FORMULATION AND EVALUATION OF A CO-DISINTEGRANT MIXTURE IN METRONIDAZOLE TABLET USING QUALITY BY DESIGN APPROACH

MAHMUD, H.S.*, YAKUBU, S.O. AND APEJI, Y.E.
Department of Pharmaceutics and Industrial Pharmacy, Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

The aim of this study was to assess the disintegrant property of Sammaz Maize starch (MS) and *Khaya senegalensis* gum (KG) and the potential effects of mixing the disintegrants to select an optimized concentration for the formulation of immediate release tablets of metronidazole manufactured via direct compression. Design of Experiments (DoE) was employed to derive the ratios for the combination and also to optimize the composition using the desirability function. The optimized formulation contained MS (1.86%) and KG (8.14%) as disintegrant. Tablets were prepared by direct compression and tablet quality tests were carried out. The formulations produced non-friable tablets (friability- 0.632-1.07 %) with sufficient hardness of between 3.9-5.4 KgF and displayed rapid disintegration between 7.5 to 11.67 seconds showing a super disintegrant property. The physical combination of MS and KG was found to be an effective co-disintegrant mixture in immediate release tablets of metronidazole and displayed novel super disintegrant characteristics.

Keywords: *Khaya senegalensis* gum, Optimized, Sammaz maize starch, Superdisintegrant
***Correspondence:** mhszubair@gmail.com, 08035903870

INTRODUCTION

Plant polymeric materials have been continually investigated as pharmaceutical raw materials. These polymeric materials are gaining interest due to their low cost, relative abundance, biodegradability and most importantly they are eco -friendly [1]. Furthermore, natural polymers have unique properties in drug delivery that have not been obtainable with other materials [2]. They have been included in pharmaceutical formulations to serve as binders in tablets, disintegrants, matrix and gel forming agents, suspending agents, thickeners to mention but a few. *Khaya senegalensis* gum, is a naturally occurring gum that exudes via incision from the trunk of *Khaya senegalensis* tree and has shown potentials for use as a tablet binder, matrix in sustained release in combination with sodium carboxymethyl cellulose and as a disintegrant in metronidazole tablets manufactured via wet granulation [3, 4]. Some studies compared the effects of two of the eight species of Khaya; Khaya *grandifolia* and *K. senegalensis* and confirmed superiority of *K. senegalensis* gum in terms of emulsifying and binding property when mechanical strength and slow dissolution rates are desired [5].

Maize (corn) starch production for pharmaceutical, food and other industrial uses have long been established. Starch is extensively used in pharmaceutical dosage forms principally as an excipient. It is used as a binder in tablet formulations typically at concentrations of 5-10 % in wet granulation, and at 15 % as a disintegrant nonetheless newer varieties that are been produced need to be assessed. One of such varieties is Sammaz -29 (SMS-29), an open pollinated unmodified maize variety produced by Institute of Agricultural Research (IAR), A.B.U Zaria. The SMS-29 starch was found to be white not requiring bleaching and showed superior binding and disintegrating ability in tablet formulations when compared with an exported variety [6]. The conventional approach adopted by most researchers to study the excipient properties of *Khaya senegalensis* gum and Sammaz maize starch (SMS) have been the one variable at a time (OVAT) approach. Formulation experiments can be planned to determine the effect of one variable at a time (OVAT), where all other independent variables are kept constant but these are more of trial and error experiments that cannot detect and quantify the interactions in which two or more variables act differently together compared to their separate effect [7], similarly, optimized composition or process parameters cannot be assured by OVAT approach [8].

Quality by Design (QbD) has therefore been introduced to evaluate cause and effect relationships thus minimizing the total number of experiments, enabling changes of experimental factors and also useful in extrapolating the data. Furthermore, it is useful in finding optimum formulation and process parameters and understanding of the interactions between combinations. The aim of this study therefore was to assess the disintegrant property of Sammaz 29 maize starch (in this context referred to as MS) and *Khaya senegalensis* gum (KG) and the potential effects of mixing the disintegrants to select an optimized

concentration for the formulation of immediate release tablets of metronidazole manufactured via direct compression. Until now, such investigations have not been carried out using the combined excipients in metronidazole tablets.

MATERIALS AND METHODS

Materials

Metronidazole powder (Central Drug House® New Delhi -110002, India), Sammaz 29 Maize starch, *Khaya senegalensis* gum powder (≤ 250µm), Microcrystalline cellulose was obtained as a gift from JRS Pharma, Germany, Magnesium stearate., Talc (BDH Chemicals, England). All other ingredients were of analytical grade.

Experimental design

Design of Experiments (DoE) was employed to optimize the concentrations of KG and MS used in combination as a disintegrant to formulate metronidazole tablets by direct compression. The concentrations of KG and MS varied from 0 – 10 % each and a total of eight experimental formulations were generated using Mixture design (Design Expert Software ver. 10). Tablets weighing 650 mg were prepared according to the formula given in Table 1 and the parameters of crushing strength (CS), disintegration time (DT) and friability (FR) were evaluated for each tablet formulation. The evaluated parameters were fitted as responses to a selected model based on the design and a series of analysis were carried out statistically to screen and select the optimal concentrations of KG and MS.

Tablet evaluation

Weight variation, thickness and diameter: Twenty (20) tablets were taken at random and weighed individually on an electronic balance (P163 Mettler instrument AG). The thickness and diameter of same tablets were determined using a digital vernier calliper (Fisher® England). The means and standard variations of these determinations were calculated.

Crushing strength: Ten (10) tablets were selected at random and their crushing strength was determined using a Monsanto hardness tester.

Friability: Ten (10) tablets were randomly selected, de-dusted and weighed (W_1) then transferred into a friabilator (Type A3R, Erweka, Germany) and operated at 25 rpm for 4 min. The tablets were collected, de-dusted and the final weight (W_2) was recorded. The percent loss in weight was calculated as friability using the formula below.

$$\% \text{ Friability} = \frac{W_1 - W_2}{W_1} X100 \quad 1$$

Disintegration: Six (6) tablets were placed in the tubes of a ZT3 Erweka disintegration apparatus containing 0.1 N HCl as the disintegration fluid, thermostated and kept constant at 37 ± 0.5 °C to mimic *in vivo* conditions. The time taken for each of the tablets to disaggregate with absence of any palpable mass was noted and the average time taken for the six tablets was recorded as the disintegration time for that formulation.

Dissolution: *In vitro* drug release studies were performed using USP apparatus 1 (basket method). The dissolution medium was 900 ml of 0.1 N HCl thermostated at 37 ± 0.5º C to simulate the gastric medium where the tablets will disintegrate. In all experiments, 5 ml aliquots were withdrawn at time points of 0, 5, 30, 60 seconds with replacement of 5 ml fresh dissolution medium (at same temperature) after each withdrawal to maintain sink condition. The samples were assayed spectrophotometrically (model UV-1800 Shimadzu, USA) at 277nm for metronidazole.

Content uniformity: To determine the amount of drug present in each tablet, 5 tablets were randomly selected from each tablet formulation and then crushed. The equivalent of one tablet (650 mg) was weighed out and dissolved in 100 ml of 0.1N HCl. The solution was shaken vigorously and then filtered. The filtrate was diluted (1 in 100) and assayed spectrophotometrically at 277 nm for metronidazole and the drug content was calculated in percentage (%). The responses evaluated are shown in Table 2.

Table 1: Tablet formula for the eight experimental formulations containing varying concentrations of KG and MS ranging from 0 – 10 %

Ingredient (% w/w)	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8
Metronidazole	30.77	30.77	30.77	30.77	30.77	30.77	30.77	30.77
Microcrystalline cellulose (MCC)	58.63	58.63	58.63	58.63	58.63	58.63	58.63	58.63
***Khaya senegalensis* gum (KG)**	2.50	5.00	0.00	7.50	5.00	10.0	10.0	0.00
Sammaz maize starch (MS)	7.50	5.00	10.0	2.50	5.00	0.00	0.00	10.0

Magnesium stearate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Talc	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

RESULTS AND DISCUSSIONS

Table 2: Experimental levels of KG and MS for each formulation and the corresponding responses obtained

Run	Component 1 A:MS%	Component 2 B:KG %	Response 1 DT (sec)	Response 2 CS (KgF)	Response 3 FR (%)
1	7.50	2.50	10.67	4.6	1.07
2	5.00	5.00	7.6	4.9	0.789
3	10.00	0.00	11.67	4.5	0.963
4	2.50	7.50	7.5	5.1	0.766
5	5.00	5.00	9.5	4.8	0.789
6	0.00	10.00	10.67	5.2	0.632
7	0.00	10.00	9.5	5.4	0.807
8	10.00	0.00	9.32	3.9	9.59

Table 3: Analysis of Variance of the three Response Variables Using the Experimental Design

Responses	source	Sum of squares	df	Mean square	F value	p-value prob>F	R squared	Adjusted. R^2	Adeq. precision
DT	**Model**	9.80	3	3.27	2.48	0.2001	0.6507	0.3886	3.908
	[1]Linear Mixture	1.29	1	1.29	0.98	0.3789			
	AB	4.61	1	4.61	3.50	0.1345			
	AB(A-B)	3.91	1	3.91	2.97	0.1599			
	Residual	5.26	4	1.32					
	Lack of Fit	0.013	1	0.013	7.170E-003	0.9379			
	Pure Error	5.25	3	1.75					
	Cor Total	15.07	7						
CS	**Model**	1.33	1	1.33	35.40	0.0010	0.8551	0.8309	11.218
	[1]Linear Mixture	1.33	1	1.33	35.40	0.0010			
	Residual	0.23	6	0.038					
	Lack of Fit	0.021	3	7.037E-003	0.10	0.9529			
	Pure Error	0.21	3	0.068					
	Cor Total	1.56	7						
FR	**Model**	19.08	1	19.08	2.38	0.1741	0.2837	0.1643	2.907
	[1]Linear Mixture	19.08	1	19.08	2.38	0.1741			
	Residual	48.18	6	8.03					

Lack of Fit	10.95	3	3.65	0.29	0.8292

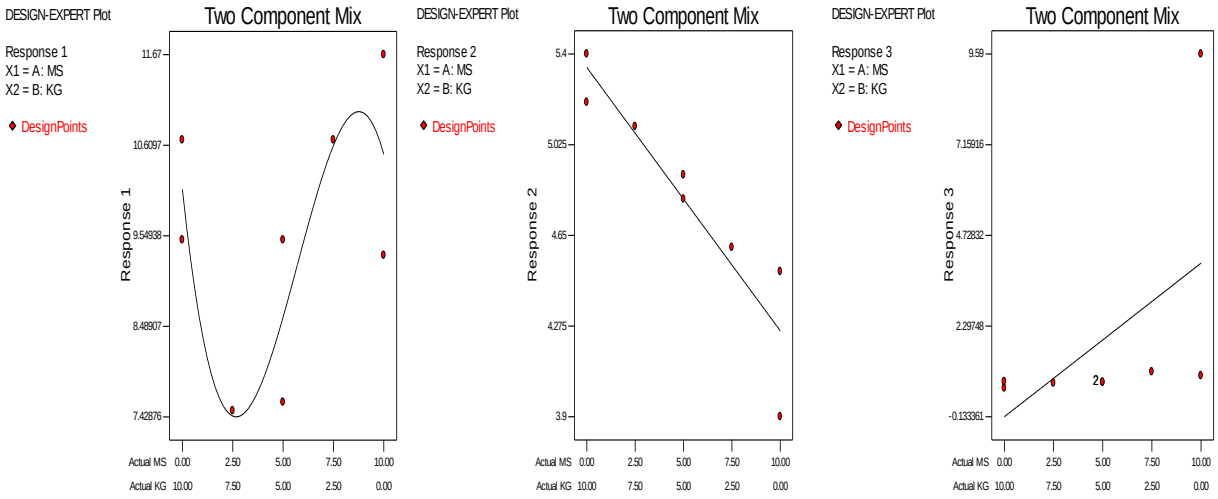

Figure 1: Contour plots of DT (a), CS (b) and FR (c)

Table 4: Prediction of the optimized composition of disintegrant mixture showing proportion of each component and the expected response

Formulation	MS	KG	DT (sec)	CS (KgF)	FR (%)	Desirability
1	1.858	8.142	7.636	5.142	0.632	0.078

DT=disintegration time, CS= crushing strength, FR= friability

Table 5: Physical properties of metronidazole tablets formulated using either MS or KG and combination of both as disintegrants

Parameters	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9 optimized
Weight variation (mg)	657±17.04	630± 16.37	634± 13.5	647±20.5	632±15.4	632±15.42	633.5±9.3	628±17.04	652.5±10.2
Diameter (mm)	12.036 ±0.02	12.03±0.02	12.02±0.01	12.02±0.02	12.06±0.01	12.03±0.01	12.04±0.01	12.03±0.02	12.03±0.01
Thickness (mm)	6.1705 ± 0.02	6.07±0.07	6.08±0.03	6.2±0.08	6.16±0.03	6.12±0.02	6.12±0.01	6.18±0.04	6.03±0.01
Dissolution t_{90} (%)	117.01	112.18	104.8	97.43	103.07	69.32	88.92	105.52	101.23

Content uniformity (%)	127	86.02	68.56	89.99	89.99	81.90	88.00	117.66	79.63

Effect of changing levels of component excipients (Factors) on DT

Tablet disintegration is important for the release of active drug component for absorption from a conventional dosage form. The BP specifies that uncoated tablets should disintegrate completely within 15 min. Table 2 depicts the results of the responses obtained for the 8 runs/formulations of metronidazole tablets manufactured via direct compression. All the formulations produced tablets that disintegrated in less than 1 minute showing an excellent super disintegrant property. Super disintegrants break up tablets in 2-10 min of contact with fluid. Generally, no perfect disintegrant exists, disintegration is said to depend on the active ingredients and excipients in the tablet formulation, method of preparation which could be either wet granulation or direct compression and also the tablet porosity [9]. The properties of the combined excipients (an amorphous MS and a semi crystalline KG in addition to the method of formulation is thought to have influenced this rapid disintegration. *Khaya senegalensis* gum (KG) has been reported to swell up to ten times its weight in water and also has a good disintegrant property in metronidazole tablets formulated via wet granulation at a concentration of 5% (DT= 9.04 min) [10].

The rapid swelling ability of Khaya gum and particle size may have a role to play in the fast disintegration action when formulated via direct compression. Likewise, swelling has been reported to be the main mechanism of disintegrant action of starch and its derivatives [11]. Formulations containing MS were found to produce hard tablets that rapidly disintegrated although the disintegration action was attributed to the disintegrant type but the rapid swelling and low cold-water solubility indices of Sammaz starch was said to have contributed to this occurrence. Substances that swell rapidly but do not dissolve to form viscous solutions that will hinder fluid uptake through porous capillaries allow disintegration to occur faster [3]. The DT values, varied across the formulations because of the changing proportions of the factors. Summary statistics of the model fitted to response DT is given in Table 3. The R^2 = 0.6507, Adequate precision of 3.908 and F value 2.48. Analysis of variance confirmed that the model is not significant at $p < 0.05$.

This means that the varied factors had no effect on the response DT. The regression equation generated for DT is given as

$$DT = 1.05MS + 1.009KG - 0.06\,MS * KG + 0.01MS * KG * (MS - KG)$$

The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. Factor A (MS) had highest regression coefficient of 1.05 relative to factor B(KG) with regression coefficient of 1.009 implying that although the effect of MS and KG were not significant on DT statistically, MS had more effect on DT, in increasing disintegration time compared to KG. This explains the slight variation of DT across the different formulations as well as the low disintegration values observed for this factor (KG) in Table 2. The interacting variables of AB (MS and KG) with a negative sign of regression estimate/coefficient suggests that the two interacting factors will lower DT. The contour plots of DT (figure 1a) further revealed that as MS increased, DT also increased and as KS increased, DT decreased. The author, Ayorinde and Odeniyi [12] mentioned that, Khaya gum and pigeon pea starch were better disintegrants than sodium starch glycolate, with the gums exhibiting better properties than the starch.

Effect of changing levels of component excipient on crushing strength (CS)

The results of CS for the 8 runs of experiment are presented in Table 2. An inconsistent variation in values of CS was observed across the different formulations. Values ranged between 3.9 to 5.4 KgF, falling within manufacturers limits of 4-8 KgF for conventional tablets except run 8 (3.9 KgF). As the concentration of KG levels increased across the formulation, CS increased. The opposite was noted for MS. Although both components have been used as binders in tablet formulations and have conferred different levels of hardness to tablets. The response CS was described by a linear model as shown in summary statistics Table 3. The model was statistically significant at $p<0.05$, with a non-significant lack of fit, the predicted R^2 was in reasonable agreement with the adjusted R^2 with a high adequate precision greater than 4, an indication that the model can be used to navigate the design space. The equation below described the response.

$$\mathrm{CS} = 0.42556 * \mathrm{MS} + 0.53444 * \mathrm{KG}$$

The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The higher regression coefficient of 0.534 coded for factor B (KG) relative to factor A(MS) with regression coefficient of 0.425 showed that the two factors had effect on the response CS. It further confirms

that KG had more effect on CS compared with MS. This therefore explains why, as the concentrations (levels) of KG increased, CS also increased. The positive signs of the components (Factors) indicate that a combination of MS and KG will improve crushing strength but where levels of KG, are higher than those of MS. The linear mixture model was used to generate the statistics of response FR (friability) on the varying factors. An F-value of 2.38 at $p<0.05$ indicated that the model is not significant relative to the noise. The lack of fit was also non-significant and implies that the linear mixture model fits. However, the precision was not adequate (2.91) because of inability of the model to navigate the design space.

$$\mathrm{FR} = +0.39849 * \mathrm{MS} - 0.013336 * \mathrm{KG}$$

The coded equation reveals that MS increased friability while KG decreased friability. Tablet friability is required to be low to ensure its physical integrity during packaging and handling. This explains why lower values of FR were obtained when the concentration of KG increased from 2.5 to 10.0% (Table 2). Besides, formulations containing more of KG had higher crushing strength i.e., harder tablets and as such are expected to be less friable.

The contour plot of DT generated from the regression equation showing the pattern of changing levels of the factors on DT, CS and FR are displayed in Figs. 1(a) – (c) respectively. Points that displayed similar response values were connected to produce contour plots. Increase in % proportion of MS from 0-10 % increased DT whereas increase in same proportion for KS decreased DT. Increasing the levels of MS from 0-10 % decreased CS, although the decrease was still within manufacturers acceptable limits for conventional tablets. Contrarily, increase in concentrations of KS increased CS. This is not surprising, as KS has been reported severally to increase tablet hardness [13]. Increasing levels of KS decreased friability while the opposite was observed with MS.

Optimization studies

The numerical optimization method was used to search the design space for a combination of factor levels that satisfies the criteria placed on each of the responses and factors using the design expert software [14]. The criteria set to predict the factors and response variables are shown in Table 4. One (1) solution with a desirability function of 0.078 was obtained as a possible combination to predict an optimized metronidazole tablet formulation containing MS (1.86%) and KG (8.14%) as disintegrants. The desirability function is a parameter used to rank the possible solutions obtained on the basis of the extent to which the criteria set for each variable is met by that solution [15, 16]. A solution having a desirability value of 1 implies that all the criteria set for the variables were achieved. The response variables of the optimized formulation were evaluated (DT= 9 sec, CS= 6.8KgF and FR= 0.51%) and model was seen to be valid for optimizing the formulation due to close similarities with the predicted values.

Further tests were carried out on the formulated tablets to assess their overall quality (Table 5). The formulated metronidazole tablets were uniform in weight with well-defined diameter and thickness. Uniformity of diameter and thickness signifies good flow of powder and uniform die fill which results to acceptable and uniform tablet weight within Pharmacopoeial limits. Like disintegration, dissolution was spontaneous with more than 75 % metronidazole released at t_{90}. However, not all formulations passed the content uniformity test within 85 -115 % of USP acceptable limits for uncoated tablets. The resulting effect of this combination/mixture was an effective reduction of disintegration time and rapid dissolution, non- friable tablets with acceptable mechanical strength. Hence, a solution for formulators in search for disintegrants with strong disintegration power.

CONCLUSION

The selection of a cubic linear model using the design expert software provided a basis for the development and optimization of a rapid disintegrant in metronidazole tablet with various properties. The optimized formulation was found to be formulation containing MS (1.86%) and KG (8.14%) as disintegrant. The effect of this combination yielded non-friable metronidazole tablets with adequate crushing strength and very low disintegration time of less than one minute. Hence, the physical combination of Sammaz maize starch (MS) and *Khaya senegalensis* gum (KG) was found to be effective as disintegrant in metronidazole tablets formulated by direct compression. The study also shows that combination of natural polymers may yield unexpected novel characteristics of excipients in tablet formulations.

ACKNOWLEDGEMENT

The authors acknowledge Prof. Mukhtar Mahmud of Department of Agronomy ABU Zaria for providing Sammaz maize grains as gift.

REFERENCES

1. OGAJI, I.J., NEP, E.I. & AUDU-PETER, J.D. (2012). Advances in natural polymers as

pharmaceutical excipients. *Pharmaceutica Analytica Acta*, **3**: 146.

2. RAIZADA, A., BANDARI, A. & KUMAR, B. (2010). Polymers in drug delivery: a review. *International Journal of Pharmaceutical Research & Development*, **2**(8)**:** 9–20.
3. MAHMUD, H.S., APEJI, Y.E. & PAUL, A.R. (2015). Evaluation of Disintegrant property of *Khaya senegalensis* L. gum (KSG) in water soluble and water insoluble drugs. *Nigerian Journal of Pharmaceutical Science*, **14**(2)**:** 14–23.
4. MAHMUD, H.S., OYI, A.R., IBRAHIM, Y.K.E. & ALLAGH, T.S. (2015). Characterization of Drug Release Profiles of Metronidazole Tablets formulated with *Khaya senegalensis* gum and Sodium Carboxyl Methyl Cellulose Matrices. *World Journal of Pharmacological Research & Technology*, **3**(6)**:** 190-201.
5. ADENUGA, Y.A., ODEKU, O.A., ADEGBOYE, T.A. & ITIOLA, O.A. (2008). Comparative evaluation of the binding properties of two species of Khaya gum polymer in a paracetamol tablet formulation. *Pharmaceutical Development & Technology*, **13**(6)**:** 473–480.
6. MAHMUD, H.S., TYTLER, B.A., SANI, A.I. & SALIHU, N.O. (2018). Investigation of Sammaz Maize Starch as a possible source of Excipient for Pharmaceutical Industry. *Journal of Pharmaceutical Research, Development & Practice*, **2**(2): 100-107.
7. DEL VECCHIO, R.J. (2014). Understanding design of experiments. Carl Hanser Verlag GmbH Co KG.
8. SINGH, B., KAPIL, R., NANDI, M. & AHUJA, N. (2011). Developing oral drug delivery systems using formulation by design: vital precepts, retrospect and prospects. *Expert Opinion on Drug Delivery*, **8**(10)**:** 1341–1360.
9. MICHAUD, J. (2002). Starch based excipients for pharmaceutical tablets. *Pharmaceutical Chemistry*, 42–44.
10. MAHMUD, H.S., OYI, A.R. & ALLAGH, T.S. (2008). Studies on some physicochemical properties of *Khaya senegalensis* gum. *Nigerian Journal of Pharmaceutical Science*, **7**(1)**:** 146–152.
11. DESAI, P.M., LIEW, C.V. & HENG, P.W.S. (2016). Review of disintegrants and the disintegration phenomena. *Journal of Pharmaceutical Science*, **105**(9)**:** 2545–2555.
12. AYORINDE, J.O. & ODENIYI, M.A. (2014). Disintegrant properties of native and modified polymers in metronidazole tablet formulations. *African Journal of Biomedical Research*, **17**(3)**:** 143–152.
13. ODEKU, OA. & ITIOLA, O.A. (2003). Evaluation of the effects of khaya gum on the mechanical and release properties of paracetamol tablets. *Drug Development & Industrial Pharmacy*, **29**(3)**:** 311–320.
14. APEJI, Y.E., OYI, A.R., ISAH, A.B., ALLAGH, T.S., MODI, S.R. & BANSAL, A.K. (2018). Development and optimization of a starch-based co-processed excipient for direct compression using mixture design. *American Association of Pharmaceutical Science &Technology*, **19**(2)**:** 866–880.
15. CHAUHAN, S.I., NATHWANI, S.V., SONIWALA, M.M. & CHAVDA, J.R. (2017). Development and characterization of multifunctional directly compressible co-processed excipient by spray drying method. *American Association of Pharmaceutical Science &Technology*, **18**(4)**:** 1293–1301.
16. CHAVAN, R.B., MODI, S.R. & BANSAL, A.K. (2015). Role of solid carriers in pharmaceutical performance of solid supersaturable SEDDS of celecoxib. *International Journal of Pharmaceutics*, **495**(1)**:** 374–384.