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THE EFFECT OF PYRODEXTRIN SOURCE, PYRODEXTRIN VARIATION AND STORAGE TEMPERATURE ON THE STABILITY OF PARACETAMOL FORMULATIONS: A STATISTICAL APPRAISAL

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ABSTRACT

One of the major problems facing the pharmaceutical industry in Nigeria is the issue of raw materials. New pyrodextrins from tacca and gladiolus starches may prove to be cheap and ideal pharmaceutical excipients for use in tablet dosage forms. However, the ability of any pharmaceutical substance to substitute imported products is its ability to compare with properties of such products. In this research, statistical parameters were used to check the properties of newly formulated pyrodextrins from tacca and gladiolus starches. Pyrodextrins derived from two tropical starches: *Tacca involucrata* (Fam: Taccaceae) and *Gladiolus actimorphantus* (Fam: Iridaceae) were used for this study. The starches were extracted using standard procedures and subjected to varying temperatures to produce pyrodextrins. The effect of pyrodextrin source, pryodextrin variation and storage temperature on the drug content of paracetamol was statistically evaluated in this study using factorial experiment in randomized complete block design (RCBD). The result of the analysis showed that the sources of the pyrodextrins and the storage temperature had much effect on the drug content of paracetamol whereas the binder concentration had little or no effect on the drug content of paracetamol.

Keywords: *pyrodextrin; source; variation; storage temperature; stability; paracetamol; statistical appraisal.*

INTRODUCTION

Starch is a widely distributed, occurring in roots, seeds and fruits of plants. Seeds usually contain 40 – 70% starch whilst roots and fruits contain between 5-25 %. The principal commercial source of starch is corn. Sources of less importance include tapioca, wheat, potatoe, canna, rye, barley, sorghum, rice, arrowroot, sago plant and oat [1-3]. The principal storage organs of cereals such as wheat, rice and maize may contain up to 70% starch, and bulbs, tubers, roots and pithy stem may contain up to 30% [3].

One of the major challenges facing the Nigerian researcher as well as our industrialists is the issue of raw material sourcing [4-6]. Many attempts at sourcing pharmaceutical raw materials have not been successful. This has largely been due to variations from batch to batch. Such batch to batch variations can only be controlled by applying statistical variables.

The use of statistical techniques in every aspect of human endeavour that requires information management, planning discovery and standards has grown in the last few decades [7, 8]. The application of these ideas have helped many researchers in the pharmaceutical sciences for instance to arrive at acceptable decisions about product quality and standardization.

A desired statistical methodology for the analysis of experimental responses, however, should be such that the most and least ambiguous information about the experiment is obtained with minimum cost [7].

Modification of starches is done by chemical treatments or other method in order to obtain combinations of properties suitable for specific applications. For many industrial applications, the properties of natural starches are changed by various treatments such as heat, acid, enzymes or oxidizing agents. Modification could be done by plant breeding, fractionation, or cross linking and hydrolysis by the use of chemicals [4].

Tacca starch is obtained from the roots of a perennial herb *Tacca involucrata*. The root tuber of this plant has been known to contain large amount of starch. The species *Tacca involucrata* is found mainly in the middle belt region of Nigeria where it serves as food in a variety of ways and also grows widely. The physiochemical properties of *Tacca involucrata* starch has been evaluated as binder in paracetamol tablets [1].

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Gladiolus starch is obtained from *Gladiolus actimmorphantus*. The plant is herbaceous with stiff, pointed and trap-shapped flattened leaves. The flower is predominantly wild and its storage organ is the corm. It grows in the savanna region of Nigeria where it is consumed locally. The plant is however, mainly valued for its ornamental purposes. The physiochemical properties of this starch have been evaluated [9].

Pyrodextrins comprise a mixture of glucose-containing oligosaccharides that is derived from the hydrohysis of starch [10]. Pyrodextrins from two sources are used in this experiment.

Materials and Methods

The starches were extracted using standard procedures and subjected to varying temperatures and length of time to produce the pyrodextrins. The pyrodextrin were thereafter subjected to chemical, physical and physicochemical tests and later evaluated as binders or disintegrants in paracetamol tablets. The yields of the starches were 57% and 45% respectively for tacca and gladiolus while the yield of pyrodextrins from the starches was 100%. All the pyrodextrins performed well as binders in concentration range of 2% - 10% weight/ volume.

In factorial experiment, the whole experiment can be performed in a completely randomized manner in which case the entire experiment can be run several times in a day by an experimenter [7, 8].

But in the experiment conducted for this research work, the complete randomization described above was restricted and the experiment was blocked. Instead of running several replications of experiment all at one time, one complete replication was run in one day – day 3 and a second complete replication was run in another day – day 7. In this case, each replication becomes a block and the design is a Randomized block Design (RCBD) with complete factorial experiment randomized in each block.

Raw tacca and gladiolus starches were dextrinized at different temperatures and time interval to give six different batches of pyrodextrins as shown in Table 1.

Table 1. *Manufacturing conditions for the pyrodextrins*

Batch	Dextrinization temperature (°C)	Dextrinization time (h)
TSDD 1	200	20
TSDD 2	200	8
TSDD 3	120	5
GSDD 1	120	5
GSDD 2	200	8
GSDD 3	200	20

TSDD = Tacca starch derived dextrin; GSDD = Gladiolus starch derived dextrin

The batches chosen for the research are TSDD1, TSDD2, GSDD2 and GSDD3. They were chosen because their dextrinization temperature was the same.

In the evaluation of pyrodextrin as binders in paracetamol tablets, the concentration of the binders were 2, 4, 6, 8, and 10% w/v for each batch of pyrodextrin corresponding to batches A, B, C, D and E respectively. In each case, the tablets were formulated by wet granulation using lactose as bulking agent, Ac-di-sol® as disintegrant and magnesium stearate as lubricant in an F3 Manesty single punch tabletting machine. Each tablet contained 500 mg of paracetamol. The formulated tablets were evaluated for weight uniformity, friability, disintegration time and hardness according the BP specification [11]. The drug content (DC) of the tablets was determined spectrophotometrically.

Determination of the absolute drug content

Ten tablets from each batch were selected at random, weighed and crushed together. A quantity of the crushed material equivalent to the calculated mean weight was weighed out and dissolved in 60 ml of 0.1 N HCl. The solution was made up to 100 ml, appropriately diluted and analysed in a spectrophotometer at 245 nm. The absorbance values were converted to concentration by reference to standard Beer's plot for paracetamol in 0.1 N HCl at 245 nm. Average of two absorbance readings were used for each batch.

Results and Discussion

Results of the evaluation of the formulated paracetamol tablets indicated that the tablets conformed to BP specification [11]. The DC analysis revealed that the content of paracetamol in the tablets ranged from 480.0 ± 6.6 mg for tablets prepared with TSDD 1 to 519.8 ± 6.6 mg for tablets prepared with GSDD 2. The results were thereafter subjected to statistical analysis, having satisfied the condition stipulated for immediate release tablets in the BP [11]. The absolute drug content is as shown in Table 2.

Table 2 : *Absolute drug content of the tablets.*

Batch	TSDD1	TSDD2	TSDD3	GSDD1	GSDD2	GSDD3
Drug content	493.4±6	493.4±6	506.9 ±6	532.9± 5	506.±3	513.2±3
Friability (%)	0.80	0.31	0.49	0.73	0.34	0.99
Disint. time (min)	1.0±0.15	0.51±0.09	0.83±0.05	1.78±0.15	0.71±0.05	0.34±0.03
Hardness (kgf)	4.67±0.45	4.17±0.62	5.17±0.24	6.0±0.20	4.67±0.50	4.33±0.25

The drug content was fairly uniform for all the batches produced as none deviated more than 10 % from the intended drug content. Similarly, the friability, the disintegration times and the hardness values were within the officially acceptable limits.

Batch

MODEL

$Y_{ijkl} = \mu + R_l + T_i + B_j + (TB)_{ij} + S_k + (TS)_{ik} + (BS)_{jk} + (TBS)_{ijk} + Z_{ijkl}$

t = 1,2, …, 3. (Temperature); i = 1,2, …., a

j = 1,2, …, 5. (Binder concentration); j = 1,2, …, b

k = 1,2 …, 4 (Pyrodextrin Source); k = 1,2, …, c

l = 1,2. (Block = day); l = 1, … r

Definition of Variables

Y_{ijkl} is the drug content of the ith temperature in the jth binder concentration of the kth pyrodextrin source for the lth day.

μ is the overall mean (universal constant) that is independent of the treatment.

T_i is the effect of the ith temperature

B_j is the effect of the jth binder concentration

S_k is the effect of the kth pyrodextrin source

R_l is the effect of the lth day (Day effect)

$(TB)_{ij}$ is the effect of the interaction between the ith temperature and the jth binder-concentration.

$(TS)_{ik}$ is the effect of the interaction between the ith temperature and the kth pyrodextrin source.

$(BS)_{jk}$ is the effect of the interaction between the jth binder concentration and the kth pyrodextrin source.

$(TBS)_{ijk}$ is the effect of the interaction between the ith temperature of the jth binder concentration and the Kth pyrodextrin source.

Z_{ijkl} is the observed error associated with Y_{ijkl}.

Assumptions and Test of Hypothesis

In statistics, to carry out a meaningful statistical analysis to the data collected, the following assumptions need to be made:

(i) NORMALITY: The normality assumptions make it possible for us to use the F-distribution in testing for the equality or otherwise of treatment mean effects.

(ii) INDEPENDENCE: The whole idea about this assumption is for “simplicity” and “tractability”.

(iii) CONSTANT VARIANCE (HOMOSCEDAS TICITY): Since in analysis of variance, we are always interested in estimating the parameters of model, our estimates are required to be Best Linear Unbiased Estimate.

TEST OF HYPOTHESIS

(a) BLOCKS

H_o: $R_1 = R_2$ Vs H_1: R_l ‘“ R_l for some l ‘“ l’

(b) TEMPERATURE

H_o: $T_1 = T_2 = T_3$ Vs HI Ti ‘“ Ti’ for some i ‘“ I’

(c) BINDER CONCENTRATION

H_o: $B_1 = B_2 = B_3 = B_4 = B_5$ Vs Hi: B_j ‘“ B_j’ for some j ‘“ j’

(d) PYRODEXTRIN SOURCE

H_o: $S_1 = S_2 = S_3 = S_4$ Vs H_1: S_k ‘“ S_k’ for some K ‘“ K’

(e) INTERACTION BETWEEN TEMPERATURE AND BINDER CONCENTRATION

H_o: $(TB)_{ij} = (TB)_{i'j'}$ Vs H_i: $(TB)_{ij}$ ‘“ (TB)i’j’ for some i ‘“ i’ and j ‘“ j’

(f) INTERACTION BETWEEN TEMPERATURE AND PYRODEXTRIN SOURCE

H_o: $(TS)_{ik}$ = TSi’k’ Vs H_1: $(TS)_{ik}$ ‘“ $(Ts)_{i'k'}$ for some i‘“i’ and K ‘“ K’

(g) INTERACTION BETWEEN BINDER CONCENTRATION AND PYRODEXTRIN SOURCE

Ho: $(BS)_{jk} = (BS)_{j'k'}$ Vs H_{1--}: $(BS)_{jk}$ ‘“ $(BS)_{j'k'}$ for some j ‘“ j’ and K ‘“ K’

(h) INTERACTION BETWEEN TEMPERATURE, BINDER CONCENTRATION AND PYRODEXTRIN SOURCE

H_o: $(TBS)_{ijk} = (TBS)_{i'j'k'}$ Vs H_1; $(TBS)_{ijk}$ ‘“ $(TBS)_{i'j'k'}$ for some i ‘“ i’, j ‘“ j’ and K ‘“ K’.

DECISION RULE

Reject H_o if F calculated > F tabulated. Accept if otherwise. The various statistical approaches used to derive the F values are summarised in Table 3 while the actual values calculated are as shown in Table 4.

Table 3 : *The skeletal analysis of variance table for a 3-Factor random effect model (factorial experiment in rcbd)*

(S.V) SOURCE OF VARIATION	d.f DEGREE OF FREEDOM	S.S SUM OF SQUARES	MS MEAN SQUARES	F F-RATIO
Block ($R_{(l)}$)	(r-1)	$SS_{(r)}$	$SS_{(r)}/r-1 = MS_{(r)}$	$MS_{(r)}$ / $F_{(r)} = MS_{(r)}$
Temperature (T)	(a-1)	$SS_{(t)}$	$SS_{(t)}/a-1 = MS_{(t)}$	$MS_{(t)}$ / $F_{(t)} = MS_{(t)}$
Binder Concentration (B)	(b-1)	$SS_{(b)}$	$SS_{(b)}/b-1 = MS_{(b)}$	$MS_{(b)}$ / $F_{(b)} = MS_{(b)}$
Pyrodextrin Source (S)	(c-1)	$SS_{(s)}$	$SS_{(s)}/c-1 = MS_{(s)}$	$MS_{(s)}$ / $F_{(s)} = MS_{(s)}$
Interaction b/w Temp & Binder Conc. $(TB)_{ij}$	(a-1)(b-1)	$SS_{(TB)}$	$SS_{(TB)}/(a-1)(b-1)$	$MS_{(TB)}$ / $F_{(TB)} = MS_{(TB)}$
Interaction b/w Binder Conc. & Source $(TS)_{ik}$	(a-1)(c-1)	$SS_{(TS)}$	$SS_{(TS)}/(a-1)(c-1)$	$MS_{(TS)}$ / $F_{(TS)} = MS_{(TS)}$
Interaction b/w Binder & Source $(BS)_{jk}$	(b-1)(c-1)	$SS_{(BS)}$	$SS_{(BS)}/(b-1)(c-1)$	$MS_{(BS)}$ / $F_{(BS)} = MS_{(BS)}$
TBS_{ijk}	(a-1)(b-1)(c-1)	$SS_{(TBS)}$	$SS_{(TBS)}/(a-1)(b-1)(c-1)$	$MS_{(TBS)}$ / $F_{(TBS)} = MS_{(TBS)}$
Error (Z_{ijkl})	(abc-1)	SS_e	$SS_e/abc-1)$	
TOTAL	(n-1) = abcr-1	$SS_{(Total)}$		

Table 4: *Anova Table for 3 X 5 X 4 Factorial Experiment In RCBD*

SOURCE OF VARIATION	d.f	S.S	M.S	F-CALCULATED	F-THEORETICAL
P	1	4335.617	4335.617	148.5993	11.97
T	2	916.867	458.434	15.7124	7.77
B	4	406.096	101.524	3.4796	5.31
S	3	1198.489	399.496	13.692	6.17
TB	8	227.211	28.401	0.9734	3.86
TS	6	1257.717	209.619	7.1845	4.37
BS	12	2480.206	206.689	7.0841	3.32
TBS	24	786.274	32.761	1.1229	2.69
Error(σ^2)	59	1721.417	29.176		
TOTAL	119	13329.966			

α = 0.001

The table of means is as shown in Table 5. The table of means is essential for the determination of the Students *t*-test that is essential for the comparison of the various pyrodextrins in terms of temperature effect and interaction with paracetamol.

Table 5: *(Two-Way) Table of Means*

	T1	T2	T3	MEANS
TSDD1	615.438	613.5	602.125	610.354
TSDD2	618.425	614.163	607.563	613.838
TSDD2	613.713	599.7	598.713	604.042
TSDD3	599.838	608.063	605.6	604.500
MEANS	**611.853**	**608.856**	**603.500**	

Pair Wise Comparison Using The Students *t*-Test

S.e = $\sqrt{\frac{2MS_e}{n}}$ = Standard Error

S.e for individual means (interactions) =

$$\sqrt{\frac{2(29.176)}{8}} = 1.71$$

Standard error for storage temperature =

$$\sqrt{\frac{2(29.176)}{32}} = 0.95$$

S.e for pyrodextrin sources = $\sqrt{\frac{2(29.176)}{24}} = 1.10$

For temperature

$T_3 < T_2 < T_1$

$H_o = T_1 = T_2 = T_3$ Vs $H_{1r} = T_i$ '" T_i' for some i '" i

Test statistic

$$t = \frac{t_i - t_i}{\sqrt{Var(t_i - t_{i'})}}$$

CONCLUSION:

$t_{T1} - t_{T2} = 2.998 < S.e\ t_{59}^{(0.995)} = 3.54$ is not significant.

$t_{T1} - t_{T3} = 8.353 > S.et_{59}^{(0.995)} = 3.574$ is significant

$t_{T2} - t_{T3} = 5.356 > 3.574$ is significant

For pyrodextrin sources

$t_{TSDD1} - t_{TSDD2} = 3.029 < S.e\ t_{59}^{(0.995)} = 4.138$; not significant

$t_{TSDD1} - t_{TSDD2} = 6.312 > 4.138$; Significant

$t_{TSDD1} - t_{TSDD3} = 5.854 > 4.138$; Significant

$t_{TSDD2} - t_{TSDD2} = 9.341 > 4.138$; Significant

$t_{TSDD2} - t_{TSDD3} = 8.883 > 4.138$; Significant

$t_{TSDD2} - t_{TSDD3} = 0.458 < 4.138$; not Significant

SUMMARY AND CONCLUSIONS

In order to make a better conclusion, significant test for difference in means was conducted using the pairwise comparison for Students' *t*-test. In drawing conclusion on this test of significance, the questions below should be answered. Where the answers are to be extracted from the result of the analysis. The questions are:

(i) Has blocking been effective?

(ii) Is the effect of each pyrodextrin source the same on the drug content?

(iii) Does the effect vary over the different storage temperatures used?

The first question is answered by saying that blocking is effective because H_o-the null hypothesis was rejected and on the whole, it means good choice of blocks.

For the second question, the effect of pyrodextrin sources is quite significantly different for the two types of starches used. This is more pronounced when the comparison is between one batch of Tacca starch and any other batch of Gladiolus starch. For example $t_{TSDD1} - t_{GSDD2}$ or $t_{TSDD2} - t_{GSDD3}$. But the effect is not significantly different when the comparison is between two batches of the same starch like $t_{TSDD1} - t_{TSDD2}$ or $t_{GSDD2} - t_{GSDD3}$. From the LSD test Tacca starch derived dextrin (TSDD) is significantly different from Gladiolus starch derived dextrin (GSDD).

The final question is whether the effect varies over the different storage temperatures used. The answer is yes for the temperature of 70 ^{0}C, because at this temperature, there is a significant difference between the effects of pyrodextrin stored under this temperature. For the pyrodextrin stored under 45 ^{0}C and 55 ^{0}C, there is no significant different between their effect on the

drug content of paracetamol. During the stability studies, the tablets were tested as well as free paracetamol which had no pyrodextrins. The tablets containing the pyrodextrins had more marked degradation at 70 °C than the free paracetamol.

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