

# Characterization of Chitosan Obtained from Snail Shell (*Achatina fulica*) and its Excipient Potentials in Metronidazole Tablet Formulation

Oluyemisi Adebawale Bamiro, Aishat Oyinkansola Salisu, Ese Mary Iyere, Olatundun Atoyegbe, Olutayo Ademola Adeleye and Lateef Gbenga Bakre

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Nigeria

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**ABSTRACT:** The aim of the work was to characterize chitosan extracted from snail shell and evaluate its use as a disintegrant and binder in metronidazole tablet formulation in comparison with standard chitosan (SC). The mechanical properties were assessed using crushing strength and friability, while the release properties were assessed using disintegration and dissolution times. The extracted chitosan (EC) was crystalline in nature and the scanning electron microscopy (SEM) showed polygonal particles with rough surface. The moisture and swelling capacity was 1.80% and 15.00%, respectively. The densities and flow properties were significantly ( $p < 0.05$ ) higher than those of the SC. As a binder, the crushing strength of formulations containing EC was higher than SC, but both formulation failed friability test. There was significant difference between the disintegration times of the metronidazole formulations containing EC and SC as a disintegrant. The result showed that EC is more effective as a binder in tablet formulations.

**Key words:** Chitosan, *Achatina fulica*, Disintegrant, Binder, Metronidazole, Dissolution

## INTRODUCTION

Wastes are discarded or unwanted materials produced after the primary use of a material. Waste was defined by Basel convention on the control of transboundary movements of hazardous wastes and their disposal as “substances or objects which are disposed of or are intended to be disposed of or are required to be disposed of by the provision of national law”.<sup>1</sup> *Achantina fulica* family Achantinidae commonly known as giant African land snail is commonly consumed in Nigeria as a delicacy. The discarded snail shells which represent waste of snails’ remnants from restaurants, eateries, or snail sellers constitute a serious degree of environmental threat. These shells are usually abandoned indiscriminately after consumption of the edible meat. Globally,

wastes are recycled in order to care for the environment and also to add positive values to the waste.<sup>2</sup> Due to huge amount these shells generated as waste, there is a dire need to recycle them in order to have a clean environment and also to generate immense economic prosperity.<sup>3</sup>

Chitosan is an organic polysaccharide obtained from the shells of crustaceans, such as crabs, shrimps and prawns.<sup>4,5</sup> Chitosan obtained by deacetylation of chitin is a biodegradable polymer of high molecular weight, composed of  $\beta$ -(1 $\rightarrow$ 4)-2-amino, 2-deoxy-D-glucopyranose.<sup>6</sup> It has gained attention due to its properties such as nontoxic, biocompatible, biodegradable, antibacterial and antimycotic effects.<sup>7-9</sup> It has been widely used in different areas such as pharmaceuticals in drug delivery, tissue engineering, waste water treatment, biotechnology, cosmetics and food processing.<sup>10-13</sup>

The aim of this study was to extract chitosan from discarded snail shells, characterize and use it as

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**Correspondence to:** Oluyemisi Adebawale Bamiro  
E-mail: bamroy67@yahoo.co.uk;  
oluyemisi.bamiro@oouagoiwoye.edu.ng; Tel: +2348023236963

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disintegrant and binder in metronidazole tablet formulation in comparison with standard chitosan.

## MATERIALS AND METHODS

**Materials.** The materials used were metronidazole powder (gift from Bond Chemicals, Nigeria), Pharmaceutical grade Chitosan (Shaanxi Dideu Medichem) and sodium hydroxide (Merck KGaA, Germany). *Achatina fulica* shells were collected from local markets in Southwest, Nigeria. All other chemicals were analytical grade

**Extraction of chitosan and characterization of its powder and granule properties.** The method of Kaewboonruang *et al.* was used for the extraction process.<sup>14</sup> The bulk density was measured by pouring the powder into a 100 ml measuring cylinder and determining it as a ratio of the weight to volume occupied. Tap density was obtained by subjecting the powder in a graduated cylinder to 100 taps by a standardized tapping procedure. The true density was determined by the fluid displacement method using n-hexane as the displacement fluid<sup>15</sup>. The angle of repose was determined by the fixed funnel method while the moisture content of the samples were determined by gravimetric analysis<sup>16</sup>. Hausner's ratio was calculated as the ratio of the tapped density to the bulk density of the samples. The percentage Carr's compressibility was calculated from the differences between the tapped density and the bulk density divided by the tapped density and the ratio expressed as a percentage. The swelling capacity was obtained by the method of Bakre *et al.*<sup>16</sup> with slight modification.

**Scanning Electron Microscopy (SEM).** The sample was mounted on aluminum stub and coated with gold in a fine coat ion sputter JFC-1100 (JOEL, Tokyo, Japan). The SEM micrograph of the sample was taken using a JSM 6100 scanning microscope (JOEL, Tokyo, Japan) at a voltage of 15KV.

**Fourier Transmission Infrared (FTIR) spectroscopy.** The samples were dried and kept in a desiccator before the FTIR analysis. Spectra were obtained by using potassium bromide disc obtained from a mixture of the chitosan powder of both the

extract and standard and dry potassium bromide on an FTIR Spectrophotometer (BX 273, Perkin-Elmer, USA).

**Differential Scanning Calorimetry (DSC).** Differential scanning calorimetric thermograms of the sample were determined on a Mettler instrument (DSC1, Toledo, USA). A 4-10 mg weight of sample were compressed into pellets in an aluminum pan and heated from 30 to 430°C at a rate of 20°C min<sup>-1</sup> under inert nitrogen atmosphere with a flow rate of 20 ml min<sup>-1</sup>. The reference used was an empty aluminium pan.

**X-ray Powder Diffraction (XRD).** The X-ray diffraction pattern of the samples was obtained with XPERT-PRO diffractometer (PANalytical, Almelo, Netherlands). The X-ray diffraction pattern was obtained at room temperature, at a voltage of 45KV and current of 40Ma using Copper (Cu) as anode material. The samples were analyzed in the diffraction angle (2θ) range of 5° to 50°.

**Elemental analysis.** Analysis of the elemental composition was done using Atomic Absorption Spectrophotometer Perkin Elmer Analyst 200 (Connecticut, USA)

**Preparation of granules.** Different batches (100g) of metronidazole granules with a basic formula containing metronidazole (60% w/w) and different concentration of extracted and standard chitosan used as a binder and disintegrant were prepared using the wet granulation. The required amount of ingredients was weighed accurately into a mortar and triturated thoroughly until a moist mass was obtained. Granules were then obtained by passing through a stainless sieve of 1000 μm (wet screening). Granules were dried in hot air oven (Shivani scientific industries Ltd) at 60°C for 1 hour. The dried granules were re-screened (dry screening) through a 710 μm mesh sieve. Using extragranular addition, the chitosan was used as disintegrant in the formulation at concentrations of 2.5% and 7.5% w/w, as binder at 5% and 10% w/w concentrations. The granules produced were stored in air tight container.

**Tablet compression.** Granules (500 mg) from each batch (Tables 1 and 2) was compressed into

tablets at compression pressure of 1tonne with a dwell time of 30 seconds using a single punch carver hydraulic hand press (Carver Inc. Wisconsin, USA) fitted with a 10.5 mm die in combination with flat

face upper and lower punches lubricated with 2% w/v dispersion of magnesium stearate in ethanol prior to compression.

**Table 1. Composition of formulations containing chitosan as a disintegrant (%w/w).**

Formulation	A	B	C	D	E
Metronidazole	60	60	60	60	60
EC	2.5	7.5	-	-	-
SC	-	-	2.5	7.5	-
PVP	5	5	5	5	5
Lactose	32.5	27.5	32.5	27.5	35

KEY: EC- Extracted chitosan, SC-Standard chitosan, PVP- Polyvinylpyrrolidone

**Table 2. Composition of formulations containing chitosan as a binder (%w/w).**

Formulation	A	B	C	D	E
Metronidazole	60	60	60	60	60
EC	5	10	-	-	-
SC	-	-	5	10	-
Corn starch	10	10	10	10	10
Lactose	25	20	25	20	30

### Mechanical and release properties of tablets

The uniformity of weight, friability (FR), crushing strength (CS) and disintegration time ( $D_T$ ) were determined according to established procedure<sup>15-17</sup>. The disintegration efficiency ratio (DER) was calculated as ratio of CSFR to friability. The dissolution was carried out according to USP<sup>18</sup> method. The dissolution medium was 900 ml of 0.1N HCL maintained at  $37\pm 0.5^\circ\text{C}$  and the absorbance was measured using a Copley UV spectrophotometer at 277 nm. The wetting time was determined by putting a tablet in a petri dish containing 10 ml of distilled water and a filter paper was placed on it. The time taken for the filter paper to get wet was taken as the wetting time. This was done in triplicate.

**Statistical analysis.** Statistical analysis was carried out using analysis of variance with computer software GraphPad Prism® 4 (GraphPad Software Inc. San Diego, USA). At 95% confidence interval, P values less than or equal to 0.05 were considered significant.

### RESULTS AND DISCUSSION

The percentage yield of chitosan from *Achatina fulica* shell was 20.5%. This is similar to the yield obtained by Kaewboonruang *et al.* from golden apple snail.<sup>14</sup> The densities of extracted chitosan (EC) were significantly higher ( $p < 0.05$ ) than those of standard chitosan (SC) (Table 3). The compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials.<sup>18</sup>

The compressibility index for EC and SC was 31.62 and 22.40% respectively. Hausner's ratio was 1.46 for EC and 1.29 for SC. The angle of repose, Hausner's ratio and compressibility index are indirect measurements of powder flowability.<sup>19</sup> Angle of repose is a characteristic property of particles related to the interparticulate friction or resistance to movement between particles.<sup>20</sup> According to United States Pharmacopeia (USP),<sup>18</sup> EC with angle of repose of  $38.73^\circ$  can be said to have a fairly good flow while SC which had a value of  $33.2^\circ$  possesses good flow.<sup>18</sup> The moisture content of the chitosan

were significantly different ( $p < 0.05$ ) with values of 1.8% and 15.52% for EC and SC respectively.

Ghannam *et al.*<sup>4</sup> also observed that the moisture content of commercial chitosan was higher than chitosan extracted from shrimp and crayfish. Low moisture content indicates that the powder will be stable on storage. The swelling capacity of EC and SC were not significantly different ( $p > 0.05$ ). Swelling is one of the mechanisms of tablet disintegration. The result obtained for the elemental

analysis of chitosan is presented in Table 4. Non-essential heavy metals such as lead and cadmium were observed to be present in EC, while only cadmium was present in SC. Their concentration was within acceptable limits. Accumulation of these heavy metals in the body over a long period can lead to toxicity. Researchers have shown the presence of heavy metals in snail shells and the presence of these metals has been used as bio-indicators of pollution.<sup>21</sup>

**Table 3. Properties of extracted and standard chitosan.**

Parameters	Extracted chitosan	Standard chitosan
Bulk density (g/mL)	0.811 ±0.001	0.550±0.017
Tapped density(g/mL)	1.186±0.014	0.709±0.013
Particle density(g/mL)	1.997±0.002	1.030±0.009
Porosity (%)	59.39±2.52	46.63±1.42
Angle of repose	38.73°±1.36	33.2°±1.29
Compressibility index (%)	31.62±0.89	22.42±3.77
Hausner's ratio	1.46±0.02	1.29±0.06
Moisture content (%)	1.8±0.01	15.52±0.02
Swelling capacity (%)	15.00±5.00	24.95±1.70

**FTIR characterization, morphology and thermal properties.** The FTIR spectroscopy are presented in Figure 1. The FTIR of the EC and SC showed peaks at 3637  $\text{cm}^{-1}$  and 3466  $\text{cm}^{-1}$  which depict the presence of an alcohol group (O–H). Peaks at 2113  $\text{cm}^{-1}$  and 2109  $\text{cm}^{-1}$  in EC and SC respectively are due to the presence an alkyne group ( $\text{C} \equiv \text{C}$ ). Peaks at 1982 and 1785  $\text{cm}^{-1}$  are attributable to an aromatic group. The FTIR of the SC showed peaks at 3242  $\text{cm}^{-1}$  which is attributable to a carboxylic group and bands at 2967  $\text{cm}^{-1}$  indicate the presence of alkane group. The Peak at 1640  $\text{cm}^{-1}$  in SC depict there is an alkene group.

The scanning electron micrograph (Figure 2) showed that the particles are polygonal in shape, but the surface of the SC is smoother than that of the EC.

The X-ray diffraction spectrum (Figure 3) showed sharp peaks with strong intensity at 22° and 27° 2 $\theta$ . It was observed that EC had more peaks compared to SC, indicating that EC is more crystalline than the SC. The DSC thermogram of

chitosan powders is shown in Figure 4. The EC showed an initial exothermic peak at 31°C indicating release of heat from the sample. This could be due to

**Table 4. Elemental analysis of chitosan.**

Elements	EC (ppm)	SC (ppm)
<b>Ca</b>	373.91	546.23
<b>Ni</b>	0.007	0.005
<b>Pb</b>	0.01	ND
<b>Zn</b>	12.21	5.43
<b>Cu</b>	2.47	1.2
<b>Cd</b>	4.59	2.18
<b>Na</b>	18.37	13.16
<b>Cr</b>	0.17	0.03
<b>K</b>	1034.9	983.80
<b>Mn</b>	5.02	5.81

a conformational change or crystallization. There was also a slightly broad and shallow endothermic peak at 38°C with small enthalpy changes which could have been due to loss of water. The SC however showed a single sharp and intense endothermic peak at 88°C.

This may be due to fast transformation with large enthalpy changes.

**Effect of chitosan as disintegrant on tablet properties.** The crushing strength and friability of tablet increased with increase in concentration of disintegrant, except EC (Table 5). Abdul Rasool *et al.* observed that when the concentration of chitosan

used as disintegrant in the formulation of furosemide tablet was increased from 3% to 15%, there was increase in hardness and friability.<sup>22</sup> The wetting time of formulations containing EC was significantly higher than those of SC. This corroborate the disintegration time of formulations containing EC

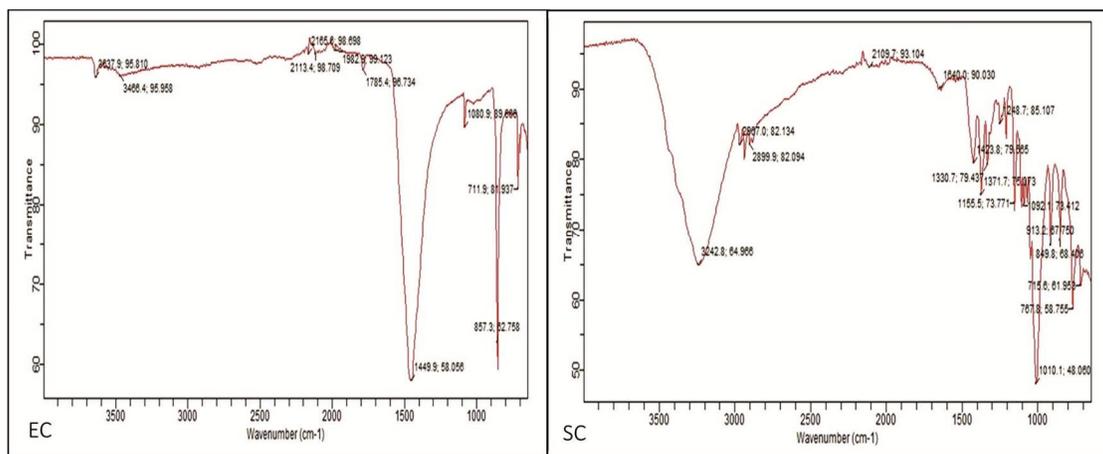


Figure 1. Fourier transform infrared spectra of EC and SC.

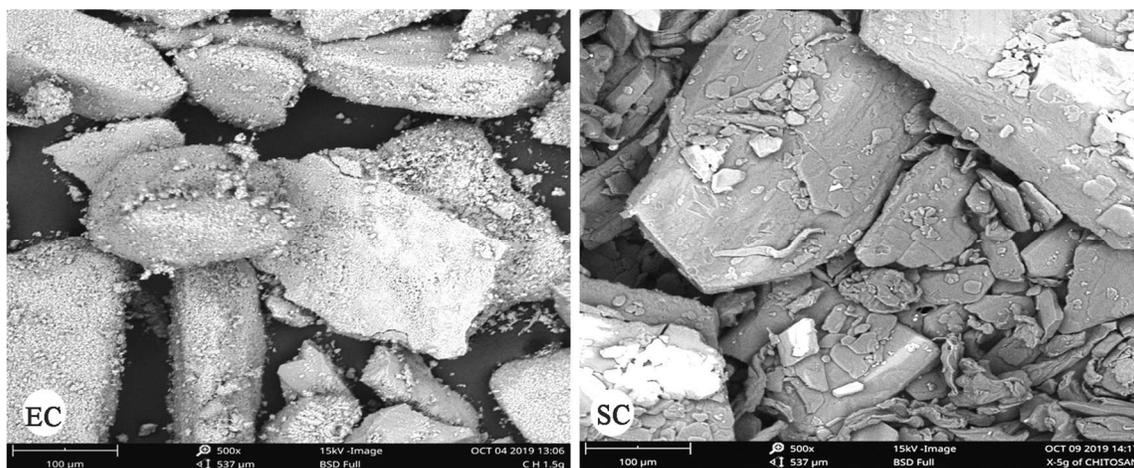


Figure 2. Scanning electron micrograph of EC and SC.

**Table 5. Tablet properties when used as disintegrant.**

Disintegrant type	Conc. (% w/w)	Weight (mg)	Friability (%)	Crushing strength (N)	Disintegration time (mins)	Wettig time (mins)	T <sub>50</sub> (mins)	T <sub>80</sub> (mins)
Control	0.0	478.8±0.01	1.1±0.22	11.37±1.34	53.38±5.76	50.45±1.12	11.50	19.00
EC	2.5	487.3±0.01	0.83±0.03	21.76±0.87	35.48±0.95	33.38±1.08	12.00	22.00
	7.5	494.1±0.01	0.67±0.01	31.36±2.51	42.33±1.21	40.33±1.56	18.00	38.50
SC	2.5	496.7±0.01	0.81±0.02	14.70±1.22	3.81±1.53	0.38±0.03	2.50	4.00
	7.5	498.3±0.01	0.99±0.03	15.29±1.56	1.02±0.27	0.21±0.01	2.50	4.00

**Table 6. Tablet properties when used as a binder.**

Binder Type	Conc (% w/w)	Tablet weight (mg)	Crushing strength (N)	Friability (%)	CSFR	Disintegration Time(minutes)	DER	T50 (Mins)	T80 (Mins)
Control	0.00	452.1± 0.0	4.71±0.9	41.37±2.35	0.11	0.32 ± 0.1	0.347	3.00	4.00
EC	5	476.3± 0.0	25.56±3.0	2.76±0.22	9.03	1.63 ± 0.4	5.54	3.00	4.00
	10	480.4± 0.0	34.50±4.7	1.67±0.10	20.68	1.33 ± 0.3	15.55	9.00	14.00
SC	5	495.0± 0.0	16.07±3.4	1.83±0.12	8.79	>60.00	NA	11.00	22.00
	10	497.0± 0.0	16.46±4.5	2.82±0.23	5.84	0.67 ± 0.4	8.71	3.00	4.00

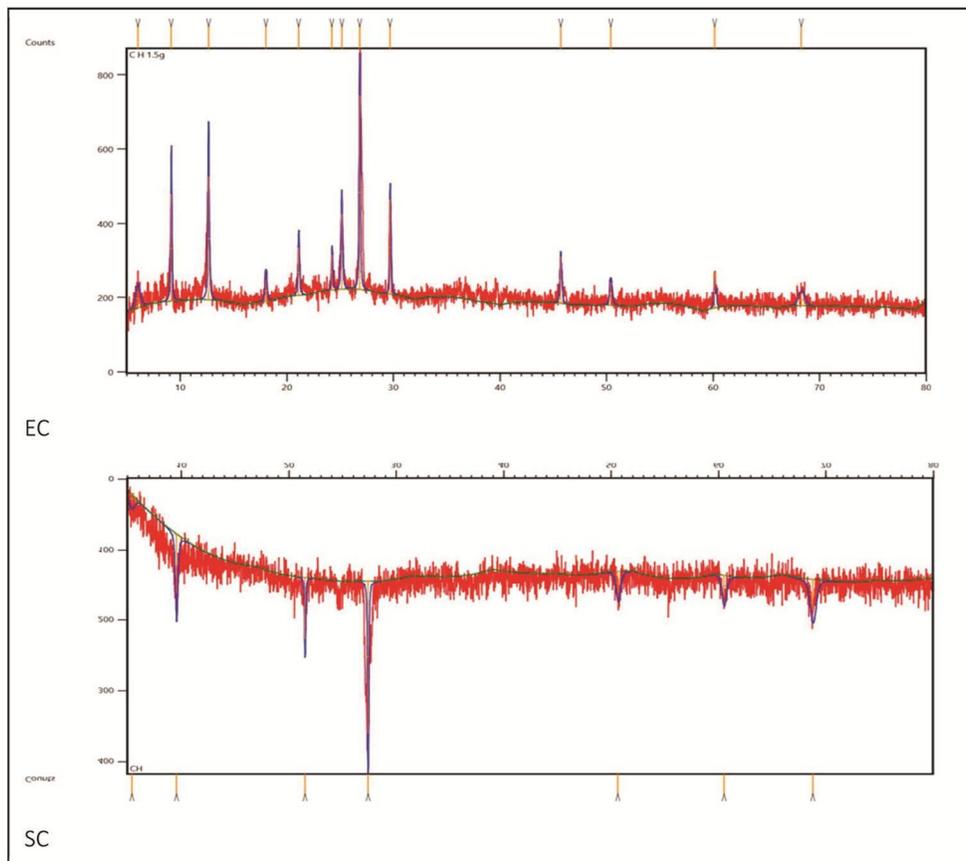


Figure 3. X-ray diffraction spectra of EC and SC.

which was also significantly higher than those containing SC. This could have been due to the high swelling capacity of SC which is one of the mechanisms of disintegration. The disintegration time of formulations containing EC was  $35.48 \pm 0.95$  to  $42.33 \pm 1.21$  which was higher than official disintegration time of 15 minutes approved for uncoated tablets.<sup>17</sup> This indicates that EC could not be used as a disintegrant for immediate release formulation. The dissolution profile of the

metronidazole tablets was assessed using  $T_{50}$  and  $T_{80}$  i.e. the time taken for 50% and 80% of the drug to be released respectively. The  $T_{50}$  and  $T_{80}$  for formulations containing SC were 2.50 and 4.0 minutes respectively at both concentrations, indicating that concentration of the SC in the formulation did not affect the release rate. The  $T_{50}$  and  $T_{80}$  for formulations containing EC was 12.00 and 22.00 minutes respectively at 2.5% concentration, while at 7.5% it was 18.00 and 38.50

minutes respectively. There was significant variation in the dissolution of formulations containing EC at different concentrations. Disintegration has been said to be an integral step in ensuring the bioavailability of drug from majority of solid dosage forms.<sup>23</sup> Formulations with SC as disintegrant released about 80% metronidazole within 4 minutes and this could have been due to the breaking up of tablets due to the high swelling of the SC, hence the release of the metronidazole.

**Effect of chitosan as a binder on tablet properties.** Crushing strength is used to determine

the strength of a tablet and it is dependent on the binder and compression pressure.<sup>24-25</sup> There was significant difference ( $p < 0.01$ ) in the crushing strength. The Pharmacopeia specification for friability is  $\leq 1\%$ .<sup>17</sup> The results in Table 6 show that all the formulations failed the friability test. High CSFR indicates stronger tablets and it provides a balance between tablet weakness and strength.<sup>26</sup> The result shown in Table 6 showed that the CSFR of formulation containing 10% w/w EC was significantly ( $p < 0.05$ ) higher than other formulations.

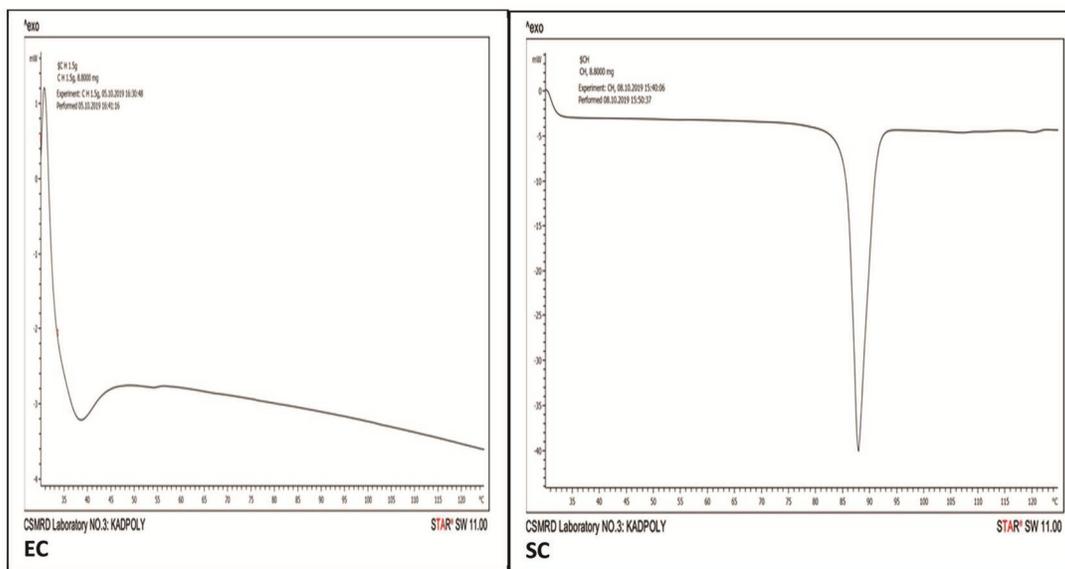


Figure 4. Differential scanning calorimetric images of EC and SC.

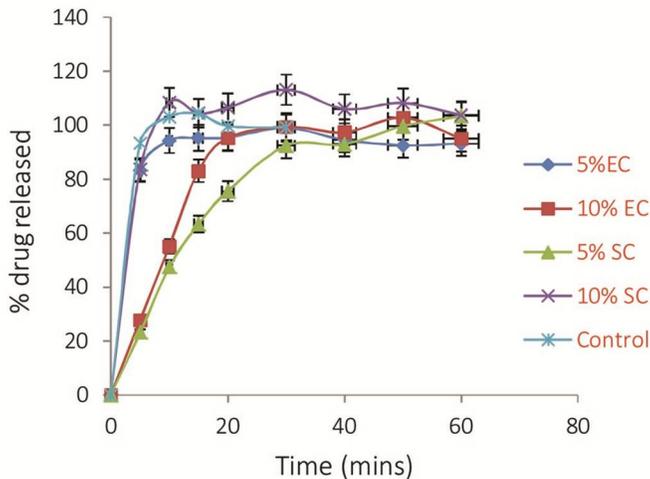


Figure 5. Dissolution profile of metronidazole tablet with chitosan as binder.

Generally all the tablets passed the disintegration time of not greater than 15 minutes specified in the compendia<sup>17</sup> except those containing 5% w/w SC. Disintegration time normally increases with increase in binder concentration, but surprisingly, the disintegration of metronidazole tablets decreased with increase in binder concentration. This may probably be due to the fact that as the concentration of chitosan increases, it leads to sufficiently high number of channels available for wicking of water which result in a higher degree of swelling. This subsequently reduces disintegration time. High disintegration efficiency ratio (DER) value indicates a better balance between disintegration and binding properties.<sup>27</sup> Formulation containing 10% w/w EC has the highest DER. Formulation without binder had the least DER, indicating a poor balance between the disintegration and mechanical properties.

The time taken for 50% and 80% ( $T_{50}$  and  $T_{80}$ ) of the drug to be released was used to assess this parameter. The dissolution profile of the tablets is presented in Figure 5. All the formulations released 80% of the active ingredient within 30 minutes as specified in the compendia.<sup>18</sup>

## CONCLUSION

Chitosan extracted from snail shell was more crystalline than standard chitosan. When used as a disintegrant in metronidazole formulation, the tablets did not disintegrate within the time stipulated by the compendia. When used as a binder, it had a better balance between disintegration and mechanical properties with the highest DER value at 10% w/w concentration.

## COMPETING INTEREST

Authors have declared no competing interest.

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