Formulation of metronidazole tablets using hydroxypropylated white yam *(Dioscorea rotundata)* starch as the binding agent.

Adenike Okunlola, Oluwaseyi O. Alade and Oluwatoyin A Odeku[*]

Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria

Technical Note

ABSTRACT

White yam starch obtained from the tubers of *Dioscorea rotundata* Poir was modified by hydroxypropylation and used as a binding agent in a metronidazole tablet formulation and compared with corn starch BP. The quantitative effects of the novel starch binder on the mechanical (tensile strength and friability) and release properties (disintegration and dissolution times) of the metronidazole tablet was analyzed using a full 2^3 factorial experimental design. The individual and interaction effects of type of starch binder (X_1), concentration of binder (X_2) and relative density (X_3) on tensile strength, friability, disintegration time and dissolution time (t_{90}) were determined. The ranking of the coefficients was $X_3 > X_2 > X_1$ on T, $X_1 > X_3 > X_2$ on F and $X_3 > X_1 > X_2$ on DT and t_{90} (time for 90% drug release) indicating that the formulation variables influence the properties of metronidazole tablets to varying degrees. This indicates that the type and concentration of starch binder as well as the compression pressure employed in table formulation need to be carefully selected to obtain tablets with the desired mechanical and drug release properties. Hydroxypropyl white yam starch could be more useful as a binder especially when tablets require high mechanical strength and faster drug release are desired.

KEY WORDS: Starch, hydroxypropylation, white yam starch, metronidazole, tablets, factorial experiment, excipients

INTRODUCTION

Polysaccharide derivatives have been researched for diverse applications in various industries. Starch, the major energy source in many plants can be widely found in cereal grain seeds (e.g. corn, wheat, rice, sorghum), tubers (potato), roots (cassava, sweet potato, arrowroot), legume seeds (peas, beans, lentils), fruits (green bananas, unripe apples, green tomatoes), trunks (sago palm) and leaves (tobacco) (1). The use of natural starch for industrial applications remains attractive because it is inexpensive, readily available,

---
[*]Corresponding author: Oluwatoyin A Odeku, Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria, E. mail: pejuodeku@yahoo.com, oa.odeku@mail.ui.edu.ng

capable of being modified and biodegradable (2, 3).

Native starch is a white powder with a bland taste and flavor insoluble in cold water. Furthermore, native starch has inherent properties that can be changed through physical and chemical modifications to produce functionally tailored starches with a wide range of properties to meet specific applications (4). Physical modification includes subjecting the starch to heat-moisture treatment and annealing, while chemical modification includes oxidation, acid thinning, hydroxypropylation and acetylation (5). Hydroxypropylation of starch is achieved through an etherification process using propylene oxide as the etherifying reagent. This process introduces hydroxypropyl groups in the polymeric chain of the starch. The highly strained three-membered epoxide ring of propylene oxide accounts for its reactive nature (6).

During hydroxypropylation, activation is achieved using alkaline reagents as catalysts to facilitate the formation of nucleophilic starch-O- alkoxide. This is followed by the reaction of the starch-O- alkoxide with the propylene oxide, leading to a bimolecular substitution to produce hydroxypropyl starch. The molar substitution is used to measure the number of propylene oxide moles substituted per anydroglucose unit (6).

Hydroxypropylation of the starch provides shelf-stability, freeze-thaw stability and cold storage stability by weakening the internal hydrogen bond strength. The decrease in the gelatinization temperature of hydroxypropylated starch is explained by the disruption of inter- and intra-molecular hydrogen bonds. Hydroxypropylation interferes with retrogradation, resulting in retention of paste fluidity with improved paste clarity and imparts desired textural properties to the starch (7). In addition, the enzymatic digestibility of starches improves after hydroxypropylation (8). Hydroxypropylation of starches from various sources including corn (9), potato (10, 11), rice (12) and wheat (13) have been reported previously.

The material and tablet formation properties of native and modified starches obtained from four different *Dioscorea* species for potential application as excipients in pharmaceutical tablet formulations have been previously reported (14, 15, 16). White yam starch has amylose: amylopectin content of 29:72 and hydroxypropylated form of the starch with the degree of substitution of 0.027% exhibited lower gelatinization and pasting temperatures than the native form of the starch (15, 16) Hydroxypropylation increased the cold water swelling and solubility of white yam starch and improved the flowability and compressibility properties of the starch. When used as a directly compressible excipient, native white yam starch formed tablets with low tensile strength, while hydroxypropyl white yam starch exhibited improved bonding characteristics and resulted in the formation of tablets with significantly ($p<0.01$) higher crushing strength with fast disintegration properties (15, 16). While the binding properties of native white yam starch has been investigated (14), the binding properties of hydroxypropyl white yam starch in tablet formulations have not been evaluated. Thus in the present study, the binding properties of hydroxypropylated white yam starch were evaluated in a metronidazole tablet formulation in comparison with corn starch using factorial design, a mathematical model that has been used for optimizing formulations (17). The factorial design is an efficient method of simultaneously estimating the relative significance of a number of variables and their interactions (17).

MATERIALS AND METHODS

The materials used were metronidazole BP (Tongxiang Zhejiang, China), corn starch BP, magnesium stearate and xylene (all from BDH, Poole, U.K), lactose BP (Pharmachem, Mumbai, India), acetone (Sigma Chemical Company, St Louis, USA) and *Dioscorea rotundata* Poir (white yam) which was obtained from local farmers in Ibadan, Nigeria and authenticated. Starch extraction from the fresh tubers of white yam has been previously reported (3, 18).

Preparation of hydroxypropylated starch

Hydroxypropyl (HP) white yam starch with a 0.027 % degree of substitution was prepared using the method described previously by Odeku and Picker-Freyer (14). Briefly, native starch (200 g) was suspended in distilled water containing sodium sulphate (30 g) stirring carefully to produce a slurry. The pH was adjusted to 10.5 using sodium hydroxide solution (5%). Propylene oxide (12% w/v of starch solid) was added and with shaking the reaction continued at 40°C for 24 hours. The reaction was neutralized with 0.2M HCl and the pH adjusted to 5.5. The starch cake was washed 5 times with distilled water, dried in a hot air oven at 40°C for 24 hours and then powdered using a laboratory mill. The hydroxypropyl content was determined by a spectro-photometric method and expressed as moles of substituent per mole of anhydro-glucose unit (19).

Preparation of the granules

Batches (200 g) of a basic formulation of metronidazole (60% w/w), lactose (30% w/w) and corn starch (10% w/w) were dry mixed for 5 minutes in a planetary mixer (Model A120, Hobart Manufacturing Co, U.K) and then moistened with 21 ml of distilled water or appropriate amounts of starch paste (2.5-10% w/w) to produce granules containing different concentrations of the starches as binder. Massing was continued for 5 minutes and the wet masses were granulated by passing the mass manually through a mesh 12 sieve (1400 μm). The wet granules were dried in a hot air oven at 50°C for 18 hours. Dried granules were sieved through a mesh 16 sieve (1000 μm) and then stored in an air tight container.

The moisture content of the granules were determined using an Ohaus moisture balance (Ohaus Scale Corporation, USA) to be between 2-4% w/w. The uniformity of mixing of metronidazole in the granules was determined using a UV/Visible spectrophotometer (Phillips Pye Unicam, PU 8610 Kinetics, Sarose scientific instruments, Cambridge, UK.) at 277 nm and found to be 99.23±2.50%.

The granule size distribution was determined by sieve analysis using (BS 410) using an Endecott sieve shaker (Octagon 200CL, Endecotts Ltd, London, UK) and granules between 500-1000μm were separated and used to formulate the tablets. The particle densities were determined using the liquid pycnometer method with xylene as the displacement fluid.

Preparation of tablets

Metronidazole granules (600 mg) were compressed for 30 seconds into tablets with predetermined loads on a Carver hydraulic press (Model C, Carver inc. Menomonee Falls, WI, US) using a 10.5 mm die and flat-faced punches lubricated with a 1% dispersion of magnesium stearate in acetone. After ejection, the tablets were stored over silica gel for 24 hours to allow for elastic recovery and hardening. The weights (w) of the tablets were determined to within ± 1 mg using a Mettler balance (PC 440) and the thickness of the tablets was measured to within ± 0.01 mm,

using a micrometer screw gauge (Moore & Wright, Sheffield, UK).The relative density of the tablets, D, was calculated using Equation 1.

$$D = \frac{w}{V_t \text{ x } \rho_s} \qquad \text{Eq. 1}$$

Where; V_t is the volume of the tablet in cm^3 and ρ_s is the particle density of the solid material in $g.cm^{-3}$.

Determination of tensile strength

The tensile strength of the tablets (T) were determined at room temperature by diametrical compression (20) using a hardness tester (Ketan Scientific & Chemicals, Ahmedabad, India). The tensile strength of the tablet was then calculated using Equation 2.

$$T_s = \frac{2L}{\pi dh} \qquad \text{Eq. 2}$$

Where; T_s is the radial tensile strength, L is the load needed to break the tablet, d is the tablet diameter and h is the tablet thickness. Results were taken only from tablets which split cleanly into two halves without any sign of lamination. The results were expressed as the mean of four determinations.

Friability testing

The friability of the tablets (n=4) was determined using a friabilator (Veego Scientific devices, Mumbai, Maharashtra, India) operated at 25 RPM for 4 minutes.

Disintegration testing

The disintegration time of the tablets (n=4) was determined in distilled water at $37\pm0.5°C$ using a disintegration tester (Veego Scientific devices, Mumbai, Maharashtra, India).

Dissolution testing

The dissolution test was performed in 900 ml of 0.1M HCL using the USPXX III basket method (Hanson Model 72RL, USA) rotated at 100 RPM, maintained at $37\pm0.5°C$. Samples (5 ml) were withdrawn at predetermined time intervals and replaced with equal amounts of fresh medium. The sample was diluted and the amount of metronidazole released was determined at a wavelength of 277 nm. Results were a mean of 3 samples.

Experiment design

Three independent process parameters binder type, binder concentration and relative density were chosen at two different concentrations, high and low, as shown in Table 1. A 2^3 full factorial design was used requiring the preparation of eight batches. The sequence of the eight experiments was randomized and the individual and interaction effects of type of binder (X_1), concentration of binder (X_2) and relative density (X_3) on tensile strength (T), friability (F), disintegration time (DT) and dissolution time (t_{90}) were determined (21). The results obtained were then subjected to regression analysis using MINITAB Version 14.2 software (Pennsylvania, U.S.A.), using the least squares method.

RESULTS AND DISCUSSION

The factorial design has been found useful in determining the effects of various formulation factors on the characteristics of drug formulations (22–24). The range of the three independent process parameters and values of tensile strength, friability and the disintegration and dissolution times, used in the calculation of the individual and interaction coefficients for the variables, are presented in Table 1, while the individual and interaction coefficients are shown in Tables 2 and 3, respectively. All the coefficients values were positive for the

Table 1 Factorial design for metronidazole formulations containing hydroxypropylated white yam starches and corn starches

BATCH CODE	CODED LEVELS			REAL VALUES			Tensile strength (MNm^{-2})	F (%)	DT (min)	t_{90} (min)
	X_1	X_2	X_3	X_1	X_2 (%)	X_3				
B_1	-1	-1	-1	Corn	2.50	0.80	0.10	1.20	1.00	30.00
B_2	+1	-1	-1	HP White yam	2.50	0.80	0.26	3.00	0.50	20.00
B_3	-1	+1	-1	Corn	10.00	0.80	0.16	0.90	2.00	35.50
B_4	+1	+1	-1	HP White yam	10.00	0.80	0.30	2.00	1.00	30.00
B_5	-1	-1	+1	Corn	2.50	0.90	0.29	0.80	13.00	42.00
B_6	+1	-1	+1	HP White yam	2.50	0.90	0.49	1.30	9.00	30.50
B_7	-1	+1	+1	Corn	10.00	0.90	0.72	0.70	26.00	49.00
B_8	+1	+1	+1	HP White yam	10.00	0.90	0.80	0.90	12.00	40.00

Table 2 The coefficients of the individual variables on the Tensile strength (T), Friability (F), Disintegration Time (DT) and dissolution time (t_{90}) of metronidazole tablets containing corn and hydroxypropylated white yam starches.

FACTOR	COEFFICIENT	T (MNm^{-2})	F (%)	DT (min)	t_{90} (min)
X_1	Effect	0.073	0.450	-2.438	-4.500
	p-value	0.109	0.070	0.289	0.035
X_2	Effect	0.105	-0.225	2.187	4.000
	p-value	0.075	0.139	0.317	0.040
X_3	Effect	0.185	-0.425	6.938	5.750
	p-value	0.043	0.075	0.108	0.028

Table 3 The interaction coefficients of the variables on the Tensile strength (T), Friability (F), Disintegration Time (DT) and dissolution time (t_{90}) of metronidazole tablets containing hydroxypropylated white yam and corn starches

FACTOR	COEFFICIENT	T (MNm^{-2})	F (%)	DT (min)	t_{90} (min)
X_1X_2	Effect	-0.018	-0.125	-1.313	0.875
	p-value	0.395	0.242	0.468	0.177
X_1X_3	Effect	-0.003	-0.275	-2.063	-0.625
	p-value	0.874	0.114	0.333	0.242
X_2X_3	Effect	0.080	0.100	1.813	0.125
	p-value	0.125	0.295	0.369	0.705

influence of binder type, binder concentration and relative density on tensile strength (T).

The positive coefficient values indicate that changing the binder from corn starch to HP white yam starch, increasing binder concentration from 2.5 to 10% w/w and increasing relative density of tablets from 0.80 to 0.90, produced tablets of higher tensile strength. This suggests that HP white yam starch exhibited greater binding since it produced tablets with higher tensile strength than corn starch, although there was no significant difference (p>0.05) in the tensile strength of the tablets. The ranking of the individual coefficients on T was in the order of $X_3 > X_2 > X_1$, indicating that relative density had the most significant effect (p< 0.05) on the tablet hardness. This is expected because the relative density of pharmaceutical materials generally increases with increasing pressure.

Increased pressure results in closer packing of the particles and the formation of more solid bonds between the particles leading to an increase in the mechanical strength of the tablets (25).

The coefficient values were positive for binder type on friability but negative for the influence
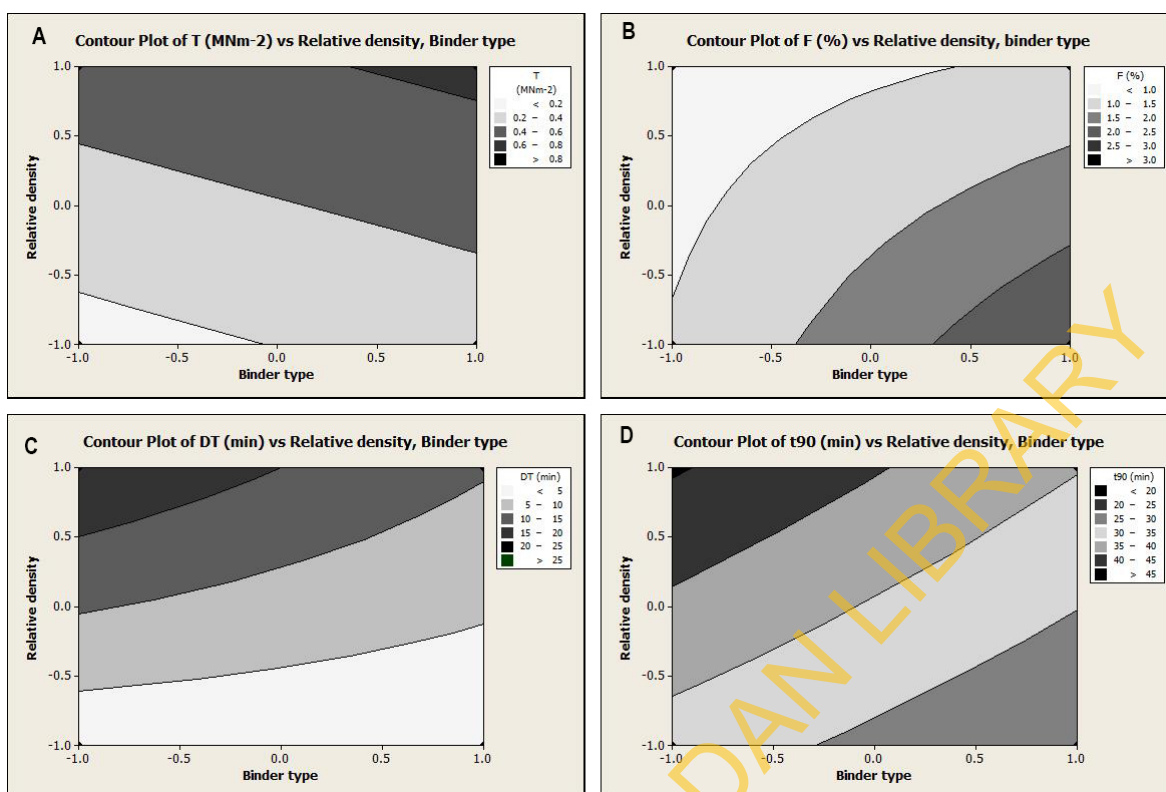
Figure 1 Contour plots showing the influence of the type of binder and relative density on the properties of the metronidazole tablets where a) is the tensile strength, T, versus relative density and binder type b) is the friability, F, versus the relative density and binder type c) is the disintegration time DT, versus relative density and binder type and d) is the dissolution time, t_{90}, versus relative density and binder type.

of starch binder concentration and relative density on friability. This indicates that as starch concentration and relative density increased, the friability (F) of the metronidazole tablets decreased. On the other hand, changing the binder type from corn starch to HP white yam starch produced tablets that were more friable, although there were no significant difference (P>0.05) in the friability of the tablets produced using the two binding agents. The ranking of the individual coefficient on F was $X_1 > X_3 > X_2$ indicating that the binder type had the highest effect on the tablet friability.

The coefficients values were positive for the influence of starch binder concentration and relative density on disintegration time (DT)

and dissolution time (t_{90} is the time it takes for 90% of the drug to dissolve) but negative for the influence of binder type. This indicates that increasing the binder concentration and tablet relative density led to an increase in the disintegration and dissolution times. This is similar to the effect previously reported for some starch binders (14, 26, 27). However, changing the type of binder from corn starch to HP white starch produced metronidazole tablets with shorter DT and dissolution times. The ranking of the effect of the coefficients on DT and t_{90} was $X_3 > X_1 > X_2$, indicating that the relative density of tablets had the most significant effect (p< 0.05) on the drug release properties.

The rankings of the interaction coefficients on T was $X_2X_3 > X_1X_2 > X_1X_3$, on friability was $X_1X_3 > X_1X_2 > X_2X_3$, on DT was $X_1X_3 > X_2X_3 > X_1X_2$ and on dissolution time (t_{90}) was $X_1X_2 > X_1X_3 > X_2X_3$. This indicates that the formulation variables interacts with each other to influence the properties of the tablets. The interaction between the concentration of the binder and relative density had the highest effect on the mechanical properties of the tablets. On the other hand, the interaction between binder type and relative density had the highest effect on the drug release properties of the tablets. The contour plots that show the effect of the type of starch and relative density on tensile strength, friability, disintegration and dissolution time are presented in Figure 1. The plots show that the relative density exerted a lesser effect than binder type on tensile strength while the binder type exerted a greater effect on friability. Contour plots for disintegration and dissolution times show that relative density had a greater influence on the release properties of metronidazole tablets than binder type. Thus, there is a need to carefully select the compression pressure and the type and concentration of starch binder employed in the formulation of pharmaceutical tablets.

CONCLUSIONS

This study showed that the mechanical and drug release properties of metronidazole tablets depended on the binder type, binder concentration and relative density of the tablets. There was considerable interaction between the independent variables as evidenced by the magnitude of the individual and interaction coefficients employed. Hydroxypropyl white yam starch could be more suitable as a binder especially when tablets with high mechanical strength but faster drug release properties are desired.

REFERENCES

1 Odeku OA, Potentials of Tropical Starches as Pharmaceutical Excipients: A Review. Starch/Stärke, 65 (1-2): 89-106, 2013. DOI: 10.1002/star.201200076

2 Wurzburg OB, Cross-linked starches, In Wurzburg OB (ed) Modified starches: Properties and uses, CRC Press, Inc., Boca Raton, FL, pp 17-53, 1986.

3 Lawal OS, Lechner MD, Hartmann B, Kulicke WM, Carboxymethyl cocoyam starch: synthesis characterization and influence of reaction parameters, Starch/ Stärke, 59: 224-233, 2007. DOI: 10.1002/star.200600594

4 Rutenberg MM, Solarek D, Starch derivatives: Production and uses, In Whistler RL, BeMiller JN, Paschall EF (eds). Starch chemistry and technology, Academic Press, NY, pp 311, 1984.

5 Yiu PH, Loh SL, Rajan A, Wong SC, Bong CFJ, Physiochemical properties of sago starch modified by acid treatment in alcohol. Amer J Appl Sci, 5 (4): 307-311, 2008.

6 Pal J, Singhal RS, Kulkarni PR, A comparative account of conditions of synthesis of hydroxypropyl derivative from corn and amaranth starch, Carbohyd Polym 43: 155-162, 2000. DOI: 10.1016/S0144-8617(00)00144-2

7 Tuschoff JV, Hydroxypropylated starches, In Wurzburg OB (ed) Modified Starches: Properties and Uses, CRC Press, Boca Raton, Fl, 90-95, 1986.

8 Liu HJ, Ramsden L, Corke H, Physical properties and enzymatic digestibility of hydroxypropylated wax, and normal maize starches, Carbohyd Polym 40: 175-182, 1999. doi.org/10.1016/S0144-8617(99)00052-1

9 Gray JA, BeMiller JN, Influence of reaction conditions on the location of reactions in waxy maize starch granules reacted with a propylene oxide analog at low substitution levels, Carbohyd Polym, 60: 147-162, 2005. doi.org/10.1016/j.carbpol.2004.11.032

10 Perera C, Hoover R, Martin AM, The effect of hydroxypropylation on the structure and physicochemical properties of native defatted and heat-moisture treated potato starches. Food Res Int, 30: 235-247, 1997.

11 Vorwerg W, Dijksterhuis J, Borghuis J, Kröger A, Film properties of hydroxypropyl starch, Starch/

Stärke, 56: 297-306, 2004. DOI: 10.1002/star.200300244

12 Seow CC, Thevamalar K, Internal plasticization of granular rice starch by hydroxypropylation: effects of phase transitions associated with gelatinization, Starch/ Stärke, 45: 85-88, 1993. DOI: 10.1002/star.19930450303

13 Wootton M, Mahdar D, Properties of starches from Australian wheats Part 3: In vitro digestibility and hydroxypropyl derivatives, Starch/ Stärke, 45: 337-341, 1993. DOI: 10.1002/star.19930451002

14 Okunlola A, Odeku OA, Evaluation of starches obtained from four Dioscorea species as binding agent in chloroquine phosphate tablet formulations. Saudi Pharm J, 19: 95 - 105, 2011. doi: 10.1016/j.jsps.2011.01.002

15 Odeku OA, Picker-Freyer KMAnalysis of the Material and Tablet Formation properties of four Dioscorea Starches, Starch/Stärke, 59: 430-444, 2007. DOI: 10.1016/j.jsps.2011.01.002

16 Odeku OA, Picker-Freyer KM, Evaluation of the material and tablet formation properties of modified forms of Dioscorea starches. Drug Dev Ind Pharm, 35 (11): 389 - 406, 2009. DOI: 10.3109/03639040902960185.

17 Gohel MC, Amin AF, Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. J Contr Rel, 51: 115-122, 1998. .doi.org/10.1016/S0168-3659(97)00102-8

18 Young AH, Fractionation of starch, In Whistler, R.L., BeMiller, J.N. and Pashall, E.F. (eds.), Starch Chemistry and Technology, 2nd Edition. Academic Press, London, pp 249-283, 1984,

19 Murphy J, Riley JP, A modified single solution method for the determination of phosphate in natural waters, Anal Chim Acta, 27:31-6, 1962. DOI:10.1016/S0003-2670(00)88444-5

20 Fell JT, Newton JM. Determination of tablet strength by diametral compression test. J Pharm Sci, 59: 688-691, 1970. DOI: 10.1002/jps.2600590523

21 Okunlola A, Odeku OA, Patel RP, Formulation optimization of floating microbeads containing modified Chinese yam starch using factorial design. J Excip Food Chem,. 3 (1): 17-25, 2012.

22 Wehrle P, Magenheim P, Benita S. The influence of process parameters on the PLA nanoparticle size distribution evaluated by means of factorial design, Eur J Pharm Biopharm, 41: 19, 1995.

23 Soares LAL, Gonzalez OG, Petrovick PR and Schmidt PC, Optimization of tablets containing high doses of spray-dried plant extract: A technical note. AAPS PharmSciTech, 6 (3): Article 46, 2005. DOI: 10.1208/pt060346

24 Rocksloh K, Rapp FR, Abu Abed S, Muller W, Reher M, Gauglitz G and Schmidt, PC Optimization of crushing strength and disintegration time of a high dose plant extract tablet by neutral network. Drug Dev Industrial Pharm, 25: 1015-1025, 2000. doi.org/10.1081/DDC-100102264

25 Itiola OA, Pilpel N, Tableting characteristics of metronidazole formulations. Int J Pharm, 31: 99-105, 1986. DOI:10.1016/0378-5173(86)90218-8

26 Dare K, Akin-Ajani DO, Odeku OA, Odusote OM, Itiola OA, Effects of pigeon pea and plantain starches on the compressional, mechanical and disintegration properties of paracetamol tablets, Drug Dev Ind Pharm, 32 (3): 357-365, 2006. DOI:10.1080/03639040500519235

27 Odeku OA, Odeniyi MA, Ogunlowo GO, Influence of ginger and banana starches on the mechanical and disintegration properties of chloroquine phosphate tablets, Asian Pac J Trop Med, 2 (1): 13 - 18, 2009.