Research Article

In Vitro Colon Specific Delivery of Optimized Degradable Alginate Beads Containing Entrapped pH-Responsive and Non pH-Responsive Drugs

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ABSTRACT

Colon specific drug dissolution studies should either mimic the fed/fasted (3 h) or pre-feed (< 3 h) small intestinal transit times because a previous study has revealed a shorter (< 3 h) small intestinal transit time after administering drug 30 or 45 min before food (pre-feed). Faecal matter/bacterial enzymes must be incorporated in colonic dissolution fluid to induce disintegration of alginate-based tablets; however alginate-based dosage form alternative that excludes faecal matter is preferable. Therefore, our aim was colon-specific dissolution study of pH-responsive Diclofenac sodium (DS) and non pH-responsive Acetaminophen (ACP) respectively as model drugs for mimicking pre-feed (< 3 h) and fed/fasted (3 h) small intestinal transit times using optimized non faecal matter-requiring degradable alginate micro beads. Swelling, degradability and dissolution studies were carried out on experimental design-optimized coated and uncoated beads. Alginate beads completely degraded (without faecal/ bacterial content) in pH 7.4 but swelled in pH 6.8. pH-responsiveness of DS was a stronger determinant of release from uncoated micro beads in SIF 6.8 than dissolution duration. However, for (coated+neusilin®) micro beads the duration of dissolution determined the quantity of drug released. In conclusion, the observed significant difference in quantity of drug released in (SIF, pH 6.8) between 2 and 3 h suggests that 2 h may be specified to mimic pre-feed state, in addition to 3 h fed/fasted state intestinal transit time during in vitro colon-specific drug dissolution studies.

Keywords: Colon delivery, alginate beads, pH-Responsive Drugs

INTRODUCTION

The NSAID DS is a known inhibitor of prostaglandin synthesis with anti-inflammatory, analgesic, antipyretic and antimicrobial activities[1]. To avoid dose-related cardiovascular adverse effects similar to those caused by celecoxib, the lowest effective dose within the shortest duration has been advocated for those on DS (NSAID) prescription including colitis[2]. Alternatively, the development of colon targeted DS formulation may assuage or preclude its adverse effects while exerting local anti-inflammatory/analgesic activity. However, the analgesic property of ACP may be a preferred option for the contraindicated use of NSAIDs in the management of Irritable bowel disease (IBD) and its pain. NSAIDs constitute a contributing factor to the pathogenesis of IBD which is linked to non-selective inhibition of COX-1 and COX-2 [3]. If the excruciating pains must be alleviated, NSAIDs or morphine avoided in IBD, then tolerable ACP preferably targeted to the colon may provide relief for a longer period of time because of direct and prolonged local contact with inflamed tissues.

Sodium alginate is a biocompatible gel-forming non-starch polysaccharide that is degradable in the microflora-rich large intestinal environment. The use of calcium alginate as a tablet matrix for colon targeting often requires the introduction of faecal matter/microbial suspension into colonic dissolution fluid to mimic the colonic physiological environment [4]. Without this the tablet may not disintegrate. In this work we thought of an alternative alginate-based dosage form (bead) that is amenable to physical degradation without requiring the incorporation of faecal suspension.

Application of optimization in drug manufacturing is a cost-effective time-saving design approach that builds enhanced quality into the dosage form. Optimization involves the identification of the effective amount of ingredients that will maximize yield. JMP software (SAS Inc USA) was used to optimize the production of DS and ACP beads in this work.

Different *in vitro* models for demonstrating drug release from formulations intended for colon targeting have been documented in literature[5, 6]. These models draw justification basically from the knowledge of gastrointestinal transit time, physiology, pH changes and the capacity of the colon microflora to degrade certain polysaccharide drug carriers [7]. An excellent review shed light on the need to design efficient *in vitro* dissolution testing of polysaccharide-based colon specific delivery methods which are predictive of *in vivo* outcome [8].

The widely accepted small intestinal transit time of 3 h constitutes the basis for conducting dissolution studies for 3 h to simulate the small intestinal environment for colon targeted drugs [9]. However, different workers have adopted dissolution models that seem to suit their dosage form design [10, 11, 12]. Typically, these models should be harmonized and categorized to ensure reliability, discriminability, reproducibility [8] and correlability with parallel *in vivo* investigations. The food effect that

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predisposes to 3 h fasted/fed intestinal transit time differs from that that leads to pre-feed transit time. Though unpopular amongst drug delivery experts, the pre-feed intestinal transit time, was reported to be shorter (approximately 100 min) than the fed/fasted [13]. This <3 h duration was due to food-occasioned gastrointestinal tract (GIT) motility. Knowledge of this time disparity could be exploited to address some *in vitro-in vivo* correlation challenges in colon targeted drug delivery systems (CTDDS) such as non-disintegration of tablets caused by early propulsion from the small intestine or inadvertent drug release caused by delayed intestinal residency [10].

In this work we have assumed that: (1) Ionization effect may enhance the dissolution of ionizable DS more than non-ionizable ACP. Consequently, DS destined to the colon should be preconditioned to spend less but optimal time in the upper intestine to forbid profuse drug dissolution caused by enhanced ionization. This concern may not apply to non-ionizable ACP, whose delayed residency in the high-pH environment of the small intestine may not occasion profuse drug release. (2) Given that the length of time spent in the small intestine may affect colon targetability of entrapped drug, discriminatory *in vitro* dissolution studies identifying CTDDS capable of delivering high cargo to the colon under pre-feed (2 h/ <3 h) or fed/fasted (3 h) transit times need to be established; so that patients are subsequently advised to take food at a ´designated time´ after drug administration, instead of the conventional take after / before food which lacks time specificity. It is possible for a small patient population on colon targeted drugs to witness small intestinal transit time (SINTT) of < 3 h. Also, the high dietery fibre-rich menu of Africans is associated with reduced SINTT [14 15]. In spite of the fractional paucity of this patient population, a drug delivery approach capable of addressing their health challenges should be a welcome development. Therefore, the aim of our present investigation was *in vitro* colon specific dissolution study evaluation of choice of pH-responsive Diclofenac sodium (DS) and non pH-responsive Acetaminophen (ACP) respectively as model drugs for mimicking pre-feed (2 h/< 3 h) and fed/fasted (3 h) (SINTT) using optimized non faecal matter-requiring degradable alginate micro beads. In brief, dissolution studies were run for 2 h in simulated gastric fluid (SGF), pH 1.2 (mimicking the stomach); 3 h (for ACP) and 2 h (for DS) in phosphate buffer simulated intestinal fluid (SIF), pH 6.8 (mimicking the small intestine) and finally 4 h and 2 h respectively in phosphate buffer pH 7.4 (mimicking the colon) for both drugs.

MATERIALS

Acetaminophen and Diclofenac sodium were kind gift samples from Juhel Nigeria Ltd. Sodium alginate (Acros Organics, USA), Neusilin® FH2 ([Magnesium aluminometasilicate] Fuji Chemical Ltd, USA), Calcium chloride dihydrate, Sodium chloride and Sodium hydroxide (BDH, England), Monobasic potassium phosphate (Hopkin and Williams Ltd, England), Conc. Hydrochloric acid (Qualikem, India). All other reagents were of Analytical grade and were used as such.

METHODS

Production of Diclofenac sodium and Acetaminophen-loaded alginate beads using factorial experimental design

A 2^3 randomized full factorial design (JMP 4.04 SAS, USA) was employed in this study. In this design, three formulation variables (polymer concentration, drug-polymer ratio and calcium chloride concentrations) were used (Table 1). The high and low concentration ranges were slotted into the software which generated 22 formulas, from where 22 batches of DS and ACP beads were produced (Table 2). In brief, the appropriate quantity of sodium alginate was dispersed in distilled water and stirred (IKA RCT, Germany) until a 1-3 % w/v smooth gel was formed. Using a 10 mL hypodermic syringe of needle size 18G, drop-wise quantities of the gel (10 mL) were introduced into 100 mL of 5-10 % w/v calcium chloride dihydrate solution in a 200 mL beaker and stirred magnetically (IKA RCT, Germany) at 150 rpm. The formed beads were further allowed a curing time of 5 min, filtered (Whatman No1 filter paper), washed with distilled water, spread on a polyethylene material and air-dried for 30 min before drying in a desiccator. The following evaluations were carried out on the 44 batches of beads: bead size, percent bead yield, entrapment efficiency and sphericity.

Table 1: Formulation design containing a set of low and high values

	Drug:Polymer	Polymer (%)	Calcium chloride (%)
Acetaminophen	0.5-2	1-3	5-10
Diclofenac sodium	0.5-1	1-3	5-10

Bead Yield

The dry beads were weighed and the % bead yield calculated using the equation below:

$$\text{Bead yield (\%)} = \frac{\text{Amount of beads produced}}{\text{Amount of starting materials}} X\ 100 \ldots\ldots\ldots\ldots\ldots Eqn\ 1$$

Bead Size Measurement

The photomicrographs of the beads were imaged using an optical microscope with a X40 objective lens. The bead size was calculated using an attached camera and software (Motic Image plus 2.0[ML], China).

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Table 2: Formulas generated by the software (MP 4.04 SAS USA)

Diclofenac sodium	Pattern	Drug: polymer	Polymer (%)	$CaCl_2$ (%)	Response	Acetaminophen	Pattern	Drug: Polymer	Polymer (%)	$CaCl_2$ (%)	Response
D1	--+	0.5	1	10		P1	+++	2	3	10	
D2	+-+	1	1	10		P2	++-	2	3	5	
D3	000	0.75	2	7.5		P3	-++	0.5	3	10	
D4	++-	1	3	5		P4	-+-	0.5	3	5	
D5	+--	1	1	5		P5	000	1.25	2	7.5	
D6	+++	1	3	10		P6	000	1.25	2	7.5	
D7	---	0.5	1	5		P7	---	0.5	1	5	
D8	+-+	1	1	10		P8	++-	2	3	5	
D9	+++	1	3	10		P9	--+	0.5	1	10	
D10	000	0.75	2	7.5		P10	+--	2	1	5	
D11	--+	0.5	1	10		P11	+-+	2	1	10	
D12	000	0.75	2	7.5		P12	+++	2	3	10	
D13	+--	1	1	5		P13	000	1.25	2	7.5	
D14	000	0.75	2	7.5		P14	000	1.25	2	7.5	
D15	-++	0.5	3	10		P15	+--	2	1	5	
D16	---	0.5	1	5		P16	000	1.25	2	7.5	
D17	-+-	0.5	3	5		P17	+-+	2	1	10	
D18	-++	0.5	3	10		P18	--+	0.5	1	10	
D19	000	0.75	2	7.5		P19	---	0.5	1	5	
D20	-+-	0.5	3	5		P20	-++	0.5	3	10	
D21	000	0.75	2	7.5		P21	000	1.25	2	7.5	
D22	++-	1	3	5		P22	-+-	0.5	3	5	

Drug content and Entrapment Efficiency

A quantity of beads from each batch representing a theoretical amount equivalent to 10 mg of drug (ACP and DS respectively) was weighed out. The beads were crushed in a mortar and 80 mL of SIF, pH 6.8 added prior to subsequent transfer to a 150 mL volumetric flask. The flask was made up to 100 mL volume, stirred and filtered (Whatman No. 1 filter paper) and 1mL further diluted to 10 mL volume with SIF. The solution was spectrophotometrically (Jenway® 6305 UV/VIS spectrophotometer, Barloworld Scientific Ltd., Essex CMB 31BWL, UK) assayed for ACP and DS content at a predetermined wavelength of 298.6 and 281 nm respectively. Triplicate determinations were made for all the batches. From the drug content the Entrapment efficiency, EE (percent fraction of the theoretical quantity of drug entrapped in the beads) was therefore calculated from the following equation:

$$EE = \frac{\text{Estimated drug content}}{\text{Theoritical drug content}} \times 100 \quad \ldots\ldots\ldots (2)$$

Out of the other dependent variables EE was chosen as the parameter for optimization. The EE values were slotted into the response column of the software and the model run; this resulted to graphs (Figures 1-2) and a Table of 125 formulas (not shown) and corresponding predicted EE values. On this premise one optimal EE value and the corresponding formula was selected for each drug and translated into a formulation. The formulations were evaluated for EE and the values compared with those of software-predicted ones. A new batch of the optimized beads was produced to contain 100 mg neusilin® FH2. The neusilin-containing beads were subsequently coated with Eudragit S-100. Coating was carried out according to the method of previous workers with modification[16]. Briefly, Eudragit S–100 solution (10 % w/v in 90 % ethanol) was atomized in the drying chamber of a pilot scale spray dryer (Buchi mini sprayer B-290, Switzerland) containing the beads until about 16 % w/w coating concentration was achieved. Swelling and dissolution studies were carried out on uncoated, coated and coated+neusilin® batches.

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Swelling studies

Swelling studies were carried out on the uncoated, coated and coated+neusilin® optimized batches respectively in pH 1.2 and 6.8 respectively[17] according to the formula below. Triplicate determinations were carried out.

$$Swelling\ index = \frac{W_2 - W_1}{W_1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Where W_2 is the weight of the swollen beads and W_1 the initial weight of the beads.

Bead Degradability without cecal suspension

About 60-100 mg quantity of beads was introduced into 100 mL of phosphate buffer (SIF pH 6.8 or 7.4) at 37±1°C in a 250 mL beaker and magnetically stirred at 100 rpm. The time for complete disintegration of beads was noted.

***In vitro* drug release Studies**

The *in vitro* drug release profiles of ACP and DS from the optimized alginate beads were studied using the Beaker method. The dissolution media consisted of simulated gastric fluid (SGF, pH 1.2) and Phosphate buffer-based simulated intestinal fluid (SIF, pH 6.8 and pH 7.4). In this study we investigated the impact of carrying out *in vitro* colon specific drug release studies in pH 1.2, 6.8 and 7.4 respectively each for 2 h, 2 h and 2 h respectively (DS), in comparison with 2 h, 3 h and 4 h respectively (ACP). Beads from each formulation representing an amount equivalent to 100 and 200 mg of DS and ACP respectively were placed inside a basket and the set-up introduced into 900 mL of freshly prepared SGF maintained at 37 ± 1°C and rotation speed of 100 rpm. 10 mL sampling at predetermined time intervals was followed by fresh equivalent volume replacement. After two hours, the SGF was replaced with 900 mL of freshly prepared SIF, pH 6.8. The dissolution in SIF, pH 6.8 was carried out for another 2 h (DS) or 3 h (ACP). The same procedure was repeated in SIF, pH 7.4 for 2 h (DS) and 4 h (ACP) respectively. Filtered (Whatman No. 1) samples were spectrophotometrically assayed for Acetaminophen or Diclofenac sodium content as earlier described. The dissolution runs were in triplicates.

***In vitro* release kinetics**

In order to understand the kinetics and mechanism of drug release in pH 6.8 and 7.4 respectively, the dissolution data obtained were fitted into various models (Zero order, First order, Higuchi and Korsmeyer-Peppas).

Zero order (concentration-independent drug release): Plot of cumulative amount of drug released vs Time.

$$C = K_0 t \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 4$$

First order: Plot of Log cumulative amount of drug remaining vs Time.

$$Log\ C_t = Log\ C_0 - \frac{K_t}{2.303t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 5$$

Higuchi: Plot of quantity of drug released vs time

$$Qt = K_H t^{1/2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 6$$

Korsmeyer-peppas release model considers drug release mechanism from polymeric matrices [18,19]. Log fraction of drug released was plotted against Log Time.

$$\frac{M_t}{M_\infty} = Kt^n \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 7$$

Where C_t=drug concentration at time t; C_0=Initial drug concentration; K_0=zero order release rate constant; K_t= first order release rate constant; K_H=Higuchi release rate constant; $\frac{M_t}{M_\infty}$=Fraction of drug released; n=the diffusional exponent which indicates the mechanism of drug release; K_{kp}= Korsemeyer-peppas drug release constant. Values of n=0.5 indicate Fickian diffusion (case 1) or square root of time kinetics; 0.5<n<1 is indicative of non-Fickian diffusion (anomalous transport); and n=1.0 indicates zero order transport (case 11 or relaxation controlled). Values of n>1.0, indicate super case II type of release. Case II generally refers to the relaxation/erosion of the polymeric chain and anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion-controlled drug release.

Scanning electron microscopy of the optimised beads

The palladium-gold-coated beads were imaged with the SEM machine (Phenome, Fei Company, USA) at x1000 and 4000 magnifications respectively.

Fourier Transform Infra Red Spectroscopy (FTIR)

Into a disc was introduced a milled quantity of a mixture of 0.4g of KBr and 0.001g of the drug powder or beads after thorough mixing. The disc was inserted into the sample compartment of the instrument and the machine operated to generate the various spectra.

Statistics

Statistical analysis was carried out using GraphPad InStart Software Inc., USA. P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Factorial experimental design

Twenty two formulations of Acetaminophen and Diclofenac sodium beads resulted from the twenty two formulas generated by the software. Subsequently, they were evaluated for % Bead yield, Encapsulation efficiency and Bead size. The results of the aforementioned parameters are shown in Table 3. The percent bead yield of the formulations ranged from 67-96 % and 27-94 % for DS and ACP respectively. There was no remarkable trend, however (+ + +) batches recorded higher bead yield than the (- - -) batches. This indicates that maximum concentration of calcium chloride was necessary for efficient cross linking and high bead yield. The average particle size of Diclofenac and Acetaminophen beads ranged between 720-1010 and 495-1488 µm respectively. These sizes appeared to be higher with higher

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concentration of calcium chloride. After drying, majority of the beads retained their spherical shapes while the others were either oblong or irregularly shaped.

Table 3: Some properties of the Twenty two Formulations generated by the software

Diclofenac sodium	Pattern	Bead Yield (%)	Bead size (µm)	Encapsulation Efficiency (%)	Acetaminophen	Pattern	Bead yield (%)	Bead size (µm)	Encapsulation Efficiency (%)
D1	--+	84	578	95.4	**P1**	+++	83.3	880	85.49
D2	+-+	79	495	83.0	**P2**	++-	58.3	820	72.54
D3	000	86	657	52.6	**P3**	-++	94	910	82.94
D4	++-	67	876	83.4	**P4**	-+-	63	720	62.47
D5	+--	76	628	75.6	**P5**	000	73	730	67.36
D6	+++	93	1016	79.3	**P6**	000	70	800	62.28
D7	---	71	751	76.6	**P7**	---	44.4	910	51.94
D8	+-+	79	784	86.7	**P8**	++-	57.4	770	75.23
D9	+++	91	1133	84.9	**P9**	--+	72.2	900	76.54
D10	000	76	956	98.7	**P10**	+--	27.8	870	62.85
D11	--+	80	808	94.1	**P11**	+-+	66.8	970	31.09
D12	000	72	1090	48.2	**P12**	+++	91.8	1010	95.51
D13	+--	72	1030	80.3	**P13**	000	80.1	740	75.13
D14	000	76	1000	70.1	**P14**	000	76	840	77.72
D15	-++	96	1304	97.8	**P15**	+--	33.3	730	51.81
D16	---	84	903	67.3	**P16**	000	86	830	70.04
D17	-+-	84	1357	85.8	**P17**	+-+	55.7	960	49.42
D18	-++	77	1390	95.1	**P18**	--+	70	870	78.17
D19	000	72	1010	95.7	**P19**	---	61.1	890	52.77
D20	-+-	81	1488	89.5	**P20**	-++	70	890	78.03
D21	000	70	1000	50.7	**P21**	000	65.3	720	77.58
D22	++-	76	1006	94.1	**P22**	-+-	72.2	740	77.96

EE was preferred for further optimization studies to determine the excipients contributing the most significant effect. Traditional formulation designs adopt the approach of varying one excipient concentration while other excipients have their concentrations constant [20]. In factorial design the interaction between one excipient and the other at different concentration levels and how it affects the dependent variables is an added advantage. In the foregoing the EE values from Table 3 were slotted into the Response column of the software and the model run. This resulted to graphical representations of the level of significance exerted by alginate, calcium chloride or drug:polymer ratio and their interactions, on the variations observed in EE. In addition 125 formulas and their corresponding predicted EE values (not shown) were also generated by the software. From these values, the optimal formula with its corresponding predicted EE was selected for Acetaminophen and Diclofenac sodium respectively. The formulas were translated into bead formulations and evaluated for EE, swelling and *in vitro* release studies.

Table 4 shows the predicted EE values of the optimized formulations from the desirability Table and the experimental EE values after formulation and evaluation. The result indicates that the predicted and experimental EE values were similar. This casts reliability on the model used to forecast the EE and on the bead formulation process.

The Whole model plot (not shown) of Actual (experimental) EE vs predicted EE resulted to 95% confidence curves and regression line passing through the sample mean line at $p<0.05$. This was an indication that the whole model plot described a significant portion of the variation in EE.

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Table 4: Experimental and predicted EE values of Optimized Acetaminophen and Diclofenac Alginate beads

Formulation	Formulation variables			Experimental	Software predicted
	Drug:Polymer	Polymer (%)	Calcium Chloride (%)		
Acetaminophen	2	3	10	92.81±3	89.85
Diclofenac sodium	0.5	3	10	89.38±2	92.08

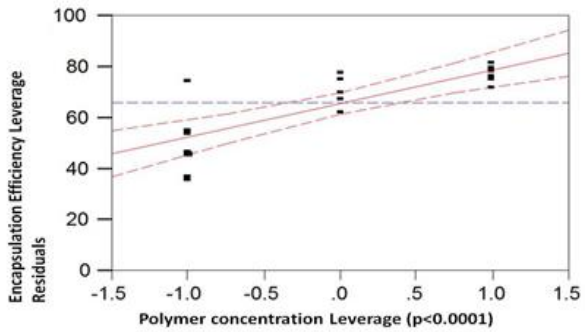

Figure 1: Plot of Encapsulation Efficiency Leverage Residuals vs Polymer concentration Leverage

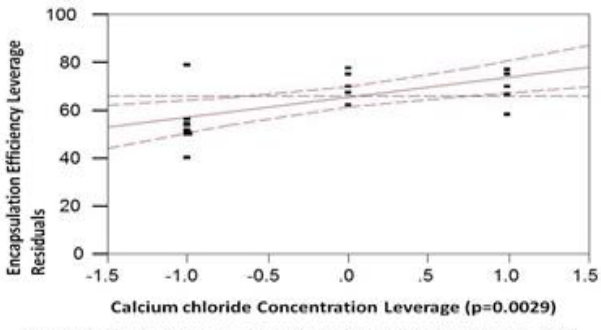

Figure 2: Plot of Encapsulation Efficiency Leverage Residuals vs Calcium chloride concentration Leverage

Figures 1-2 are representations of the level of significance contributed by the independent variables. Unfortunately, DS formulations did not have graphs with p values less than 0.05, therefore they were not treated further. On the other hand ACP had graphs that portrayed significance, thus we concentrated on them.

The leverage plots for polymer concentration (Figure 1), Calcium chloride concentration (Figure 2) and Drug:polymer ratio*polymer interaction (not shown) also had the 95% confidence curves and regression line passing through the sample mean line. This shows that they significantly ($p<0.05$) contributed to differences in EE. Future production and scale-up could therefore incorporate proportions of materials with the potential to yield optimum EE.

Swelling Studies

The result of swelling studies of the optimized formulations as shown in Figure 3, indicate that, in SGF the (coated + neusilin®) batches of DS beads did not undergo swelling while the uncoated and coated batches swelled minimally. For ACP beads, the coated and coated+neusilin® batches recorded no swelling whereas the uncoated batch had minimal swelling. Within alkaline media swelling index improved, with the uncoated beads ranking highest in fluid imbibition. Little or no swelling in acidic pH in contrast to generous fluid sorption and swelling in alkaline fluid is consistent with previous report[21]. At low pH the carboxylic group in alginate exists mostly as COOH. However, at higher pH it exists as -COO^- upon ionization. This culminates in weak H-bonding association between polymer chains and electrostatic repulsion of –COO^- groups, consequently swelling ensues[21, 22]. Generally, coating and the inclusion of neusilin® were intended to reinforce gastric protection thereby controlling the rate of fluid sorption.

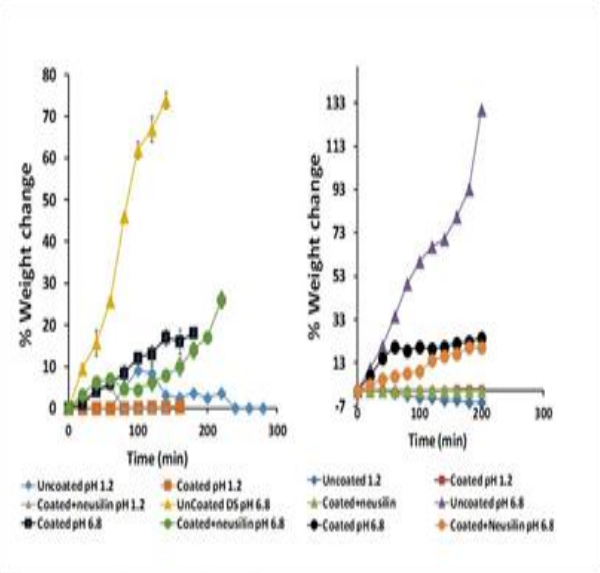

Figure 3: Swelling profile of Diclofenac sodium and Acetaminophen beads in acidic and alkaline media

Degradability of beads

Figure 4 is representative of the degradability behaviour of the beads in alkaline media. In pH 7.4 the DS and ACP beads underwent complete degradation in less than 2 h. When the experiment was conducted in pH 6.8 the beads did swell but suffered no significant degradation. These results stand out as the first report to reveal that in phosphate buffer pH 7.4, alginate beads could degrade without the inclusion of human or rat faecal matter. Before now the incorporation of rat cecal suspension as *in vitro* degradation trigger of polysaccharide matrix-based tablets for colon targeting has been an established dissolution procedure[4, 23]. The particulate size of these beads favoured their amenability to degradation in pH 7.4 medium without requiring addition of colon bacteria

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degradants. Therefore, we posit that drug-loaded alginate beads which are easy to produce, less excipient/equipment-demanding and inexpensive are preferred alternatives to alginate matrix-based tablets intended for colon specific delivery. This observation reinforces to limelight the formulation usefulness of alginate beads, as dissolution experiments can seamlessly be conducted without the extra cost and tedious inclusion of enzymes or cecal suspensions into the simulated colonic fluid (SIF, pH 7.4).

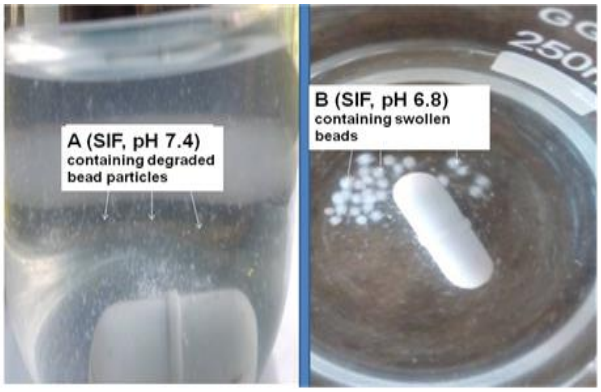

Figure 4: Degraded (A) and undegraded (B) beads

***In vitro* Drug Release**

Graphical representations of the *in vitro* release profiles of DS and ACP alginate beads are shown in Figures 5 and 6 respectively. For both drugs, release was minimal and without significant quantitative difference in SGF. Typically, Eudragit® S-100 coating and the matrix-bound-neusilin® FH2 provided a hydrophobic barrier against fluid ingress.

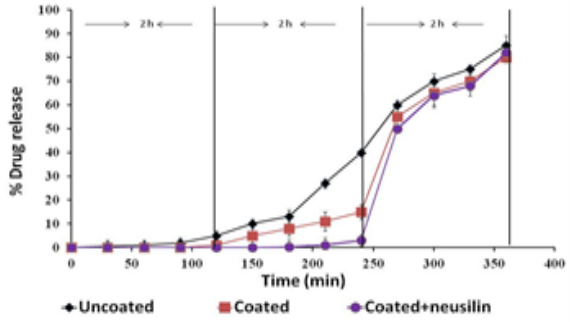

Figure 5: Release profile of Diclofenac sodium from Alginate beads

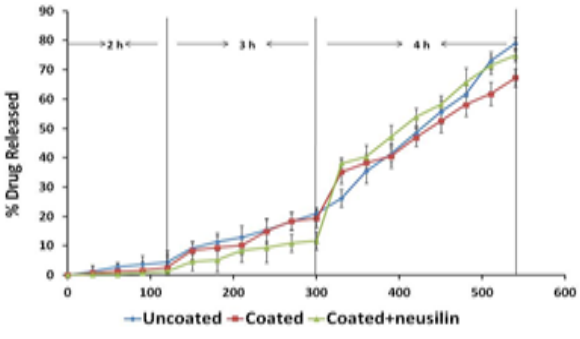

Figure 6: Release profile of Acetaminophene from Alginate beads

When the dissolution medium was changed to SIF, pH 6.8 ACP beads (coated + neusilin®) released more ($p<0.05$) drug (11 %) in 3 h than those of DS (3%) in 2 h. Although (coated) ACP beads equally released more drug than those of DS, the difference was however not significant. For the uncoated beads the quantity of DS released in 2 h was about one and a half times those of ACP released in 3 h. The diffusion of the soluble drugs through the swellable alginate matrix began with fluid sorption, polymer swelling and relaxation, drug dissolution and diffusion [24, 25]. Calcium alginate appreciably swells if the aqueous medium has a PK_a above the PK_a of alginate [21]. Similarly, DS is insoluble in acidic solution of up to PK_a 4 but dissolves in water and alkaline pH due to enhanced ionization [26]. Consequently, DS beads (uncoated) released higher amount of drug ($p<0.05$) within 2 h than did ACP beads that were subjected to 3 h dissolution duration. Apparently, pH-responsiveness of DS was a stronger determinant of release in pH 6.8 medium than release duration. Some workers have also reported pH-induced ionization and increased dissolution of drugs [27]. On the other hand the behavior of ACP has a physicochemical basis. Although a weak acid with a PK_a of 9.51 it does not ionize in acidic, basic or neutral pH, and therefore its solubility is non pH-dependent [28].

When replaced with pH 7.4 medium, further swelling and gradual degradation and dissolution occasioned faster drug release. Thus the already swollen DS beads containing dissolved drugs liberally released copious quantity of DS within 2 h. Although DS beads released more drugs (in 2 h) than ACP (in 4 h), the difference was not significant. However, the pH-responsiveness of DS still played a role.

With regard to (coated +neusilin®) beads, Eudragit® S-100 coating provided permeation barrier; during coating the polymer film may have coated both the hydrophilic alginate and the hydrophobic neusilin at especially the bead periphery. This Eudragit coating probably conferred enhanced hydrophobic behavior on the already hydrophobic neusilin, thereby ascribing controlled permeability. Essentially, longer dissolution duration instead of ionization effect impacted drug release in pH 6.8 medium. Thus ACP beads with longer dissolution testing time of 3 h witnessed higher drug release [29, 30]. Since some CTDDS are often programmed to release minimal drug in the stomach and small intestine through coating and other means efficient *in vitro* dissolution models ought to be optimized to ensure potential approximation with *in vivo* expectation.

The postprandial/fasted state drug dosing is thought not to cause significant variation in the small intestinal transit time. However, these two conditions (fed and fasted) do not paint the complete picture of human prandial conditions[13]. Thus other works have investigated not only the effect of fed and fasted but also pre-feed conditions on the small intestinal transit time[13, 31]. Administration of food 30 min after radiolabeled enteric coated

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erythromycin tablet ingestion (pre-feed) resulted to about 50 % reduction in bioavailability, attributed to shorter transit time in the small intestine[31]. Ingestion of food 45 min after oral administration of radiolabeled non-disintegrating tablets (pre-feed) also gave rise to shorter transit time (approximately 100 min) than fed and fasted conditions. Arguably, running a parallel *in vitro* dissolution experiment simulating the small intestine for 3 h may not correlate with *in vivo* outcome under a pre-feed state. This is because transit to the ileocecal region within the short transit time of 2 h predisposes the dosage form to be probably laden with high drug cargo in the colon environment characterized by paucity of aqueous content. Furthermore such shorter transit time is due to early propulsion caused by gastro-colonic response[32]. Thus in real life setting significant patient population may sometimes encounter SINTT shorter than 3 h due to timing of food intake during drug administration. For immediate release formulations SINTT may not be of grave concern, however, for CTDDS and enteric dosage forms, where residency in the small intestine may be delayed or accelerated by phase 111 house keeper waves SINTT becomes critical.

Mesalamine tablet disintegration and passing of whole tablet with feces[32-34] is attributed to intestinal transit variation that relates to timing of food. If food intake is delayed for up to 90 min the fed and fasted conditions are reverted to[35]. Under pre-feed dosing condition patient advice may be, ´take drug 30-45 min before food´. For a CTDDS to be efficacious in more patient populations the profound impact of timing of food must be revisited.

Kinetics and mechanisms of drug release.

The dissolution data of ACP and DS were fitted into various kinetic models to give an insight into the release kinetics and mechanism of release. The kinetic models were evaluated using R^2 linearity fit values. The results in Table 5 show the R^2, K and 'n' values obtained from the zero order, first order, Higuchi and Korsmeyer Peppas model plots. The release of ACP from the beads followed zero order, First order and Higuchi release kinetics as per their high linearity, with higher rate constants favoring release in pH 6.8. The >1.0 Korsmeyer Peppas ´n´ values recorded by all the batches was an indication of super case 11 mechanism. Super case II is characterized by prolonged acceleration of fluid sorption. This longer time acceleration event is a dimensional anomaly caused by the superimposition of fickian diffusion that is followed by the progressing case II boundaries that culminate in polymer relaxation [36]. Zero order release kinetic is characterized by constant release ideal in controlled drug release systems.

Table 5: The various release models and their release parameters

		Acetaminophen								Diclofenac sodium							
		Zero order		First order		Higuchi		Korsmeyer-Peppas		Zero order		First order		Higuchi		Korsmeyer-Peppas	
		R^2	K_0	R^2	K_1	R^2	K_H	R^2	N	R^2	K_0	R^2	K_1	R^2	K_H	R^2	N
Uncoated	pH 6.8	0.99	0.08	0.97	-0.0005	0.98	2.3	0.99	1.1	0.94	0.35	0.92	-0.002	0.93	9.5	0.46	2.7
	pH 7.4	0.99	0.25	0.95	-0.0025	0.99	10	0.99	2.5	0.98	0.27	0.96	-0.0045	0.98	9.4	0.83	0.97
Coated	pH 6.8	0.94	0.08	0.94	-0.0004	0.92	2.4	0.83	1.2	0.99	0.11	0.98	-0.0005	0.99	3.0	0.89	2.5
	pH 7.4	0.99	0.16	0.97	-0.0014	0.98	6.6	0.96	1.3	0.98	0.27	0.96	-0.0037	0.98	9.4	0.99	1.5
Coated+neusilin	pH 6.8	0.95	0.05	0.94	-0.0004	0.96	1.5	0.88	1.6	0.86	0.03	0.85	0.0001	0.84	0.88	-	-
	pH 7.4	0.99	0.19	0.98	-0.0019	0.99	7.7	0.97	1.5	0.96	0.96	0.93	-0.0046	0.96	11	0.99	1.5

K_0: zero-order release rate constant; K_1: first-order release rate constant; K_H: Higuchi release rate constant; R^2: regression line value; n: Korsmeyer -Peppas value.

Morphology and DSC of the uncoated Beads

Figures 7 and 8 show the SEM images of ACP and DS beads. Whereas images A and C represent X4000 magnification, B1, B2, D1 and D2 represent X1000 magnification. The striking observation on the bead surfaces is the presence of fissures and holes of different micro dimensions, difficult to visualize with ordinary optical microscopy. Furthermore, higher magnification unveiled numerous smaller cracks and holes on A and C that would otherwise be difficult to observe with lower magnification. The protuberances on B1, B2 and D2 and the convolutions on D1 may have developed during the ionic cross linking process between calcium ions and the alginate polyanions. Other particles on the surfaces may be drug crystals. The cracks and fissures could have developed during drying and moisture evaporation. In aqueous medium these crevices and pores become water inlet channels that trigger swelling of beads in alkaline pH.

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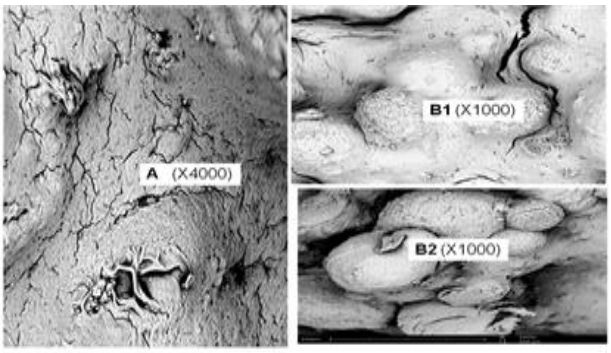


Figure 7: SEM omages of the surface morphology of ACP beads

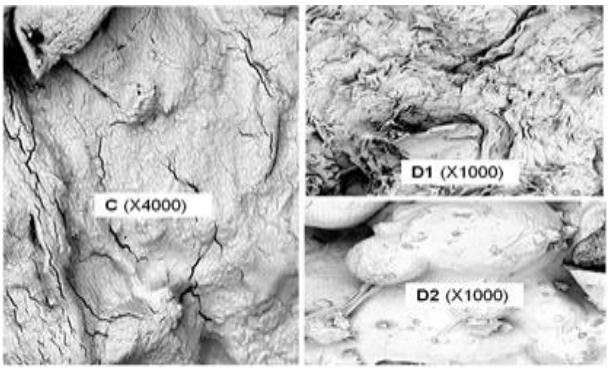


Figure 8: SEM images of DS beads showing surface morphology

Optical microscopy of representative beads is shown in Figure 10. Evidently, elucidation of surface morphology is not possible from these images, except for clues about bead shape and diameter. Prior to drying, the fresh beads mostly came out with very spherical shapes; however during drying alterations set in that caused them to assume oblong, spherical or even rough shapes.

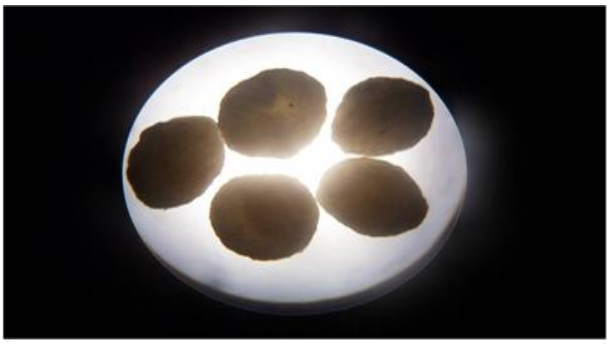
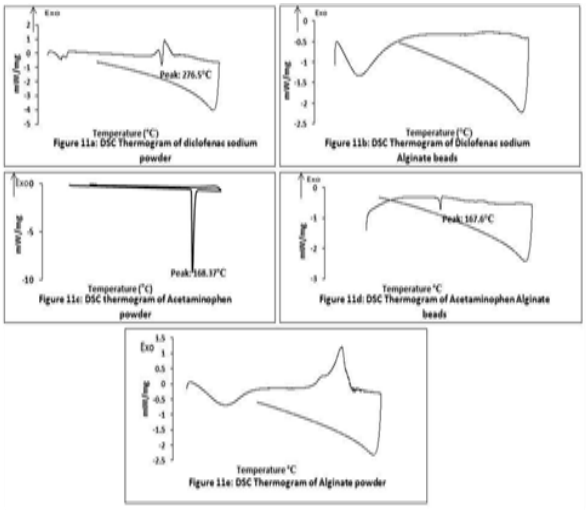


Figure 9: Photomicrograph of Diclofenac sodium (DS) beads

Figures 10 (a-e) shows the DSC thermograms of DS powder, uncoated neusilin-containg DS bead, ACP powder, uncoated neusilin-containing ACP bead and sodium alginate powder. In Figure 11a, an endothermic sharp peak representing the melting peak (276.5^{0}C) of DS was followed by an exothermic peak of probably its decomposition product. Tudja et al [37] reported that the heating rate and atmospheric investigation condition during DSC affect melting peaks; lower heating rates give room for more decomposition products than higher heating rates. Within the bead containing neusilin® FH2 the DS peak disappeared. The presence of neusilin® an amorphous compound may have amorphosized the drug[38]. Acetaminophen peak did not disappear, however it became diminished probably due to incomplete amorphosization.



Figures 10a-e: The DSC thermographs of DS, ACP, alginate & the beads

FTIR spectral studies

In Figures 11 and 12 the observed wave numbers on DS and ACP spectra were within 1500-2000 cm^{-1}, characteristic of double bonds (C=C, C=0) and 3000-3500 cm^{-1} of OH and N-H stretches. When the drugs were incorporated into the alginate or alginate and neusilin matrix the resultant peaks were still within the above mentioned wave number ranges. This underpins physical without chemical or deleterious interactions.

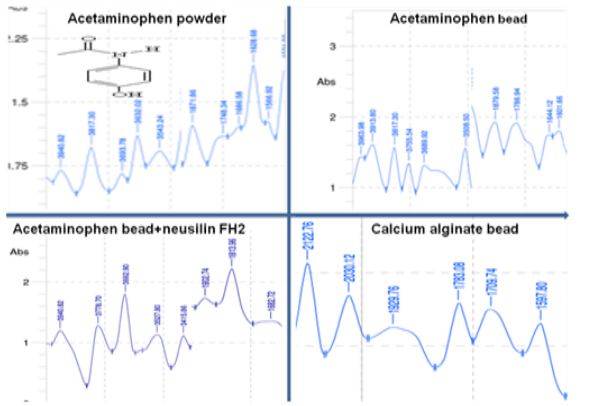


Figure 11: FTIR spectra of Acetaminophen beads

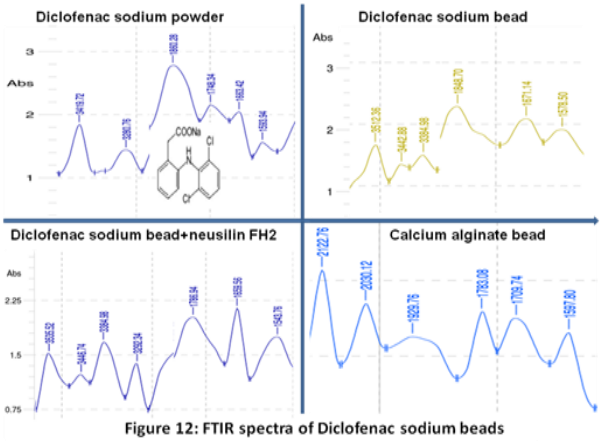


Figure 12: FTIR spectra of Diclofenac sodium beads

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CONCLUSION

Optimized alginate beads only swelled in SIF pH 6.8 but degraded in pH 7.4 without faecal matter/enzyme degradants. PH-responsiveness of Diclofenac sodium was a stronger determinant of release from uncoated micro beads in SIF 6.8 than dissolution duration. However, for (coated+neusilin®) micro beads the duration of dissolution determined the quantity of drug released. In conclusion, alginate bead-based formulations will effectively degrade in dissolution medium (pH 7.4) simulating the colon without warranting inclusion of faecal suspension. The observed significant difference in quantity of drug released between 2 and 3 h suggests that 2 h may be specified for mimiking pre-feed state, in addition to 3 h fed/fasted state intestinal transit time.

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