ACECLOFENAC DELIVERY BY MICROENCAPSULATION USING LBL SELF-ASSEMBLY FOR DELAYED RELEASE

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ABSTRACT

Colonic targeting has gained increasing interest over the past years, not just for the transport of drugs for the treatment of local diseases associated with the colon but also for its potential for transporting peptides and proteins, particularly low molecular weight peptide drugs. Without protection, such peptide drugs are usually digested within the gastric and small intestinal sections.

In the present work Layer-By-Layer (LBL) self-assembly was utilized to make Aceclofenac single bilayer microcapsules produced by sequential adsorption of positively charged chitosan and negatively charged Pectin on the external surface of negatively charged Aceclofenac microcrystals. Taguchi approach was applied to determine the best concurrence of composition factors that is concentration of chitosan, pectin, centrifugation speed and incubation time. The microcapsules were characterized for encapsulation efficiency, particle size, zeta potential, scanning electron microscopy and *in-vitro* release kinetics.

Surface electric potential of Aceclofenac microcrystals was found to be negative with zeta potential -1.39 mV, in acetate buffer of pH 4. The primary and the secondary deposit layer of chitosan and pectin was found to have a positive and negative charge with zeta potential of +5.57 mV and -22.8 mV respectively. The sequential changing of surface zeta potential after each deposition is a satisfactory indication of the LBL self-assembly of the oppositely charged polyelectrolytes.

The average size and encapsulation efficiency of the optimized single bilayer microcapsules (F5) was found to be 20µm and 63.83%, respectively. The *ex-vivo* percentage cumulative drug release of (F5) in Phosphate buffer pH 6.8 containing 2-4% w/v colonic fecal matter of male albino rat was found to be 98.40%. The optimized batch of microcapsules showed first order release kinetics (R^2= 0.950) in presence of colonic fecal matter.

Keywords: Aceclofenac, Chitosan, Pectin, Rat caecal contents, colonic drug delivery.

INTRODUCTION

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides (Chaurasia and Jain, 2003).

Site-specific delivery and release of drugs will not only be useful to achieve systemic therapeutic effects (after absorption), but also for topical applications, for instance, for the treatment of inflammatory bowel disease, ulcerative colitis, and colon cancer, to name the most prevalent.

There are various approaches to accomplish colon targeting or reliable drug release into the caecum or colon among which, one approach is to design prodrugs that release the effective drug after enzymatic cleavage at certain predetermined sites of the intestine. Other means include coatings or embedding of the drug with film or matrix forming polymeric materials that are biodegradable by enzymes of the microflora in the caecum or colon (Satyanarayana *et al*., 1998).

Microencapsulation is a process of applying relatively thin coating to small particles of solid drug microparticles to modify and delay the drug release from different solid dosage form (Allen *et al*., 2005). LBL self-assembly technique follows the phenomenon of sequential adsorption of oppositely charged Polyelectrolytes, which is mainly due to the electrostatic force of attraction between charged polyelectrolytes. Different charged components, including linear polymers, enzymes, polyelectrolytes, and inorganic nanoparticles, are frequently used in the process of microencapsulation (Ye *et al*., 2005, Biguccia *et al*, 2008).

Controlled release systems, are designed to release drug *in vivo* according to predictable rates that can be verified by *in vitro* measurements. The solid drug particles of controlled release systems which act as a core material must have minimum solubility at pH in which coating procedure was carried out for the development of controlled release systems, and have maximum solubility under the conditions at which controlled release is to take place. The release behavior of coat can be modified by changing the thickness of coating layers. Thicker coating of polymers leads to longer release times. Mostly, the release behavior of controlled release system is controlled by some intrinsic factors like the polymer layer thickness,

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which is generally adjusted by increasing or decreasing the number of polymer layers. But increasing the coating layer number in LBL self-assembly procedure is a time consuming process and also leads to about 10-20% loss of drug to be encapsulated (Arida *et al*., 2007; Ye *et al*., 2005).

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) showing potent anti-inflammatory and analgesic properties. Given by the oral route, Aceclofenac is absorbed into the systemic circulation in a short time with peak plasma concentrations being achieved in 1 to 3 hours. The high concentration with fast absorption of Aceclofenac produces unwanted effects in the gastrointestinal tract (GIT) such as dyspepsia, abdominal pain, nausea, gastritis and ulcerative stomatitis (Radhika *et al*., 2009, Trivedi *et.al,* 2008).

Pectin is a polysaccharide that acts as a connecting layer in the cell wall of all plants. Pectin is formed by the esterification of the polygalacturonic acid, in which all monomeric units of galacturonic acid is connected to each other with 1α-4 linkages. The gel forming property of the pectin mainly depends on the extent of degree of esterification (Ashford *et al*., 1994).

Chitosan is a partly deacetylated chitin, which is a basic polymer having a number of free amino groups. Chitosan generally swells and easily dissolves in the pH range 1-5 is not soluble in basic pH and is resistant to intestinal fluids. The intestinal microflora in the caecum is highly active. The difference between ileum and caecum in bacterial counts per milliliter is 10^2 to 10^9. These colonic bacteria degrade natural polysaccharide polymers in colon and bring about the release of active drug content in colon (Shimono *et al*., 2002).

The present study investigated the colonic delivery of Aceclofenac microcapsules prepared by LBL self-assembly for the local treatment of diseases associated with the colon which cause inflammation and pain.

MATERIALS AND METHODS

Materials

Aceclofenac (MW: 354.2) was obtained from Hab Pharmaceuticals and Research Ltd., Dehradun as a gift sample. Chitosan (MW: 15,000) was purchased from Sd-fine Chemicals Limited, Mumbai, India. Eudragit S-100 was purchased from Rohm Pharma, Darmstadt, Germany. Pectin was obtained as a gift sample from HiMedia Laboratories Ltd, Mumbai, India.

Preparation of Aceclofenac microcapsules

Method

Different charged polymers were used as coating materials on Aceclofenac microcrystal through the LBL technique in acetate buffer of pH 4. The process of sequential adsorption was carried out in several steps. Accurately weighed 150mg of Aceclofenac microcrystals were taken in 20mL of acetate buffer of pH 4 in which the drug was insoluble. Chitosan (0.5-5mg/ml) was added slowly into the beaker in which the drug was already dispersed with mild stirring. The suspension was allowed to stir continuously on a magnetic stirrer for 15-30 minutes so that all the microcrystals were uniformly coated. Coated microcrystals were then separated by centrifugation at 5000 rpm for 5 minutes. Separated microcapsules were then washed with distilled water to remove traces of adsorbed drug on surfaces. The microcapsules were then resuspended in 20ml of pectin solution (0.5-5 mg/ml) and the above steps were repeated to prepare single bilayer microcapsules (Pargaonkar *et al*., 2007).

Experimental design (Taguchi method)

Taguchi is a statistical method of optimizing the best combination of levels of experimental factors. The Taguchi method utilizes a generic signal-to-noise (S/N) ratio to predict the variation present within the different batches. The L-9 array design was applied, which requires nine experiments with four parameters at three levels of each (tables 1 and 2). The interaction was neglected (Dobrzanski *et al*., 2007). There are two S/N ratios which are commonly used for optimization of experimental levels of different factors.

(i) Smaller-The-Better: This is calculated by the following equation $n = -10 \text{ Log}_{10}$ [average of sum squares of all obtained value]

(ii) Larger-The-Better: For optimization, when ideal value is larger-the-better, the following equation can be used $n = -10 \text{ Log}_{10}$ [average of sum squares of reciprocal of all obtained values]

Evaluation of self-assembled microcapsules

Particle size

The average size of single bilayer microcapsules was measured by using Photon Correlation Spectroscopy (PCS) coupled with Malvern zetasizer (Pargaonkar *et al*., 2007).

Zeta potential measurements

Prediction of the reversal of charge after deposition of each polyelectrolyte was determined by the electro-phoretic mobility of microcapsules in a U tube at 25^{o}C, using Malvern Zetasizer (Pargaonkar *et al*., 2007).

Surface morphology

Shape and surface morphology study of the single bilayer microcapsules was carried out by using scanning electron microscopy (SEM) (LEO-430, Cambridge and UK). A drop of sample was put on double adhesive tape, which was struck to an aluminum stub. The sample was allowed

to evaporate. The stubs were then coated with gold under an argon atmosphere. Photographs were taken at 500 times magnification. SEM photographs of uncoated drug microcrystal's single layer microcapsules, and double layered microcapsules were taken (Pargaonkar *et al*., 2007).

Determination of encapsulation efficiency

Microcapsules (100 mg) were dissolved in 30 ml of ethanol:acetone solvent system in the ratio of (2:1) and centrifuged at 2000 rpm for 2-5 minutes and filtered .The filtrate was analyzed for drug content; this filtrate was diluted up to appropriate dilution; and for the determination of Encapsulation efficiency the following formula was used (Radhika *et al*., 2009).

$$\text{Encapsulation efficiency}\,(\%) = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

In vitro drug release studies

The dissolution profile of single bilayer microcapsules was examined in pH 0.1N HCl for 2 h and in phosphate buffer of pH 7.4 for 4 h by using rotating basket method as per USP XX/NFXV Tablet dissolution tester. Single bilayer microcapsules containing aceclofenac (150mg) was filled into gelatin capsule and coated with Eudragit S-100. This capsule was placed in the basket and the dissolution was carried out in 0.1 N HCl for 2 h and in phosphate buffer solution of pH 7.4 for the remaining period at 50 rpm and 37±0.5°C temperature. With the help of 5 mL pipette samples were withdrawn at predetermined time periods and replaced with equal volume of buffer solution. The absorbance was taken by using UV double beam spectrophotometer at 274 nm

Ex vivo drug release in presence of colonic fecal matter of male albino rat

Two rats were killed by spinal traction and their colonic matter was transferred into pH 6.8 Phosphate buffer, continuously supplied with CO_2 to provide anaerobic condition. The drug release studies were carried out in a beaker on magnetic stirrer (200ml) containing 100ml of dissolution medium connected with a conical flask with the help of 'U' tube containing sodium carbonate and citric acid in distilled water for supply of CO_2 to provide anaerobic condition during experiment (Krishnaiah *et al*., 2002; Prasad Rama *et al*., 1998).

Drug release kinetics

To predict the release kinetics of optimized formulations, graphs were plotted as cumulative amount of drug released vs. time (Zero order-equation), log cumulative percentage of drug remaining vs. time (first order-equation), and cumulative percentage of drug released vs. square root of time (Higuchi's model-equation), (Costa and Lobos, 2001).

RESULTS

Particle size

On the basis of results given in table 3, single bilayer microcapsules formed by LBL technique should have minimum particle size for better formulation. From (fig. 1a) it is clear that at level A-2, B-2, C-3 and D-2 the average size of microcapsules will be minimum. They were found to be in the range of 19.0µm -21.5µm. In order to minimize variability the estimated log effect should be lower. The particle size will be smaller when levels are, A- Negligible, B-2, C-3 and D-1 (fig. 1b). In

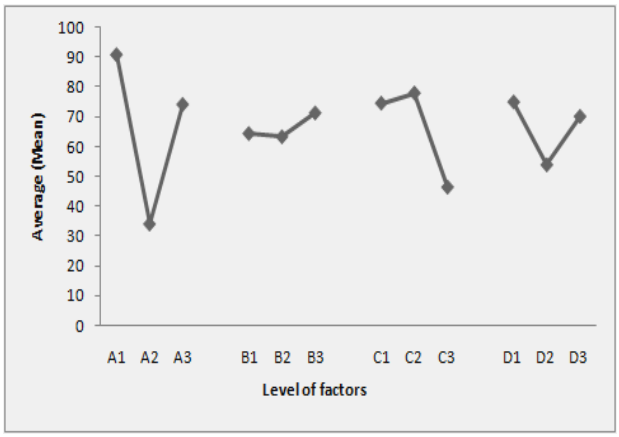
Table 1: Level of process parameters

Variables	Levels		
	Low	Medium	High
A-concentration of chitosan	-1 (0.5mg/ml)	0 (2.5mg/ml)	+1 (4.5mg/ml)
B- concentration of pectin	-1 (0.5mg/ml)	0 (2.5mg/ml)	+1 (4.5mg/ml)
C- incubation time	-1 (15min.)	0 (20min.)	+1 (30min.)
D-centrifugation speed	-1 (5000rpm)	0 (8000rpm)	+1 (10000rpm)

Table 2: Taguchi L_9 (3^4) orthogonal array

Batches	X1	X2	X3	X4
F1	-1	-1	-1	-1
F2	-1	0	0	0
F3	-1	+1	+1	+1
F4	0	-1	0	+1
F5	0	0	+1	-1
F6	0	+1	-1	0
F7	+1	-1	+1	0
F8	+1	0	-1	+1
F9	+1	+1	0	-1

this work, a smaller size is the indication of a better performance. Therefore, the smaller-is-better option for optimum size of microcapsules was preferred. Results of S/N ratio indicates that at level A-1, B-3, C-Negligible, D-3 (fig. 1c) particles having smaller size can be obtained.



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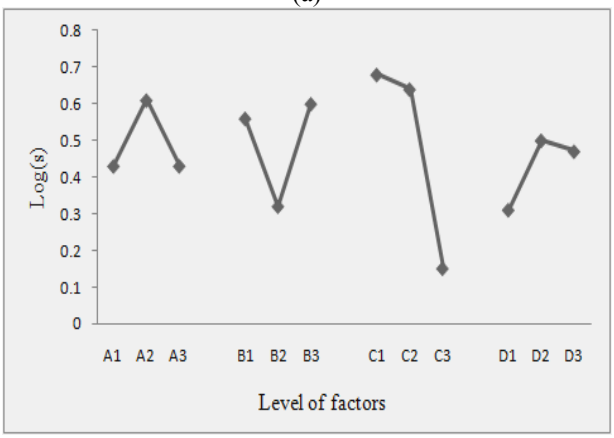

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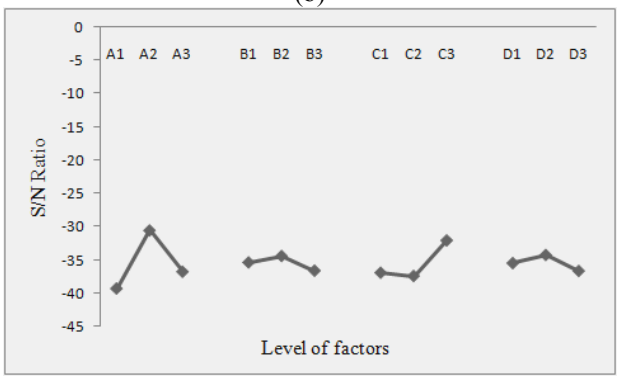
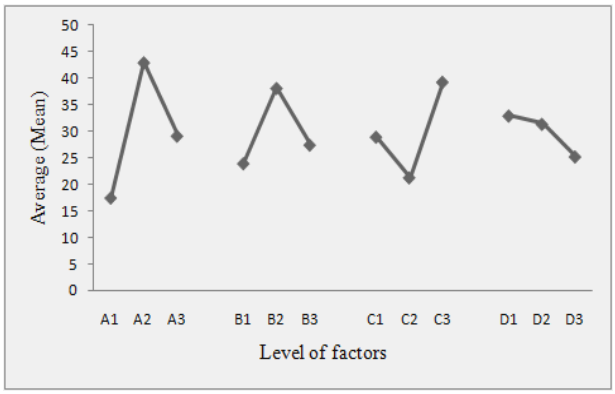


(c)

Fig. 1: Estimated factors effects of average, log(s) and S/N Ratio on particle size.

Encapsulation efficiency

On the basis of results given in table 4, microcapsules formed by LBL technique should have high encapsulation efficiency for better formulation. From (fig. 2a), it is clear that at level A-2, B-2, C-3 and D-1 delete encapsulation efficiency of microcapsules will be high and was found to be in the range of 62.0-65.5%. In order to minimize variability the estimated log effect should always be low and then encapsulation efficiency will be high, when levels are taken as A-3, B-3, C-2 and D-1(fig. 2b). In this work, the encapsulation efficiency of microcapsules should be high for better results. Therefore, the larger-is-better option was selected for optimum encapsulation efficiency. Results of S/N ratio indicate that at level A-2, B-2, C-3, D-2 (fig. 2c) high encapsulation efficiency can be obtained.



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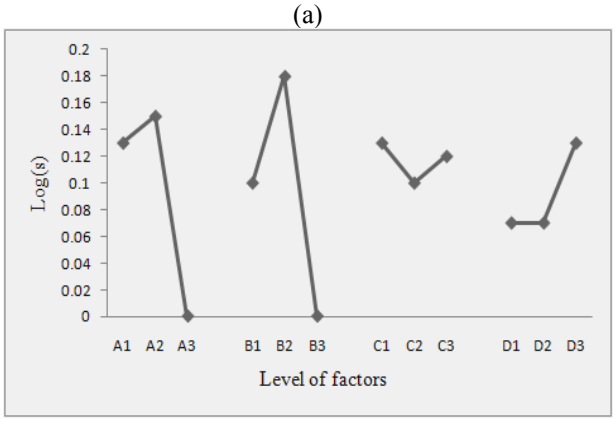

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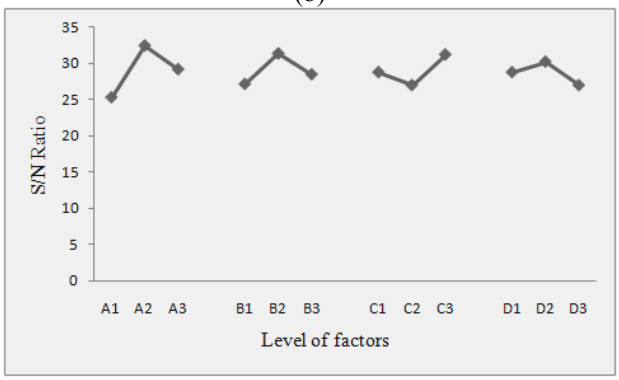

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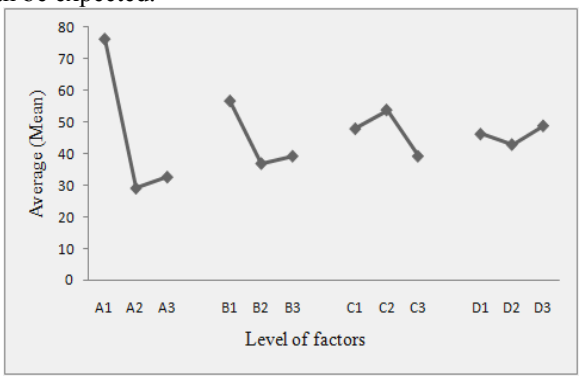
Fig. 2: Estimated factors effects of average, log(s) and S/N Ratio on encapsulation efficiency

In vitro drug release at pH 7.4

On the basis of results given in table 5, microcapsules formed by LBL technique must shield drug release in GIT

and release the drug in colon to confirm colon targeting of microcapsules. From (fig. 3a) it is clear that at level A-2, B-2, C-3 and D-2, the drug release from microcapsules will be lowest. In order to minimize variability the estimated log effect should always be low, that is drug release will be low when levels are taken as A-1, B-1, C-2 and D-1 (fig. 3b). In this work, the drug release of microcapsules will be lowest for better results. Therefore, the smaller-is-better option was selected for optimum drug release. Results of S/N ratio indicate that when levels were taken as A-2, B-3, C-3, D-1 (fig. 3c) then a higher degree of protection of drug from release in GIT can be expected.



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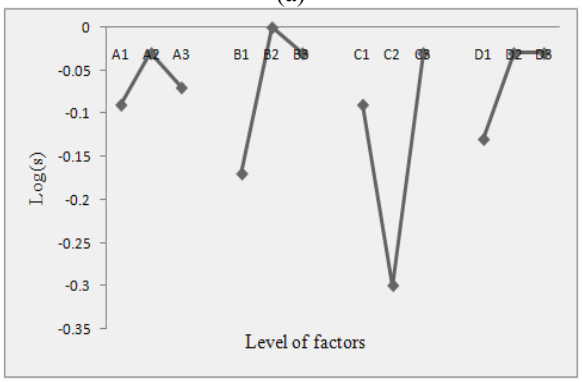

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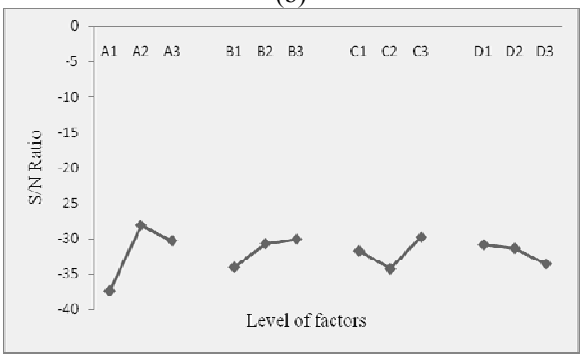


(c)

Fig. 3: Estimated factors effects of average, log(s) and S/N Ratio on *in vitro* drug release.

From (fig. 4) it is clear that no drug release was observed for 2h in 0.1N HCl due to coating of Eudragit S-100 onto the capsule in which single bilayer microcapsules were filled for oral drug delivery for colon targeting were taken. For next 4 h drug release studies were carried out at pH 7.4 because Eudragit S-100 starts dissolving at above pH 7.0 which would be the case in the ileum part of intestine.

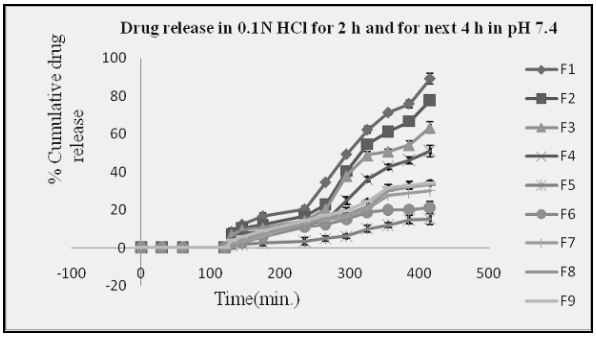


Fig. 4: *In vitro* drug release of formulations (F1-F9).

On the basis of results of Taguchi design (table 6 and 7) F5 formulation was selected as the one having all the optimum values. It means when concentration of chitosan and pectin was at medium level and when incubation time was high with low centrifugation speed a better formulation was formed

DISCUSSION

Formulation F5 provides better protection of drug release from microcapsules in GIT than other formulations. This is mainly due to formation of interpolymer complex between chitosan and pectin at medium concentration level in acidic pH, because chitosan is ionized at acidic pH and to some extent, pectin too. Both materials are soluble at this pH and an interaction was observed between the carboxyl groups of the pectin and the amino groups of the chitosan. In basic medium, the protection is offered by insolubility of chitosan at higher pH. The incubation time was found to be high to ensure uniform coating of microcrystals so that the maximum amount of drug from microcapsules was released in colon. At higher centrifugation speed the coated drug particles formed clumps which was not redispersed but at lower centrifugation speed micro-capsules were easily separated and redispersed (fig. 5).

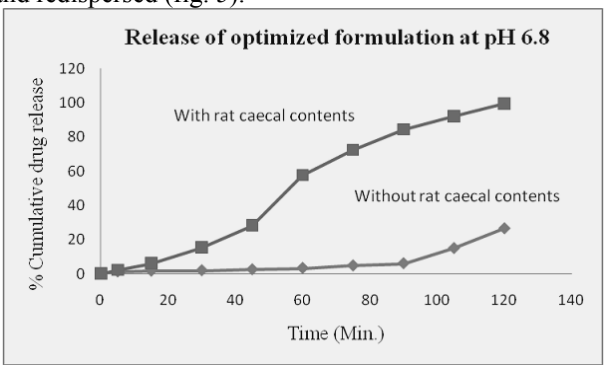


Fig. 5: Drug release in presence and absence of rat caecal content.

Table 3: Experimental data (optimization of particle size)

S. No.	Observed particles size			Mean	S.D*	Log of S.D*	S/N Ratio
1	100	110	107	105.66	5.13	0.71	-40.48
2	86	85	90	87	2.64	0.421	-38.79
3	80	78	81	79.66	1.52	0.181	-38.02
4	55	45	43	47.66	6.42	0.807	-33.61
5	19	19.5	21.5	20	1.32	0.120	-26.03
6	37	48	32	35	8.13	0.91	-31.94
7	40	41.5	38.5	40	1.50	0.17	-32.04
8	80.5	83	86	83.16	2.75	0.43	-38.40
9	105	95	98	99.33	5.13	0.71	-39.94

*S.D Standard deviation

Table 4: Experimental data (optimization of encapsulation efficiency)

S. No.	Encapsulation efficiency			Mean	S.D*	Log of S.D*	S/N Ratio
1	12.5	13.5	15	13.66	1.25	0.096	23
2	20.5	18	17.5	18.66	1.60	0.204	26.9
3	21	18.5	20	19.83	1.26	0.10	26
4	22	24.5	25	23.83	1.60	0.204	27.6
5	62	65.5	64	63.83	1.75	0.243	36.9
6	40	41.5	42	41.16	1.04	0.017	33
7	35.0	34.5	33.0	34.16	1.04	0.017	30.9
8	32.0	33.0	30.5	31.83	1.25	0.096	30.4
9	20.5	21.0	22.0	21.16	0.76	-0.119	26.5

*S.D Standard deviation

Table 5: Experimental data (optimization of in-vitro drug release)

S. No.	Cumulative %Release at pH 7.4			Mean	S.D*	Log of S.D*	S/N Ratio
1	89.0	88.0	88.5	88.9	0.5	-0.30	-38.23
2	78.0	77.5	76.0	77.16	1.04	0.01	-37.95
3	63.0	62.50	61.0	62.16	1.04	0.01	-36.02
4	50.0	51.50	50.50	50.66	0.76	-0.11	-34.20
5	15.0	14.50	16.50	15.33	1.04	0.01	-23.67
6	20.0	21.50	22.0	21.16	1.04	0.01	-26.49
7	31.0	30.5	29.50	30.16	0.76	-0.11	-29.66
8	34.50	32.50	33.50	33.33	1	0	-30.50
9	35.0	34.0	33.50	34.16	0.76	-0.11	-30.70

*S.D Standard deviation

Surface electric potential of Aceclofenac microcrystals was found to be negative with zeta potential -1.39 mV, in acetate buffer of pH 4. The primary and the secondary deposit layers of chitosan and pectin were found to have a positive and negative charge with zeta potential of +5.57 mV and -22.8 mV respectively. The sequential changing of Surface zeta potential after each deposition is a satisfactory indication of the LBL self-assembly of the oppositely charged polyelectrolytes.

Shape and surface morphology studies of the single bilayer microcapsules were carried out by using scanning electron microscopy (SEM) indicating that the average particle size of optimized formulation was 20µm (fig. 6).

The release profiles of optimized formulation were explained by a model that represents systems where drug diffusion occurs through a polymeric structure, $M_t/M_\infty = k_t^n$

(r^2=0.950) where M_t/M_∞ is the fractional release of the drug, t is the release time, k is a constant, and n is the release constant, indicative of the mechanism of drug release. From (table 8), it was clear that optimized formulation followed first order release kinetic (r^2=0.950) and Quasi-Fickian diffusion mechanism (n=0.017) in the presence of colonic fecal matter of male albino rat.

Table 6: Summary of analyses of factor effects

Factor	Mean	Log(s)	S/N Ratio
Particle size			
A	0	_	-1
B	0	0	+1
C	+1	+1	_
D	0	-1	+1
Encapsulation efficiency			
A	0	+1	0
B	0	+1	0
C	+1	0	+1
D	-1	-1	0
***In vitro* drug release**			
A	0	-1	0
B	0	-1	+1
C	+1	0	+1
D	0	-1	-1

Table 7: Final optimized parameters values

Factor	Optimized level
A	0 (Medium concentration of chitosan)
B	0 (Medium concentration of pectin)
C	+1 (High incubation time)
D	-1 (Low centrifugation speed)

REFERENCES

Allen LV, Popovich NG and Ansel HC (2005). Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. BI Publication, Delhi, p.265.

Arida AI, Moawia M and Tabakha A (2007). Encapsulation of ketoprofen for controlled drug release. *Eur. J. Pharm. Sci*., **66**: 48-54.

Ashford M, Fell J, Attwood D, Sharma H and Wood P (1994). Studies on pectin formulations for colonic drug delivery. *J. Cont. Rel*., **30**: 225-232.

Biguccia F, Luppia B and Cerchiarac T (2008). Chitosan/pectin polyelectrolyte complexes: selection of suitable preparative conditions for colon-specific delivery of vancomycin. *Eur. J. Pharm. Sci.,* **3**: 435-444.

Chourasia MK and Jain SK (2003). Pharmaceutical approaches to colon targeted drug delivery systems. *J. Pharm. Pharm*. Sci., **6**: 33-66.

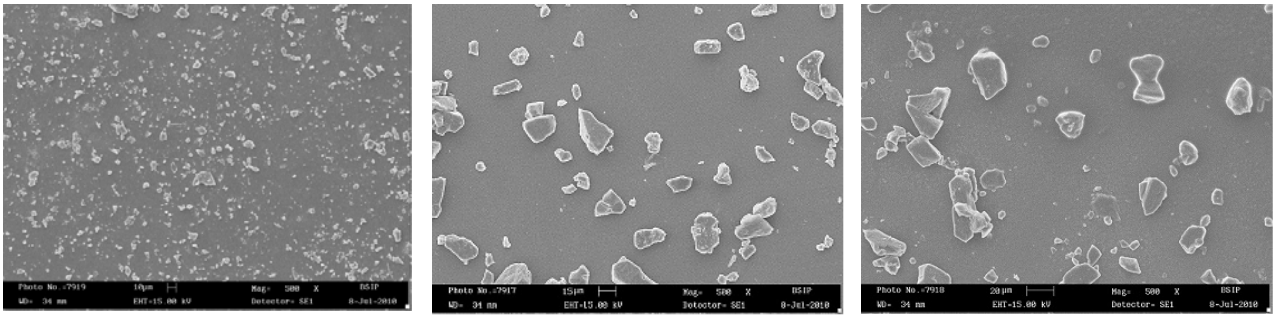
Costa P and Lobo SMJ (2001). Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.,* **13**: 123-133.

Dobrzanski LA, Domagala J and Silva JF (2007). Application of taguchi method in the optimization of filament winding of thermoplastic composites. *Arch. of Mat. Sci. and Engg*., **28**(3): 133-140.

Krishnaiah YSR, Reddy PR and Satyanarayana V (2002). Studies on the development of oral colon targeted drug delivery systems for metronidazole in the treatment of amoebiasis. *Int. J. Pharm.,* **236**: 43-55.

Pargaonkar N, Lvov MY, Li N and Villiers DM (2005). Controlled release of dexamethasone from microcapsules produced by polyelectrolyte layer-by-layer nanoassembly. *Pharm. Res*., **22**: 826-835.

Prasad Rama YV, Satyanarayana S and Krishnaiah YSR (1998). Evaluation of guar gum as a compression coat



(A) Uncoated drug crystals (B) Microcapsules after first coating (C) Microcapsules after second coating

Fig. 6: (A) Uncoated drug crystals, (B) Microcapsules after first coating, (C) Microcapsules after second coating.

Table 8: *In vitro* release kinetics parameters of optimized formulation in presence of rat caecal contents

Batch	Zero order		1st order equation		Korsmeyer Peppas equation		Higuchi equation	
	k	R^2	k	R^2	N	R^2	K	R^2
F5	0.798	0.921	0.019	0.950	0.017	0.773	7.48	0.756

for drug targeting to colon. *Int. J. Pharm*., **171**: 137-146.

Radhika RP, Luqman M and Borkhatania HC (2009). Preparation and evaluation of delayed release microspheres of aceclofenac. *Asian J. Pharm. Sci*., **56**: 221-225.

Satyanarayana S, Krishnaiah YSR, Rama Prasad YV and Rao S (1998). Evaluation of guar gum as a compression coat for drug targeting to colon. *Int. J. Pharm.,* **171**: 137-146.

Shimono N, Takatori T, Ueda M, Mori M and Higashi Y (2002). Chitosan dispersed system for colon-specific drug delivery. *Int. J. Pharm*., **245**: 45-54.

Trivedi P, Verma A and Garud N (2008). Preparation and characterization of aceclofenac microspheres. *Asian J. Pharm. Sci*., **2**: 110-115.

Ye S, Wang C, Xingxing L and Zhen T (2005). Deposition temperature effect on release rate of indomethacin microcrystals from microcapsules of layer-by-layer assembled chitosan and alginate multilayer films. *J. Cont. Rel*., **106**: 319-328.