The synergistic effect of *Landolphia owariensis* latex and Eudragit® L-100-coated capsules on the *in vitro* controlled release of metronidazole for possible colon targeting

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The overall objective of this present investigation was to determine the synergistic potentiality of *Landolphia owariensis* latex (LOL) and Eudragit® L-100 as hydrophobic polymeric agents in ensuring controlled drug release for possible colon-targeted delivery of metronidazole. Metronidazole granules were prepared by the wet granulation method and manually encapsulated. The entire capsule surface was first coated with Eudragit® L-100 (primary coating). Secondly half (50%) or five-sixth (5/6 or 83%) of the capsule surface was coated atop the primary coating with LOL (secondary coating). Two different granule size fractions were isolated and compared. Parallel gradient i*n vitro* drug release studies were carried out in media of pH 1.2, 6.8 and 7.4, respectively. The dissolution data were subjected to kinetic treatment. Results showed that the least quantity of drug release took place at pH 1.2 followed by pH 6.8, with greatest drug release taking place at pH 7.4. Capsule surface coated with LOL significantly ($P<0.05$) affected drug release and time of release. Matrix former (binder) concentration had no significant ($P<0.05$) effect on drug release, but particle size did. More than one drug release mechanisms were operational and most capsules with 50% surface coated with LOL recorded higher Dissolution efficiency (DE) but lower Mean dissolution time (MDT) than those of 83%. In conclusion, the use of Eudragit® L-100 and LOL as primary and secondary capsule coatings respectively have demonstrated competence in controlling drug release and thus may hold promise at preferentially targeting metronidazole to the colon against amebic diseases.

Key words: *Capsule surface coating, colon targeting, dissolution medium, drug release kinetics, metronidazole*

INTRODUCTION

The oral route has remained the most convenient route of drug administration[1] for conventionally administered drugs. Controlled drug delivery, intended for spatial targeting or sustained release, has largely remained a huge arena for industrial research/production and laboratory investigations.[2] Colon targeting is a special type of controlled drug delivery approach designed for oral local site-specific delivery of some drugs direct to the colon. This is adopted, when avoidance of the gastric environment will beneficially save protein and peptide drugs[3] from the deleterious potential of the stomach's low pH/gastric enzymes in order to intelligently optimize systemic concentration upon disposition in the colon; or delay release until the colon is accessed for the local treatment of colitis and colorectal cancer.[4]

Amebiasis is an infection of the large intestine caused by *Entamoeba histolytica*, a single-celled protozoan parasite that causes 34-50 million symptomatic infections each year and is responsible for up to 100,000 deaths.[5-7] Metronidazole, the drug of choice for intestinal amebiasis, can be delivered to the colon for its effective action against *E. histolytica*. It is rapidly and completely

absorbed from its conventional tablet form. Although these tablets provide a minimal amount of metronidazole for local action in the large intestine which is still effective in the relief of amebiasis, undesirable systemic side effects occur upon their administration.[8]

Researches are ongoing in this area (colon targeting) of drug delivery; with a number of approaches documented in literature.[9-10] Some of these approaches have one attendant limitation or the other. The pH-dependent approach, though has found commercial application, is still reported to have shortcomings, prominent of all is large variation in gastrointestinal tract (GIT) pH. Hence the need for effective polymer modification, that should be able to optimally makeup for these shortcomings. The use of polymers or matrix formers in combination with one or more pH-dependent polymers could cushion the drastic influence of variable GIT pH and further prolong drug release.

Apart from the complementary significance of a candidate polymer or matrix former its economic advantage may project it in preference to others. For this purpose we thought of combining a pH-sensitive polymer and edible locally available hydrophobic polymeric latex derived from *Landolphia owariensis* in coating capsules intended for colon targeting. The fear of drug interaction with our newly derived polymer is allayed by the fact that the polymer and even the pH-dependent polymer will have little or no direct contact with the drug mentronidazole.

Its potential application in self-emulsifying oil formulations (SEOFs), and *in vitro* potential use in colon-targeted drug delivery, respectively, have previously been evaluated.[11-12] The overall aim of this present investigation was to determine the synergistic potentiality of LOL and Eudragit® L-100 as hydrophobic capsule coatings in ensuring delay and prolonging of drug release for possible colon-targeted delivery of metronidazole. Some objectives of this work include, coating of the capsules initially with Eudragit® L-100 and later with *Landolphia owariensis* latex (LOL) and evaluating the effect of percent surface of capsule coated with LOL, particle size and matrix former concentration on *in vitro* drug release.

MATERIALS AND METHODS

Materials
This work employed the following materials, LOL (the tree is located in the botanical garden of Botany department, University of Nigeria, Nsukka; identified by the botanical garden attendant and the latex tapped and processed in our laboratory), sodium hydroxide, hydrochloric acid, potassium dihydrogen phosphate (BDH, England), Eudragit® L-100 (Evonic, Germany), acetone, hexane, ethanol (Riedel-de Haen, Germany), methylcellulose (MC 25mpa.s. USP, FLUKA, Germany), metronidazole powder (a kind gift from Rajrab Pharmaceuticals Nigeria Ltd). All other reagents were of analytical grade and were used as provided by the manufacturer.

Methods
Precipitation of latex from Landolphia owariensis stem
The method of Adikwu and Ossai[13] was employed but with modification. Cuts were made on the stem and the white latex collected with a 1L beaker. The tapped latex was then diluted with two times its volume of water before filtering through a muslin cloth. Sufficient acetone was then introduced into it to precipitate the latex. It was later dried in the oven for 4 h at 60°C and stored in a desiccator until further use.

Preparation of metronidazole granules by wet granulation
The wet granulation method was employed in the production of metronidazole granules. The appropriate quantities of metronidazole and lactose were weighed and blended thoroughly as shown in Table 1. The matrix former, methylcellulose was also weighed, formed into a suitable granulating fluid and the entire mixture kneaded in a porcelain mortar and pestle. The damp mass formed was then forced through sieve no. 10 (1.7-mm mesh) and dried at 50⁰C for about 1 h. The dry mass was also forced through sieve no. 16 (1.0-mm mesh) and stored in a desiccator until further use.

Particle size separation
Three sieves (with a collector pan beneath sieve no. 52) of decreasing aperture and tightly fitted to each other in this order: no.16, 25, 52 and a collector pan were used.[14] The dry granulation was emptied into sieve no. 16 and shaken through the rest of sieves, so that a batch was retained by number 25, a second batch by number 52, and a third batch by the collector pan. Thereafter the different fractions were separately collected in a polyethylene material and stored in the desiccator for further use as follows:
- The mean size of granules that passed through sieve no. 16 but retained on number 25=0.84mm
- The mean size of granules that passed through sieve no. 25 but retained on number 52=0.47mm
- The mean size of granules that passed through sieve number 52 but retained on the pan=0.25mm undersize.

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

Table 1: The wet granulation formula

	4% methylcellulose Amt of Ingred		1% methylcellulose Amt of Ingred (mg)	
	Per cap (mg)	x300 cap (g)	Per cap (mg)	x300 cap (g)
Metronidazole	100	30	100	30
Lactose	140	42	147.5	44.25
Methylcellulose	10	3	2.5	0.750
Total	250	75	250	75

Only the MPG and those of 0.47 mm (FOS) were stored and used in this work for further studies.

Encapsulation of granules
A 250-mg quantity of the granules was weighed and manually filled into a 250-mg hard gelatine capsule. This was done for the two particle sizes

Preparation of dissolution medium
A 0.4% w/v of sodium hydroxide solution was prepared in purified water. Then 0.301 g of potassium dihydrogen phosphate (KH_2HPO_4) was dissolved in one litre of purified water. The sodium hydroxide solution was gradually introduced into the KH_2HPO_4 solution to achieve a pH of 6.8 or 7.4. Dissolution medium of pH 1.2 (0.1 N HCl) was equally prepared by diluting particulate appropriate quantity of conc. Hydrochloric acid with 1L to give a pH of 1.2.[15-16] In each case a pH meter was used to ascertain the pH.

Preparation of coating solution
The coating solution was prepared by dispersing 33 g of LOL in hexane in a 100-ml beaker to achieve a concentration of 33% w/v. Solubilization of the dispersion was facilitated by stirring and warming at a temperature of 50⁰C. Similarly a 20% w/v dispersion of Eudragit® L-100 in ethanol was also prepared.

Coating of the capsules
A candidate capsule was preweighed in an analytical balance (Metler, England) and the weight noted. Primary coating of the capsule with Eudragit® L-100 was carried out prior to secondary coating with LOL. Each capsule was pierced at one end with a hypodermic needle to provide a firm support. The capsule was then dipped into the Eudragit® L-100 coating solution for 3-5 sec and removed. It was dried under a fan at room temperature and allowed to equilibrate for 24 or more hours. Thereafter, it was reweighed and then disengaged from the needle while the piercing spot was sealed off with a drop of Eudragit® L-100 coating solution. Subsequently the Eudragit® L-100-coated capsules (UC) were further coated (atop 50 or 83% of their surfaces) with LOL (LC) by dipping into the hexane dispersion of LOL, dried as above and stored for about 1-2 weeks. A 50% capsule coating (FC) meant having approximately half of the pre-coated capsule further coated with LOL while 83% coating (EC) meant coating approximately 5/6 of the capsule surface with LOL. The % surface coatings were visually determined rough estimates. The choice of 83% by the authors was to have a reasonably variable coating difference from 50% coating. Seventy-five percent coating was initially considered by the authors because it was easier to delineate from the capsule. However, 83% was chosen only because it provided a higher coating area than either 50 or 75%. Table 2 shows a summary of the coating details. It should be noted that the concentrations of Eudragit® L-100 or LOL coatings were limited to a range of 5-10% w/w of the capsule.

Table 2: w/w % Concentration of *Landolphia owariensis* latex as a secondary coating over 50 or 83% capsule surface

1% methylcellulose as binder *Particle size of granules(mm)		4% methylcellulose as binder Particle size of granules(mm)	
0.47	Multiparticulate	0.47	Multiparticulate
w/w % concentration of LOL coating over capsule surface			
9.1(C1)	10.4(C4)	10.1(C7)	10.4(C10)
7.1(C2)	5.5(C5)	5.2 (C8)	6.4(C11)
0(C3)	0(C6)	0(C9)	0(C12)

C1, C4, C7 and C10=Batches with 50% capsule surface coated with LOL (FC); C2, C5, C8 and C11=Batches with 83% capsule surface coated with LOL (EC); C3, C6, C9 and C12=Batches with capsule surface coated with only Eudragit® L-100 (control, UC); *The particle sizes as earlier stated are approximate mean values; 0.47=Encapsulated granules of 0.47 mm mean particle size; Multiparticulate=Encapsulated granules containing a conglomerate of three particle size granules: 0.25, 0.47 and 0.84 mm

Dissolution studies
Dissolution studies were carried out using the rotating basket method (VEEGO, India) in a medium of volume 900 ml (pH 1.2) and temperature 37±1⁰C. The equipment was switched on to rotate at a speed of 100 rpm. At predetermined time intervals 5-ml samples of the dissolution medium were withdrawn and assayed spectrophotometrically (UV/VIS, Unico, USA) after appropriate dilution at 277 nm. Meanwhile, 5 ml of a fresh medium was used to refresh the dissolution medium.

The dissolution was run for 120 minutes at pH 1.2 (0.1N HCl). Two hours was chosen to mimic the average gastric emptying time.[15] At the end of the 120 mins, the equipment was switched off to allow for dissolution medium replacement. pH 1.2 medium was replaced with 900 ml of a second dissolution medium, pH 6.8. Thereafter the capsule was reinstated in the basket and dissolution run as at before but for 3 h. Three hours was also chosen because the reported average intestinal transit time is 3-4 h.[17] At the end of 180 mins the medium was again discarded and replaced with a third medium of pH 7.4 to mimic the ileocecal pH[18] and the same process repeated but this time until the capsule released all or nearly all the drug. The tests were carried out in duplicates and statistical analysis (UNIANOVA and Student 't' test) carried out using SPSS 16.0 INC statistical software at $P<0.05$ level of significance.

Kinetics of drug release
In order to examine the kinetics of drug release from the coated capsules, the release data (at pH 7.4) were fitted to the following power law (exponential) equation,[19-21] often used to describe the drug release behavior from polymeric systems:

$$M_t/M_f = kt^n \tag{1}$$

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

Where, M_t/M_f is the fraction of drug released at time t, k is the coefficient (release rate) constant which accounts for the structural and geometrical properties of the matrix or tablet, and n is the diffusional exponent indicative of

the mechanism of drug release. For a cylindrical gel values of the exponent $n=0.5$, $0.5<n<1$, and $n=1.0$ indicate Fickian diffusion (case 1), non-Fickian diffusion (anomalous transport), and zero-order transport (case 11 or relaxation controlled), respectively.[22] While $n>1.0$, indicates super case II type of release. Case II generally refers to the erosion of the polymeric chain and anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled drug release.

The data were also further fitted to zero order, first order, Hixson Crowell and Kitazawa release kinetics.[20,23-24] Mean dissolution time (MDT or MDT$_{invitro}$) was calculated using the integral method while Dissolution Efficiency (DE) was calculated from the dissolution curve at t$_{60\%}$ using the trapezoidal technique. The mean *in vitro* dissolution time (MDT$_{invitro}$) is the mean time for the drug to dissolve under *in vitro* dissolution conditions. This was calculated using the following equation:[25]

$$MDT_{invitro} = \int_0^{to} (M_{to} - M(t))dt / M_{to} \qquad (3)$$

M$_\infty$ = Quantity of drug contained in the capsule at zero time
M$_t$ = Fraction of drug released at time t

RESULTS

Investigation of the effect of capsule surface coated with LOL, binder (matrix former) concentration and particle size, on drug release were carried out. Since gradient release technique was adopted the % cumulative drug released at pH 1.2, 6.8 and 7.4, respectively, were integrated as a uniform continuous release data and plotted against time to yield a single-line graph as shown in Figures 1-4. The various T$_{1.2}$, T$_{6.8}$, T$_{7.4}$, D$_{1.2}$, D$_{6.8}$ and D$_{7.4}$ values for all the batches are equally represented in Figure 5. The values represent the cumulative time (T) taken to attain cumulative maximum drug (D) release at the three different media respectively within the dissolution period. Results indicated that the rate and quantity released were slower and greater respectively in pH 7.4 than in pH 1.2 or 6.8. Univariate analysis of variance revealed that capsule surface coated with LOL had a significant ($P<0.05$) effect on both quantity of drug released (D$_{7.4}$) and time of release (T$_{7.4}$). In addition a post hoc multicomparism (LSD) test further revealed that while 83% capsule surface coating demonstrated greater effect ($P<0.05$) on quantity of drug released than 50% and control, it also significantly ($P<0.05$) prolonged (T$_{7.4}$) drug release more than the control (but not 50% capsule surface coating). Independent samples *t* test indicated that while binder (matrix former) concentration had no significant ($P<0.05$) effect on drug release particle size did.
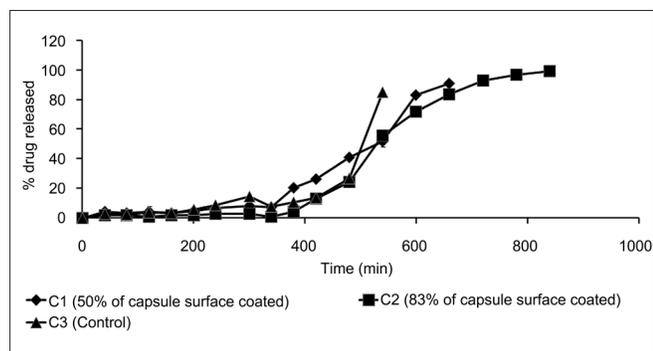

Figure 1: Effect of capsule surface coated with LOL on drug release from encapsulated 0.47 mm granules containing 1% w/w methylcellulose
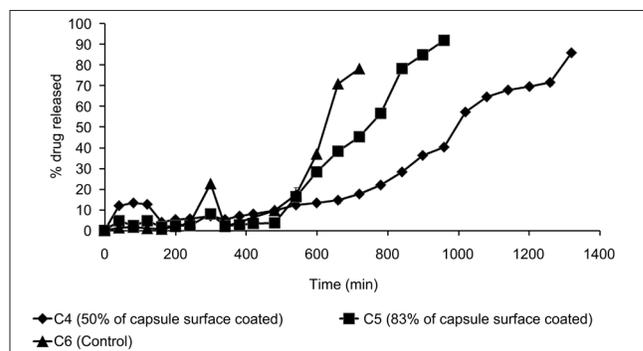

Figure 2: Effect of capsule surface coated with LOL on drug release from encapsulated 0.47 mm granules containing 4% w/w methylcellulose
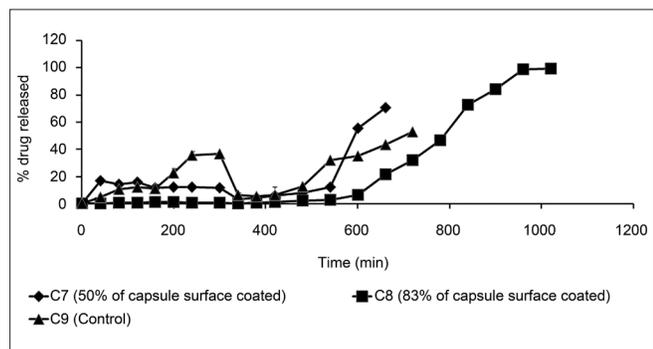

Figure 3: Effect of capsule surface coated with LOL on drug release from encapsulated multiparticulate granules containing 1% w/w methylcellulose
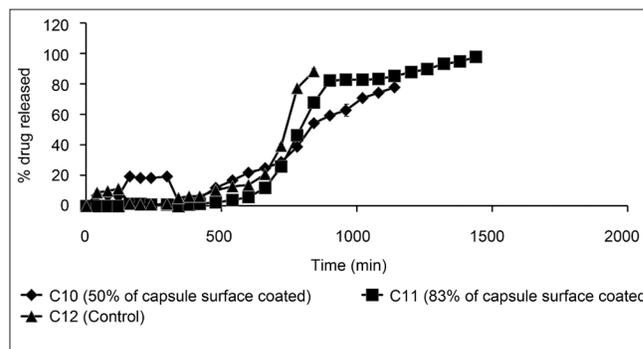

Figure 4: Effect of capsule surface coated with LOL on drug release from encapsulated multiparticulate granules containing 1% w/w methylcellulose

The graphical representation of Korsemeyer-Peppa's and other release models were not shown for want of space; however, their release kinetic parameters are presented in Table 3. As can be seen, C1 released drug by Zero order, Hixson-Crowell and gave a good fit for Korsemeyer-Peppas model which confirmed the type of operational release mechanism. C2 showed a good fit with Higuchi, zero order and Korsmeyer-Peppas release mechanisms. C4 fitted well into Higuchi, Zero order and Hixson-Crowell; in addition it also fitted into Korsmeyer-Peppas model, with an "*n*" value of 0.54. The release profile of C5 fitted into Zero order and Hixson-Crowell models but C6 only Hixson-Crowell. The release profile of C7 did not have good fit for any of the release models (kinetics); its dissolution curve in Figure 3 indicated two distinct slopes, with one being approximately six times more than the other.
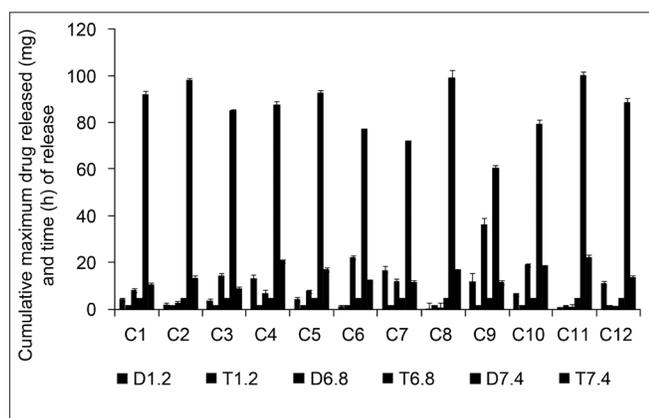

Figure 5: The various cumulative maximum drug released (D) and the time of release (T) at media pH 1.2, 6.8 and 7.4, respectively

DISCUSSION

In vitro drug release at pH 7.4 was to predictably parallel *in vivo* drug release in the colon. The higher $D_{7.4}$ and $T_{7.4}$ values of most of the EC capsules reflected the predominant influence of polymer concentration-based hydrophobicity; which offered efficient structural and permeability barrier to the capsule. The synergistic barrier constitution of LOL secondary coating and Eudragit L-100 primary coating resulted not only to, high $D_{7.4}$ and $T_{7.4}$ but also the retention of some or most parts of their shapes postdissolution.

Consequently this created a conducive platform for sustained drug release, which presents a possible potential advantage of wider drug spread from the ileocecal to the other parts of the lengthy colon. A combination of initial delay or minimal release and subsequent prolonging of drug release were attributes possessed by this formulation approach that, involved double layers of polymer coating. A suitable colon-targeted formulation dosage form should be sufficiently gastroresistant to delay and subsequently promote prolonged drug delivery to the colon, in spite of precarious gastrointestinal barriers and forces that must be negotiated. This is therapeutically significant in such disease conditions as amebiasis.

The colon is known to be a site where comparatively limited absorption of drugs takes place, since highly absorptive cells like villi are absent.[26] Therefore prompt deposition of drug from the dosage form at the ileocecal region or beginning of the ascending colon may be less preferred to a gradual release process. This is mostly important for some disease conditions that are associated with and traverse the sigmoid or descending portions of the colon. In amebic infections the trophozoites of the microorganism *E. histolytica* reside in the

Table 3: The various release models and their release parameters

Mc (%)		Higuchi		Zero order		First order		Hixson crow		Korsemeyer-peppas		Kitazawa		DE	MDT
		K_H	R^2	K_0	R^2	K_1	R^2	K_{HC}	R^2	N	R^2	K_{K1}	K_{K2}	(%)	(H)
C1	1%	89	0.94	15.9	0.98	-0.18	0.89	0.45	0.96	1.10	0.98	0.2	0.8	62.4	4.0
C2	1%	87	0.96	13.6	0.95	-0.23	0.93	0.43	0.87	1.2	0.97	0.6	nd	32.8	3.8
C3	1%	101	0.68	21.6	0.78	-0.21	0.67	0.70	0.90	2.1	0.99	1.6	<0.1	20.0	2.5
C4	4%	11	0.97	1.85	0.99	-0.04	0.84	0.38	0.99	0.54	0.95	nd	nd	100	1.5
C5	4%	62	0.92	9.53	0.96	-0.09	0.84	0.35	0.96	1.54	0.85	0.2	0.4	55.6	5.9
C6	4%	72	0.81	12.7	0.88	-0.10	0.80	0.50	0.98	1.62	0.94	0.4	0.1	43.0	4.2
C7	1%	67	0.7	12.6	0.78	-0.09	0.74	0.51	0.88	1.4	0.77	0.6	<0.1	23.0	1.3
C8	1%	66	0.84	9.96	0.90	-0.15	0.69	0.41	0.98	2.4	0.96	0.3	2.0	36.0	3.6
C9	1%	46	0.91	8.07	0.95	-0.05	0.95	0.35	0.94	1.1	0.85	0.1	<0.1	66.8	3.8
C10	4%	43	0.95	6.17	0.98	-0.05	0.94	0.22	0.97	1.2	0.98	<0.1	<0.1	100	1.4
C11	4%	53	0.89	6.8	0.89	-0.08	0.92	0.26	0.86	1.9	0.91	0.4	0.2	41.6	9.1
C12	4%	56	0.70	9.37	0.77	-0.09	0.70	0.33	0.91	1.1	0.65	<0.1	0.6	57.6	5.3

% MC=%w/w methyl cellulose concentration; nd=No defined difference in K-value; K_0=Zero order; K_1=First order rate constant; K_H=Higuchi rate constant; N=Korsemeyer-Peppas slope value; K_{HC}=Hixson-Crowell rate constant; K_k=Kitazawa rate constant; C1=Capsules with 50% surface coated with LOL containing 0.47 mm granules and 1% methylcellulose; C2=Capsules with 83% surface coated with LOL containing 0.47 mm granules and 1% methylcellulose; C3=Capsules with 0% surface coated with LOL containing 0.47 mm granules and 1% methylcellulose; C4=Capsules with 50% surface coated with LOL containing 0.47 mm granules and 4% methylcellulose; C5=Capsules with 83% surface coated with LOL containing 0.47 mm granules and 4% methylcellulose C6=Capsules with 0% surface coated with LOL containing 0.47 mm granules and 4% methylcellulose; C7=Capsules with 50% surface coated with LOL containing MPG and 1% methylcellulose C8=Capsules with 83% surface coated with LOL containing MPG and 1% methylcellulose; C9=Capsules with 0% surface coated with LOL containing MPG and 1% methylcellulose; C10=Capsules with 50% surface coated with LOL containing MPG and 4% methylcellulose; C11=Capsules with 85% surface coated with LOL containing MPG and 4% methylcellulose; C12=Capsules with 0% surface coated with LOL containing MPG and 4% methylcellulose

lumen of the cecum and large intestine and also adhere to the colonic mucus and epithelial layers.[27] Therefore, longer transit time and wider distribution of the drug would be appropriately required.

It is also one of the most potent cytotoxic cells known, for its ability to destroy human tissues.[27,28] Infection occurs when the cyst form of the parasite is ingested with contaminated food or water. After excysting to form the trophozoite in the small intestine, the ameba can colonize the bowel lumen, invade through the intestinal epithelium to cause colitis or liver abscess, or form cysts that are excreted with the stool to start a new round of infection.[27,29] So a preferred colon-targeted dosage form that will be able to slowly release its metronidazole content as it transits the entire colon should be potentially able to have good lethal contact with either the trophosoites or cysts. If there is a good widespread contact between the metronidazole and the cysts, there may be a complete eradication of the cysts and avoidance of cross-infection. In addition its antibacterial effect on the trophozoites may prevent the possibility of cytotoxicity.

Almost in all cases, based on visual observation, the initial erosion of the LC capsules started from those small portions uncoated with LOL (i.e., portions with only Eudragit® L-100 coatings). Gradual erosion of this end prompted ingress of fluid into the encapsulated granules and possible time-based gel formation, capable of drug release retardation.[30-32] For FC capsules, the eroded portion exceeded that of EC. In addition UC capsules had the largest erodible portion exposed to the dissolution medium. In the course of dissolution therefore, complete erosion of the Eudragit® L-100 coated surface took place, resulting in total capsule collapse. This total collapse of control capsules was not the case with any of the EC or FC capsules which retained part of their structures, thus acting as partial reservoir systems.

The vulnerability of the UC capsules (control) to total collapse could result to burst or premature release and unprecedented dose-dumping. It has been reported that drug release above 30% within 1 h of dissolution creates the chances of dose-dumping.[16] This may explain in part the limitation of pH-dependent polymer approach. This means that secondary or outer coating with LOL offered advantage over a single primary coating with only Eudragit® L-100. Thus, lending additional credence to previous report that coating with only pH-sensitive polymers is associated with higher failure rates.[17] Some workers have also adduced that the pH-dependent polymer approach has shown lack of site-specificity because of inter/intrasubject variation and the similarity of the pH between the small intestine and the colon.[33-34] In this work the use of this additional coating agent (LOL) was to augment and make up for this deficiency.

Another factor worthy of note is the possible prevalent hydrodynamic conditions imposed by the rotating basket during dissolution. The capsule-containing basket rotated in the clockwise direction with the capsule resting within it. Sometimes the capsule may be positioned with one end facing the same direction throughout a dissolution course; some other times this may not be so. However if the eroded portion faced the direction of rotation, hydrodynamic forces may impinge more on that end through influx and pressure of fluid, thus triggering faster drug release from the capsule. Some other times the eroded capsule portion may not be permanently on one direction, in which case a different effect may be observed. Kumar et al,[35] reported the effect of hydrodynamic forces on the release of drugs from matrix and reservoir systems. EC capsules are more likely to experience less hydrodynamic impact than FC and UC capsules.

Particle size played a time-based role on the release profile of metronidazole from the coated capsules. MPG capsules witnessed more evident prolonging of drug release than those of FOS since they provided a better platform for the delay of drug release from the capsules for longer times ($T_{7.4}$). The intracapsular packing of these MPG may have been tighter than the monosized FOS; this could have created lower theoretical intracapsular bulk density.[14] Possibly, higher void spaces in FOS granules may have predisposed them to elicit faster ingress of greater quantity of fluid than MPG granules[32] thus promoting faster drug diffusion; hence the shorter $T_{7.4}$ values recorded.

Although it was observed that particle size affected the dissolution pattern, mere increase in surface area of the drug or its granules may not always guarantee a corresponding increase in dissolution rate.[32] Rather it is the increase in the effective surface area or the area exposed to the dissolution medium and not the absolute surface area that is directly proportional to the dissolution rate.[36] According to the Nernst-film model theory,[36] under the influence of no reactive chemical forces, when a solid particle is immersed in a liquid it initially forms a solution at the interface, forming a thin stagnant layer or film around the particle; subsequently diffusion of drug from this layer takes place at the boundary to the bulk of the fluid. Relating this to our coated capsules, the permeation of fluid into the capsule was the most crucial factor, followed by formation of drug solution upon interaction with the dissolution medium and subsequent diffusion into the bulk solution. Relatively wide intracapsular void spaces may probably encourage more fluid distribution and permeation into the spaces and granules, respectively, consequently resulting to more drug dissolution. It should be recalled that for the LC capsules, fluid permeation was mostly unidirectional via the erodible Eudragit® L-100-coated end.

Kinetics of drug release

The "n" values of C1 and C2 were above 1, an indication of super case 11 transport (strong presence and predominance of zero-order release). The high regression line (R^2) value of 0.98 confirms zero order as the predominant release

mechanism in operation. Although Hixson-Crowell release kinetics was evident in C1 it was minimal in comparison with zero order. It could be due to release taking place mostly through an orifice than from different parts of the capsule surface. Such occurrence of different release mechanisms appeared to be more common than in tablets where drug release rather takes place simultaneously from all over the tablet surface. The common zero order release model that described the release pattern of C1 and C2 may be attributed to the relative similarity in their dissolution curves [Figure 1] and zero-order rate constant (K_0) values [Table 3].

Zero order release means drug release taking place independent of time and concentration while Hixson-Crowell release mechanism[23] also known as cube root model describes changes in the surface area of the tablet/capsule where progressive dissolution of matrix takes place as a function of time.[37] Coating of the capsules with LOL evidently preconditioned the release process by introducing different release mechanisms. The control capsule (C3) revealed no good fit with any of the release models. It may not be unconnected with multisource release concomitantly taking place from the capsule surface. It was earlier pointed out that all the LC capsules maintained most part of their structural shapes up to the limit of the secondary coating. Furthermore the two extreme Kitazawa k-values of >1.0 and <0.1 recorded by C3 connoted sharp changes in the dissolution process. In comparison, C2 and C1 which did not have such extreme Kitazawa values as C3 showed evidence of more release kinetics.

$0.5 < n < 1.0$ implies anomalous (non-Fickian) transport and refers to a combination of both diffusion and erosion controlled drug release.[38-39] By this is meant drug diffusion taking place concurrently with progressive slow erosion of matrix/gel surfaces. C4 with "n" value of 0.54 interestingly witnessed the most prolonged drug release ($T_{7.4} = 17$ h) with evidence of "no-defined" (nd) Kitazawa (K) values. "No defined" Kitazawa values, in turn implied absence of sharp slope changes in the dissolution curve. Slope changes are due to sudden changes in rate of drug release. In addition, the zero order good fit observed in C4 was typical of a perfect sustained drug release where slow release of

drug takes place independent of time and concentration. Design of an oral dosage form to release its drug content over extended period and at a constant rate (zero order) that is independent of concentration is the expected ideal in sustained release technology. Some workers[40-41] produced asymmetric membrane capsules and tablets which successfully controlled drug release for a long time. Some of them[42] who worked with asymmetric membrane (cellulose acetate coated) capsules reported drug release taking place by zero order kinetics. This was attributed to the insoluble and semipermeable nature of the modified capsule surface.

As can be observed the K_0 value of C4 was approximately one-ninth that of C5 which is in tandem with its longer $T_{7.4}$ value. The presence of distinct slopes in C7 may portend the absence of predominant release mechanism/s. On the other hand C8 fitted into Hixson-Crowell and Korsmeyer-Peppas release kinetics. Although the linearity (R^2 value) of the Korsmeyer-Peppas plot was 0.96 and the "n" value unusually as high as 2, there was no good zero-order fit. On further examination it was observed that one of the Kitazawa values was 2.0 (and that was the highest) while the other was 0.3. The two extreme variant Kitazawa k-values corroborated the sudden sharp change in drug release as depicted by an initial slow drug release for about 1 h prior to the abrupt but steady and prolonged release as shown in Figure 3. An interplay between this sharp difference in slope values and impact of % capsule surface coated with LOL may have contributed to this aberrant case of high Korsmeyer-Peppas "n" value (of above 1) without a good fit with zero order kinetics.

It is becoming more evident that a dissolution curve with sustained release pattern and occasional minimal slope changes that are not sharp, may accommodate a number of predominant release mechanisms. This was the case with C10 which fitted into Higuchi, zero order, Hixson-Crowell and Korsmeyer-Peppas release kinetics. On the contrary sharp changes may engender poor fits as reflected in C11 and C12 (control) which lacked good fit.

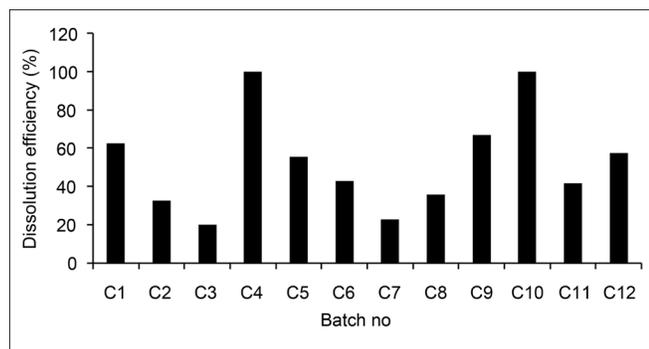Figures 6 and 7 show the graphical representation of dissolution efficiency (DE) and mean dissolution time

Figure 6: Chart representation of the dissolution efficiency values of 0.47 mm (C1-C6) or multiparticulate (C7-C12) granules
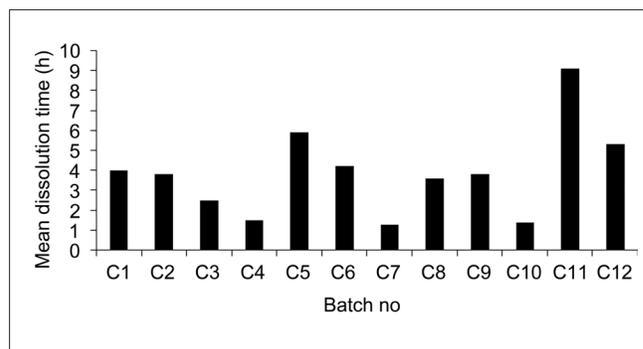
Figure 7: Chart of mean dissolution time of capsules containing 0.47 mm (C1-C6) or multiparticulate (C7-C12) granules

(MDT). C4 and C10 gave the highest DE of 100% and one of the least MDT of 1.5 and 1.3 h respectively. Incidentally these two batches were capsules with 50% of their surfaces coated with LOL and the only two batches that witnessed good fit with up to four release mechanisms. Their high DE correlated well with their low MDT. It should be noted that their $T_{7.4}$ were close, i.e., 21 ± 1.4 and 19 ± 2.8 h, respectively. With the exception of C7 all the FC capsules recorded above 50% DE. Having 50% of the capsule surface coated with LOL therefore may have predisposed the capsules to higher DE and lower MDT than EC capsules. With the exception of C1 which almost had the same MDT with C2 the rest of the EC capsules indicated higher MDT than FC. This is in line with their general longer $T_{7.4}$ and higher coating-occasioned extended drug release. Some workers[39] have reported the presence of lipophilic binder as being responsible for higher MDT values. The hydrophobic character of LOL, the candidate coating agent contributed to this observation. Generally in controlled drug delivery higher MDT should be an indication of prolonging of drug release and is recommended in colon-targeted drug delivery for efficient drug release and improved contact time within the colonic region; however, this should also be complemented by acceptable DE. DE describes the efficiency of drug dissolution.

CONCLUSIONS

The complementary effect of the two hydrophobic polymers evidently controlled metronidazole release; with FC capsules exerting a greater significant ($P<0.05$) effect on drug release. Particle size significantly ($P<0.05$) affected drug release while, matrix former concentration did not. Coating of capsule surface predisposed to mostly more than one release kinetics. Most FC capsules recorded higher DE but lower MDT than those of EC. Therefore Eudragit® L-100 and LOL employed as primary and secondary coating agents, respectively, synergistically impacted on most of the capsules to delay and later release greater quantity of drug for a prolonged period of time at pH 7.4, thus buttressing their potentiality in targeting of drugs to the colon. Further research work to attempt *in vivo* correlation is being contemplated.

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