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Formulation and Evaluation of Levamisole Colon Targeted Tablets

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Abstract: The aim of the present study was to develop colon targeted drug delivery system for Levamisole using Eudragit L-100 and cellulose acetate phthalate (CAP). Levamisole is an anthelminthic and immunomodulator belonging to the class of imidazothiazole derivatives. It is a white to pale cream powder, odorless and soluble in water. Levamisole is rapidly absorbed from GIT and its half life is 4.4 to 5.6 hrs which is major limitation for levamisole. Due to its less $t_{1/2}$, doses of levamisole produced severe reactions in the GIT. Hence matrix formulation containing various proportions of Eudragit L-100 and CAP were prepared by direct compression technique. All the formulations were evaluated for in-process quality control tests and the in-vitro drug release study was undertaken at 37 ± 0.5^0c in 0.1N HCl for 2hrs followed by 7.4 p^H phosphate buffer for 3hrs and 6.8 p^H phosphate buffer for 19hrs. Results indicated that drug release has been retarded more by using Eudragit L-100 than by using CAP and 1:1 ratios of Eudragit L-100 and CAP. Formulation LM-4 (Eudragit L100 -5 % w/v) seems to quiet promising for colonic drug delivery with only 7.6 % drug release in first 5hrs. After 24 hrs cumulative percent drug release for LM-4 was found to be 98.4%. Drug release kinetics revealed that, drug release from LM-4 follows zero order release with fickian diffusion. Whereas Levamisole formulations did not release drug in stomach and small intestine but delivered drug to the colon resulting in slow absorption of drug and making drug available for local action in the colon.
Key words: Colon specific drug delivery system, CDDS, Esemoprazole, cellulose acetate phthalate, entric coated tablet.

Introduction

Among all the routes of administration that have been explored for the development of controlled release systems the oral route has by far achieved the most attention and success. That is due in part to the ease of administration as well as to the fact that gastrointestinal physiology offers more flexibility in dosage form design than most other routes.

The scientific framework required for development of a successful oral controlled drug delivery dosage form consists of an understanding of three aspects of the systems. Namely

1. The physiochemical characteristics of the drug.
2. Relevant gastrointestinal anatomy and physiology.
3. Dosage form characteristics.

Controlled drug delivery system that provides continuous delivery of the drug for a predetermined period with predictable and reproducible kinetics and known mechanism of release. [1]

Targeting drugs directly to the colon is advantageous in the topical treatment of colonic disease such as ulcerative colitis, Crohn's disease and for the oral delivery of peptides and other liable drugs. Absorption of drug molecules form the colon like other regions of GIT is a result of a complex series of events. Successful colonic uptake of a drug species require both enzymatic stability and has to transport from the mucosal surface to the venous and or lymphatic capillaries located in the sub-mucosa. The colonic epithelial permeability is insufficient to allow for the transport rate required for a therapeutic delivery. Then the co administration of an absorption enhancing agent offers a potential means of overcoming this barrier mostly through the use of chemical enhancers [2]. These agents are roughly sub charted into categories of chelating agents, non steroidal anti inflammatory agents (NSAIDS), surfactants (mostly as mixed micelles), phenothiazenes and a general class of molecules which include fatty acids, acylcarnitive acylamino acids and dicarboxylic acid.

Comparison of their rate of onset and recovery of a treated mucosa has also been made. Fatty acids have strong and fast reactivity and allow for a fast recovery of barrier functions. Bile salts and salicylates are moderate and fast acting agents with fast barrier functions recovery. Strong surfactants and chelating agents have strong or moderate reactivity and a slow recovery of barrier function and solvents such as dimethyl sulfoxide and ethanol have moderate reactivity and act primarily as agents to improve drug miscibility in an aqueous environment. There are other potential enhancers which may be more colon specific such as ethylacetoacetate which must be first metabolically transformed to emanine. [3]

Several chemical enhancers including sodium taurocholate and Sodium ethylene diamino tetra acetate (Na-EDTA) oelic acid, Polyoxyethylated nonionic surfactants, Citric acid and dihydroxy bile salts open the paracellular OJC function. Deoxycholate, a dihydroxy bile salts, may also disrupt OJC function as well as stimulate cellular uptake through transcellular transport. [4] Agents which affect the local production of NO through the nerve stimulation at the ileocolonic junction will increase the epithelial permeability by decreasing the paracellular resistance will increase the colonic drug absorption. Another component released during the nerve stimulation is substance P, possibly through activation of cycloxygenase pathway, also appears to modify the absorption across the colonic mucosa NSAIDS at high concentration can also act as cation chelators, their action appears to be more complex than a direct chelation effect.

Levamisole, is an anthelminthic and immunomodulatory belonging to a class of synthetic imidazothiazole derivatives. Levamisole has been used in humans to treat parasitic worm infections. It has been studied in combination with other forms of chemotherapy for colon cancer, melanoma, and head and neck cancer[5]. The objective of this study is to develop formulations using a combination of time and pH dependent system for delivering Levamisole to the colon and to sustain the release of the drug using Eudragit and Cellulose Acetate Phthalate as an enteric coating polymer so as to reduce the dosing frequency of the drug and to demonstrate its site specificity in the colon. [6]

Materials and Methods

Materials

Levamisole was obtained from Drugs India Pvt ltd (India). Micro crystalline cellulose was obtained from Himedia. Poly ethylene glycol 400 was obtained from Fischer scientific. Cellulose Acetate Phthalate (CAP) and Eudragit L-100 was purchased from Drugs India Pvt ltd (India). All the remaining ingredients and chemicals utilized were of analytical grade.

Methods

Standard graph for Levamisole

Step – 1: **Preparation of standard stock solution:**

An accurately weighed quantity of 100 mg of levamisole was taken in a 100 ml standard flask. To this equal volume of water was added and made up to the volume.

Step – 2: **Preparation of sample solution:**

Different aliquots (0.0, 0.2, 0.4,…… , 1.0 mL) of Levamisole were accurately measured from the above primary stock and transferred into a series of 100 mL volumetric flasks and volume made up to the mark with water. Then all dilutions were scanned by UV Spectrophotometer at 213nm against blank and the results were tabulated and a plot was drawn between concentration (µg/ml) on x-axis and absorbance (nm) over y-axis.

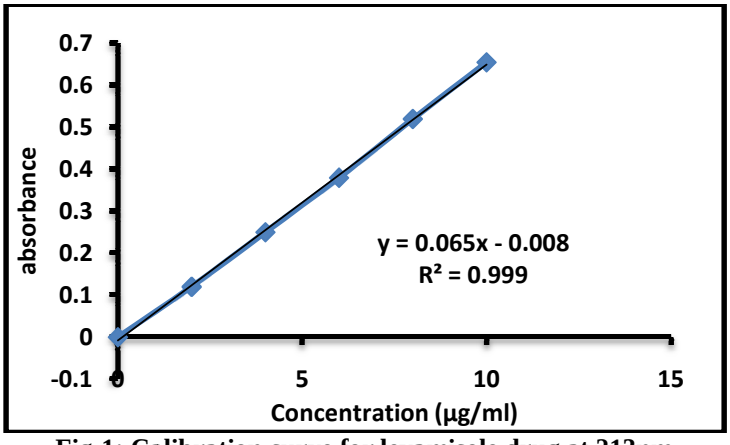

Fig-1: Calibration curve for levamisole drug at 213nm

Drug-Excipient compatibility study: FT-IR spectroscopy

FT-IR patterns were studied by Shimadzu 8400S, Japan FT-IR spectrometer. The samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 (Sample: KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. The scans were obtained at a resolution of 4 cm^{-1}, from 4000 to 400 cm^{-1}.

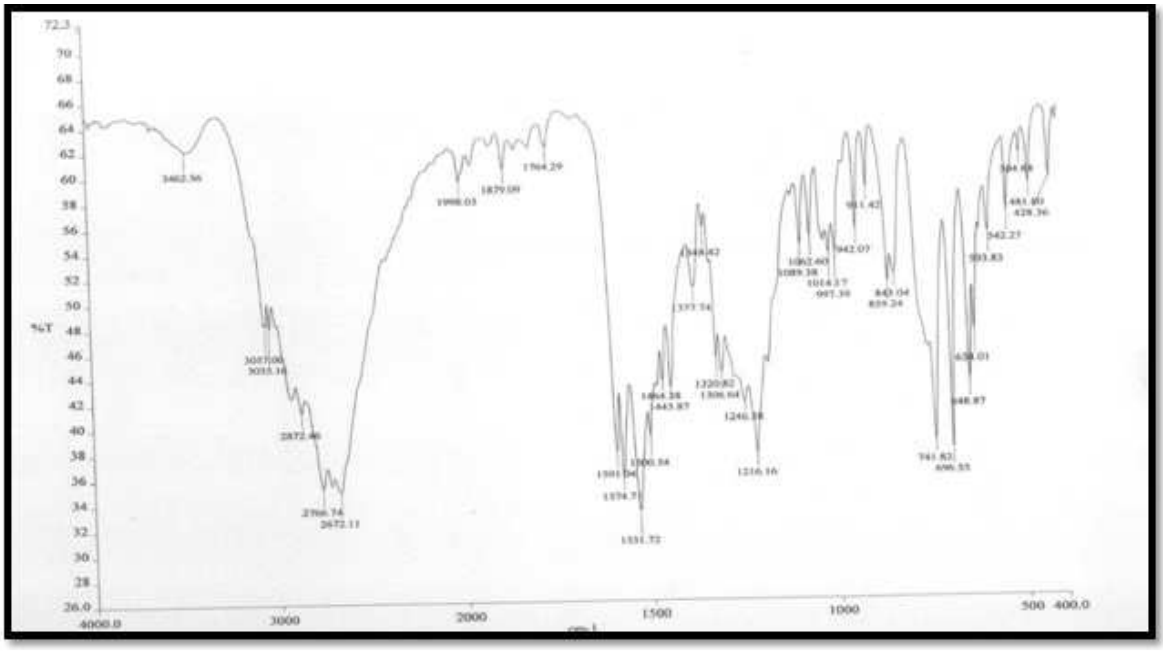

Fig-2: FT-IR Spectra of Levamisole drug

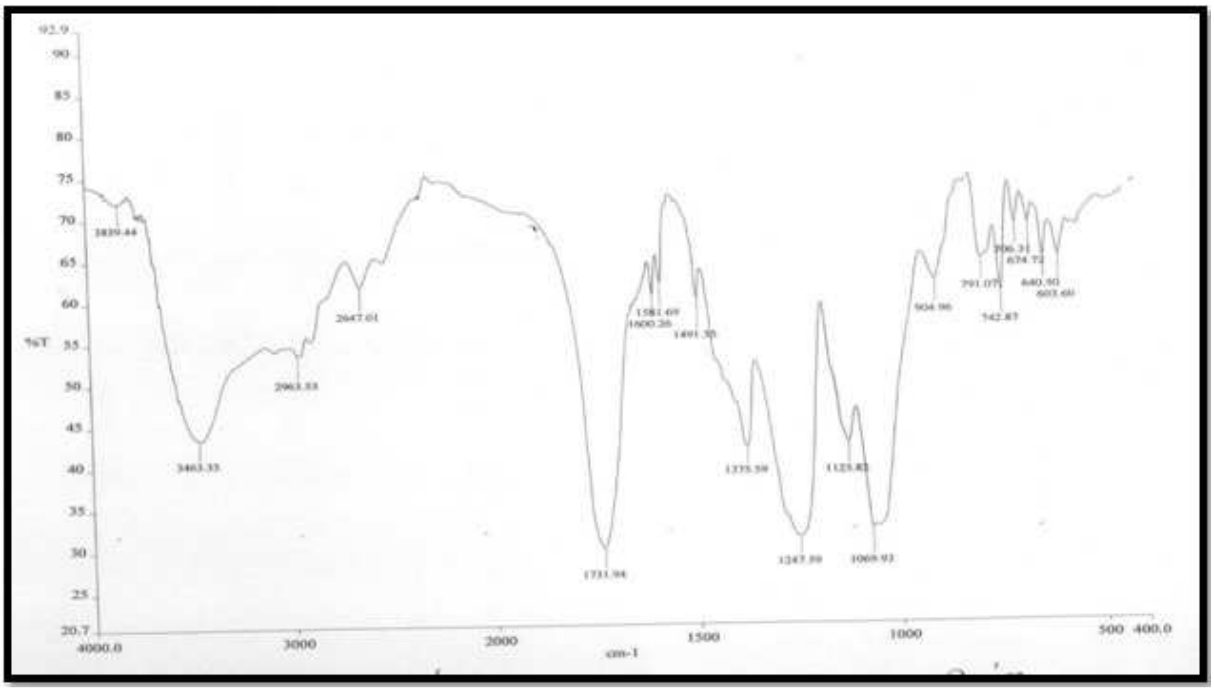

Fig-3: FT-IR Spectra of Cellulose acetate phthalate

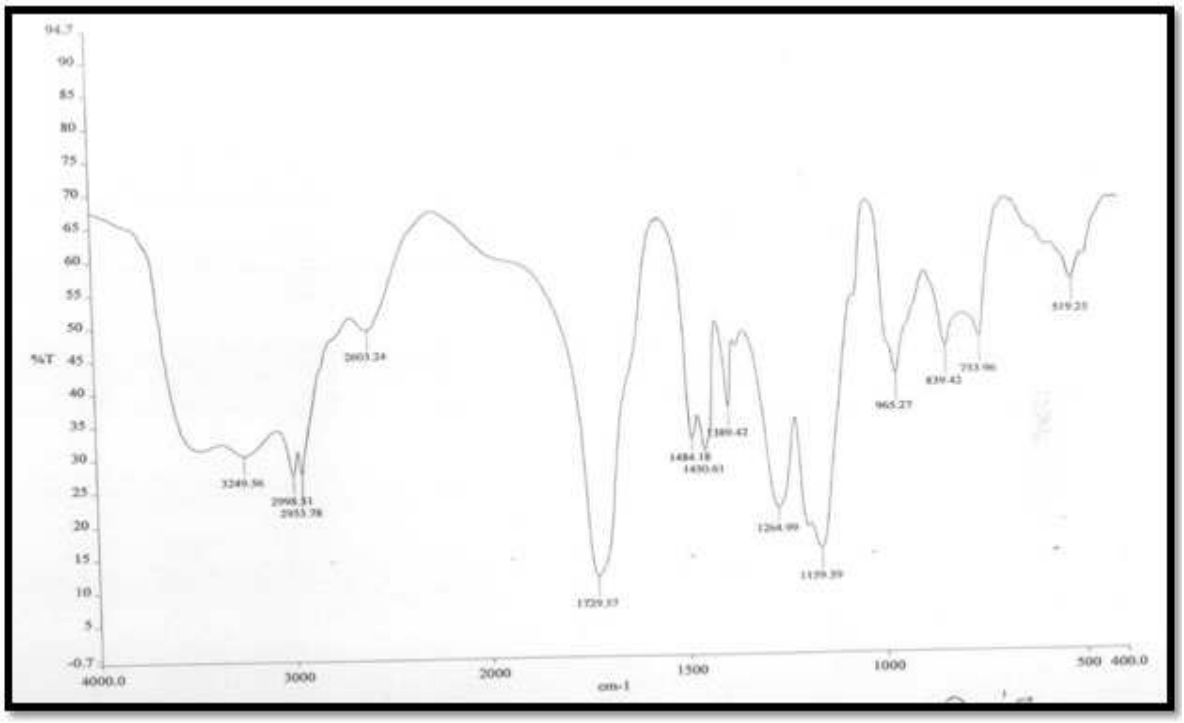

Fig-4: FT-IR Spectra of Eudragit

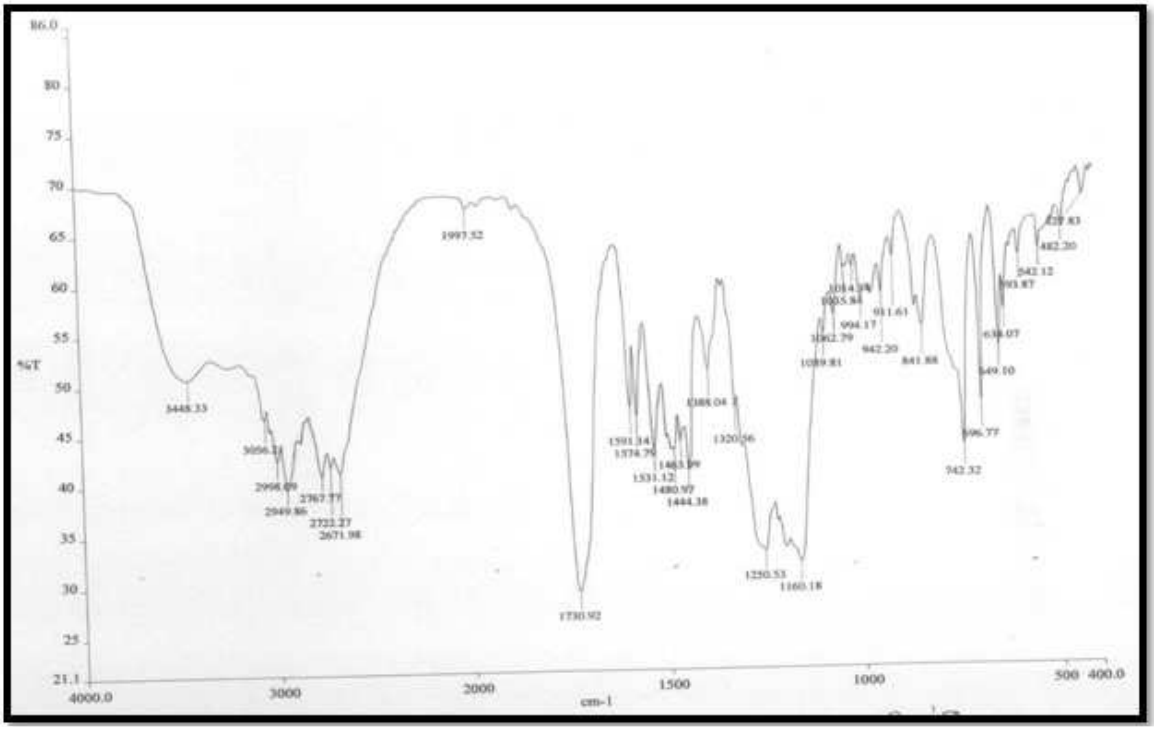

Fig-5: FT-IR Spectra of Levamisole + Cellulose acetate phthalate + Eudragit

Preparation of Core Tablets

All the formulation was prepared by direct compression method. The drug (150mg/tablet) and other excipients used in the formulation shown in table-1 were passed through No. 60 sieve prior to compression. Powder blends were prepared using a cone mixer for 15 min. Then talc was added and mixed for another 5 min. The required quantity of

the ingredients for preparing the sustained release formulations were compressed using a rotary tablet punching machine equipped with 13mm capsule shaped, concave punches (Cadmach). The batch size of each formulation was 50 tablets. [7]

Table-1: Formulation table for levamisole core tablets (mg)

Formulation	Drug	MCC	Talc	Magnesium stearate	Core Weight
LM-1	150	690	100	60	1000
LM-2	150	690	100	60	1000
LM-3	150	690	100	60	1000
LM-4	150	690	100	60	1000
LM-5	150	690	100	60	1000
LM-6	150	690	100	60	1000
LM-7	150	690	100	60	1000
LM-8	150	690	100	60	1000
LM-9	150	690	100	60	1000

Evaluation of core tablets

Pre-compression parameters

Pre-compression parameters such as Bulk density, Tapped density, Angle of repose Carr's compressibility index, Hausner's ratio were performed for the powder mixture and the results were tabulated in table-3. [8-10]

Post-Compression Parameters

After compression of desired doses of drug and its excipients into suitable tablet dosage form, each batch was subjected to the evaluation parameters such as weight variation, tablet dimensions such as thickness, diameter, hardness, friability, drug content and was tabulated in table-4. [11-16]

Coating of Core Tablets

After formulation and evaluation of core tablets all the batches were subjected to prior coating according to the table given below.

Table-2: Composition of enteric coated tablets

Formulation	Eudragit L -100 (% w/v)	CAP (% w/v)	Eudragit L -100 & CAP (% w/v)	PEG (% v/v)	Acetone (ml)
LM-1	-	2%	-	2%	100
LM-2	-	4%	-	2%	100
LM-3	-	6%	-	2%	100
LM-4	5 %	-	-	2%	100
LM-5	10%	-	-	2%	100
LM-6	15%	-	-	2%	100
LM-7	-	-	5% (1:1)	2%	100
LM-8	-	-	10% (1:1)	2%	100
LM-9	-	-	15% (1:1)	2%	100

Evaluation of Enteric Coated Tablets

Hardness Test:

The hardness of the coated tablets was measured using same procedure as described earlier with the help of Monsanto hardness tester. The hardness of various formulations was shown in table -4.

Weight Variation Test:

The weight variation test was carried out for the coated tablets using the same procedure as described earlier and the results were reported in the table 4. [17, 18]

***In-vitro* Dissolution Profile of Levamisole Coated Tablets:**

In vitro drug release studies for the prepared tablets were conducted for a period of 24 hours using USP type-II (Paddle) dissolution apparatus (Lab India) at 37±0.5^{o}C and 75 rpm speed using pH 1.2 buffer for initial 2 h, phosphate buffer of pH 7.4 up to 22h as dissolution medium. At predetermined interval of time, 10 ml of sample was withdrawn from the dissolution medium and replaced with fresh medium to maintain the volume constant. After filtration and appropriate dilution, the sample solutions were analyzed at 213 nm for Levamisole by UV-visible spectrophotometer. The amount of drug present in the samples was calculated and the results were reported in tables 5.

Mathematical modeling for drug release profile

The cumulative amount of Levamisole released from the formulated tablets at different time intervals were fitted in to several kinetic models such as Zero order kinetics, first order kinetics, Higuchi model and Korsemayer-peppas model to characterize mechanism of drug release. [19-25]

Results and Discussion

Table-3: Pre compression parameters for Levamisole drug powders

Formulations	Bulk density(g/cm^3)	Tapped density(g/cm^3)	Hausner's ratio (%)	Carr's index (%)	Angle of repose(^{0})
LM-1	0.64	0.75	1.17	14.67	$23^0.73^1$
LM-2	0.65	0.73	1.12	10.96	$22^0.12^1$
LM-3	0.68	0.74	1.09	8.11	$22^0.66^1$
LM-4	0.63	0.71	1.13	11.27	$21^0.18^1$
LM-5	0.61	0.68	1.11	10.29	$24^0.12^1$
LM-6	0.57	0.7	1.23	18.57	$23^0.11^1$
LM-7	0.61	0.71	1.16	14.08	$22^0.93^1$
LM-8	0.66	0.74	1.12	10.81	$22^0.15^1$
LM-9	0.60	0.72	1.20	16.67	$21^0.17^1$

Table-4: Post compression parameters for Levamisole powders

Formulation code	Hardness Kg/cm^2	Thickness (mm)	Weight variation (mg)	Friability (%)	Content uniformity (%)
LM-1	4.5	5.2	998	0.61	97.0
LM-2	4.7	4.9	1000	0.72	98.2
LM-3	5.1	5.0	1005	0.73	99.8
LM-4	4.6	5.1	1002	0.65	97.5
LM-5	5.3	4.8	999	0.80	99.1
LM-6	4.9	5.2	995	0.69	98.5
LM-7	5.5	4.9	1002	0.78	97.7
LM-8	5.2	5.0	1004	0.85	99.6
LM-9	5.0	5.2	998	0.82	98.5

Table-5: In-vitro drug release data for Levamisole colon targeted tablets

Medium	Time (Hrs)	LM-1	LM-2	LM-3	LM-4	LM-5	LM-6	LM-7	LM-8	LM-9
0.1M HCl	1	3.6	1.9	1.2	2.5	2.1	1.8	3.7	2.0	2.3
	2	6.2	3.5	3.4	5.4	4.3	3.2	6.4	4.1	3.3
6.8 Phosphate buffer	5	16.5	10.8	8.6	7.6	7.2	4.8	18.3	12.9	10.4
	6	25.7	23.8	19.9	24.3	21.4	17.8	24.2	22.5	19.6
	9	38.5	32.4	29.4	32.1	30.2	26.3	39.0	31.3	20.5
	12	59.2	48.8	46.2	55.6	44.4	42.1	56.4	45.4	43.4
	15	73.5	66.2	58.6	69.8	61.5	52.3	72.1	66.7	56.0
	18	89.4	78.4	72.1	83.4	73.4	69.3	86.2	79.2	75.2
	21	98.4	88.3	80.4	90.4	87.2	78.4	97.5	89.0	83.2
	24	__	97.2	94.2	98.4	96.3	91.2	__	98.1	96.0

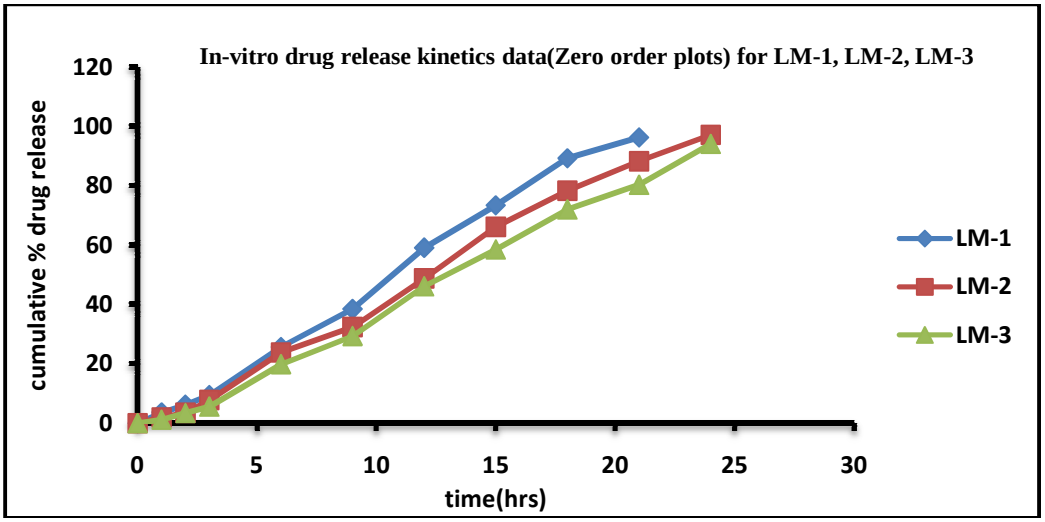

Fig- 6: In-vitro drug release kinetics data (Zero order plots) for LM-1, LM-2, LM-3

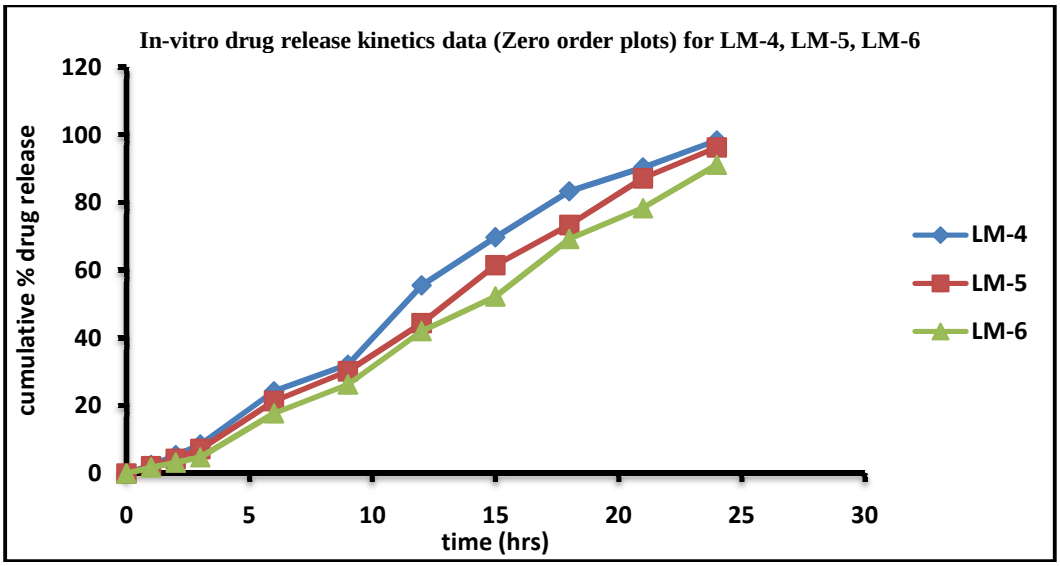

Fig-7: In-vitro drug release kinetics data (Zero order plots) for LM-4, LM-5, LM-6

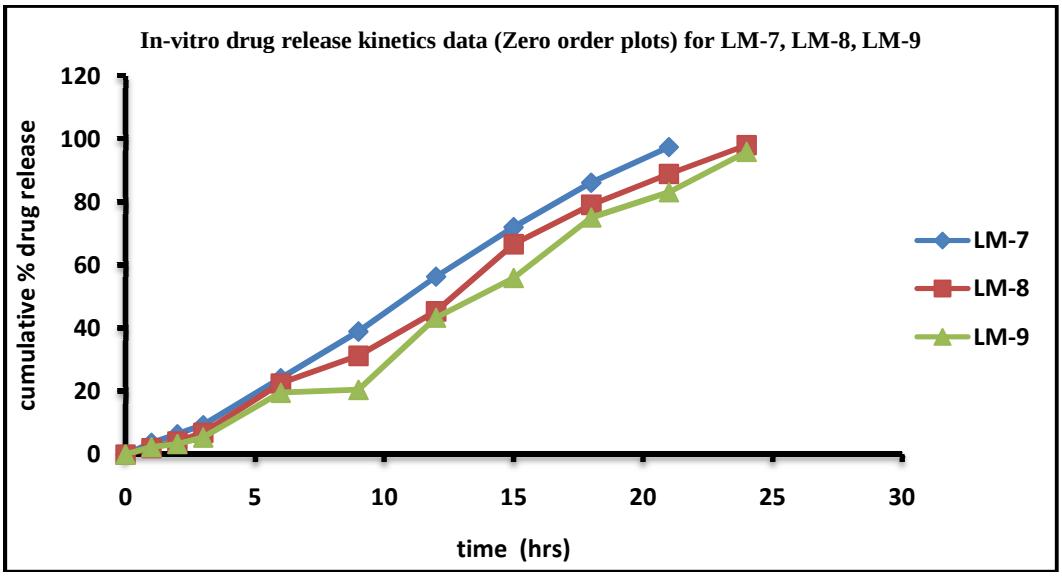

Fig-8: In-vitro drug release kinetics data (Zero order plots) for LM-7, LM-8, LM-9

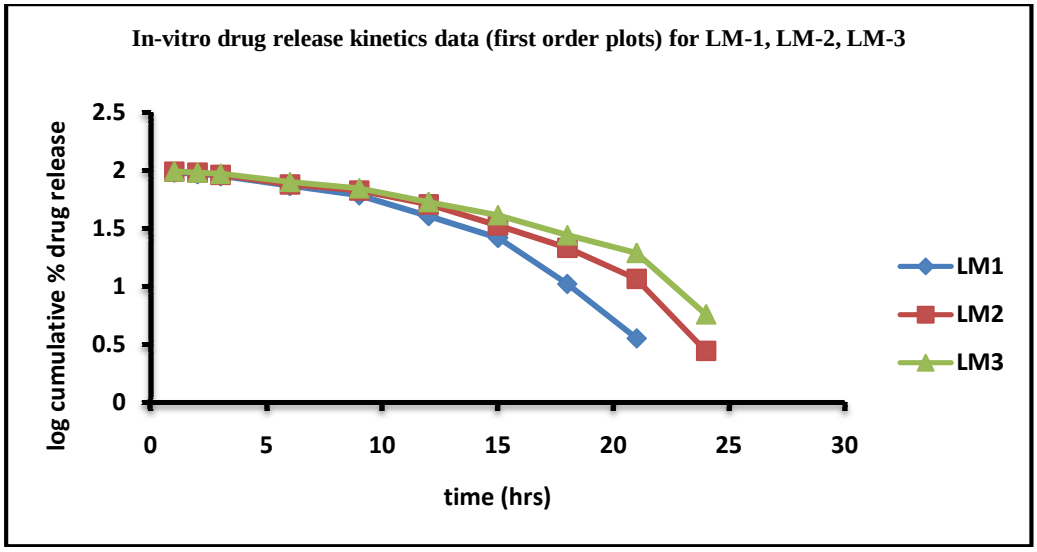

Fig-9: In-vitro drug release kinetics data (first order plots) for LM-1, LM-2, LM-3

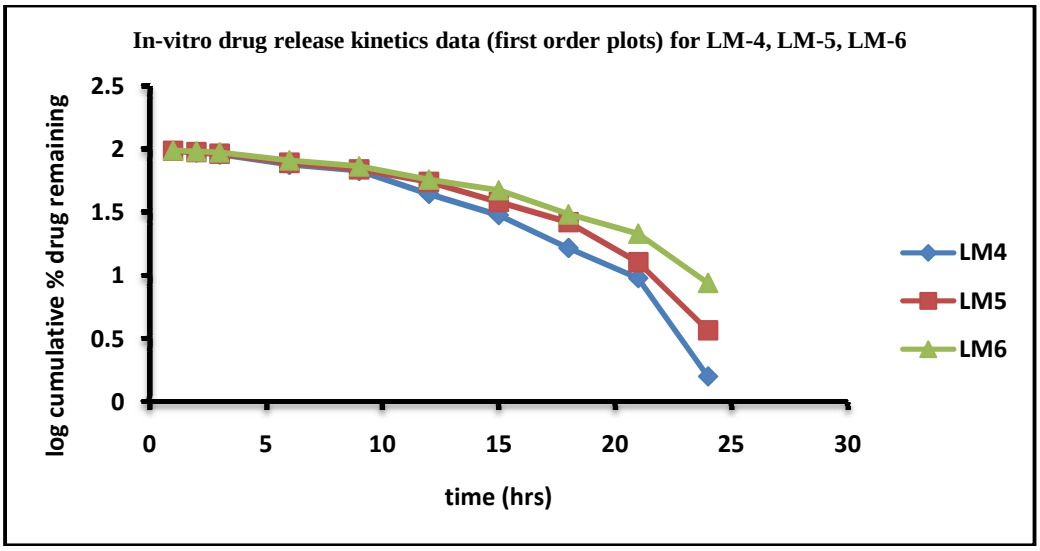

Fig-10: In-vitro drug release kinetics data (first order plots) for LM-4, LM-5, LM-6

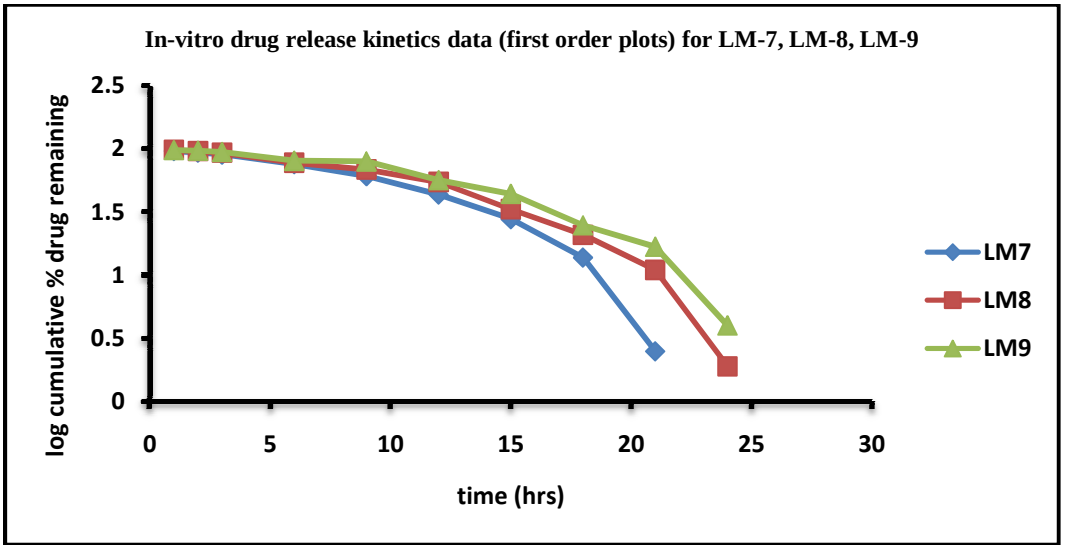

Fig-11: In-vitro drug release kinetics data (first order plots) for LM-7, LM-8, LM-9

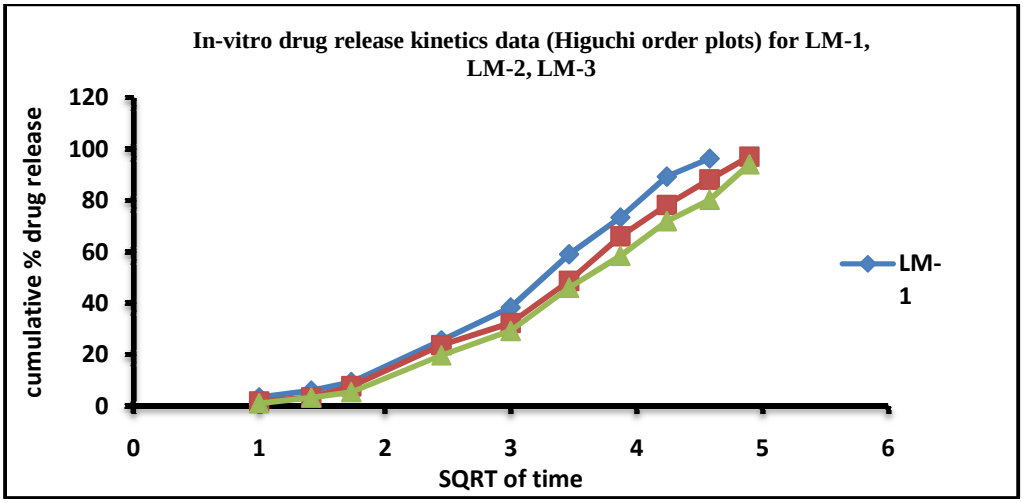

Fig-12: In-vitro drug release kinetics data (Higuchi order plots) for LM-1, LM-2, LM-3

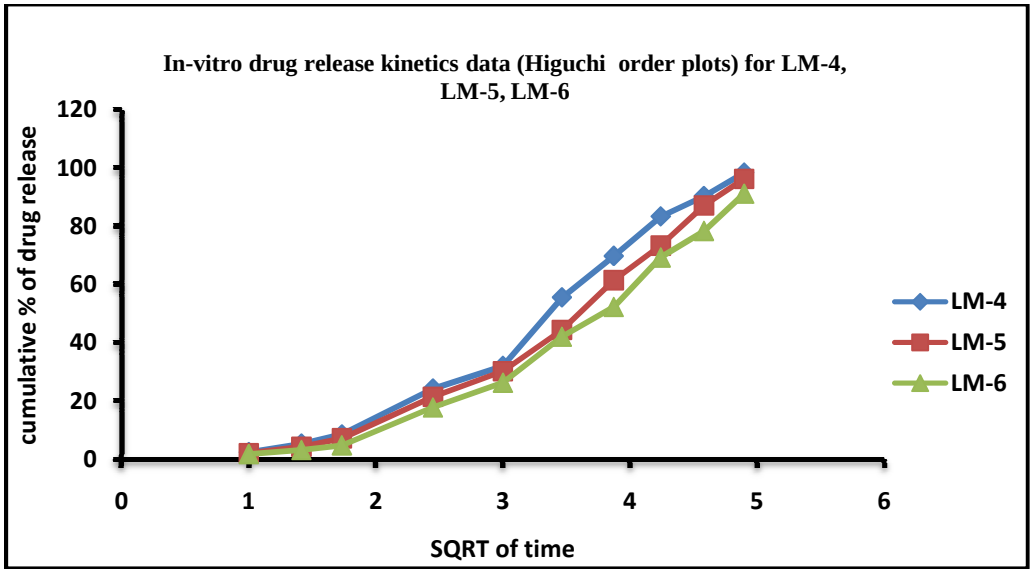

Fig-13: In-vitro drug release kinetics data (Higuchi plots) for LM-4, LM-5, LM-6

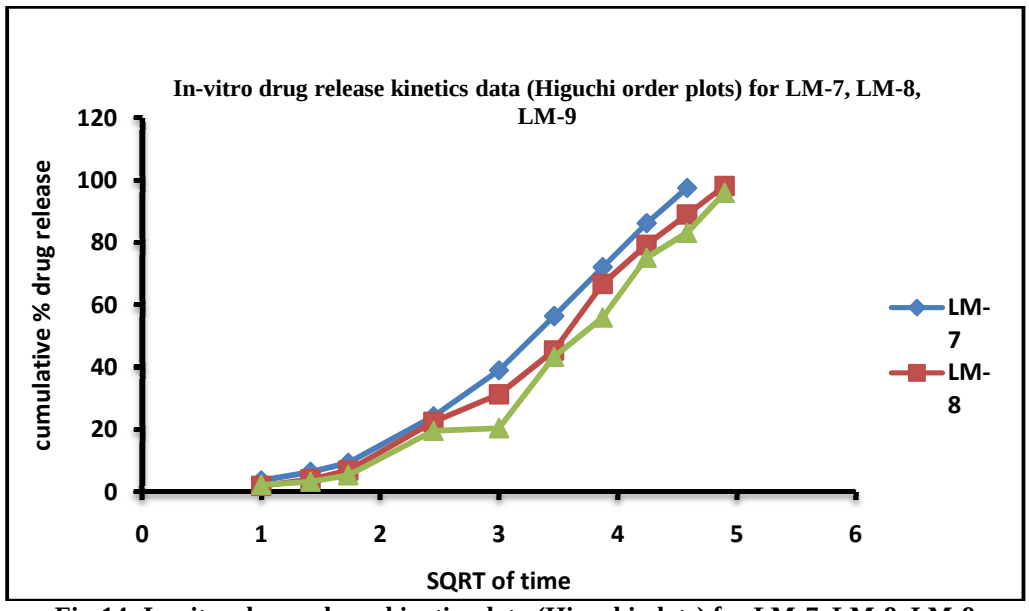

Fig-14: In-vitro drug release kinetics data (Higuchi plots) for LM-7, LM-8, LM-9

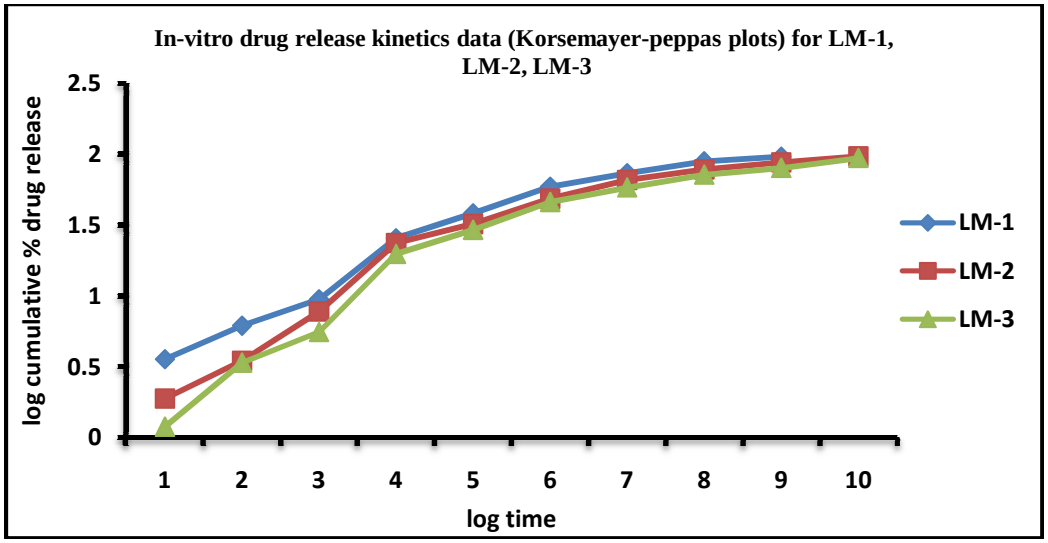

Fig-15: In-vitro drug release kinetics data (Korsemayer-peppas plots) for LM-1, LM-2, LM-3

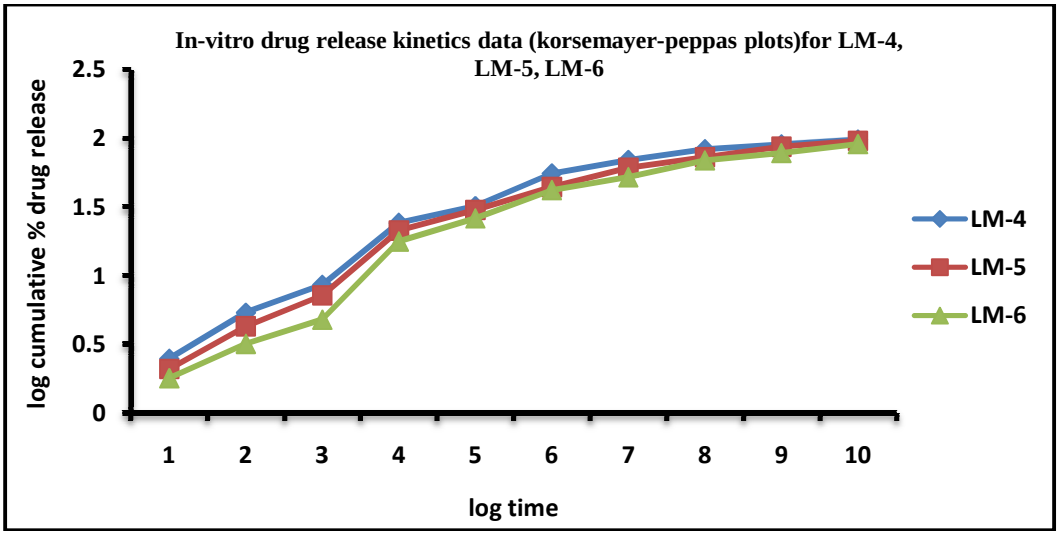

Fig-16: In-vitro drug release kinetics data (Korsemayer-peppas plots) for LM-4, LM-5, LM-6

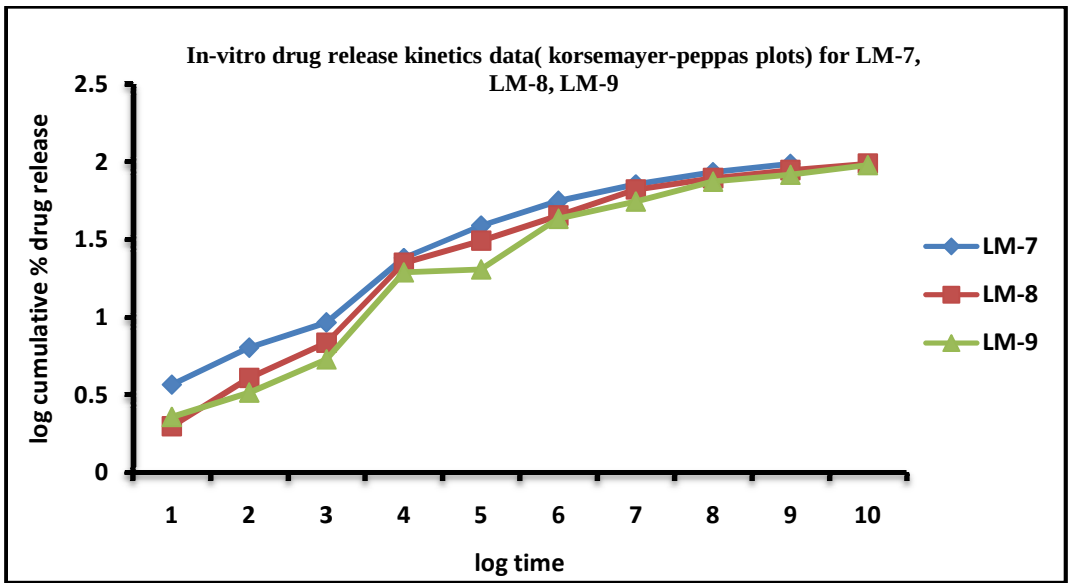

Fig-17: In-vitro drug release kinetics data (Korsemayer-peppas plots) for LM-7, LM-8, LM-9

Table-6: Parameters and determination coefficients of release profile from Levamisole colon targeted tablets

Formulation code	Correlation Coefficient values (R^2)				Diffusion Exponent value (n)
	Zero Order	First order	Higuchi	Korsemayer-peppas	
LM-1	0.9942	0.8877	0.96	0.93	0.18
LM-2	0.9944	0.8641	0.97	0.89	0.19
LM-3	0.996	0.873	0.97	0.89	0.20
LM-4	0.9887	0.8543	0.97	0.90	0.17
LM-5	0.9958	0.8533	0.97	0.91	0.18
LM-6	0.9937	0.8904	0.96	0.91	0.19
LM-7	0.9925	0.8329	0.97	0.94	0.18
LM-8	0.997	0.8308	0.97	0.90	0.19
LM-9	0.9831	0.8347	0.95	0.93	0.19

Table-7: Stability results of levamisole colon targeted tablet (organoleptic properties)

(Batch -1) week	Temperature and relative humidity (25°C/60%RH)		
	Size and shape of tablets	Goss nature of tablets	Color of tablets
0	Concave	Smooth	White
2	Concave	Smooth	White
4	Concave	Smooth	White
6	Concave	Smooth	White
8	Concave	Smooth	White
10	Concave	Smooth	White
12	Concave	Smooth	White

Table - 8: Stability results of levamisole colon targeted tablet (Batch-2)

(Batch -2) Week	Temperature and relative humidity (40°C/70%RH)		
	Size and shape of tablets	Goss nature of tablets	Color of tablets
0	concave	Smooth	White
2	concave	Smooth	White
4	concave	Smooth	White
6	concave	Smooth	White
8	concave	Smooth	White
10	concave	Smooth	White
12	concave	Smooth	White

Table-9: Stability results of levamisole colon targeted tablet (Batch-3)

(Batch -3) Week	Temperature and relative humidity (60°C/80%RH)		
	Size and shape of tablets	Goss nature of tablets	Color of tablets
0	concave	Smooth	White
2	concave	Smooth	White
4	concave	Smooth	White
6	concave	Smooth	White
8	concave	Smooth	White
10	concave	Sticky	Slightly yellowish
12	concave	Sticky	Slightly yellowish

Table-10: Post compression parameters for Levamisole tablets after stability studies

Parameter	LM-1	LM-2	LM-3	LM-4	LM-5	LM-6	LM-7	LM-8
Appearane	Flat concave white	Flat concave white	Flat concave white	Flat concave white	Flat concave white	Flat concave white	Flat concave white	Flat concave white
Dimension (mm) Diameter Thickness	19mm 5.2	19mm 4.9	19mm 5.0	19mm 5.1	19mm 4.8	19mm 5.2	19mm 4.9	19mm 5.0
Weight variation Avg.wt Result	995.5mg ± 0.15 Comply	998.6mg ± 0.25 comply	995.3mg ± 0.18 comply	996.3mg ± 0.59 comply	1000.5mg ± 0.26 comply	1004.7mg ± 0.26 comply	1002.4g ± 0.16 comply	1000.5g ± 0.14 comply
Hardness	4.5kg/cm^2	4.7kg/cm^2	5.1kg/cm^2	4.6kg/cm^2	5.3kg/cm^2	4.9kg/cm^2	5.5kg/cm^2	5.2kg/cm^2
Friability	0.61%	0.72%	0.73%	0.65%	0.80%	0.69%	0.78%	0.85%

Table-11: Stability studies In-vitro dissolution profile of LM-4

S.NO	Medium	Time		% drug release of LM-4		
			Initial	Batch-1 (25°C/60%RH)	Batch-2 (40°C/70%RH)	Batch-3 (60°C/80%RH)
1	0.1M HCl	1	2.5	2.4	2.1	1.9
2		2	5.4	5.2	4.8	4.2
3	6.8 Phosphate buffer	5	7.6	8.3	7.9	6.9
4		6	24.3	24.1	23.8	22.6
5		9	32.1	31.4	30.7	29.1
6		12	55.6	54.6	54.1	53.8
7		15	69.8	68.8	68.1	67.3
8		18	83.4	81.4	79.9	78.8
9		21	90.4	89.4	89.1	88.2
10		24	98.4	97.9	97.6	96.1

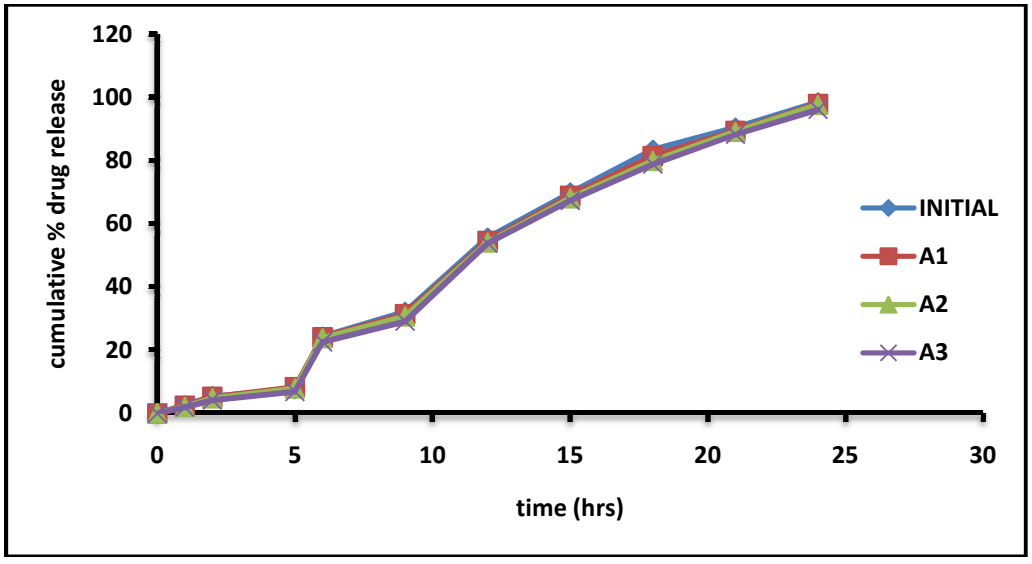

Fig- 18: Stability data graph for LM-4 formulation

A1= Batch-1 (25^{0}c/60% RH),
A2= Batch-2 (40^{0}c/70% RH),
A3= Batch-3 (60^{0}c/80% RH)

Discussion

Pre-Compression Evaluation Parameters

FT-IR Studies

In this studies fig-2 demonstrates the FT-IR spectra of pure drug (Levamisole) which shows characteristic peaks at 741.82, 1216.16, 1531.72, 2672.8 and 2872.1 cm^{-1} represents C-Cl stretching, C-N stretching, N-O asymmetric stretching, O=H stretching, C-H stretching respectively which were responsible peaks for the action of levamisole. Hence when fig-2 (pure drug-levamisole) was compared with fig-3,4,5(drug with mixture of excipients) it was observed that there is no characteristic change in the above peaks which represents that there were no incompatibilities with the excipients utilized in the formulation of colon targeted tablets.

Micromeritic Parameters

Flow properties are the important concern in the formulation and industrial production of tablet dosage form. Results of micromeritic measurements such as bulk density, tapped density, angle of repose, Carr's index, and Hausner's ratio for drug alone and physical free flowing powder mixture are represented in the tables- 3. From the results it was observed that levamisole drug alone due to its amorphous nature shows poor flow properties when compared to its physical mixture, which shows good flow properties.

Post-Compression Evaluation Parameters

Results of post-formulation parameters such as tablet dimensions (thickness, diameter) Hardness, Friability, Weight variation and Drug content uniformity were represented in tables- 4 (before coating) and table-10 (after coating). There should be certain amount of strength or hardness and resistance to friability for the tablet, so that tablet should not break during handling. However, it has also effect on drug dissolution. Average hardness of Levamisole colon targeted tablet ranges from 4.5 to 5.5 kg/cm^2. Friability studies of colon targeted tablet are in the range of 0.61 % to 0.85 %. This indicates that acceptable resistance is shown by the tablets to withstand handling.

In-Vitro Dissolution Studies

In-vitro dissolution studies has been performed for 24 hrs and the results revealed that, drug release has been retarded more in LM-4, LM-5, LM-6 (prepared by using Eudragit L-100) when compared to LM-1, LM-2, LM-3 (Prepared by using CAP) and LM-7, LM-8, LM-9 (Prepared in 1:1 ratios of CAP & Eudragit L-100) drug retardation may be due to the use of Eudragit L-100, in formulations LM-4, LM-5,LM-6. The influence of use of different methacrylate co-polymers on drug retardation is generally in the following order in the formulation of levamisole colon targeted tablets.

Eudragit L-100 > CAP > Eudragit: CAP (1:1)

Out of the nine formulation (Prepared by using Eudragit L-100, CAP and Eudragit L-100 : CAP (1:1), LM-4 showed best release profile in 24 hrs with 98.4% drug release and the results are tabulated in table-5.

In-Vitro Drug Release Kinetics

Although model independent methods are simple and easy to apply, they lack scientific justification. Hence different model dependent approaches (Zero order, First order, Higuchi, Korsemayer-Peppas plots) were performed for dissolution profile comparison of all colon targeted tablets (fig 6-17). The results of these models indicate all colon targeted tablets follows zero order as "best fit model". This is due to previously proved fact depending on R^2 value obtained from model fitting. From the results it was found that formulations prepared with eudragit L-100 showed better retardation when compared to other formulations. Diffusional exponent 'n' and mechanism of diffusional release from swellable colon targeted tablets indicates that the drug release mechanism was found to be fickian diffusion.

Stability Studies

The stability tests were conducted on LM-4 which is considered to be the best. The formulation was analyzed for its organoleptic properties and dissolution profile for a period of 12 weeks. The results showed that the colour and gross nature of tablets were slightly changed for batch -3 (which is kept at 60^0c/80 % RH). No changes were found for batch -1 (which is kept at 25^0c / 60 % RH) and batch -2(which is kept at 40^0c / 70 %RH). The percentage drug release was found to be slightly decreased after twelve weeks for batch -3, shown in fig-18.

Conclusion

The present study was carried out to investigate the colon targeted release of levamisole using methacrylate co-polymers. In the same study the ability of methacrylate co-polymers for targeting the drug release in colon has been carried out. From the results it was found that formulation liberation. All the pre-compressional and post-compressional parameter were evaluated and found to be within the limits. Among all the formulation of Levamisole, LM-4 with 5 % Eudragit L-100 emerged to be best one, because it exhibits the maximum percentage drug release of 98.4 %. In –vitro kinetics for LM-4 showed that, the drug release mechanism was found to be fickian diffusion. So, Levamisole may be formulated as colon targeted tablets, which may increase the patient compliance and decreased reactions in GIT with increase in half life.

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