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Ex-vivo evaluation of crab shell chitosan as absorption enhancer in ciprofloxacin tablet formulation

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This study was aimed at evaluating crab shell chitosan as absorption enhancer in ciprofloxacin tablet formulation using the *ex-vivo* model. Six batches of ciprofloxacin tablets containing varying concentrations of crab shell-derived chitosan ranging from 0 to 5% ^{w}/w at 1% ^{w}/w intervals were produced. Batch CTS-0 containing no chitosan served as the control. The crushing strength, friability, disintegration time, dissolution profile and permeation profile of all the batches were determined. Friability was not significantly affected but the crushing strength and disintegration time of tablets decreased with increase in concentration of chitosan. There was no significant difference in the cumulative percent drug released in 1 h but the cumulative percent drug permeated in 4 h increased with increase in the concentration of chitosan. It increased from 68% (when no chitosan was added) to 81.8% (when 5% ^{w}/w chitosan was incorporated). The polymer caused a faster onset of drug release but the eventual total drug released was not significantly influenced. It also improved the permeation of the released drug. This study correlates with *in-vivo* bioavailability study because the usual oral bioavailability of ciprofloxacin without absorption enhancer is 70%. Hence, crab shell chitosan at concentration of 5% ^{w}/w could increase the absorption of ciprofloxacin from 70 to 82%. The study suggests the use of the chitosan at this concentration to improve the absorption of ciprofloxacin.

Key words: Crab shell chitosan, ciprofloxacin, dissolution, permeation, absorption.

INTRODUCTION

Ciprofloxacin is a fluoroquinolone antibacterial with a wide spectrum of activity (Campoli-Richard et al., 1998). It inhibits bacterial growth and replication by interfering with the action of DNA gyrase (topoisomerase II) and topoisomerase IV. A peak plasma concentration of 2 - 3 µg/mL occurs within 2 h after an oral administration of 500 mg dose. It has an oral bioavailability of about 70% (Chambers, 2004). Drugs have been classified into four in the Biopharmaceutic Classification System (BCS) by Amidol et al. (1995). This classification is based on biopharmaceutical properties of solubility and permeability which are the two properties used in assessing

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Table 1. Tablet formula.

Ingredient	Batches					
	CTS-0	CTS-1	CTS-2	CTS-3	CTS-4	CTS-5
Ciprofloxacin (%)	80	80	80	80	80	80
Maize starch (%)	10	10	10	10	10	10
Lactose (%)	5	4	3	2	1	0
Acacia (%)	3	3	3	3	3	3
Chitosan (%)	0	1	2	3	4	5
Talc (%)	1.5	1.5	1.5	1.5	1.5	1.5
Mg stearate (%)	0.5	0.5	0.5	0.5	0.5	0.5
Tablet weight (mg)	625	625	625	625	625	625

bioavailability of orally administered drugs. Ciprofloxacin has unique characters that are intermediate between BCS classes II and III (Wu and Benet, 2005). This implies that the solubility is not as good as that of class III drugs and the permeability is not as good as that of class II drugs. Hence, the drug has both solubility and permeability limitations resulting to its incomplete absorption.

Chitosan has been employed in many drug delivery systems (Hu et al., 2013; Yin et al., 2009). It is a hydrophilic polymer with disintegrant property. Its effect on drug release from tablets is intermediate between the effect of corn starch and that of sodium starch glycolate (Ritthidej et al., 1994). It is also capable of causing temporary increase in the permeability of intestinal mucosa promoting drug permeation (Kos et al., 2008). Since the oral route requires that a drug dissolves in the gastrointestinal fluid and then penetrates the epithelial cells of the intestinal mucosa to get into systemic circulation (Ashford, 2007), the presence of chitosan in a formulation might exert an effect on both drug release from tablets and drug permeation across intestinal membrane leading to improved drug absorption.

Ciprofloxacin is the most widely used of the fluoroquinolones, though it has the lowest bioavailability (Vance-Bryan et al., 1990). Chitosan is deacetylated product of chitin, the second most abundant poly-saccharide. Chitin is a major component of the shells of crustaceans which are abundantly available at all coastal regions as wastes from seafood (Olorunsola et al., 2015). Chitosan has the potential of positively influencing both drug release and permeation. This study evaluates crab shell chitosan as absorption enhancer in ciprofloxacin tablet formulation using the *ex-vivo* model. The chitosan is incorporated in the tablet formulation and the effect of the polymer on the drug release and intestinal permeability evaluated.

MATERIALS AND METHODS

The materials used were: ciprofloxacin powder (Hopkin & Williams, England), maize starch B.P. (BDH Chemicals, England), lactose (BDH Chemicals, England), acacia gum (BDH Chemicals, England), talc (BDH Chemicals, England) and magnesium stearate (BDH Chemicals, England).

Crab shell collection and chitosan extraction

Shells of *Callinectes gladiator* were obtained from Oron, Akwa Ibom State, Nigeria. They were sun-dried for five days to remove moisture from the shells. The dried shells were crushed in a mortar and then powdered using laboratory blender (Christison, United Kingdom). Twenty five gramme sample of the powdered shell was weighed and transferred into a 250 ml capacity beaker. Chitin was obtained by deproteination with 100 ml of 4% ^{w}/v NaOH and demineralization with 135 ml of 1% ^{w}/v HCl; and chitosan was derived from the chitin by deacetylation with 100 ml of 50% ^{w}/v NaOH using the methods described by Olorunsola et al. (2015).

Preparation of granules

Six batches of granules were prepared using the wet granulation method based on Table 1. Each batch was prepared using 3% ^{w}/w acacia gum as binder and 10% ^{w}/w maize starch as disintegrant. Batch sizes of 50 tablets were prepared with each tablet containing 500 mg ciprofloxacin.

The weighed quantities of ciprofloxacin, maize starch B.P and lactose were dry-mixed for 5 min and then moistened with mucilage of acacia (binder). The wet mass was screened through a 2.0 mm mesh and dried in a hot air oven (Gallenkamp, Germany) at 60°C for 1 h. The dried granules were then screened again through a 1.0 mm mesh.

Preparation of tablets

The required quantities of chitosan were added to appropriate batches such that batches: CTS-0, CTS-1, CTS-2, CTS-3, CTS-4 and CTS-5 contained 0, 1, 2, 3, 4 and 5% ^{w}/w chitosan respectively. The chitosan was gently blended with the granules over a period of 3 min. Talc and magnesium stearate were also weighed and gently blended with the granules over a period of 3 min. The granules were then compressed at a pressure of 60 KN using a single punch tableting machine (Erweka, Germany).

Tablet evaluation

Uniformity of weight

Twenty (20) tablets from each of the batches were individually weighed using an analytical weighing balance (Mettler, Germany).

The mean weight and the standard error of the mean were calculated.

Crushing strength

The crushing strength of five tablets from each batch was determined with Mosanto hardness tester (Laboratory Tree Co., India). It was done by holding tablet between a fixed anvil and a moving jaw. The load was gradually increased until the tablet just fractured. The applied force was recorded and the mean crushing strength was calculated.

Friability

Ten (10) tablets were dusted, weighed together and then subjected to abrasion test using Roche frabilator (model TAR 10, Erweka, Germany) operated at 25 rpm for 4 min. The tablets were then dusted properly and reweighed collectively. The difference in weight was determined and the friability value was calculated. The procedure was carried out thrice.

Disintegration time

Six tablets from each batch were subjected to disintegration test in a freshly prepared 0.1 N HCl at 37°C using the BP disintegration apparatus (Erweka, Germany). The disintegration time was taken to be the time when no particle remained inside the basket of the disintegration apparatus.

***In-vitro* drug release**

A tablet was placed in the dry basket of the U.S.P. dissolution apparatus (UNICO Shanghai Instrument, China) containing 900 ml of 0.1 N HCl thermostatically maintained at 37 ± 0.5°C. The apparatus was set to a rotational speed of 100 rpm for 1 h. A 10 ml sample was taken at 10 min interval with subsequent replacement with equal volume of the dissolution medium. Each withdrawn sample was filtered and the absorbance was taken at 277 nm using UV spectrophotometer (UNICO Shanghai Instrument, China). Cumulative percent drug released was obtained and then plotted against time.

Isolation of the absorption tissue

The animal (pig), housed in a cross-ventilated room (temperature of 25 ± 2.5°C) was sacrificed in accordance with internationally accepted laboratory animal use and the guidelines and rules for animal experimentation. Six portions of approximately same diameters and length of 15 cm were cut out from the small intestine of the freshly sacrificed pig and used immediately for the permeation study.

***Ex-vivo* permeation study**

Drug permeation study was performed using the method described by Sharma et al. (2013). The segments of the small intestine were used as donor chambers. Each segment was tied at one end and filled with 5 ml of simulated intestinal fluid (pH 6.8). A tablet was introduced into each of the chambers and the segment was then tied at the other end. The donor chamber was immersed into a dissolution apparatus containing 900 ml of the simulated intestinal fluid (the receptor medium). The temperature was maintained at 37 ± 0.5°C and the apparatus was set to operate for 5 h. Samples were withdrawn at 30 min intervals from the receptor medium with replacement using pure medium. The samples were diluted, filtered and the absorbance was taken at 277 nm using a UV spectrophotometer (UNICO Shanghai Instrument, China). A graph of cumulative percent drug permeated was plotted against time. The time for 50% drug permeation ($t_{50\%}$) was read from the plot. Value of steady state drug flux (J) and permeation coefficient (K_p) were calculated using Equations 1 and 2 respectively.

$$J = \frac{dQ}{dtA} \quad (1)$$

where dQ is the change in the quantity of drug permeated (µg) through the membrane of surface area A (cm^2) within time dt (min). The value of dQ/dt was estimated from the slope of the straight line portion of the graph.

$$Kp = \frac{J}{C} \quad (2)$$

Where, J is the drug flux and C is initial concentration of the drug in the donor compartment. The percent drug permeated in 4 h was also recorded.

Statistical analysis

Data obtained from tablet evaluation were expressed as mean values ± standard error of the mean. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison test using GraphPad Instat-3 software. The differences in cumulative percent drug permeated in 4 h were explored using the Chi-square test. Significance of difference was taken at *p - values* less than 0.05.

RESULTS

The physical properties of the tablets are shown in Table 2. The crushing strength decreased with increase in concentration of chitosan. There was no significant difference in the friability values of the different batches. The disintegration time decreased with increase in the concentration of chitosan. However, the values were not significantly different. The batch containing 3% ^{w}/w chitosan had the highest (CS/FR)/DT value.

The dissolution profiles of the different tablet batches are shown in Figure 1. All the batches gave over 75% drug release within 1 h and there was no significant difference in the amount of drug released over this period.

The plot of cumulative percent drug permeated versus time is shown in Figure 2. The permeation coefficient, the time taken for 50% drug permeation and % drug permeated in 4 h are shown in Table 3. The permeation coefficient increased and the time for 50% drug permeation decreased with increase in the concentration of chitosan. The percent ciprofloxacin permeated per time increased with increase in the concentration of chitosan. The control (tablet without chitosan) gave 68.0%

Table 2. Physical properties of tablets.

Batch	Weight (mg)	Crushing strength (kgf)	Friability (%)	Disintegration time (min)	(CS/FR)/DT
CTS-0	628 ± 0.15	5.2 ± 0.75	0.36 ± 0.00	10.54 ± 0.12	1.37
CTS-1	631 ± 0.18	5.0 ± 0.63	0.34 ± 0.01	10.44 ± 0.21	1.40
CTS-2	626 ± 0.13	5.0 ± 0.89	0.33 ± 0.02	10.30 ± 0.18	1.47
CTS-3	628 ± 0.24	4.8 ± 0.56	0.30 ± 0.01	10.26 ± 0.26	1.55
CTS-4	636 ± 0.24	4.6 ± 0.56	0.31 ± 0.04	10.21 ± 0.23	1.45
CTS-5	630 ± 0.18	4.4 ± 1.20	0.33 ± 0.01	10.14± 0.24	1.44

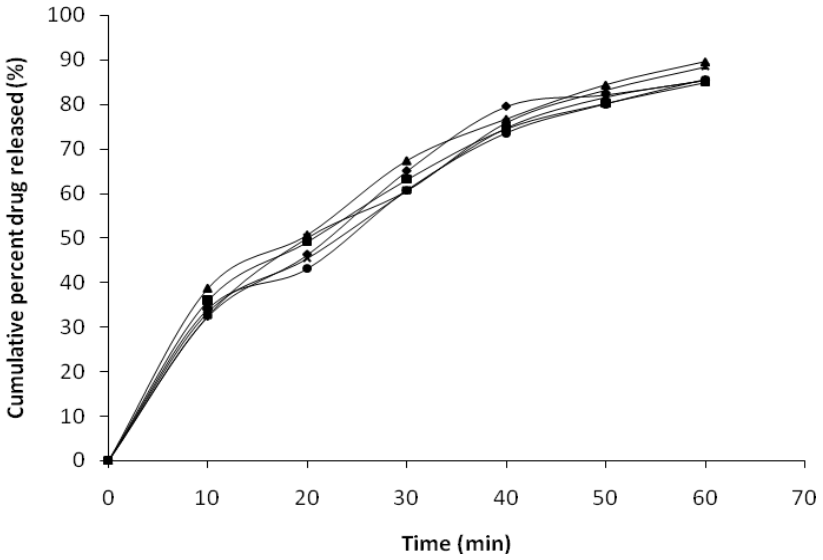

Figure 1. Plot of cumulative percent ciprofloxacin released versus time. Key: CTS-0 (♦), CTS-1 (■), CTS-2 (▲), CTS-3 (×), CTS-4(✱), CTS-5 (●).

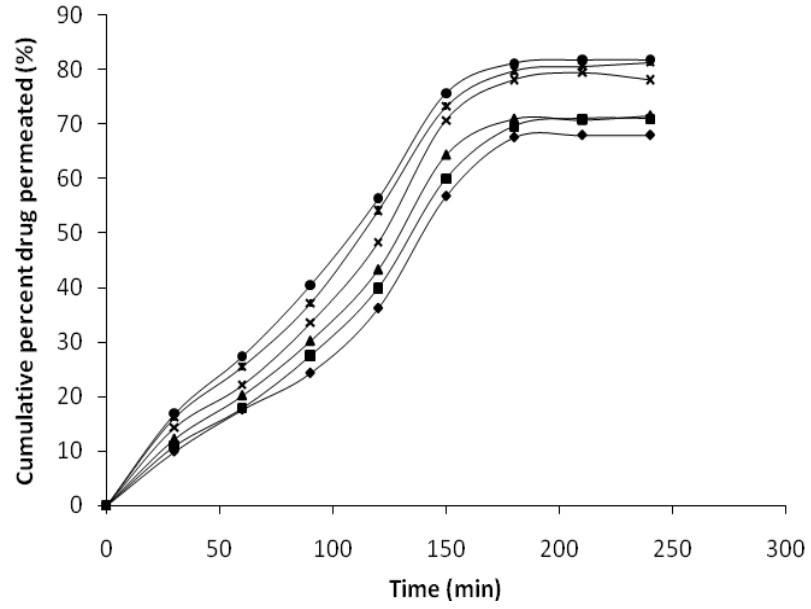

Figure 2. Plot of cumulative percent ciprofloxacin permeated versus time Key: CTS-0 (♦), CTS-1 (■), CTS-2 (▲), CTS-3 (×), CTS-4(✱), CTS-5 (●).

Table 3. Permeation parameters.

Batch	Permeation coefficient (cm/s)	Time for 50% drug permeation (min)	Amount of drug permeated in 4 h (%)
CTS-0	29.22×10^{-6}	137.5	68.0
CTS-1	39.06×10^{-6}	135.2	71.2
CTS-2	46.50×10^{-6}	128.2	71.6
CTS-3	50.22×10^{-6}	122.1	78.2
CTS-4	55.32×10^{-6}	113.7	81.3
CTS-5	56.42×10^{-6}	104.8	81.8

ciprofloxacin drug permeation, CTS-1 (tablet with 1% ^{w}/w chitosan) gave 71.2% permeation and CTS-5 (tablet with 5% ^{w}/w chitosan) gave 81.8% permeation in 4 h.

DISCUSSION

Batch CTS-0 which contained no chitosan served as the control. Weight uniformity is an essential property of a tablet formulation since it guarantees the provision of a uniform dosing of medication. The variation expressed by the different batches of the tablets as shown in Table 2 indicates conformity to the standard for weight uniformity. The USP 31 - NF 26 (2008) states that not more than two of individual weight should deviate from mean weight by more than 5% and none should deviate by more than 10% for tablets of mean weight greater than 324 mg. All the tablet batches passed the test for uniformity of weights.

The normal range of crushing strength for conventional tablet is 4 - 7 kgf and the crushing strength of all the tablet batches fell within this range. The decrease in crushing strength of tablets with increase in concentration of chitosan is an indication that the presence of chitosan reduced tablet strength perhaps by decreasing the amount of plastic deformation occurring during compression (Uhumwangho et al., 2006). The test for friability of tablet measures the ability of the tablet to withstand abrasion during packaging, handling and shipping and a friability value of less than 1% is needed for a tablet to pass friability test (Alderborn, 2007). All the tablet batches passed the test and the values for the different batches were not significantly different. Hence, while chitosan decreases tablet strength in terms of hardness, it does not significantly affect tablet strength in terms of friability.

From the study, the disintegration times for all the batches conform to the standard value of 1-15 min for uncoated tablets. The decrease in the disintegration time of the tablets with increase in the concentration of chitosan is in consonance with the work of Ritthidej et al. (1994) where disintegrant property of chitosan was reported. Since tablet disintegration is the initial step of dissolution, chitosan promotes rapid onset of drug release. The crushing strength – friability – disintegration time index [(CS/FR)/DT] provides a measure of the overall quality of tablets (Alebiowu and Itiola, 2003). The increase in this index with increase in the concentration of chitosan up to 3% ^{w}/w shows that this concentration produced tablets with the best quality. There was a decrease in the index with further increase in the concentration of chitosan beyond 3% ^{w}/w. It could be inferred that the decrease in the strength of tablet above this chitosan concentration did not lead to corresponding decrease in the disintegration time.

For a conventional tablet to pass dissolution test, at least 75% of the drug must be released within 1 h (USP 31 – NF 26, 2008). All the batches passed the test for dissolution and there was no significant difference in the amount of drug released in 1 h. The increase in percent ciprofloxacin permeated in the presence of chitosan can be linked to the ability of the polymer to increase permeability of intestinal mucosa (Yin et al., 2009). The possible mechanism employed is the opening of tight junction in the mucosa wall and also the widening of paracellular route (Sonia and Sharma, 2011). There was a continuous increase in the cumulative percent drug permeated with increase in the concentration of chitosan; with 5% ^{w}/w chitosan causing an increment from 68% (when no chitosan was added) to 81.8%. While the cumulative percent drug permeated in 4 h with respect to tablets containing 1 - 3% ^{w}/w chitosan were not significantly different from that of tablet without chitosan, the cumulative percent drug permeated with respect to tablets containing 4 and 5% ^{w}/w chitosan were significantly different.

This study correlates with *in-vivo* bioavailability study because the oral bioavailability of ciprofloxacin without permeation enhancer is 70% (Chambers, 2004). Hence, crab shell chitosan at concentration of 5% ^{w}/w could increase the bioavailability of ciprofloxacin from 70 to 82%. *In-vivo* bioavailability study can be carried out to obtain the pharmacokinetic parameters of these formulations.

Conclusion

The presence of chitosan improves the overall quality of ciprofloxacin tablet. The polymer brings about a faster

onset of drug release without significantly affecting the total drug released. It promotes the permeation of the released drug across biological membrane. This study suggests the use of crab shell chitosan at concentration of 5% ^{w}/w of tablet to improve the absorption of ciprofloxacin. Further study in form of *in-vivo* evaluation is recommended to validate this conclusion.

Conflict of Interests

The authors have not declared any conflict of interest.

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