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The Application of Factorial Experimental Design in Capsule Coating for Potential Colon Targeting of an Antiamebic Drug

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Authors' contributions

Authors may use the following wordings for this section: This work was carried out in collaboration between all authors. Authors NCO and AC designed the study. Author NCO performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NCO and AC managed the analyses of the study. Author NCO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The application of factorial experimental design to evaluate the effect of particle size, capsule surface coating and binder concentration on the *in vitro* controlled release profile of metronidazole from encapsulated granules.

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Methodology: Metronidazole granules were prepared by the wet granulation technique and encapsulated in hard gelatin capsule shells. Eudragit® L-100 and *Landolphia owariensis* latex served as primary and secondary coatings respectively on 50 or 75% of capsule surface. The three formulation factors (% capsule surface coating, matrix former concentration and particle size) were subjected to a 2x3x4 factorial design experiment using the software (JMP 4.0.4, SAS Inc. USA). Gradient drug release studies were conducted in three media; firstly in media of pH 1.2 for 2 h, pH 6.8 for 3 h and finally pH 7.4 until exhaustion of drug release. The drug release data were subjected to kinetic treatment to establish operational release kinetics such as zero order, first order, Higuchi, Hixon Crowell and Kitazawa, while the power law enabled the prediction of mechanism of drug release.
Results: Results showed that % capsule surface coated with *Landolphia owariensis* latex and particle size significantly ($p<0.05$) contributed to time of drug release (T7.4) at pH 7.4. In tandem with this, maximum amount of drug released (D7.4) at pH 7.4 was significantly ($p<0.05$) affected by particle size alone. A few batches were characterized by anomalous transport while over 80% were associated with super case 11 type of release.
Conclusion: We therefore conclude that, factorial experimental design identified *Landolphia owariensis* latex coating and particle size of granules as being chiefly responsible for drug release variations.

Keywords: Landolphia owariensis; release kinetics; metronidazole; colon targeting; release mechanism.

1. INTRODUCTION

Colon specific drug delivery is a manipulative targeting technique that ensures the deposition and release of dosage form-borne drug in the colon for predetermined local activity or systemic absorption. Its uniqueness is underscored by the tasking nature of the dosage form design and therapeutic significance of colon physiology. In spite of the absence of absorption-enhancing villi, its length and the presence of lymphoid tissues ascribe a physiological advantage to it [1,2].

Some approaches have been suggested and adopted for colon-specific drug-targeting. They include pH-dependent polymer approach, time-dependent approach, pro-drug approach and bacteria enzyme-degraded polymer approach [3]. These adaptive approaches exploit the physiological or anatomical characteristics of the gastrointestinal tract. The time-dependent approach is designed to adapt to the gastrointestinal tract (GIT) transit time. The pH-dependent approach takes advantage of the variable pH of the GIT while the pro-drug approach and enzyme-degraded polymer approach utilize the degradation potential of the colonic bacteria enzymes. Drugs targeted to the colon may be aimed at treating inflammatory conditions of the colon, colorectal cancer, crohn's disease [4] or to improve the bioavailability of poorly absorbed peptide and protein drugs.

Metronidazole is an antiprotozoan drug used in the treatment of parasitic protozoa diseases, including amoebiasis, giardiasis, vaginosis, trichomoniasis, pelvic inflammatory disease, etc. Amoebiasis the most common and severe in the tropics, due to sanitation and socioeconomic status is caused by ingestion of food or water contaminated with cists of *Entamoeba histolytica*. Asymptomatic amoebiasis involves trophozoites arising from the cists, which potentially bore through the large intestine to cause ulcer and/or invade other

tissues like the liver. Symptomatic amoebiasis includes amoebic dysentery and non-dysenteric amoebic colitis. Oral ingestion of 250 or 500 mg of metronidazole is rapidly absorbed with peak plasma level concentration of 6-12 microgram/mL within 2-3 h. It is widely distributed and mechanism of action attributed to a reduction of metronidazole by bacterial reductases to a metabolite which interacts with the DNA to impede further replication [5]. One-third of normal dose is administered to patients with hepatic impairment. Colon-specific targeting of metronidazole is therefore aimed at achieving local contact with the infective agent. In this way dose reduction may be accomplished, systemic absorption reduced and high local antiprotozoan activity guaranteed.

Careful choice of polymer is important for the fabrication of a robust dosage form with optimum capability to deliver drug as appropriate. The pH-dependent polymer approach in some cases has been reported to discharge drug content before reaching the colon [6, 7]. In such cases the incorporation of a hydrogel or hydrophobic polymer in the tablet matrix may prolong transit time and forestall premature drug release. Most enzyme-degraded polymers are hydrogels with hydrophilic properties that permit early drug diffusion. Therefore the introduction of coating or blending with release retardants might be useful in controlling drug release.

Landolphia owariensis (LO) latex is a potential secondary plant metabolite derived from a climber that produces edible fruits. Some of its physicochemical and formulation properties have been previously evaluated [8,9]. It has a tensile stress of 182.7Nmm^{-2}, endothermic melting temperature peak of 98.6ºC; forms smooth dispersion in most non-polar solvents but insoluble in polar solvents including water. It was chosen in this study because of its hydrophobicity and pH-independence.

Since the majority of orally administered metronidazole is excreted through the urine, colon targeting may optimize administered dose and avoid unwanted urinary excretion of some fractions. Colon targeting will greatly minimize the wide biological distribution to several tissues, including where it is not needed, with the envisaged advantage of reducing or averting reported cases of resistance [5].

For a novel technique to translate from the laboratory to industry cost-effectiveness of excipients and final drug product is a crucial factor for consideration. Some drug products in the market are expensive probably because of high cost of excipients that should have been used in reduced amount. Ultimately every pharmaceutical company would prefer techniques that inform the use of minimum excipient concentrations with optimal effect. This is what optimization is all about. In optimization the least quantity of ingredients with the best effect is determined during preformulation studies and subsequently incorporated as part of the commercial product formula. Full factorial experimental design is one of the optimization methods we sought to employ in this work.

Therefore, the objective of our present investigation was to formulate encapsulated metronidazole granules and coat the capsules for possible targeted delivery to the colon. JMP (4.0.4, SAS Inc. USA) statistical software was employed in evaluating the effect of different capsule surface polymer coatings (0, 50 and 75%), matrix former concentration (1 and 4%) and particle size (0.30, 0.45, 0.80 mm and multiparticulate) on drug release from capsules. Our present investigation included four particle sizes and LOL (*Landolphia owariensis* latex) coating thickness of 12-20% w/w.

2. MATERIALS AND METHODS

2.1 Materials

Potassium dihydrogen phosphate, hydrochloric acid, sodium hydroxide (BDH, England), Eudragit L-100 (Evonic, Germany), acetone, hexane, ethanol (Riedelde Haen, Germany), methylcellulose (MC 25mpa.s. USP, FLUKA, Germany), metronidazole powder (Rajrab Pharmaceuticals Nigeria Ltd., Ilorin Nigeria). *Landolphia owariensis* latex was sourced and processed in our laboratory.

2.2 Methods

2.2.1 Processing of Polymer

Landolphia owariensis latex was tapped from the plant located in our botanical garden and processed in our laboratory as previously reported [7]. The processed *Landolphia owariensis* latex was stored for further studies.

2.2.2 Factorial experimental design and preparation of granules

The three formulation factors were subjected to a 2x3x4 factorial design experiment as shown in the software's (JMP 4.0.4, SAS Inc. USA) feed Table template of Table 1. The software was commanded to replicate only once, thus resulting to a total of 48 randomized formulation options (24 duplicates). Granules were prepared by the wet granulation technique, as previously reported [7]. The formulation formula is represented inTable 2

Table 1. Full factorial design template of the factors and their levels

Name	Role	Values			
Matrix former concentration	Continuous	1%		4%	
% capsule surface coated	Categoric	0%	50%	75%	
Particle size	Categoric	0.30	0.45	0.80	Multiparticles

Table 2. Formulation formula for 250 mg metronidazole granules

Binder concentration (w/w %)	Metronidazole (mg)	Lactose (mg)	Methylcellulose (mg)	Total (mg)
4	100	140	10	250
1	100	147.5	2.5	250

2.2.3 Size separation

Three sieves were fitted together in descending order of mesh size, 1.0, 0.6 and 0.3 mm respectively, and the granulation shaken through sieve 1.0 mm and received on a collector pan.

The mean size of granules that passed through sieve 1.0 mm but retained on 0.6 mm= 0.80 mm.

The mean size of granules that passed through sieve 0.6 mm but retained on 0.3 mm= 0.45 mm.

The mean size of granules that passed through sieve 0.3 mm but retained on the pan= 0.3 mm. The granule sizes were obtained by summing the two sieve aperture sizes and dividing by two.

An equal fraction of each of the three particle sizes were mixed together to yield 250 mg of the blend, otherwise called multiparticulate granules (multiparticles).

2.2.4 Encapsulation of granules and coating of capsules

A 250 mg quantity of the granules was weighed and manually filled into #2 hard gelatin capsules. Two coating solutions were prepared: 10% w/v ethanol solution of Eudragit® L-100 and 33% w/v hexane dispersion of LOL. Primary capsule coating was achieved with Eudragit® L-100 prior to secondary coating with LOL. Each capsule was wholly dipped into Eudragit® L-100 dispersion and air-dried for 24 h. Subsequently the Eudragit® L-100-coated (ELC) capsules were dipped into hexane dispersion of LOL and allowed to air-dry for 2 weeks. When approximately one-half length of the ELC capsules was dipped into the LOL dispersion the batches were called 50% (FIP) capsule coating. Similarly when approximately three quarter length of the ELC capsules was dipped into the LOL dispersion they were called 75% (SEP) capsule coating. The total weight of the Eudragit® L-100 coating was limited to 5-10% w/w and LOL, 12-20% w/w

2.2.5 Dissolution studies

Dissolution was conducted as previously reported [7]. Parallel drug release studies were carried out on the coated capsules using the gradient method at three different pH media respectively as follows, 2 h in 0.1N HCl (pH 1.2), 3 h in phosphate buffer (pH 6.8) and lastly phosphate buffer (pH 7.4) until exhaustion of drug release.

2.2.6 Kinetic studies

The drug release kinetics was studied on the release data of pH 7.4. The data were fitted to zero order, first order, Higuchi, Hixson Crowell and Kitazawa release kinetics [10-12]. Furthermore the mechanism of drug release was evaluated by fitting the release data to the following exponential equation [13,14] often used to describe drug release behavior from polymeric systems:

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

$$\text{Log}\ (M_t/M_f) = \text{Log}\ K + n\text{Log}\ t \quad (2)$$

Where M_t/M_f is fraction of drug released at time t, K is the coefficient (release rate) constant which takes into consideration the structural and geometric properties of the matrix and n is the diffusional exponent which indicates the mechanism of drug release. 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

[15]. Model independent approach, mean dissolution time (MDT) was calculated using the integral method [16]:

$$MDT = \int_0^{\infty} (M\infty - M(t))dt/M\infty \quad (3)$$

2.2.7 Factorial experimental design/Statistical analysis

The two response output data (cumulative amount of drug released (D7.4) at pH 7.4 and the maximum time (T7.4) of release in pH 7.4) were keyed into the response column of the software (JMP 4.0.4, SAS Inc. USA) and the Fit model run. Statistical significance was considered at ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Factorial experimental design

The Whole model plot (Fig. 1) of the Actual versus Predicted T7.4 had a regression line and confidence curves that fell below the mean line. This explains significant ($p<0.05$) variations in T7.4. From the leverage plots it was only % capsule surface coating, particle size and % capsule surface coating-particle size interaction respectively that significantly ($p<0.05$) contributed to T7.4 variation. Therefore, matrix former concentration that showed no significant ($p<0.05$) effect was excluded and the model rerun. Consequently, after exclusion % capsule surface coating and particle size respectively maintained significant ($p<0.05$) contributions (Figs. 2 and 3). Exclusion of the insignificant factors fine-tuned the first Whole model plot (Figure not shown), thus resulting to a new Whole model plot (Fig. 1) and minimal changes in root mean square error (RMSE) and R^2 values. RMSE is a measure of the random noise size and the standard deviation of the process noise that presumes that the un-estimated effects are negligible [17]. This low value is suggestive of other possible important minor effects apart from the main effects from each factor. The R^2 value of 0.70 indicates that the model explained nearly 70% of the variations in the T7.4 data [18].

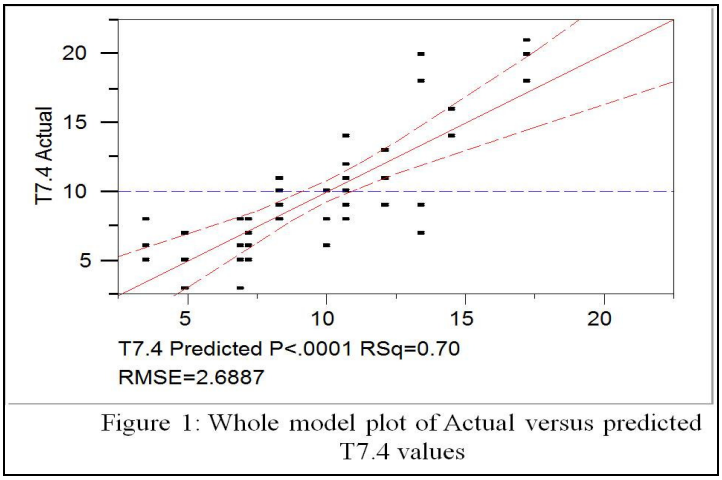

Figure 1: Whole model plot of Actual versus predicted T7.4 values

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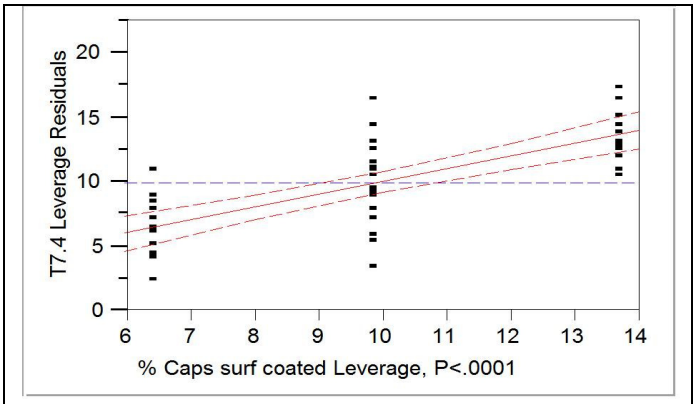

Figure 2: Leverage plot for % capsule surface coating

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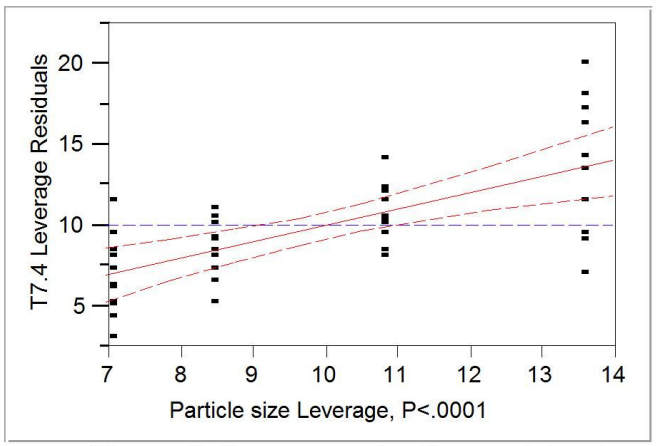

Figure 3: Leverage plot for particle size

Fig. 3. Leverage plot for particle size

The ANOVA (analysis of variance) result on Table 4 indicates a low p-value (prob F) of less than 0.0001 [17,18]. The F test probabilities in the effects tests showed that % capsule surface and particle size with p values of <0.0001 respectively contributed significantly ($p<0.05$) to T7.4 variations.

At 0 % capsule surface coating and particle size of 0.30 mm (Fig. 4a), the Predicted T7.4 was 4.9375. However, when % capsule surface coating was at the 75% setting and particle size at the multiparticulate setting, the corresponding maximum desirability became 0.794317. This means that the T7.4 increased from 4.9375 h to 17.27083 h at the most desirable setting (Fig. 4b). In Table 3 the output grid table of Predicted values and their corresponding rank of Desirability or acceptability, further elucidated this observation.

Table 3. Output grid table of predicted T7.4 and D7.4 and their corresponding desirability values

	T7.4			D7.4		
Capsule surface coating	**Particle size**	**T7.4**	**Desirability**	**Particle size**	**D7.4**	**Desirability**
0%	0.30	4.9375	0.21347601	0.45	87.5416667	0.73864729
0%	0.45	3.52083333	0.15113174	0.80	88.9166667	0.7666558
0%	0.80	7.27083333	0.31642695	Multipart	76.0166667	0.50985479
0%	Multipart	10.0208333	0.44044793			
50%	0.30	8.375	0.36571867			
50%	0.45	6.95833333	0.30256566			
50%	0.80	10.7083333	0.47224655			
50%	Multipart	13.4583333	0.60379055			
75%	0.30	12.1875	0.54213496			
75%	0.45	10.7708333	0.47515715			
75%	0.80	14.5208333	0.65623819			
75%	Multipart	17.2708333	0.79431686			

Table 4. Statistical result of factor effects on the time of drug release at pH7.4 (T7.4)

Summary of fit for T7.4				
R square				0.701156
R square Adj				0.66558
Root mean square error				2.688711
Mean of response				10
Observations (or Sum Wgts)				48
Analysis of variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	712.3750	142.475	19.7084
Error	42	303.6250	7.229	Prob > F
C. Total	47	1016.0000		<.0001

Effect tests					
Source	**Nparm**	**DF**	**Sum of squares**	**F ratio**	**Prob > F**
% Caps surf coated	2	2	420.87500	29.1095	<.0001
Particle size	3	3	291.50000	13.4409	<.0001

From the coefficients, extracted from parameter estimates table (not shown) the model equation was derived between the response (T7.4) and the formulation factors:

$$Y=b_0 +b_1X_1+b_2X_2 +b_3X_3+ b_4X_4+ b_5X_5 \quad (4)$$

$$Y=10+0.13X_1 - 3.6X_2 \quad (5)$$

Where b_0 = intercept, b_1 and b_2 are coefficients, X_1 =% capsule surface coating, X_2 =particle size.

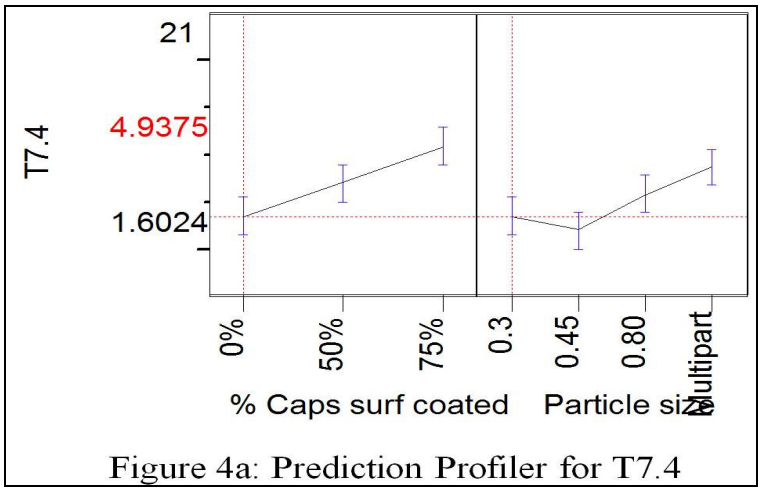

Fig. 4a. Prediction profiler for T7.4

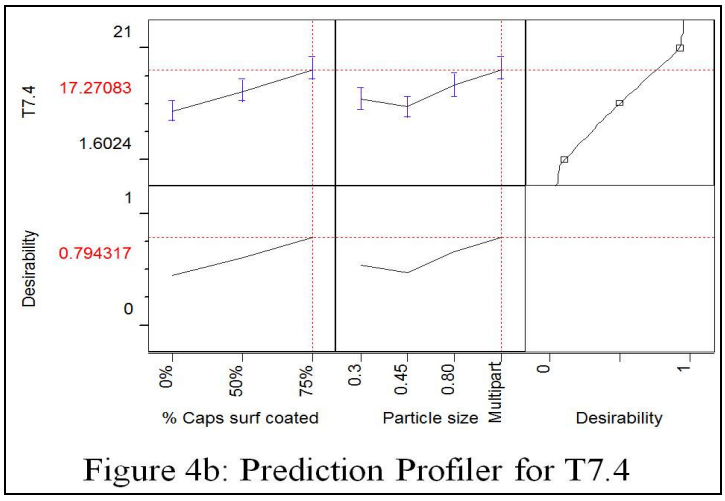

Fig. 4b. Prediction profiler for T7.4

The first Whole model leverage plots (Figure not shown) of Actual versus Predicted D7.4 was statistically significant ($p<0.05$). Similarly, the leverage plot for particle size was statistically significant ($p<0.05$). However, since other factor effects were not significant they were excluded from the Fit model and rerun with only particle size. This resulted in a new Whole model plot as shown in Fig. 5. Fig. 6 shows that the final leverage plot for particle size had significant effect ($p<0.05$). In the prediction profiler plots (Figs. 7a and b), the maximum Desirability was 0.766656 at the multiparticulate size. This shows that the D7.4 increased from 86.91667% at the center of the factor ranges to 88.91667 % at the most desirable setting. The increase from 86-88 % was a small increment, compared to 4-17 h, in T7.4. The time of release was therefore a more critical factor for consideration than the maximum amount of drug released. In addition, two formulation factors significantly complemented, in determining variations in T7.4.

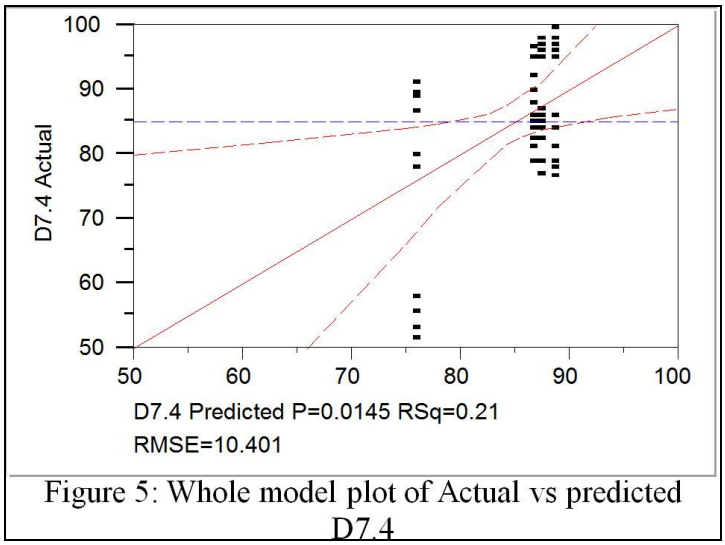

Fig. 5. Whole model plot of actual vs predicted D7.4

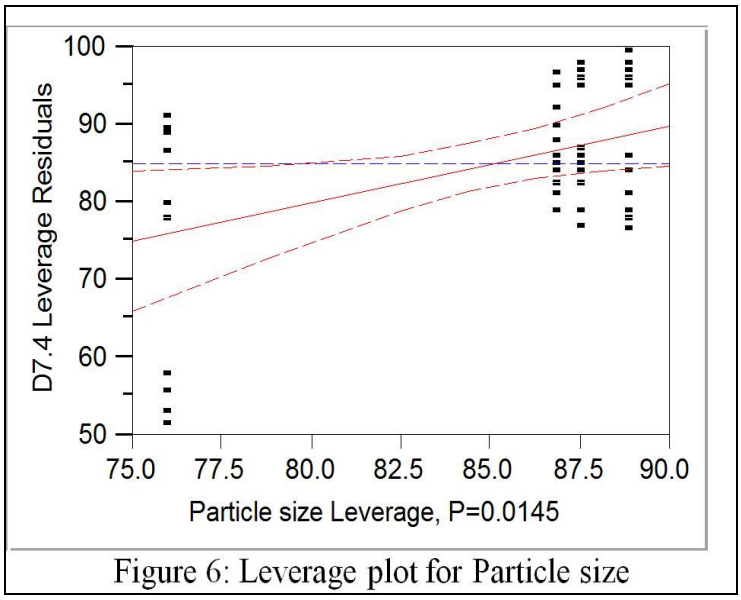

Fig. 6. Leverage plot for particle size

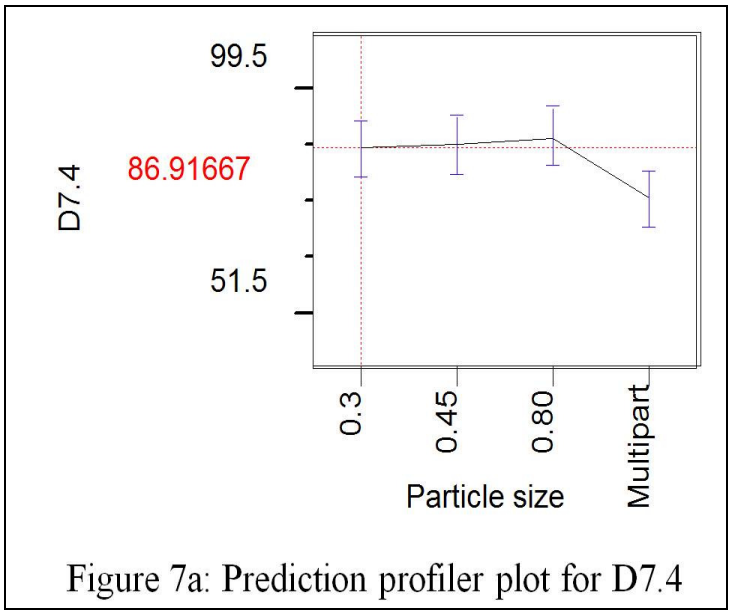

Fig. 7a. Prediction profiler plot for D7.4

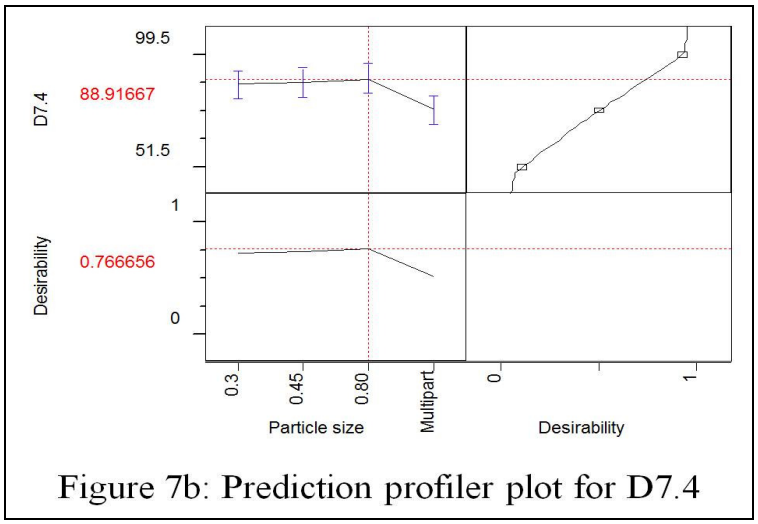

Figure 7b: Prediction profiler plot for D7.4

Fig. 7b. Prediction profiler plot for D7.4

The model equation for D7.4 is, $Y= b_0 +b_1X_1 = 84.85-8.83X_1$ (6)

Where b_1=the coefficient of X_1 (particle size).

Table 5. Statistical result of factor effects on cumulative amount of drug released (D7.4)

Summary of fit for D7.4				
R square				0.211011
R square Adj				0.157216
Root Mean square error				10.40083
Mean of response				84.84792
Observations (or Sum Wgts)				48
Analysis of variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	1272.9806	424.327	3.9225
Error	44	4759.7992	108.177	Prob > F
C. Total	47	6032.7798		0.0145

Effect tests					
Source	**Nparm**	**DF**	**Sum of squares**	**F ratio**	**Prob > F**
Particle size	3	3	1272.9806	3.9225	0.0145

3.1.2 Release kinetics

The dissolution profiles (figures not shown) suggest that, in the most part SEP capsules achieved slowest release compared to the control (without LOL coating). Table 6 shows the release kinetics and mechanism of release results. The software used during this study design generated 48 formulations, which were 24 duplicates; hence the 24 batches in Table 6. In Table 6 we considered linearity (R^2) values of 0.90 and above as acceptable; values of 1.0 would be most linear. About half of the batches indicated Higuchi and zero order kinetics

while a few, first order. Drug release from almost all the batches was by Hixon-Crowell kinetics. The two kitazawa K values were suggestive of the existence of at least two distinct slopes. On the other hand, in cases where distinct slopes could not be delineated we indicated it as "nd" (no distinct slope). In a couple of cases some K values were less than 0.1(<0.1); where this coexisted with a second higher value, as in batches 5, 11, 14, 17 and 24 respectively there was a remarkable absence of most other release kinetics. Batches 7, 8 and 9, with n values of 0.61, 0.93 and 0.93 respectively, depicted anomalous release transport, characterized by erosion and fickian diffusion simultaneously in operation. However, most of the batches recorded n>1.0, implying super case 11 release mechanism.

3.2 DISCUSSION

3.2.1 Factorial experimental design

From the factorial design results D7.4 was significantly ($p<0.05$) affected by only particle size (Table 5) while T7.4 was significantly ($p<0.05$) affected by % capsule surface coating and particle size (Table 4). In a way this establishes valid particle size-dependent release [19].

In site-specific drug targeting the pharmacokinetic profile of the drug is altered [20]. In the case of metronidazole direct activity against the infective agent is locally fostered without initial contact with the systemic circulation. Colon targeting does not only involve spatial deposition of drug but also its temporal control at the target site. Spatial deposition deals with the local environment where, targeting is sought while temporal control involves length of time of drug activity [20]. A well fabricated dosage form for colon targeting should prevent or significantly minimize drug release until regional contact is made with the ileo-cecal region where, abrupt drug release takes place [21] and continues with the colonic content transit process. Copious drug concentration deposited at the colon would justify prospective and effective pharmacological activity. Importantly, availability of optimal drug quantities for local therapeutic activity against protozoan infections will be anticipated.

Since T7.4 was significantly affected by % capsule surface coating and particle size, these two factors should be considered prior to formulation. This affirms why maximum achievable desirability was at multiparticulate size and 75% capsule surface coating. The two factors were responsible for the minimal drug release at pH 1.2 and 6.8 media respectively, and prolonged release at colonic (pH 7.4) medium. Temporal consideration in drug targeting is imperative to ensure adequate contact period between the drug and the infective agent. *Entameba hystolitica* is a protozoa that causes amebiasis. When cysts of *Entameba hystolitica* are ingested they form sporozoites in the intestine. Consequently more cysts formed via reproduction are either excreted, form colonies in the large intestine or bore through the lumen walls to cause ulceration and infect nearby tissues like the liver [5]. Since some of the cysts get excreted it means that they are apparently available throughout the different segments of the colon. Therefore, their clearance is contingent on the liberal spread of metronidazole throughout the colon. Hence, the need for controlled release requiring gradual, drug deposition. The mean T7.4 value of 10 h predicts the possibility of colonic drug release throughout the colon within that time purview. This may probably institute a lethal eradication of the cyst in the lumen and a possible preclusion of feco-oral re-infection. In addition, vulnerability to ulceration of the colon or cross tissue infection may be averted. The availability of at least 84 % of drug slowly transiting with colon contents over 10 h may also arrest the incidence of amoebiases. The idea is that in the large intestine the capsule may discharge drug as haustral pressure and movements drive it on. As was earlier

pointed out, absorption through the colon walls is difficult due to the viscous consistency of the fluid caused by high water absorption capacity of the colon and inefficient mixing [22].

Is there any rationale for colon targeted metronidazole formulation? Conventional oral metronidazole tablets must be systemically absorbed prior to tissue availability. A 250 or 500 mg dose is rapidly absorbed and widely distributed with peak plasma level concentration of 6-12 microgram/mL within 2-3 h [5]. Although in amoebic dysentery oral ingestion provides effective cure, however unnecessary non-specific distribution to other tissues may raise concerns of toxicity or even drug resistance, especially for susceptible organisms in tissues with sub-lethal concentrations. In colon specific delivery 100 mg or less of metronidazole may be adequate to achieve what 500 mg achieves in conventional oral administration. As a consequence, dose reduction may reduce or prevent associated toxicity (especially in hepatic impairment [5] and reduce production costs. The present investigation is not advocating complete withdrawal of conventional forms of metronidazole because of their immediate release benefits. In amoebic dysentery a start dose of 400 or 500 mg dose should be subsequently followed by colon targeted formulations. Twelve hourly administrations may suffice to achieve satisfactory cure.

3.2.2 Release kinetics

Drug release from the capsules was a function of time-dependent softening and gradual erosion of the non LOL-coated portion of SEP or FIP, permeation of fluid and erosion of granules. Permeation of fluid into the encapsulated granules initiated granule hydration and drug dissolution. Diffusing drug molecules were then entrapped in a saturation layer within the capsule with a thickness that depended on the extent of capsule erosion, granule erosion and/or fluid permeation. Consequently, the capsules were exhausted of constituent granules but maintained most parts of their shapes. However, erosion of capsule surface was only restricted to the portion of the capsule surface that was not coated with LOL.

Zero order kinetic, was remarkable amongst some of the batches. This was an indication of concentration-independent drug release. The obvious presence of Hixon-Crowell release kinetic is attributed to the structural packing of the intact granules and the semi-impervious LOL-coated capsule surface. The coated capsules behaved nearly like a coated cylindrical controlled release tablets that allowed restricted permeation of fluid through one or more orifices. Progressive dissolution of matrix as a function of time, typical of Hixon-crowell kinetic, governed drug release extensively. Over 80% of the batches were associated with Super case II release mechanism. This means characteristic relaxation-controlled release due to reduction in attractive forces between polymer chains [23]. In other words drug release was strongly influenced by the relaxation rate of polymer chains and their erosion [23,24,25].

The mean dissolution time (Fig. 8) was between 0.8 and 6 h; lower values were recorded by the control batches while higher values by mostly 75% capsule surface coating. Lower values are indicative of faster release due to the absence of LO coating.

Table 6. The various release models and their release parameters

[1]Batch no	[2]MFC (%)	[3]LOL (%)	Higuchi		Zero order		First order		Hixson Crowel		Mechanism of release		Kitazawa	
			K	R^2	K	R^2	K	R^2	K	R^2	n	R^2	K	K
B1	1	12-20	63	0.80	10.2	0.87	-0.11	0.58	0.45	*0.96*	1.94	0.85	0.2	2.2
B2	4	12-20	62	0.83	10.2	*0.90*	-0.09	0.78	0.39	*0.97*	1.43	0.88	0.1	0.9
B3	1	12-20	59	0.79	9.5	0.86	-0.09	0.71	0.36	*0.96*	1.38	0.83	0.2	1.6
B4	4	12-20	77	*0.92*	12.2	*0.93*	-0.11	0.96	0.45	0.88	1.80	0.90	0.2	<0.1
B5	1	0	73	0.69	13.5	0.77	-0.13	0.60	0.62	0.86	1.5	0.88	<0.1	1.6
B6	4	0	108	*0.99*	21.1	*0.95*	-0.20	0.99	0.54	*0.95*	1.21	0.95	Nd[4]	Nd
B7	1	12-20	91	*0.95*	18.2	*0.95*	-0.21	0.95	0.43	*0.94*	0.61	0.96	0.3	0.6
B8	4	12-20	70	*0.92*	12.2	*0.97*	-0.11	0.83	0.17	*0.98*	0.93	0.89	0.2	0.6
B9	1	12-20	61	*0.90*	9.58	*0.94*	-0.13	0.68	0.27	*0.97*	0.93	0.96	Nd	Nd
B10	4	12-20	50	0.77	8.12	0.84	-0.07	0.61	0.32	*0.97*	1.21	0.91	1.3	0.1
B11	1	0	101	0.68	21.6	0.78	-0.21	0.67	0.70	*0.90*	1.22	0.80	1.6	<0.1
B12	4	0	72	0.81	12.7	0.88	-0.10	0.80	0.50	*0.98*	1.62	0.94	0.4	0.1
B13	1	12-20	58	0.84	8.5	*0.90*	-0.16	0.64	0.33	*0.98*	1.84	0.93	0.1	1.6
B14	4	12-20	64	0.79	10.4	0.85	-0.09	0.80	0.40	*0.93*	1.45	0.87	0.9	<0.1
B15	1	12-20	39	*0.94*	5.5	*0.98*	-0.05	0.91	0.20	*0.93*	1.32	0.99	Nd	Nd
B16	4	12-20	47	0.83	6.7	0.89	-0.08	0.67	0.23	*0.98*	1.22	0.91	Nd	Nd
B17	1	0	116	0.83	20.9	0.89	-0.25	0.77	0.72	*0.93*	1.99	0.90	1.1	<0.1
B18	4	0	70	*0.92*	12.0	*0.97*	-0.09	0.89	0.45	*0.99*	1.39	0.96	0.3	<0.1
B19	1	12-20	84	*0.96*	13.6	*0.95*	-0.14	*0.98*	0.49	0.85	1.36	0.77	0.4	<0.1
B20	4	12-20	23	0.59	3.8	0.70	-0.03	0.56	0.21	*0.91*	1.31	0.86	<0.1	0.4
B21	1	12-20	29	*0.93*	4.07	*0.96*	-0.02	0.72	0.18	*0.94*	1.32	0.93	Nd	Nd
B22	4	12-20	15	0.68	2.39	0.77	-0.01	0.72	0.23	*0.94*	1.25	0.72	Nd	Nd
B23	1	0	46	*0.91*	8.07	*0.95*	-0.05	0.95	0.35	*0.94*	1.09	0.85	0.1	<0.1
B24	4	0	56	0.70	9.37	0.77	-0.09	0.70	0.33	*0.91*	1.07	0.77	<0.1	0.6

[1]= the software randomly suggested 48 formulae (batches), ie 24 duplicate batches.
[2]= w/w % concentration of methyl cellulose (matrix former) concentration
[3]=Total w/w % amount of LOL coated on the capsule surface
[4]=No definite slope

50% capsule surface coating, 1% matrix former concentration, 0.30 mm particle size (B1); 50% capsule surface coating, 4% matrix former concentration, 0.30 mm particle size (B2); 75% capsule surface coating, 1% matrix former concentration, 0.30 mm particle size (B3); 75% capsule surface coating, 4% matrix former concentration, 0.30 mm particle size (B4); 0% (control) capsule surface coating,1% matrix former concentration, 0.30 mm particle size (B5); 0% (control) capsule surface coating, 4% matrix former concentration, 0.30 mm particle size (B6); 50% capsule surface coating, 1% matrix former concentration, 0.45 mm particle size (B7); 50% capsule surface coating, 4% matrix former concentration, 0.45 mm particle size (B8); 75% capsule surface coating, 1% matrix former concentration, 0.45 mm particle size (B9); 75% capsule surface coating, 4% matrix former concentration, 0.45 mm particle size (B10); 0% (control) capsule surface coating,1% matrix former concentration, 0.45 mm particle size (B11); 0% (control) capsule surface coating, 4% matrix former concentration, 0.45 mm particle size (B12); 50% capsule surface coating, 1% matrix former concentration, 0.80 mm particle size (B13); 50% capsule surface coating, 4% matrix former concentration, 0.80 mm particle size (B14); 75% capsule surface coating, 1% matrix former concentration, 0.80 mm particle size (B15); 75% capsule surface coating, 4% matrix former concentration, 0.84 mm particle size (B16); 0% (control) capsule surface coating,1% matrix former concentration, 0.80 mm particle size (B17); 0% (control) capsule surface coating, 4% matrix former concentration, 0.80 mm particle size (B18);50% capsule surface coating, 1% matrix former concentration, multiparticulate size (B19); 50% capsule surface coating, 4% matrix former concentration, multiparticulate size (B20); 75% capsule surface coating, 1% matrix former concentration, multiparticulate size (B21); 75% capsule surface coating, 4% matrix former concentration, multiparticulate size (B22); 0% (control) capsule surface coating,1% matrix former concentration, multiparticulate size (B23); 0% (control) capsule surface coating, 4% matrix former concentration, multiparticulate size (B24).

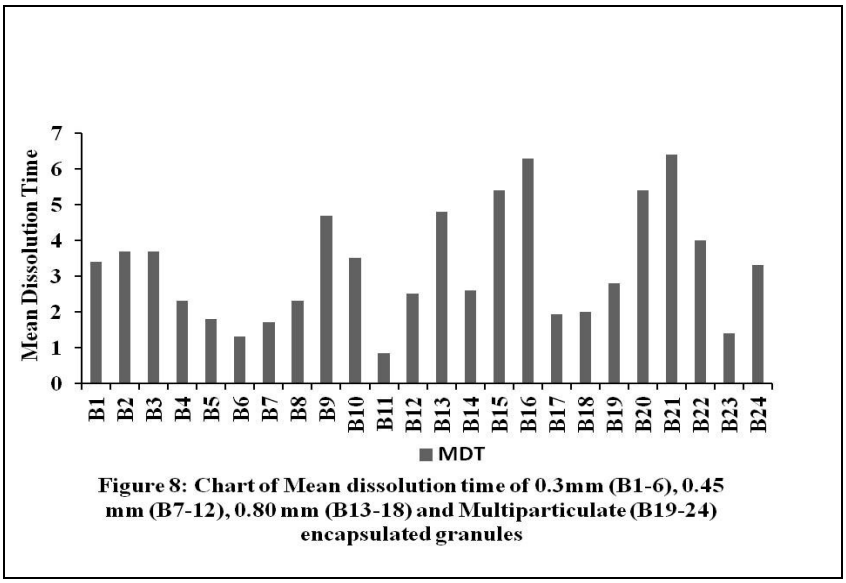

Figure 8: Chart of Mean dissolution time of 0.3mm (B1-6), 0.45 mm (B7-12), 0.80 mm (B13-18) and Multiparticulate (B19-24) encapsulated granules

Fig. 8. Chart of mean dissolution time

4. CONCLUSION

Optimization approach was adopted in the evaluation of different factor effects on the time and quantity of drug released at colon pH 7.4% capsule surface coated with *Landolphia owariensis* latex and particle size significantly ($p<0.05$) affected time of drug release, while amount of drug released was significantly ($p<0.05$) affected by only particle size. In other words, matrix former concentration (binder) did not significantly contribute to either time or quantity of drug released. Extensive Hixon-Crowel release kinetic was observed in most batches. A few batches were characterized by anomalous transport while over 80% were associated with super case 11 type of release. In conclusion, optimization approach elucidated *Landolphia owariensis* latex coating and particle size of granules as being chiefly responsible for drug release variations.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

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