

## Development and Evaluation of Metronidazole Microspheres using Starch Isolates of Maize Genotypes as Sustained Release Polymer

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### Abstract

Genetic engineering of maize plants for improved yield, drought and pest resistance has received considerable attention in agricultural research. This work aims to develop metronidazole microspheres using starches obtained from genetically modified maize cultivars as controlled release polymers. Metronidazole microspheres were prepared by ionotropic gelation method using polymer blend of starches (A and B) isolated from genetically modified maize grains and sodium alginate. The microspheres were characterized using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). A 3<sup>2</sup> factorial design was employed using the entrapment efficiency, time taken for 50% (T<sub>50</sub>) and 90 % (T<sub>90</sub>) drug release as dependent variables while A, B and polymer-drug ratio were independent variables. SEM reveals that the formulations are polyhedral, hard and discrete with a smooth surface. Metronidazole microspheres formulations containing starch isolates from maize genotypes had significantly higher (p<0.05) entrapment efficiency. Formulations containing a blend of starch and alginate showed a more sustained release than the formulations having only alginate. Values of T<sub>90</sub> ranged between 6.12±3.20 to 47.13±7.01 hrs suggesting a sustained release of the drug. Generally, drug release from the microspheres was through erosion and polymer relaxation. The effect of type of polymer on the dissolution times was more significant (p<0.05) than those of polymer: drug ratio. This result shows that starches obtained from genetically modified maize grains can be employed as sustained release polymers in the formulation of metronidazole microspheres.

**Keywords:** Maize starch, Genetic modification, Metronidazole microspheres, Release properties.

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### 1. Introduction

Advances in drug delivery technologies have led to the development of multi particulate systems such as microspheres, microcapsules and microbeads with numerous benefits to drug delivery and disease management. Multi particulate delivery systems offer a wide and uniform drug distribution in the gastrointestinal tract which reduces gastric mucosa irritation and prolong drug

delivery. Microspheres consist of protein and biodegradable polymers which are free flowing with particle size of usually between 1-1000 μm (1). The spherical shape and micro-size make them to be easily dispersed in the body when injected and also offers a means of delivery of drugs directly into the bloodstream. Microspheres offer a means to targeted drug delivery with specificity and reduce plasma drug fluctuations resulting in minimal toxicity and side effects. They are used to control drug release, reduce dosing frequency and improve patient compliance. They also reduce the

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risk of dose dumping compared to single unit dosage forms like polymeric matrix tablets (2). A wide range of synthetic and natural polymers have been employed in microsphere formation (3-5). However, the search for newer carrier polymers which are biocompatible and biodegradable remains a challenge. There has been a growing interest in the use of starch as carrier polymers in targeted drug delivery systems probably due to its wide abundance, cheapness, non-toxic property and biodegradable products. It consists of glucopyranose monomers and hydrolyzes to form D-glucose (6-8).

Maize (*Zea mays*) or corn is a major staple food grown all over the world particularly Asia, Latin America and Africa. It is the third most important cereal worldwide after wheat and rice and it is commonly referred to as the cereal of the future due to its high nutritional value and the diverse use of its by-products (9). Genetic engineering (breeding) of maize plant for improved yield, high starch and nutrient contents, enhanced tolerance to pest, drought, weed competition, heat stress, cold temperature stress and herbicides has received considerable attention in agricultural research. These breeding programs have led to the production of Pro vitamin A-rich maize genotype (PVA 39) and drought resistance genotype (IWD 15) by International Institute of Tropical Agriculture in Nigeria. This work aims to develop metronidazole microspheres using starches obtained from PVA 39 and IWD 15 genotypes as release-retardant polymers. The model drug used in this study is Metronidazole, an antibiotic listed in the WHO list of Essential medicines. It is a 5-nitroimidazole derivative which is active against anaerobic bacteria and certain parasites. It has an elimination half-life of about 6.5 h and it is usually administered as a 200 mg tablet after every 8 hours (10). The formulation of a controlled release metronidazole tablet would be a better alternative to the conventional tablet dosage form. In this study, starches isolated from the maize cultivars will be used (alone and in combination with alginate) as controlled release polymers in metronidazole microspheres which will be characterized and analyzed for its drug release properties. Sodium alginate is a natural polysaccharide composed of blends of D-mannuronic and L-guluronic acid. Alginates form gels and cross-

link by exchanging the sodium ions with divalent cations ( $\text{Ca}^{2+}$ ) from calcium chloride. This results in stacking of the guluronic groups to form an egg-box structure. The alginate monomers dimerize and form bridges with many other chains resulting in gel network (11).

## 2. Materials and methods

### 2.1. Materials

The materials used were metronidazole powder BP (Lifeline Pharmaceuticals, Mumbai), sodium alginate (Sigma - Aldrich GmbH) and Calcium chloride (Tianjin Kermel Chemical Reagent Co., Ltd Tianjin city China). Maize grains cultivars (IWD 15 and PVA 39) were obtained from International Institute of Tropical Agriculture, Nigeria. Starch isolation was done using established method (12). All other reagents used were of analytical grade.

### 2.2. Extraction of Starch

Starch was extracted using the method earlier reported by Bakre *et al.*, 2021 (13). Grains of IWD 15 and PVA 39 were washed with water to remove foreign materials. The washed grains were soaked in sodium metabisulphite solution (0.75% w/v) for 24 h and crushed using a blender. The starch dispersion was sieved using a calico cloth and left to stand at room temperature overnight. The supernatant was discarded and the sediment (starch) was washed with distilled water then air-dried for 24 h. The dried starch then was pulverised in a mortar and coded as A and B for starches isolated from PVA 39 and IWD 15.

### 2.3. Pre-formulation studies

Pre gelatinized starch from the maize cultivars was prepared using the method of Bakre *et al.* (14). A 20% w/v aqueous starch slurry was heated on a water bath and stirred continuously for 10 min until a white paste was obtained. Microsphere formulations were prepared using the pre gelatinized starch alone and in combination with sodium alginate at curing time of 30 min. Stable microspheres were formed only with blends of pre gelatinized starch and sodium alginate.

### 2.4. Preparation of metronidazole microspheres

The metronidazole microspheres were

**Table 1.** Composition of microsphere formulation.

Materials	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Metronidazole (g)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sodium alginate (g)	3.0	6.0	9.0	1.5	4.0	7.2	1.5	4.0	7.2
B (g)	–	–	–	1.5	2.0	1.8	–	–	–
A (g)	–	–	–	–	–	–	1.5	2.0	1.8
Calcium chloride (g)	5	5	5	5	5	5	5	5	5
Distilled water (mL)	100	100	100	100	100	100	100	100	100
Polymer: drug	1:1	2:1	3:1	1:1	2:1	3:1	1:1	2:1	3:1
Polymer: polymer	–	–	–	1:1	2:1	4:1	1:1	2:1	4:1

Key: A-starch extracted from genetically modified cultivars PVA 39; B-starch extracted from genetically modified cultivars IWD 15.

prepared by ionotropic gelation method according to Table 1. A blend of pre gelatinized maize starch and sodium alginate was dispersed in 100 mL distilled water and homogenized for 15 min using a magnetic stirrer. Metronidazole (3g) was then added and stirred until a homogeneous dispersion was obtained. The pre gelatinized starch/sodium alginate-drug dispersion (25 mL) was added dropwise through a syringe fitted with a 18 G needle into 50 mL of 5 % w/v of calcium chloride and stirred for 30 min. The microsphere beads formed were allowed to remain in the calcium chloride solution for 30 min to ensure further cross linking and completion of the curing process. The microspheres were decanted, washed with distilled water and dried in the oven at 40 °C for 12 h.

## 2.5. Characterization of microspheres

### 2.5.1. Surface morphology analysis

The scanning electron microscope ZEISS EVO18 (Germany) was used to analyze the surface morphology of the samples with a current of 7 mA for 90 s.

### 2.5.2. Differential scanning calorimetry

The instrument used was Mettler instrument (DSC1, Toledo, USA). DSC thermograms were obtained by heating sample pellets at a flow rate of 20 mLmin<sup>-1</sup> under inert nitrogen (15).

### 2.5.3. Swelling index evaluation

A 100 mg quantity of microspheres was soaked in 20 mL phosphate buffer (pH 7.4) for 3

hours and the weight noted. Swelling index was calculated using the equation 1:

$$\text{Swelling index (\%)} = \frac{\text{Change in weight (mg)}}{\text{Original weight (mg)}} \times 100 \quad (\text{Eq. 1})$$

### 2.5.4. Entrapment efficiency

Microspheres (50 mg) were crushed in a glass mortar with a pestle and suspended in 10 mL of phosphate buffer, pH 7.4. The solution was filtered after 24 hours and the filtrate was diluted appropriately with phosphate buffer, pH 7.4 and analyzed at 340 nm using UV/VIS spectrophotometer.

### 2.5.5. Drug Release Study

The dissolution test apparatus (Model NE4-COPD; Copley Scientific Limited, Nottingham, UK) was employed at a speed of 50 rpm. The dissolution medium was phosphate buffer (900 ml), pH 7.4 maintained at 37±0.5 °C. A 100 mg quantity of the microspheres was placed in the dissolution medium. Samples (5 ml) were taken at intervals and replaced with same volume of fresh medium. The withdrawn samples were analyzed at a wavelength of 340 nm on a UV-Vis spectrophotometer. Determinations were made in triplicates.

## 2.6. Kinetics and Mechanism of Drug Release

The *in vitro* drug release data were fitted into various kinetics models to determine the kinetics and mechanism of drug release from the formulation ( Eq. 2-6) (16-20).

$$\text{Zero-order: } Q=K_0 t \quad (\text{Eq. 2})$$

**Table 2.** 3<sup>2</sup> Factorial design for metronidazole microspheres formulations.

Batch	Variable levels		Real values		Response			
	X1	X2	X1	X2	Entrapment efficiency (%)	T50 (hrs)	T90 (hrs)	Swelling Index
			(polymer type)	(polymer: drug)				
F1	-1	-1	Alginate	1:1	89.6±0.02	0.60±0.11	6.12±0.25	3.6±0.01
F2	-1	0	Alginate	2:1	73.0±0.01	21.59±1.01	47.13±0.13	2.1±0.11
F3	-1	+1	Alginate	3:1	49.7±0.12	6.06±0.72	13.15±0.01	1.9±0.01
F4	0	-1	B +Alginate	1:1	25.7±0.25	7.17±0.43	17.78±0.21	2.4±0.02
F5	0	0	B +Alginate	2:1	86.1±0.03	7.92±0.17	18.98±0.09	3.1±0.01
F6	0	1	B +Alginate	3:1	85.6±0.01	10.37±0.61	21.38±0.18	3.2±0.03
F7	+1	-1	A +Alginate	1:1	77.0±0.02	5.48±0.23	12.79±0.24	1.1±0.01
F8	+1	0	A +Alginate	2:1	84.8±0.06	8.37±0.36	22.30±0.61	2.4±0.00
F9	+1	+1	A +Alginate	3:1	55.7±0.21	7.57±0.15	18.55±0.82	1.9±0.01

First order:  $\text{Log } Q = \text{log}Q_0 - K_1 t/2.303$  (Eq. 3)

Higuichi model:  $Q = k_H t^{1/2}$  (Eq. 4)

Hixson:  $(100-Q)^{1/3} = 100^{1/3} - k_S t$  (Eq. 5)

Korsmeyer-Peppas:  $Q/Q_0 = kt_n$  (Eq. 6)

The mechanism of drug release was obtained by plotting log cumulative percentage of the drug release (first 60% drug release) versus log time. The parameter Q is the amount of drug released after time t, Q<sub>0</sub> is the initial concentration of drug, Q/Q<sub>0</sub> is the fraction of drug released, k<sub>H</sub>, k<sub>1</sub>, k<sub>0</sub>, k<sub>s</sub> and k, represent the release constants for Higu-chi, first-order, zero-order, Hixson and Korsmeyer-Peppas models respectively. The parameter, n Korsmeyer-Peppas equation represents the drug release mechanism. For a cylindrical tablet, values of n equals to 0.5 indicates a Fickian diffusion mechanism, 0.50<n<1 represents non-Fickian transport, n=1 is Case II transport or typical zero-order, and n>1 indicates super case II transport.

### 2.7. Experimental Design

A 3<sup>2</sup> factorial design was employed using two factors at three levels. All the nine possible combinations are shown in Table 2. The dependent variables were entrapment efficiency, the time taken for 50% (T<sub>50</sub>) and 90 % (T<sub>90</sub>) drug release while the polymer-drug ratio and polymer types were taken as independent variables.

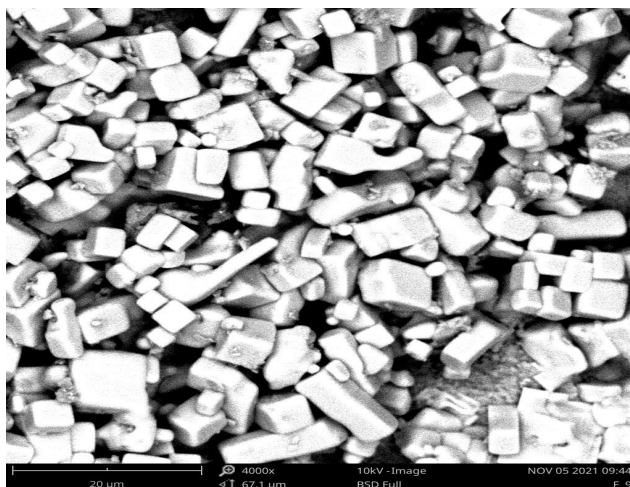
### 2.8. Data analysis

The data obtained were subjected to a two-way analysis of variance (ANOVA) in GraphPad Prism©4 (Graphpad Software Inc. San Diego, CA). P≤0.05 was considered significant at 95% confidence interval. Contour and surface plots were generated using Minitab 19 Statistical Software (Minitab Inc., USA).

## 3. Results and discussion

### 3.1. Characterization of Metronidazole microspheres

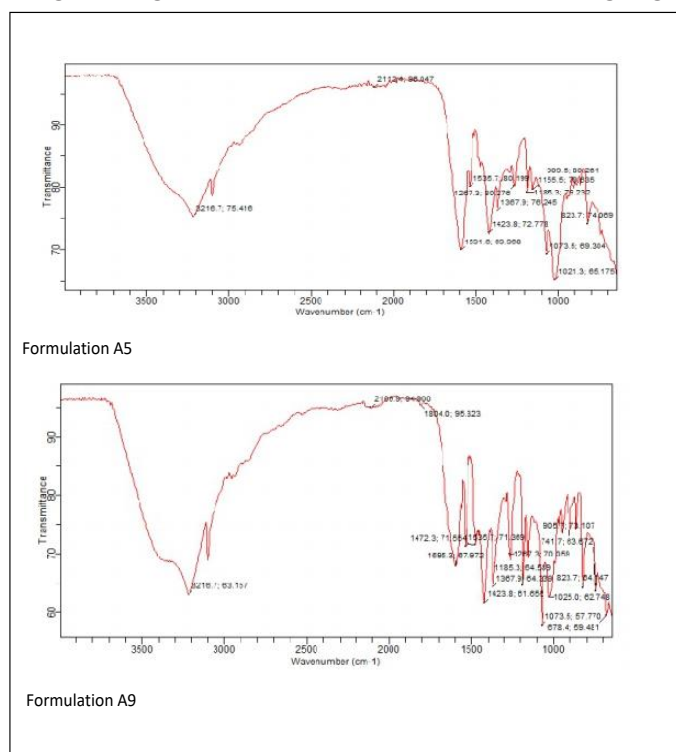
The ionotropic gelation technique has been predominately used for encapsulation of drugs because it is simple, fast, economical, easy to implement and does not use organic solvents (21). The microspheres formed by the ionotropic gelation method were sufficiently hard and discrete with a smooth surface (Figure 1). Swelling of microspheres has been identified with drug release. Formulation F1 has the highest swelling index. The swelling index generally increased with increase in the amount of polymer for formulations containing starch-alginate blend. Metronidazole microspheres containing starchB-alginate blend showed significantly higher swelling index (p<0.05) than those containing sodium alginate alone as the polymer. This could be as a result of pre-gelatinization of the starches isolated from the genotypes which invariably enhanced the swelling index of microspheres (22). Figure 2 shows that the spectra of microsphere formulations containing alginate and



**Figure 1.** SEM image of Formulation F9 (magnification  $\times 4000$ ).

starches isolated from the genotypes showed absorption peaks at  $3210\text{cm}^{-1}$  (O-H Stretch), C-O bend ( $1074.35\text{ cm}^{-1}$ ), C-N stretch ( $825.53\text{ cm}^{-1}$ ) and N-O stretch ( $1533.41\text{ cm}^{-1}$ ). These peaks are characteristic of pure metronidazole powder (23). This indicates that no new chemical moiety was formed and also confirms that no interaction occurred between the polymer and the drug. Table 2 presents the entrapment efficiency, an important parameter for evaluating drug loading of the mi-

crocapsule formulations. The highest entrapment efficiency was exhibited by formulation F1. A high value of entrapment efficiency is desirable because fewer drugs will be lost during preparation, which is important for the commercial production. The range of the entrapment efficiency of the formulations was between  $25.7\pm 0.25$  and  $89.6\pm 0.02\%$  and it was observed that microspheres containing the starches have higher entrapment efficiency than those containing alginate alone at polymer:



**Figure 2.** FTIR Spectrum of Formulations containing alginate and starches isolated from the genotypes.



**Table 3.** Release kinetics parameters for microsphere formulations.

Formulations	Power law n	Drug release models			
		R2			
		Zero order	First order	Higuchi	Hixson
F1	1.20	0.590	0.835	0.839	0.758
F2	0.89	0.690	0.685	0.881	0.717
F3	1.26	0.927	0.998	0.972	0.972
F4	1.09	0.851	0.960	0.922	0.889
F5	1.07	0.813	0.865	0.941	0.867
F6	1.14	0.924	0.946	0.964	0.942
F7	1.20	0.867	0.942	0.986	0.921
F8	1.03	0.763	0.862	0.890	0.813
F9	1.11	0.841	0.904	0.964	0.886

drug ratios of 2:1 and 3:1. Entrapment efficiency decreased with increase in polymer: drug ratio for metronidazole microspheres containing alginate alone and a similar trend was observed when polymer concentration was increased by two fold in formulations containing the starches but when the concentration was increased to three fold, less of the drug was entrapped.

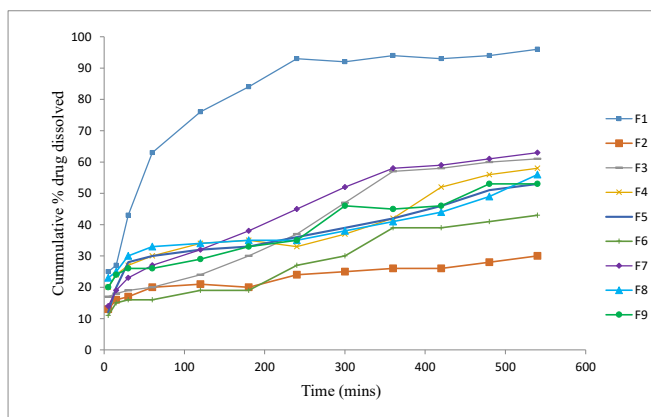
### 3.2. *In vitro* drug release studies

Microspheres drug release depends on the extent of cross linking, morphology, size and

the density of the drug as well as the presence of excipient. The release parameters and drug release profile of the microsphere formulations are presented in Table 2 and Figure 3.  $T_{50}$  and  $T_{90}$  values obtained showed that all the formulations exhibited controlled release of the drug. Generally, formulations containing a blend of starches and alginate showed a more sustained release than the formulations having only alginate especially when combined at a polymer- drug ratio of 1:1 and 3:1. In formulations containing alginate alone (F1-F3) and a blend of alginate with starch A (F6-F9),

**Table 4.** The possible association between entrapment efficiency as well as dissolution times ( $T_{50}$  and  $T_{90}$ ) of microsphere formulation and different variables.

Dependent Variable	Source	Degree of freedom	Sum of Squares	Mean Square	F value	P value
Entrapment (%)	X1	1	6.2017	2.196	10.12	0.003
	X2	2	0.389	6.202	28.58	0.465
	Residual	4	0.800	0.217		
	Total	8	7.675			
	Corrected Total	8	1.085			
$T_{50}$ (hrs)	X1	2	6.669	3.334	21.590	0.007
	X2	2	0.389	0.194	1.259	0.377
	Residual	4	0.618	0.154		
	Total	9	426.560			
	Corrected Total	8	7.676			
$T_{90}$ (hrs)	X1	2	50.427	25.213	16.551	0.012
	X2	2	2.580	1.290	0.847	0.494
	Residual	4	6.093	1.523		
	Total	9	2479.740			
	Corrected Total	8	59.100			

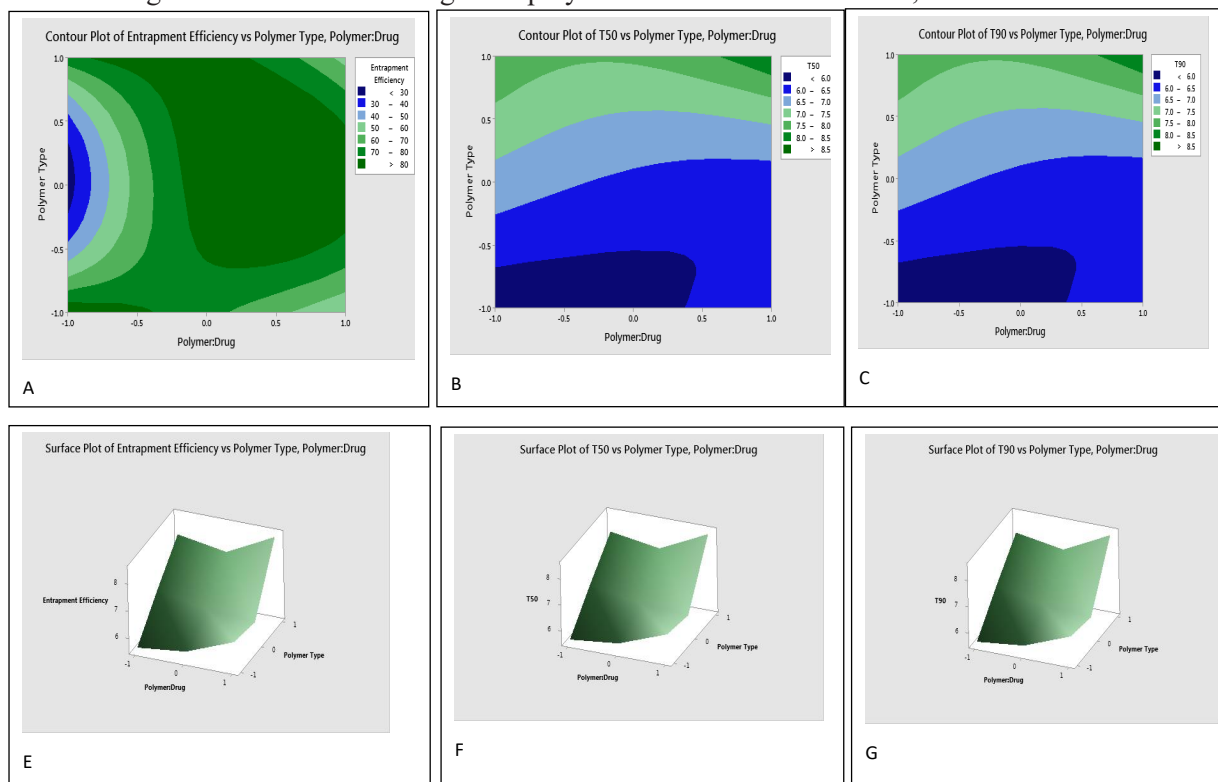


**Figure 3.** Dissolution profile of Metronidazole microspheres.

the effect of increase in polymer concentration on drug release was similar. A 2- fold increase in polymer concentration slowed down drug release while a 3- fold increase caused a faster release. This is consistent with the observation of Wang *et al.*, 2002 (24) who reported the existence of a threshold polymer concentration in ketoprofen loaded PLGA based microsphere at which the system becomes sufficiently hydrophilic to allow for penetration of substantial amount of water with resultant drug dissolution. Also the degree of poly-

mer chain entanglement decreases resulting in a weakened polymer gel network. All these contribute to increased permeability so that release is less retarded at 3 fold increase in polymer concentration. The threshold of starch B however appears to be higher than that of A because drug release was still retarded at 3- fold increase in polymer concentration.

The values for time taken for 90 % drug release was in the range of between  $6.12 \pm 3.20$  to  $47.13 \pm 7.01$  h. Overall, formulation F2 containing



**Figure 4.** Contour and surface plots showing effect of independent variables on Entrapment Efficiency, T<sub>50</sub> and T<sub>90</sub>.

alginate alone provided a more sustained release of the drug while F6 with polymer: drug ratio (2:1) produced the highest sustained release effect among formulations containing a blend of alginate and genetically modified starches. This may probably be due to the fact that the high amount of the starch reduces the porosity of the gel matrix which consequently slowed down drug release. The long duration of drug release from all the formulations containing starches from maize genotypes may justify the potential use of the starch polymers as sustained release modifiers.

Table 3 shows the result of the simulation of drug kinetics using the various release models. It reveals that all the formulations fit into the Higuchi model which represents the best model for drug release. In general, the diffusion exponent,  $n$ , in the Korsmeyer-Peppas model was greater than 1 which suggests super case transport mechanism in which case drug release is controlled by erosion and drug relaxation. However, only formulations F2 exhibited non-fickian diffusion.

### 3.3. Experimental Design

From the design of experiment, mathematical models were derived to predict the value of responses to formulation factors. Table 4 shows the results of the two way analysis of variance (ANOVA) for the regression model. The result indicates that the dissolution times ( $T_{50}$  and  $T_{90}$ ) and entrapment efficiency significantly depends on the type of polymer ( $p < 0.05$ ). In addition, the effect of type of polymer on the dissolution times

was more significant than those of polymer-drug ratio. The interactive term  $X_1X_2$  showed positive effects on all response variables studied indicating that their values increased when both factors were simultaneously increased. The polymer-drug ratio did not significantly affect ( $p > 0.05$ ) the entrapment ratio and the dissolution times. The  $F$ -value for  $T_{50}$  and  $T_{90}$  were 21.59 and 16.55 respectively. The determination coefficients,  $R^2$  and  $R^2$  (Adj) which represents the fitting reliability of the regression models were calculated to be 0.920 and 0.839 respectively for  $T_{50}$ . This indicates that approximately 92% of the variance is attributed to the variable and suggests a low significance of the models. Figure 4 represents the surface and contour plots which afford a two dimensional view of the relationship between a response variable and two independent variables. Generally, areas with darker colour indicates stronger response. The plots reveal that polymer-drug ratio and polymer type strongly interacted to increase the dissolution times ( $T_{50}$  and  $T_{90}$ ) and entrapment efficiency.

### 4. Conclusion

Our results showed that blend of starches obtained from maize genotypes and sodium alginate could be useful in the preparation of spherical discrete microspheres using ionotropic gelation method which could significantly control the release of metronidazole.

### Conflict of Interest

None declared.

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