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FORMULATION AND EVALUATION OF TINIDAZOLE-LOADED ALGINATE MICRO-BEADS FOR COLON DELIVERY

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ABSTRACT

The colon is a site that allows for both systemic and local delivery of drugs. Colon-targeted drugs are more effective therapeutically with reduced systemic effects. In this study, we developed tinidazole microbeads using a combination of lipids, surfactants and synthetic polymers to produce extended release formulation of tinidazole beads and to minimize the systemic side effects. Tinidazole loaded microbeads were formulated using emulsification ionic gelation technique. The resulting beads were coated differently with Eudragits and Kollicoat. They were evaluated for drug content, encapsulation efficiency, particle size and morphology, thermal analysis, *in vitro* in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.2) and *in vivo* release properties. The encapsulation efficiency range was within 47 ± 0.08 to 93.6 ± 0.02 % and the particle size was within 32.7 ± 0.08 to 44.4 ± 0.09 µm. Batch T_4 was the best batch of the formulations as it gave the highest EE and *in vitro* release. The swelling index result obtained, showed that batch T1 did not swell in pH 1.2, while batches T2-T4 showed swelling ability. The *in vivo* studies showed that after 24 h, the uncoated batch T4 achieved peak serum concentration of 293.37 µg/ml, while the marketed sample achieved peak serum concentration of 188.21 µg/ml at 24 h. The characterization studies showed that the alginate beads could be considered as potential systems for colon delivery of tinidazole for use in the treatment of amoebic infection.

KEYWORDS: **Alginate beads; Colon delivery; Tinidazole; Kollicoat®; Eudragits®.**

INTRODUCTION

The effect of a drug in a patient depends on the pharmacological properties associated with the drug. These effects occur as a result of the interaction between the drug and receptors at the site of action. The efficacy of this drug-target interaction is dependent on the ability of the drug to be delivered to its site of action at a concentration and rate that causes minimum side effects and maximum therapeutic effects [1]. The essence of the targeted drug delivery (TDD) is to deliver the medication only to areas of interest within the body [2]. The major difference between the TDD and the conventional drug delivery (CDD) is that the former gets released in a dosage form, while the latter functions by the absorption of drugs across the biological membrane [3]. The conventional dosage forms are associated with certain disadvantages. For instance, the parenteral delivery of drug is highly invasive with ephemeral effects. Although the oral administration of drugs is popular and appropriate, it cannot be used for certain drugs such as protein due to the poor absorption associated with oral route [3].

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The technology associated with the TDD has been refined in such a way as to take into consideration certain factors such as bioavailability, drug absorption processes, pharmacokinetic process and timing for optimal drug delivery [1]. The major requirement associated with targeted drug delivery system include: retain, evade, target and release [4]. The drug should be able to be loaded into an appropriate drug delivery vehicle, ability to escape the secretion of the body that may degrade it which may lead to long retention time. The use of different DDS is dependent on the different sites of interest within the body [4]. There are two major strategies that are used to target a drug to its desired organ [3]. The passive targeting involves the accumulation of drug around the specific site. It is usually found in tumor tissue and in the treatment of microbial infections such as leishmaniosis, candidiasis and brucellosis [3]. The active targeting involves the interaction between the ligand receptor. Research has made it possible to target drug delivery into the colon. It is desirable in the treatment of amebiasis, ulcerative colitis, crohn's disease and colonic cancer [5].

Tinidazole is the drug of choice used in the treatment of acute and chronic amoebiasis and other protozoal disease [6]. The elimination half-life is 12 – 14 h, and it's usually excreted via urine (20-25 %) and feaces (12 %) [7]. The associated side effects include seizure, vaginal itching, nausea, vomiting, constipation and stomach cramps. When taken orally as a conventional tablet, it causes gastric distress [7]. For peptides, the colon is believed to be the suitable absorbing site due to the fact that it has less diversity and intensity of digestive enzymes, protection of peptides from hydrolysis, enzymatic degradation in duodenum and jejunum [8] and enhanced systematic bioavailability and long residual time [9]. The use of pH sensitive polymer coated drug delivery to the colon is one of the primary approaches for CDDS. During fasting, the pH range of the stomach is between 1 to 2, although it increases after eating [10]. In the proximal and distal small intestine, the pH is 6.5 to 7.5 respectively. The differences in pH levels necessitates the use of pH dependent polymers. The pH dependent polymers used in colon specific drug delivery are insoluble in low pH levels but become increasingly soluble as pH rises [11].

Among the different approaches to achieve targeted drug release to the colon, the use of polymers especially the ones which are degraded by colonic bacteria hold great promise. Recently, gastro-retentive systems for treating bacterial infections have shown special interest. The prolongation of the local availability of tinidazole has been reported to be an important factor in the treatment of amoebic dysentery [12]. This formulation would be beneficial in delivery higher concentrations of tinidazole in the colon ensuring better microorganism eradication. The aim of this study was to develop tinidazole microbeads using a combination of lipids, surfactants and synthetic polymers employing emulsification ionotropic gelation technique to target the colon for local actions, produce extended release formulation of tinidazole beads and to minimize the systemic side effects.

MATERIALS AND METHODS

Materials

The following materials were used as procured: Sodium alginate (Thermo Fisher Scientific Inc., USA), Olympic Pure Sunflower Oil (Ren Solsikkeolje, UK), Moringa oil (a batch prepared in Pharmaceutical Technology and Industrial Pharmacy), Triacetin, Kolliphor EL, Kolliphor P188, Kollicoat MAE 100 P (a kind gift from BASF, Ludwigshafen, Germany), Neusilin (Fuji Chemical Ind. Co. Ltd, Japan), PEG 6000 and Calcium Chloride Dihydrate ($CaCl_2.2H_2O$) (Qualikems, India), Eudragit® S100 And Eudragit® L100 (Evonik Industries, Germany), Magnesium stearate (Alfa Chemical Corp, New York, USA), Talc (Honeywell Specialty Chemicals, Germany), Tinidazole (A kind gift From Pauco Pharmaceuticals Ltd Awka, Nigeria), All other reagents were of analytical grade and were used as procured.

Preparation of the tinidazole emulsion

Using the formula in Table 1, tinidazole o/w emulsion was prepared. The emulsion formed from the various batches were introduced into different concentrations of calcium chloride (5 %, 10 %) through an orifice diameter of 0.6 × 25 mm. A curing time of 30 mins was observed, after which the beads were decanted and dried under room temperature. The beads formed from the various batches either collapsed after drying or had the drug leached out that gave a very weak bead that collapsed on touching. However, an emulsion formed from only sunflower oil without KP188 (batch C5) produced a stable and strong microbeads. This formula was then optimized for the experiment. Moreover, beads formed from the different concentrations had no difference. Therefore 5 % concentration of calcium chloride was chosen.

Formulation of the tinidazole beads using the optimized formula

The tinidazole emulsion was prepared using the formula shown in Table 2. Sodium alginate (NaAG), Kolliphor EL (KEL), Kolliphor P188 (KP188), sunflower oil (SFO), tinidazole and deionized water was used to prepare an emulsion for each batch. The emulsion was introduced drop-wise into a 5 % $CaCl_2$ solution using a syringe. The formed beads were allowed to cure for 30 min, and then washed with distilled water to remove excess cross linking agent and then dried at room temperature [13].

Preparation of coated drug loaded microbeads

Weighed quantities of excipients listed in Table 3 were dissolved in ethanol (temperature of the solution was raised to 37 ± 0.5 °C) to which a weighed quantity of drug loaded beads from a set of batches were dipped into for 5 min each and allowed to dry at room temperature for 2 h. Another set of batches of drug loaded beads were similarly dipped into a different ethanol solution (temperature of the solution was raised to 37 ± 0.5 °C) containing weighed quantity of Kollicoat MAE 100P for 5 min and allowed to dry at room temperature for 2 h.

Characterization of microbeads

Particle size and morphology

The particle size of drug loaded microbeads from various batches were determined by introducing each batch of microbead on a slide and viewed under a Hund® Binocular microscope (Weltzlar, Germany) attached with a Motic image analyzer (Moticam, China) at ×400 magnification.

Thermal analysis of drug loaded beads

Melting transitions and changes in heat capacity of the pure sample of tinidazole, sunflower oil, drug loaded T4 and drug loaded Kollicoat coated T4 microbeads were determined using a Differential Scanning Calorimeter (Netzsch DSC 204 F1, Geratebau, GmbH, Selb, Germany). About 1 mg of each sample was weighed into aluminum pan, hermetically sealed and the thermal behavior determined within the range of 20- 500 °C, at a heating rate of 10 K/min under a 20 ml/min nitrogen flux [14].

Swelling Index of drug loaded beads

Weighed quantities of T1, T2, T3, and T4 each were put into buffer pH 1.2, 6.8 and 7.2 and their weights were taken at 0.5, 1, 1.5 and 2 h. The swelling index of the drug loaded beads was calculated using the formula:

$$\text{Swelling index} = \frac{\text{final weight} - \text{initial weight}}{final\ weight} \times 100 \quad ..1$$

Drug content and encapsulation efficiency

A 20 mg quantity each of T1 and T2, and 21 mg quantity each of T3 and T4 were weighed and individually put into 3 mls of acetone and vortexed for 0.5 –1 h until the drug loaded beads disintegrated [15, 16]. The solution was made up to 10 ml using buffer pH 7.2. It was filtered and diluted 100 fold using the same buffer. The absorbance was read off using UV/VIS spectrophotometer at 310 nm. The encapsulation efficiency was calculated using the formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual drug content}}{Theoretica\ \ drug\ content} \times 100 \quad \ldots\ldots\ldots\ldots\ 2$$

In vitro drug release

Weighed quantity of drug loaded beads, equivalent to 5 mg each from various batches was individually wrapped in a dialysis membrane and secured tightly in a magnetic stirrer stand. The dissolution medium was simulated intestinal fluid (pH 7.2) maintained at 37 ± 0.5 °C. The wrapped beads were immersed in the dissolution medium set at 150 rpm and 1ml sample was drawn at 30 min interval, and the volume of the dissolution medium was maintained by replacing with 1ml of the buffer. The absorbances of solutions were measured at 280 nm using the UV-spectrophotometer (Spectrumlab 752S, Hitachi, Japan) and a calibration curve was plotted between concentration of drug (µg/ml) on x-axis v/s absorbance on y-axis to get the linearity and regression equation [17]. The above procedure was repeated also for each batch on buffer pH 1.2, 6.8 and 7.4.

In vivo release study

Clinically normal wistar rats weighing 100- 120 g were prepared for the experiment. Earlier the rats were acclimatized to the new environment, housed separately in metabolic cages. They were allowed free access to food and water throughout the study. The rats were divided into six groups of six rats each. The microbeads were fed to the rat. Prior to the administration, blood samples were drawn from the retro-orbital venous plexus of the rats. Then, with reference to their weights, the calculated dose volumes were administered to the rats orally using separate 1ml calicula. Batches of T4 (uncoated, coated and kollicoat coated) having the highest % EE, pure tinidazole (positive control) and marketed tinidazole were administered at a dose of 45 mg/kg and the control group received the unloaded

microbeads. After drug administration, drug samples were drawn from the retro-orbital venous plexus of the rats at 2, 8, 12, 24, 36, 48, 60, and 72 h collected in EDTA tubes, centrifuged at 4000 rpm for 25 min and their sera were diluted with buffer 7.4 and analysed spectrophotometrically at 300 nm. The Ethical Approval Number: H15REA156. Approval date: 3rd September, 2016, Expiry date: 3rd September, 2019.

Statistical analysis

The measurements were done in replicates and data analyzed by descriptive statistics and student t-test using Excel Microsoft Office, Version 2007 and Graph Pad Prism, version 6. Statistical difference between means considered significant at ($p < 0.05$).

RESULTS

Particle size, morphology analysis and encapsulation efficiency

The microbeads were characterized for particle size as shown in Table 4. The particle size ranged from 32.7 to 44.5 µm. T3 had the highest particle size of 44.5 µm, while T1 had the lowest particle size of 32.7 µm. The particle size of T2 was higher than T1 without a significant difference ($p < 0.05$), while the particle size of T3 was higher than T4, without a significant difference ($p < 0.05$). The particle size showed that among the batches, there was no significant difference ($p < 0.05$). The photomicrographs are shown in Figures 1-4.

Encapsulation efficiency

High encapsulation efficiency is very important to reduce drug wastage [12]. Encapsulation efficiency of optimized formulation T2 was 93.6 ± 0.02 %, whereas T1 microbeads showed the least encapsulation efficiency of 47.90 ± 0.02 %, with a significant difference ($p < 0.05$). For formulations T3 and T4, formulation T3 gave an EE of 74.90 ± 0.02, while T4 gave an EE of 84.60 ± 0.01, without a significant difference ($p < 0.05$). This may be due to possible leakage of drug from alginate microbeads.

Thermal analysis

The DSC measurements were carried out in order to determine the thermotropic behavior of the drug and optimized batches. It is used in determining the effects of excipients on the physicochemical properties of the drug especially in the determination of incompatibilities between the drug and the excipients. DSC patterns of tinidazole, sunflower, uncoated T4 and collicoat T4 are shown in Figures 5-8 respectively. The DSC melting peaks of tinidazole is similar to the melting point reported by Kuldeep and co-workers [18]. As shown in Figures 7 and 8, the thermogram of uncoated T4 and Kollicoat T4 showed a broad peak of 122.2 °C and 122.5 °C respectively. As the heat flows into the pure sample of tinidazole, bonds holding the molecules break up and the sample melts. This is an endothermic process as heat is required and the temperature at which this occurred was at 128.5 °C which is seen as a sharp peak in Figure 6. This melting peak showed that the tinidazole used was pure and crystalline. In the DSC of the formulations shown in Figures 7 and 8, the uncoated T4 showed a broad peak which signifies the melting point of the active constituents; tinidazole. The broad rounded peak signifies a reduction in crystallinity of the drug [18]. Reduction in enthalpy suggests less crystallinity of lipid matrix [19., 20]. However, as a result of the presence of excipients, there was a shift in the melting peaks to 122.2 °C for uncoated T4 and 122.5 °C for T4 Kollicoat coated T4. However, the latent heat of fusion for both formulations was different as 570.1 J and 361.6 J was reported for uncoated T4 and Kollicoat coated T4 respectively. There was a second exothermic peak in the thermogram of the formulations (Figures 7 and 8). The thermogram of sunflower oil showed a broad rounded exothermic peak at 188.1 °C corresponding to the polymerization of the fatty acid components of the sunflower oil. The results obtained from the DSC thermograms, showed compactibility between the drug and the excipients.

Swelling index

The swelling index of the 4 batches are presented in Table 5. Batch T1 did not swell in pH 1.2, while batches T2-T4 showed swelling ability. For batches T2-T3, there was swelling and disintegration within 30 min in phosphate buffer pH 6.8 and 7.2. This shows that the microbeads are stable in acidic pH and will not likely release the drug in this medium.

In vitro release studies

The *in vitro* release was performed on all the batches (coated, uncoated and kollicoat coated) in different buffers. The percentage drug release was plotted against time. In pH 7.2, there was a burst release within the first 2 h for uncoated batch T3 (86.23 %) but for the uncoated batch T4, there was a decrease within the first 2 h. At 3.5 h, there was a burst release (99.45 %).

In vivo release study

The Table 6 represents the *in vivo* release studies. It was observed that after 24 h, the uncoated T4

Table 1: Formula for preparation of the tinidazole emulsion

Batch Code	Quantities per unit dose %w/v									%w/w
	MO	SFO	TR	NS	MS	KP188	KEL	Water	NaAG	Drug loading mg/g of total mixture.
C1	70	-	-	-	-	1	1	25	3	250
C2	40	30	-	-	-	1	1	25	3	250
C3	-	70	-	-	-	1	1	25	3	250
C4	-	70	-	-	-	-	2	25	3	250
C5	-	70	-	-	-	-	1	25	3	125
C6	-	-	69	2	-	1	1	25	3	125
C7	-	30	39	-	2	1	1	25	2.5	125
C8	-	39	30	2	-	1	1	25	2.5	125

Key: MO- Moringa oil, SFO- Sunflower oil, TR- Triacetin, NS- Neusilin, MS- Magnesium Stearate, KP188- Kolliphor P188, KEL-Kolliphor EL, NaAG- Sodium Alginate.

Table 2: Quantities of materials used for the optimized bead formulation

Batch code	SFO (%w/v)	KP188 (%w/v)	KEL (%w/v)	De-ionised water (%w/v)	NaAG (%w/v)	Target weight (%w/w)	Drug loading mg/g of the total mixture (%w/w)
T1	70	-	2	25	3	20	125
T2	70	1	1	25	3	20	125
T3	43.3	-	1	25	3	80	125
T4	22	-	1	25	3	80	125

Key: SFO- Sunflower oil, KP188- Kolliphor P188, KEL-Kolliphor EL, NaAG- Sodium Alginate.

Table 3: Quantities of coating materials

Excipients	Quantities (g)
Neusilin	0.20
PEG 6000	0.40
Eudragit S100	1.00
Eudragit L100	1.00
Talc	0.25
Kollicoat MAE 100P	3.00

Table 4: Particle size and Encapsulation efficiency (mean ± SD)

Batches	Particle size (µm)	Encapsulation efficiency (%)
T1	32.70 ± 0.08	47.80 ± 0.08
T2	39.12 ± 0.12	93.60 ± 0.02
T3	44.40 ± 0.09	74.90 ± 0.02
T4	43.20 ± 0.08	84.60 ± 0.01

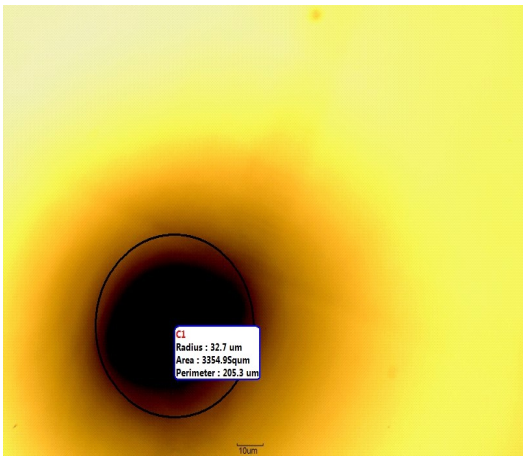

Figure 1: Photomicrograph of Batch T1

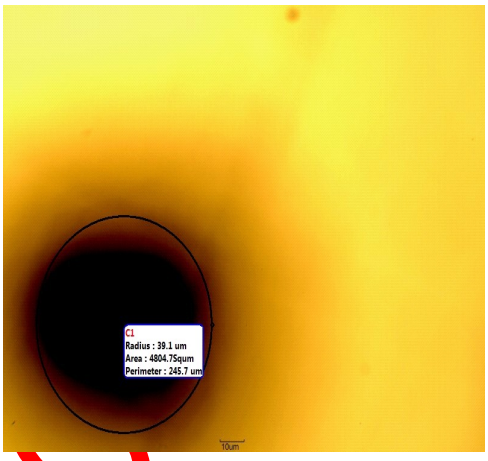

Figure 2: Photomicrograph of Batch T2

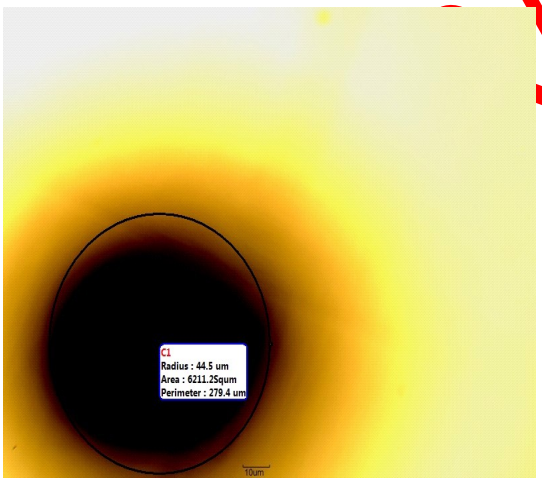

Figure 3: Photomicrograph of Batch T3

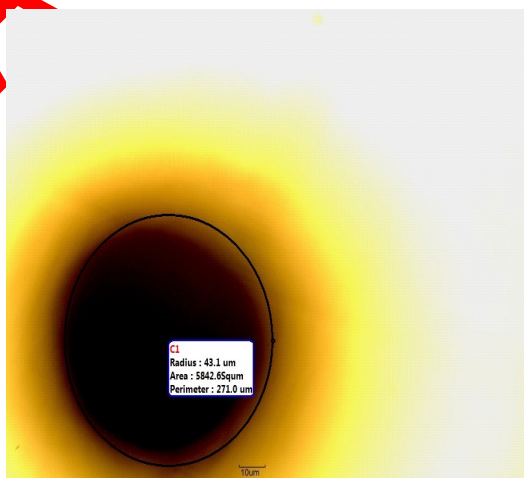

Figure 4: Photomicrograph of Batch T4

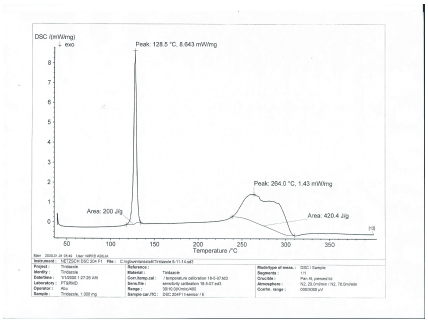

Figure 5: DSC thermogram of tinidazole

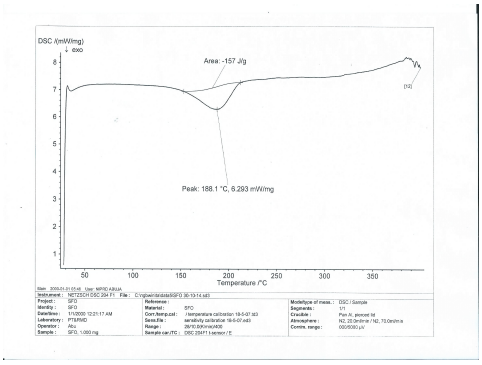

Figure 6: DSC thermogram of Sunflower oil

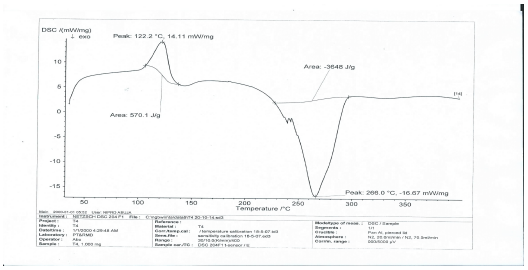

Figure 7: DSC thermogram of uncoated T4

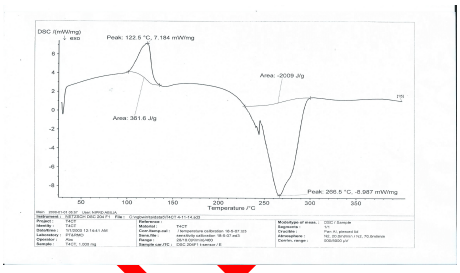

Figure 8: DSC thermogram of Kollicoat coated T4

Table 5: Swelling index

Batch code	Swelling index (%)									
	pH 1.2			pH 6.8				pH 7.2		
	0.5 h	1 h	1.5 h	2 h	0.5 h	1 h	1.5 h	0.5 h	1 h	1.5 h
T1	-	-	-	-	0	60	180	0	0	-
T2	-	20	-	10	322	-	-	-	-	-
T3	-	11.11	-	0	455.6	-	-	-	-	-
T4	-	33.3	-	44	333.3	-	-	-	-	-

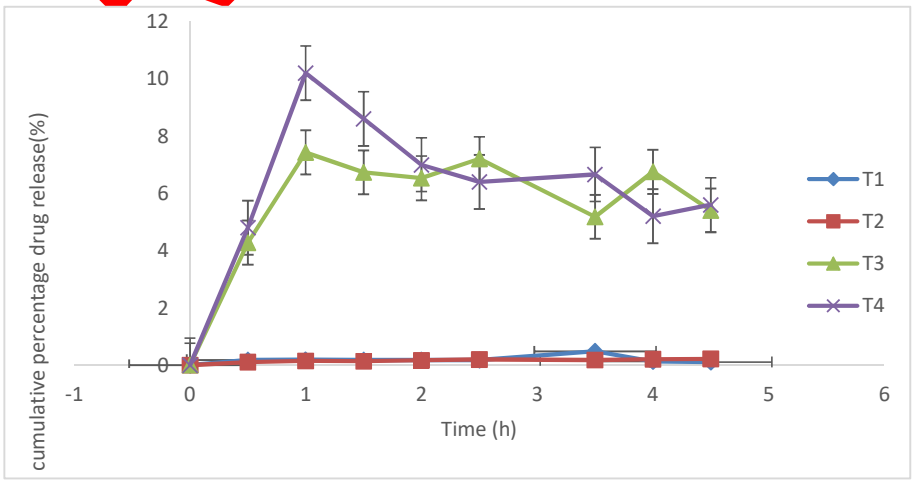

Figure 9: *In vitro* release of uncoated batches T1, T2, T3 and T4 in phosphate buffer pH 7.2.

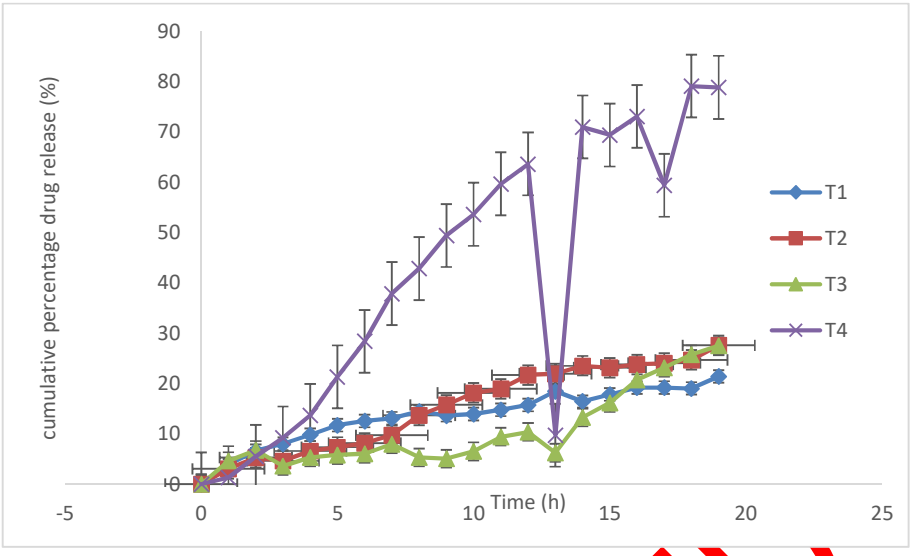

Figure 10: *In vitro* release of coated eudragit batches T1, T2, T3 and T4 in phosphate buffer pH 7.2.

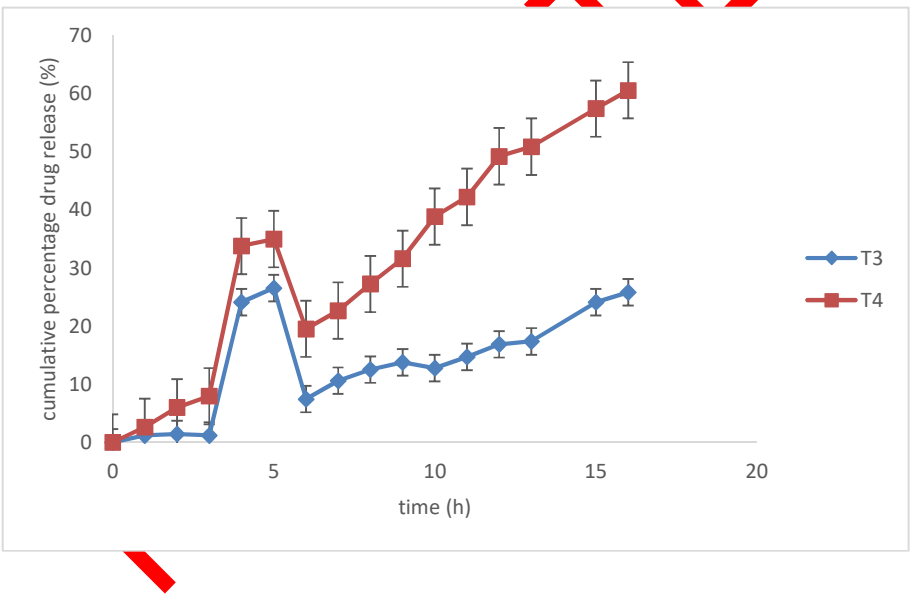

Figure 11: *In vitro* release of the Kollicoat coated T3 and T4 in pH 7.0.

Table 6: *In vivo* release studies.

Batch name	Time interval	K value	Mean drug release	Mean serum concentration	SD	COV
Pure sample	0	-	-	0	0	0
	2	0.0588	5.49	274.4	2.75	50.13
	24	0.0588	4.69	234.5	2.27	48.32
	48	0.0588	7.7	348.8	2.97	38.50
	72	0.0588	6.33	316.3	-	-
Kollicoat coated T4	0	-	-	0	0	0
	2	0.0588	4.19	209.61	0.61	4.63
	8	0.0588	3.24	161.99	0.11	3.34
	12	0.0588	2.94	147.11		
	24	0.0588	3.66	182.82	2.41	65.7
	48	0.0588	3.77	188.35	0.3	7.98
	72	0.0588				
Marketed sample	0	-	-	0	0	0
	2	0.0588	4.25	212.37	1.06	24.95
	8	0.0588	2.32	116.07	1.00	43.02
	12	0.0588	3.63	181.34	0.52	14.37
	24	0.0588	3.76	188.21	3.85	102.4
	48	0.0588	6.071	303.57	1.92	31.67
	72	0.0588	10.42	520.98	3.56	34.16
Uncoated T4	0	-	-	0	0	
	8	0.0588	4.19	209.47	0.76	18.07
	12	0.0588	5.48	273.81	0.16	2.96
	24	0.0588	5.87	293.37	-	-
	48	0.0588	3.81	190.48	-	-
	72	0.0588	5.71	285.71	-	-
Eudragit coated T4	0	-	-	0	0	0
	8	0.0588	3.95	197.7	0.74	18.77
	12	0.0588	4.73	1.04	1.04	22
	24	0.0588	4.09	0.95	0.95	22.30

achieved peak serum concentration, and after 48 h, the pure tinidazole gave highest serum concentration, while the marketed sample and Kollicoat coated T4 at 72 h, had not achieved their peak plasma concentrations yet. Although the marketed sample had a higher concentration than the Kollicoat coated T4 at 72 h, the Kollicoat coated T4 gave the best release among the formulations.

DISCUSSION

The composition of the emulsion had an effect on the particle size. It was observed that as the concentration of oil used in preparing the emulsion decreased, the particle size decreased. The result also showed that the particle size increased with increase in drug-polymer ratio. This could be due to the increase in polymer concentration. Kuldeep and co-workers [18] reported similar result using mebendazole for colon targeting. Although, the particle size of T2 was higher than T1, there was no significant difference ($p < 0.05$). The particle size of T3 (44.40 ± 0.09) was higher than that of T4 (43.20 ± 0.08), without a significant difference ($p < 0.05$).
The encapsulation efficiency of formulation T2 was higher than T1 with a significant difference ($P < 0.05$), while formulation T4 had a higher EE than T3, without a significant difference ($p < 0.05$).

The DSC melting peaks of tinidazole is similar to the melting point reported by Kuldeep and co-workers [18]. As shown in Figures 7 and 8, the thermogram of uncoated T4 and Kollicoat T4 showed a broad peak of 122.2 °C and 122.5 °C respectively. As the heat flows into the pure sample of tinidazole, bonds holding the molecules break up and the sample melts. This is an endothermic process as heat is required and the temperature at which this occurred was at 128.5 °C which is seen as a sharp peak in Figure 6. This melting peak showed that the tinidazole used was pure and crystalline. In the DSC of the formulations shown in Figures 7 and 8, the uncoated T4 showed a broad peak which signifies the melting point of the active constituents; tinidazole. The broad rounded peak signifies a reduction in crystallinity of the drug [18]. Reduction in enthalpy suggests less crystallinity of lipid matrix [19., 20]. However, as a result of the presence of excipients, there was a shift in the melting peaks to 122.2 °C for uncoated T4 and 122.5 °C for T4 Kollicoat coated T4. However, the latent heat of fusion for both formulations was different as 570.1 J and 361.6 J was reported for uncoated T4 and Kollicoat coated T4 respectively. There was a second exothermic peak in the thermogram of the formulations (Figures 7 and 8). The thermogram of sunflower oil showed a broad rounded exothermic peak at 188.1 °C corresponding to the polymerization of the fatty acid components of the sunflower oil. The results obtained from the DSC thermograms, showed compactibility between the drug and the excipients.

The swelling studies showed that Batch T1 did not swell in pH 1.2, while Batches T2-T4 showed swelling ability at different pH. According to Kuldeep and co-workers (2015), the results they obtained showed that percent degree of swelling was increased with increase in polymer concentration [18].

The *in vitro* studies showed that for Kollicoat coated batches of T3 and T4, there was an increase of drug release in pH 7.0, while for Eudragit coated batches of T1, T2, T3 and T4 in same pH, there was a highly significant increase in release for batch T4. From the results obtained, the effects of the excipients as well as their behavior in different pH can be seen, thus showing that release depends on the nature of the excipients [20].

CONCLUSION

This study has demonstrated the feasibility of formulating tinidazole alginate micro-beads by the emulsification ionic gelation technique for the treatment of acute and chronic amoebiasis. The formulated microbeads exhibited relatively better *in vitro* release profiles than the reference with a significant difference ($p < 0.05$). Further work has to be carried out on the pharmacokinetic and pharmacodynamics studies.

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