are presented in Table 1 and 2 respectively. Composition of matrix embedded formulations of EL100 and ES100 (both at 25% w/w and 50% w/w of drug) with EC in the ratio 3 : 2 and 4 : 1 are listed in Table 3. The prepared tablets from all the batches were found to be of good quality with acceptable physical characteristics (Table 1 to 3). The low value of weight variation, optimal hardness and friability, and high degree of drug content uniformity suggest that wet granulation is an acceptable method of manufacturing matrix embedded formulations of indomethacin. Since the drug is poorly soluble (< 3.5 μg/ml) in gastric pH, dissolution was carried out in distilled water for the first two hours followed with phosphate buffer (pH 7.4) for the remaining period of study. This medium was considered the most suitable as the drug was freely soluble at this pH and it also mimics the alkaline environment of small intestine and colon. For some formulations, release rate studies were also done in simulated GI fluid pH without enzymes (Table 4).

For an ideal colon targeted drug delivery system, the drug release should be prevented in the stomach and small intestine (residence time of 3 h) (Ibekwe et al. 2004). Release of drugs must be completed within the residence time in the colon of about 12 h. Therefore, a 14 to 18 h

Table 1: Composition and physical properties of tablet formulations containing Eudragit L100 or S100 alone

Formula	IEL(0.25)	IEL(0.5)	IES(0.25)	IES(0.5)
Components[a]				
Indomethacin	50 mg	50 mg	50 mg	50 mg
EL100	25%	50%	–	–
ES100	–		25%	50%
Physical properties				
Drug content (mg/tab)[b]	99 ± 0.5	100.3 ± 0.5	98.6 ± 0.7	98.5 ± 1.0
Weight variation (%) [c]	± 3.0	± 3.0	± 2.8	± 2.9
Hardness (Kg/cm^2)[d]	2.5 ± 0.2	2.2 ± 0.4	2.4 ± 0.5	2.2 ± 0.4
Friability (%)[e]	< 0.5%	< 0.6%	< 0.1%	< 0.2%

[a] Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives. [b] % w/w of the drug content. [c] ±max % variation. [d] mean of triplicate with s.d. [e] mean of 20 tablet

Table 2: Composition and physical properties of tablet formulations containing Eudragit L100 and S100 in combination

Formula	IEL1ES1(0.25)	IEL1ES1(0.5)	IEL3ES2(0.25)	IEL3ES2(0.5)	IEL2ES3(0.25)	IEL2ES3(0.5)
Components[a]						
Indomethacin	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg
EL100	12.5%	25%	15%	30%	15%	30%
ES100	12.5%	25%	10%	20%	10%	20%
Physical properties						
Drug content (mg/tab) [b]	99.5 ± 0.4	99.2 ± 0.5	100 ± 0.5	100.1 ± 0.5	100.3 ± 0.5	99.6 ± 0.7
Weight variation (%) [c]	± 4.0	± 4.0	± 3.8	± 4.1	± 3.0	± 0.8
Hardness (Kg/cm^2) [d]	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	3.0 ± 0.2	2.2 ± 0.4	2.1 ± 0.5
Friability (%)[e]	< 0.5%	< 0.4%	< 0.3%	< 0.5%	< 0.6%	< 0.1%

[a] Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives. [b] % w/w of the drug content. [c] ±max % variation. [d] mean of triplicate with s.d. [e] mean of 20 tablet

Table 3: Composition and physical properties of tablet formulations containing Eudragit L100/S100 with ethylcellulose

Formula	IEL3EC2(0.25)	IEL3EC2(0.5)	IEL4EC1(0.25)	IEL4EC1(0.5)	IES3EC2(0.25)	IES3EC2(0.5)	IES4EC1(0.25)	IES4EC1(0.5)
Components[a]								
Indomethacin	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg
EL100	15%	30%	20%	40%	–	–	–	–
ES100	–	–	–	–	15%	30%	20%	40%
EC	10%	20%	5%	10%	10%	20%	5%	10%
Physical Properties								
Drug content[b] (mg/tab)	98 ± 0.5	99.3 ± 0.5	98.5 ± 0.7	98.5 ± 1.0	99.5 ± 0.4	99.2 ± 0.5	101 ± 0.5	98.1 ± 0.5
Weight variation[c] (%)	± 2.0	± 3.2	± 2.8	± 2.7	± 4.5	± 4.9	± 3.9	± 4.6
Hardness[d] (Kg/cm^2)	2.7 ± 0.2	2.4 ± 0.4	2.3 ± 0.5	2.4 ± 0.4	2.1 ± 0.2	2.2 ± 0.2	3.1 ± 0.2	3.0 ± 0.2
Friability[e] (%)	$< 0.6\%$	$< 0.7\%$	$< 0.2\%$	$< 0.1\%$	$< 0.8\%$	$< 0.3\%$	$< 0.3\%$	$< 0.5\%$

[a] Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives. [b] % w/w of the drug content. [c] ±max. % variation. [d] mean of triplicate with s.d. [e] mean of 20 tablet

Table 4: Release kinetics data from different plots for selected formulations in simulated GI fluid pH (without enzymes)

Formula	Release kinetics parameters				
	r[a]	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]
IEL4EC1(0.25)	0.9814	0.002	2.42	4.9	13.3
IES4EC1(0.25)	0.9991	0.002	1.92	8.2	25.5
IEL3ES2(0.25)	0.9811	0.048	1.09	3.6	15.3
IEL2ES3(0.25)	0.9881	0.014	1.59	3.8	14.3

[a] Correlation coefficient. [b] Release rate constant. [c] Diffusional exponent indicative of the release mechanism. [d] Time for 10% of the drug release (h). [e] Time for 90% of the drug release (h)

extended release formulation with an initial lag time of about 4–6 h is usually considered suitable for colon targeting. Further, this time lag would ensure the passage of the formulation intact though to the distal ileum or proximal colon without appreciable drug loss.

2.1. Effect of polymer type and proportion

The release profiles from matrix tablets containing Eudragit L100 and S100 at total polymer proportion of 25% w/w of drug, i.e., IEL(0.25) and IES(0.25) respectively, are shown in Fig 1a. The release profile from the corresponding 50% w/w polymeric matrix [IEL(0.5) and IES(0.5)] are shown in Fig 1b. Between EL100 and ES100 comparative higher retardation was obtained in the case of ES100. This can be attributed to the fact that EL100 dissolves at a pH less than 7.0 resulting in higher swelling and erosion in phosphate buffer pH 7.4 (Mehta et al. 2001). Increasing the polymer proportion from 25% w/w to 50% w/w considerably retarded the overall rate of drug release from the matrix. The release kinetics were found to follow super case-II release (Ritger and Peppas 1987) indicating rate of release increased with time as the rate of polymer dissolution increases in higher pH (7.4).

The calculated $t_{10\%}$ values (Table 5) for the two formulations [IEL(0.25): 2.1 h, IES(0.25): 3.5 h] show that better retardation in drug release in the initial period was obtained in case of IES(0.25) as compared to IEL(0.25). A statistically significant difference ($p < 0.05$) was obtained when paired t-test for means was done for the dissolution profiles of IEL(0.25) and IES(0.25). But doubling the polymer proportion in the matrix to 50% w/w did not retard the initial release significantly with calculated $t_{10\%}$ of 2.8 h and 3.7 h respectively for IEL(0.5) and IES(0.5). The $t_{90\%}$ values for these formulations varied between 11.2 h for IEL(0.25) (fastest release) to 22.6 h for IES(0.5) (slowest release). Thus, it may be concluded that except for IES (0.5), all the other formulations, IEL(0.25), IES(0.25) and IEL(0.5) showed 90% drug release within 12–19 h and can serve as controlled release matrices for colon specific drug delivery.

2.2. Effect of polymer combination

The release profiles from the matrix tablets containing both EL100 and ES100 in relative ratios of 3 : 2, 1 : 1,

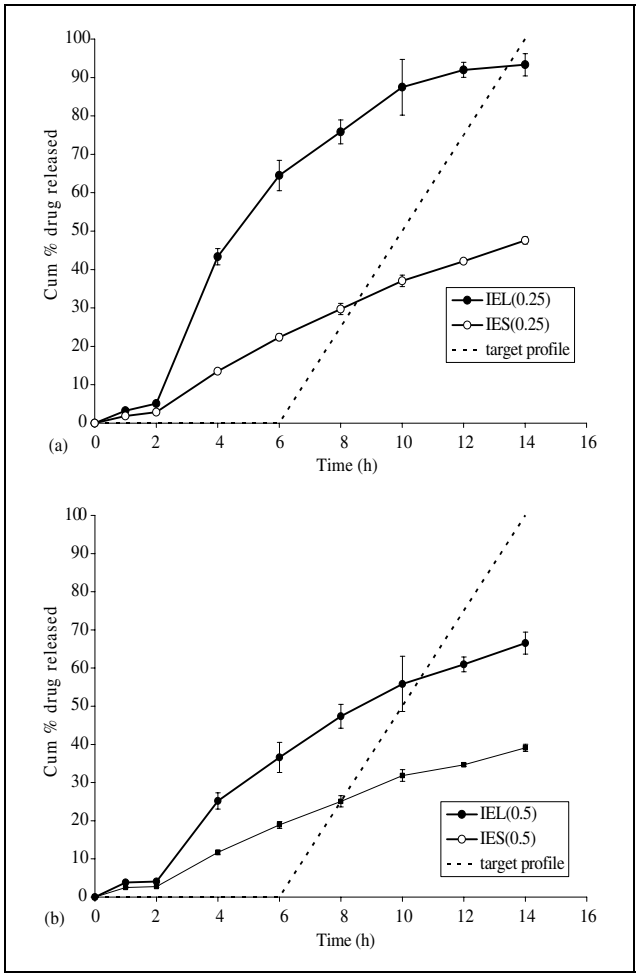

Fig. 1: Release profile of indomethacin from EL100 or ES100 matrix tablets at total polymer proportion of (a) 25% w/w of drug and (b) 50% w/w of drug. Each data point represents the average of two dissolution trials done in duplicate with standard deviation

Table 5: Release kinetics data from different plots for EL100/ ES100/EC matrix tablets

Formula	Release kinetics parameters				
	r[a]	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]
IEL(0.25)	0.9130	0.520	1.39	2.1	11.2
IES(0.25)	0.9750	0.014	1.39	3.5	19.0
IEL(0.5)	0.9480	0.110	1.32	2.8	17.3
IES(0.5)	0.9760	0.010	1.35	3.7	22.6
IEL3ES2(0.25)	0.9050	1.324	1.66	2.8	7.5
IEL1ES1(0.25)	0.9531	0.144	1.96	2.9	14.3
IEL2ES3(0.25)	0.9800	0.151	1.67	2.5	13.8
IEL3ES2(0.5)	0.9921	0.320	1.70	2.3	9.9
IEL1ES1(0.5)	0.9360	0.123	1.93	2.6	15.4
IEL2ES3(0.5)	0.9836	1.541	1.62	2.2	15.7
IEL3EC2(0.25)	0.9921	0.006	1.70	2.9	16.5
IEL4EC1(0.25)	0.9140	0.026	1.57	2.3	7.6
IEL3EC2(0.5)	0.9995	0.025	1.26	4.1	19.0
IEL4EC1(0.5)	0.9690	0.010	1.74	3.0	17.5
IES3EC2(0.25)	0.9976	0.029	1.05	3.8	26.5
IES4EC1(0.25)	0.9980	0.170	1.50	3.4	14.8
IES3EC2(0.5)	0.9976	0.030	1.05	4.2	28.5
IES4EC1(0.5)	0.9850	0.160	1.30	3.4	22.6

[a] Correlation coefficient. [b] Release rate constant. [c] Diffusional exponent indicative of the release mechanism. [d] Time for 10% of the drug release (h). [e] Time for 90% of the drug release (h)

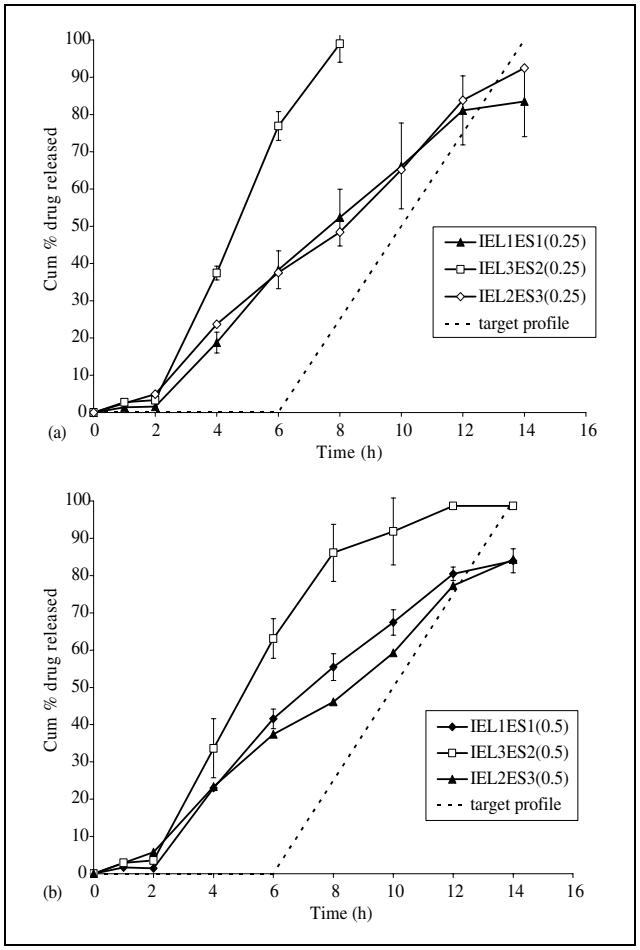

Fig. 2: Release profile of matrix tablets of indomethacin containing combination of EL100 and ES100 in varying ratios at total polymer proportion of (a) 25% w/w of drug and (b) 50% w/w of drug. Each data point represents the average of two dissolution trials done in duplicate with standard deviation

2 : 3 respectively, i.e., IEL3ES2(0.25), IEL1ES1(0.25), IEL2ES3(0.25), at total polymer proportion of 25% w/w of drug, are shown in Fig. 2a. An increase in the proportion of ES100 (relative to EL100) indicated no significant difference in the initial release parameters but affected the overall release kinetics. The $t_{10\%}$ value was found to be 2.8, 2.9 and 2.5 h, respectively for these formulations. The duration of drug release was extended from 8 h for IEL3ES2(0.25) to 15 h for IEL1ES1(0.25) with increase in relative proportion of ES100. Thus, retardation in indomethacin release from these matrices was found to depend on the relative proportion of ES100 in the polymer matrix. However, there was no significant difference ($p < 0.05$) between the two formulations IEL1ES1(0.25) and IEL2ES3(0.25) with respect to their release behavior.

When these matrices were compared at similar relative polymer ratios at total polymer proportion of 50% w/w of drug, i.e., IEL3ES2(0.5), IEL1ES1(0.5), and IEL2ES3(0.5) release profiles that were quite similar to those obtained in case of 25% w/w of the drug were observed (Fig. 2b). When the dissolution data of the different formulations at polymer proportions of 25% w/w and 50% w/w of drug was compared using one way ANOVA for means, the difference was statistically insignificant ($p < 0.05$, F_{Calc} less than F_{Crit}). The calculated $t_{10\%}$ and the $t_{90\%}$ values (Table 5) are indicative of this. The results indicated no advantage upon increasing the total polymer content in the matrix. Since the 'n' values obtained (Table 5) for these series of formulations were in the range of 1.62 to 1.96, it can be concluded that erosion of the Eudragit matrix in alkaline pH was the primary mechanism of drug release.

2.3. Effect of ethyl cellulose in polymeric matrix

Effect of EC in EL100 or ES100 matrix was studied at both 25% and 50% w/w level of the polymer. In these formulations the Eudragit to EC ratio was varied as 3 : 2 or 4 : 1. Incorporation of EC in the matrix retarded the release rate when compared to EL100 alone or ES100 alone at both 25% and 50% w/w levels (Fig. 1a and b). The release profiles from the matrix tablets containing EL100 and EC in the ratio 3 : 2 or 4 : 1 at total polymer proportion of 25% w/w of drug, i.e., IEL3EC2(0.25) and IEL4EC1(0.25) are shown in Fig. 3a and corresponding to 50% w/w of the drug [IEL3EC2(0.5) and IE4EC1 (0.5)] are shown in Fig. 3b. It was observed that increasing the relative proportion of EC from 20% as in the case of [IEL4EC1(0.25) or IEL4EC1(0.5)] to 40% [IEL3EC2(0.25) or IEL3EC2(0.5)] retarded the initial release and also extended the total duration of release (Fig. 3a and b; Table 5). The $t_{10\%}$ increased from 2.3 h for IEL4EC1(0.25) to 2.9 h for IEL3EC2(0.25) while $t_{90\%}$ increased significantly from 7.6 h for IEL4EC1(0.25) to 16.5 h for IEL3EC2(0.25).

In case of IEL4EC1(0.5) and IEL3EC2(0.5), with total polymer proportion of 50% w/w of drug, the $t_{10\%}$ increased from 3.0 h for IEL4EC1(0.5) to 4.1 h for IEL3EC2(0.5) and $t_{90\%}$ increased from 17.5 h for IEL4EC1(0.5) to 19.0 h for IEL3EC2(0.5). The increase in total polymer content in these formulations probably resulted in the formation of a tight non-porous matrix allowing for very slow penetration of external media and slower release rates.

The release profiles from the matrix tablets comprising of ES100 and EC (3 : 2 and 4 : 1 ratios) at total polymer proportion of 25% and 50% w/w of drug, are shown in Fig. 4a and 4b respectively. The formulations IES3EC2(0.25) and

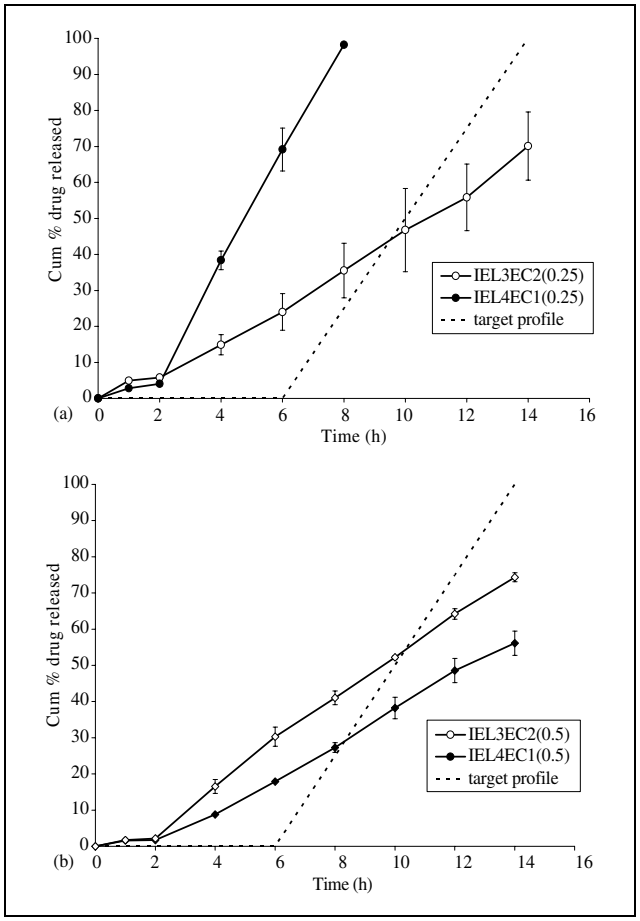

Fig. 3: Release profile of matrix tablets of indomethacin containing combination of EL100 and EC in varying ratios at total polymer proportion of (a) 25 %w/w of drug and (b) 50% w/w of drug. Each data point represents the average of two dissolution trials done in duplicate with standard deviation

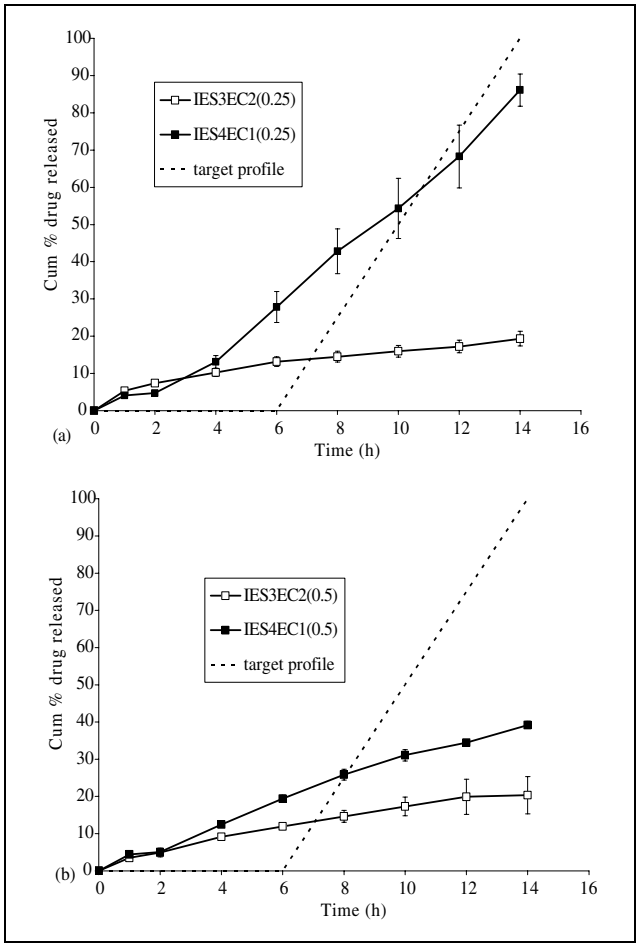

Fig. 4: Release profile of matrix tablets of indomethacin containing combination of ES100 and EC in varying ratios at total polymer proportion of (a) 25% w/w of drug and (b) 50% w/w of drug. Each data point represents the average of two dissolution trials done in duplicate with standard deviation

IES4EC1(0.25) were marginally different in terms of initial release as is evident from the $t_{10\%}$ values (Table 5) for the two formulations (3.8 h and 3.4 h respectively). At the same time, the retarding effect of EC seems to be more pronounced in the former case as may be observed in the $t_{90\%}$ values [26.5 h for IES3EC2(0.25) and 14.8 h for IES4EC1(0.25)]. Since the release extended beyond 26.5 h (much higher than the targeted value of 14–16 h) for IES3EC2(0.25), this formulation was not considered a suitable one.

From the release profiles of matrices at polymer proportion of 50% w/w of drug in similar relative ratios, i.e., IES3EC2(0.5) and IES4EC1(0.5), the calculated $t_{10\%}$ values of 4.2 h for IES3EC2(0.5) and 3.4 h for IES4EC1(0.5) indicating good retardation in the initial release (Table 5). A statistically significant difference ($p < 0.05$) was obtained when paired t- test for means was done for the dissolution profiles of IES3EC2(0.5) and IES4EC1(0.5). However, the $t_{90\%}$ values for the two formulations (IES3EC2(0.5): 28.5 h, IES4EC1(0.5): 22.6 h) showed a considerable deviation from the theoretical target release.

The release mechanism from EL100 and ES100 matrices in combination with EC was found again to be super case II release indicating swelling followed with erosion of the polymer at higher pH as the primary mechanism of drug release. Also, ES100 matrices retarded the over all release better than EL100 matrices as ES100 (due to lower percentage of methacrylic acid units) dissolves at relatively higher pH than EL100.

2.4. Effect of simulated GI fluid pH (without enzymes) on release kinetics

During the course of gastrointestinal transit, drug may be exposed to various pH conditions ranging from 1.2 in the stomach to 7.0 in the intestine. Therefore, selected formu-

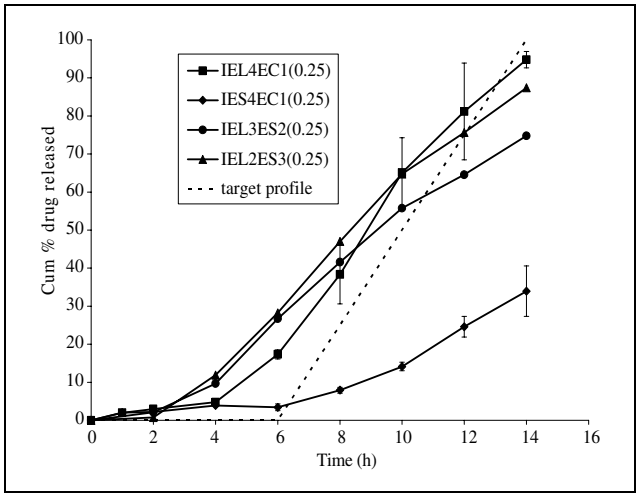

Fig. 5: Release profile of indomethacin from selected formulations in simulated GI fluid pH (without enzymes). Each data point represents the average of two dissolution trials done in duplicate with standard deviation

lations were taken for release studies in changing pH. The release kinetics were calculated from zero to 4th h and then from 4th h onwards until the end of the study. The release profiles for IEL4EC1(0.25), and IES4EC1(0.25) are shown in Fig 5. The calculated $t_{10\%}$ values (Table 4) for the two formulations (IEL4EC1(0.25): 4.9 h, IES4EC1(0.25): 8.2 h) shows a significant difference in the initial release behavior of the drug from the two matrices ($p < 0.05$). This also indicates the influence of a gradient pH change on the release rate and rate kinetics of drug from different matrices. A lag time of about 8.2 h as observed in the latter case may be beneficial in certain cases when GI transit times are very high or targeting to the remotely terminal part of the colon is desired. However, the calculated $t_{90\%}$ values for the two formulations (IEL4EC1(0.25): 13.3 h, IES4EC1(0.25): 26.5 h) indicated an unacceptable slow release rate for IES4EC1(0.25). Therefore, it was concluded that IEL4EC1(0.25) was the better formulation of the two in this case. Amongst the other two formulations studied, i.e., IEL3ES2(0.25) and IEL2ES3(0.25), it was observed that both formulations showed similar release rate behavior as evident from the $t_{10\%}$ values, calculated based on first 4 h release data, (IEL3ES2(0.25): 3.6 h and IEL2ES3(0.25): 3.8 h) and $t_{90\%}$, calculated based on 4th h to last time point release data, (IEL3ES2(0.25): 15.3 h and IEL2ES3(0.25): 14.3 h). These formulations showed good time lag in initial drug release and attained complete release within 14–16 h, implying their potential application as colon specific drug delivery systems.

2.5. Effect of storage on the release profile and batch reproducibility

No significant difference was observed in the release profile of different batches of each matrix formulation, indicating that the manufacturing process employed was reliable and reproducible. Also, the release kinetics remained unaltered up to one year of storage and there were no changes in the tablet characteristics, suggesting that indomethacin was stable in the designed matrices.

3. Experimental

3.1. Materials

Indomethacin was obtained as a gift sample from Ajanta Pharma Ltd, Mumbai, India Eudragit (both L100 and S100) was obtained as gift samples from Rohm Pharma, Germany and ethylcellulose (N-22 cps, Aqualon, USA) was purchased from Signet Chem., Mumbai, India. All other chemicals, excipients and solvents used were of either analytical or pharmaceutical grade.

3.2. Characterization of bulk drug

The bulk drug was characterized by various official tests of identification (Indian Pharmacoepia, 1996) and was analyzed in phosphate buffer pH 7.4 by UV spectrophotometric method at 320 nm. The IR spectrum obtained [Jasco Infrared spectrophotometer; model- IR Report 100] was compared with that of the standard. Effects of various formulation excipients (EL100, ES100, ethyl cellulose, talc, magnesium stearate, and ethyl alcohol) on the stability and UV absorbency profile of the drug were also studied.

3.3. Method of analysis for various test samples

The analytical method used was based on spectrophotometric estimation of drug using UV-visible spectrophotometer (*model – V570, Jasco Corporation, Tokyo, Japan*) at 320 nm in phosphate buffer pH 7.4. The calibration curve for indomethacin ranged from 5 to 50 μg/ml in phosphate buffer pH 7.4.

3.4. Matrix embedded formulation preparation

Matrix embedded formulations of indomethacin with pH responsive release was prepared by wet granulation technique. The manufacturing procedure employed in brief was as follows: Accurately weighed quantities of presieved drug and polymer(s) were mixed thoroughly and granulated with minimum volume of ethyl alcohol. The wet granules were sieved though # 60 mesh and the dried granules were mixed with 1% w/w (of the dried granules) mixture of talc and magnesium stearate (3 : 1) and compressed using 5 mm punches on a 16 station rotary tablet compression machine (Cadmach, Ahmedabad, India). Composition of designed tablets are presented in Tables 1 to 3.

3.5. Physicochemical characterization of designed formulations

The designed formulations were studied for their physicochemical properties like weight variation, hardness, friability and assay. For estimating weight variation, 20 tablets of each formulation were weighed using an electronic balance (*AG135, Mettler Toledo, GMBH, Greifensee, Switzerland*). The hardness of 10 tablets was measured using Monsanto (standard type) tablet hardness tester. Friability was determined by taking 20 tablets in a *Campbell Electronic Friabilator* for 4 min at 25 rpm.

For estimation of drug content, ten tablets were crushed and powdered. The aliquot of powder equivalent to 10 mg of drug was weighed and dissolved in methanol: phosphate buffer pH 7.4 (1 : 10) mixture. The resultant solution was filtered and suitably diluted with phosphate buffer (pH 7.4) and analyzed spectrophotometrically at 320 nm. From the absorbance value drug content was calculated on average weight basis.

3.6. In vitro release studies

In vitro dissolution studies were carried out using USP Type II (paddle method) apparatus (*Electrolab TDT-08L, Mumbai, India*) at 75 rpm. The dissolution was carried out for the first two hours in distilled water (500 ml). Then, 200 mL of phosphate buffer concentrate (4.75 g of KH_2PO_4 and 1.07 g of NaOH in distilled water) was added to raise the total media volume to 700 ml and pH to 7.4 for the remaining period. At predetermined time intervals, a 10 ml sample was withdrawn and replaced with fresh dissolution media. The samples were filtered, suitably diluted and analyzed using the UV method discussed earlier. The release studies were conducted in duplicate and the mean values along with the SD were plotted against time.

3.7. Effect of simulated GI fluid pH (without enzymes) on release

The release profile was also studied in a medium of changing pH. The initial condition was 350 ml of 0.1 N HCl (pH 1.2) for 0–2 h. At the end of 2nd hour, 190 ml phosphate buffer (3.75 g of KH_2PO_4) and 60 ml of 0.5 M NaOH in distilled water was added to raise the pH of the media to 4.5 and total dissolution media volume to 600 ml. At the end of 4th h, pH was raised to 7.4 by adding 300 ml phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water). At predetermined time intervals, a 10 ml sample was withdrawn and replaced with fresh dissolution media. After appropriate dilutions, the samples were analyzed by the UV method discussed earlier.

3.8. Evaluation of release rate kinetics

In order to understand the mechanism of drug release from these formulations, the cumulative percentage drug release data was treated according to the power equation given by Ritger and Peppas (1987) to elucidate the mechanism of release.

$$M_t/M_\infty = Kt^n \quad (1)$$

Where, M_t/M_∞ is fraction of drug released at any time 't'; K is release rate constant incorporating the structural and geometric characteristics of the tablets; n is the diffusional exponent, indicative of the release mechanism. [The value of n for a cylinder is 0.45 for Fickian release, > 0.45 and < 0.89 for non- Fician release, 0.89 for case II release and > 0.8 for super II release]. The dissolution data from 2 h onwards was used for analysis for formulations studied in distilled water for 2 h followed with phosphate buffer pH 7.4. In case of dissolution data obtained from simulated GI fluid pH (without enzymes) first 4 h dissolution data and from 4th h data up to last time point data was analyzed separately. The values of K, n, $t_{10\%}$ and $t_{90\%}$ (time required for 10% and 90% of drug release in h respectively) and 'r' (correlation coefficient value), as obtained by fitting the dissolution data in Eq. (1) of designed formulations are given in Tables 4 and 5. The correlation coefficient and regression analysis was done using MS office 2003 Excel work book.

3.9. Batch reproducibility and stability on storage

Three batches of each formulation were prepared and their respective dissolution rates were evaluated under the same conditions. The best formulation of each type was studied after 6 months and 1 year for the effect of storage in ambient conditions on the stability and release profiles of drug from the different formulations respectively. The tablets were sealed in airtight cellophane packets and were stored under ambient conditions (tem-

perature of 25 °C, relative humidity of 65%). The *in vitro* release profile for each was studied as per the specification enlisted in previous sections and compared with its original release profile.

3.10. Data analysis

The difference in the release data for the different formulations was compared using paired t-test for means and one-way analysis of variance (ANOVA) at 5% level of significance using Microsoft Office 2003, Excel package.

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