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Influence of Incorporation of *Pleurotus tuber-regium* Powder on the Release Characteristics of Acetaminophen Tablets formed with certain Acrylate Methacrylate Copolymers as Binders

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This study investigated the effects of incorporation of *Pleurotus tuber-reguim* powder on the release profiles of acetaminophen tablets. *Pleurotus tuber-reguim* powder was prepared from the mushroom sclerotium. The powdered sclerotia was bleached with 3.5% w/v sodium hypochlorite solution, slurred with ethanol, washed with water and dried at 50°C for 24 h. Using wet granulation technique, varying concentrations of the fine powder (0-25% w/w) were used intra- and extra-granularly to prepare various batches of acetaminophen tablets with 5% w/v Eudragit RL-100 or RS PO as binder. The tablets were evaluated for hardness, friability, disintegration and dissolution. The tablets formed hard compact with acceptable hardness values ranging from 5.0-9.7 kp which decreased with increasing concentration of *P. tuber-regium* powder. Only the batches of tablets without disintegrants met the official specification for friability with values ≥ 0.69 %. *Pleurotus tuber-regium* powder inclusion lowered the disintegration times of the matrix tablets with increasing concentrations. There was marked increase in the release of the drug from the matrix tablet with increase in the concentrations of the *P. tuber-regium* powder irrespective of the mode of disintegrant incorporation or polymer type. The intra-granular incorporated batches of tablets exhibited a higher drug release within 6 h when compared with the extra-granular incorporated tablets.
***P. tuber-regium* powder serving as a swelling agent when incorporated intra-granularly in matrix tablet system has the potential application for modulating or enhancing drug release at appropriate concentrations.**

Keywords: *P. tuber-regium,* excipients, disintegrants incorporation, drug release

INTRODUCTION

Pharmaceutical excipients are essential components of a drug product. They may be employed as bulking agents, diluents/fillers, binders, disintegrants, lubricants, *et cetera* [1]. Synthetic polymers such as pre-gelatinized starch, sodium carboxymethyl starch, polyvinylpyrollidone and carboxymethyl cellulose have been used extensively as excipients in the formulation of solid dosage forms as disintegrants. However, they are expensive and require high foreign exchange to purchase, thus making manufacturing of pharmaceuticals very expensive.

In recent times, emphasis has shifted to the search and development of natural products as alternatives to the synthetic ones in the formulation of various dosage forms [2]. Natural products are cheap, locally sourced and readily available. They do not require sophisticated and expensive techniques for extraction, above all, they are chemically inert, non-toxic, eco-friendly and biodegradable [3,4]. Their

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renewable sources if cultivated also give them an added advantage [5]. Some naturally occurring polysaccharides such as starch from both cereals and legumes or gums have been studied as disintegrants in pharmaceutical formulation of solid dosage forms and they have demonstrated potential applications as excipients in solid dosage forms [6-8].

Pleurotus tuber-regium (Basidiomycota) is a tropical sclerotial mushroom [9]. The mushroom and sclerotium (underground tuber) are eaten in Nigeria for their nutritional and medicinal values [10,11] as well as for their taste and aroma [12]. The sclerotium is a compact mass of hardened fungal mycelium containing food reserves which help the fungi survive environmental extremes [13]. It is spherical to ovoid in shape measuring up to ≥30 cm in diameter [14]. The local use of the sclerotium powder as a soup thickener because of its ability to swell in water and add bulk to the soup instigated a preliminary study into the swelling ability of the mushroom powder and it was found to be comparable with maize starch BP as a tablet disintegrant [15]. However, a search in the literature showed that there is no previous report on the influence of mode of incorporation of *P. tuber-regium* powder on the drug release characteristics of matrix tablets. The objective of this study was thus to investigate the effect of incorporation of *P. tuber-regium* powder on the tablet properties and drug release from acetaminophen matrix tablets formulated with certain acrylate methacrylate copolymers (Eudragit RL-100 and RS-PO) as binders.

EXPERIMENTAL

Materials

Acetaminophen (Paracetamol) powder (William Ransom & Sons PLC, Hitchin Hertfordshire England), Eudragit RL-100 and Eudragit RS PO (Rohm Pharma GmbH, Darmstadt, Germany), maize starch BP and talc (BDH Chemicals, UK), lactose (Merck Darmstadt, Germany), 3.5 %w/v sodium hypochlorite (Reckitt and Colman Nig. Ltd., Lagos). *Pleurotus tuber-regium* (Bursaraceae) tubers were purchased locally from a market in Benin City and processed into powder in our laboratory. All sieves were BSS (Endecotts Ltd. London, England) and water was double distilled.

Methods

Preparation of *Pleurotus tuber-regium* powder

Dry tubers of *P. tuber-regium* were processed into powder using the method previously reported by Iwuagwu and Onyekweli [15]. The dark brown outer skin of the sclerotia was scraped off with a knife and the white inner tissue was cut into small pieces. The pieces were ground into powder in a dry manual mill and further reduced using a Fitz mill (Manesty Machines, UK). The fine white powder was bleached with 3.5 % w/v sodium hypochlorite solution. The wet mass was slurred with ethanol in a stainless steel vessel and left to stand in a water bath at a temperature of 60 °C with continuous stirring for a period of 60 min. The slurry was then squeezed using a fine muslin cloth and the resulting wet mass washed with excess water and dried in an oven at 50 °C for 24 h. The resulting powder was passed through a 212 µm laboratory sieve to obtain smooth fine powder. The percentage yield of the powder was calculated.

Physicochemical tests of *Pleurotus tuber-regium* powder

Organoleptic properties

The texture, colour and odour of the powder were noted.

Solubility

The solubility profile of a 100 mg quantity of the powder was determined in 2 ml of water at ambient temperature.

Swelling capacity

The swelling capacity of *P. tuber-regium* powder was estimated using the method of Iwuagwu and Onyekweli [15] and compared with maize starch BP. About 5 g of the powders with a tapped volume (V_i) in a 100 ml measuring cylinder was dispersed with 85 ml of distilled water and thereafter made up to volume with more water. The dispersion was allowed to stand for 24 h and the volume of the sediment (*Vm*) noted. The swelling capacity was computed using equation 1.

$$Swelling\ capacity(\%) = \frac{[Vm - Vi]}{Vi} \times 100 \ldots . Equation 1$$

Preparation of Eudragit binding solution

A 5 % w/v solution of Eudragit RL-100 was prepared by solvating 2.5 g in 50 ml of ethanol. The dispersion was stirred and left overnight for complete solvation. The procedure was adopted using Eudragit RS PO. Sufficient quantities of both solutions were subsequently used for wet massing acetaminophen powder during the granulation process.

Preparation of acetaminophen granules

Table 1 shows the formula used in the preparation of acetaminophen granules. The granules were prepared by the wet granulation method and a total of twenty-two batches were prepared. Batches A1 and A2 were prepared with Eudragit RL-100 and Eudragit RS-PO respectively with no disintegrant added while batches B-I were prepared in a similar manner with the two polymers but with the intra-granular incorporation of *P. tuber-regium* in varying amounts to batches B-E and extra-granularly to batches F-I. Batches J and K were prepared similarly but with 5 % w/w maize starch powder as disintegrant incorporated intra-granularly and extra-granularly respectively as standard disintegrant for control.

For each batch, the drug was dry mixed with lactose (filler) and the corresponding amounts of disintegrant for the intra-granular batches. The dry mix was wet massed with sufficient binder solution or mucilage and then passed through a 1.40 mm sieve and dried at 60 ºC for 30 min in a hot air oven (Gallenkamp, UK). The dry mass was passed through an 850 µm sieve and further dried for 30 min. At this stage, the granules of the extra-granular batches were mixed intimately with their corresponding amounts of disintegrant in readiness for compression.

Compatibility studies

Drug-excipient compatibility was investigated by carrying out DSC and FTIR analyses on the granules of Eudragit RL-100, Eudragit RS-PO and acetaminophen powder. The DSC analysis was carried out using the Netzsch DSC 204F1 Phoenix apparatus (Netzsch Germany). Four milligrams of the sample was weighed into an aluminium pan. The seal was pierced and calibration of the calorimeter was carried out with indium. Heating of the sample was carried out at the rate of 10 °C per min from 30 to 450 °C under nitrogen at a flow rate of 70 ml/min while FTIR analysis of the sample was done using FTIR-4100 Spectrophotometer (Shimadzu Co. Japan). Using the potassium bromide (KBr) tablet method, 5 mg of the sample was blended with KBr to 200 mg. The powder was

compressed using a sigma press into a tablet shape. The tablet was placed in the sample compartment and the IR scan was carried out at 4000 - 500 cm^{-1} range.

Table 1: Formula of prepared acetaminophen granules and tablets

Mode of incorporation	Batch Code	Drug (mg)	Binder (% w/v)		Disintegrant mg (% w/w))		Lactose (mg)	Total (mg)
			Eudragit		*P. tuber-regium*	Maize starch		
			RL-100	RS-PO				
INTRA-GRANULAR	A1	500	5	-	-	-	150	650
	A2	500	-	5	-	-	150	650
	B1	500	5	-	25 (5)	-	125	650
	B2	500	-	5	25 (5)	-	125	650
	C1	500	5	-	50 (10)	-	100	650
	C2	500	-	5	50 (10)	-	100	650
	D1	500	5	-	75 (15)	-	75	650
	D2	500	-	5	75 (15)	-	75	650
	E1	500	5	-	125 (25)	-	25	650
	E2	500	-	5	125 (25)	-	25	650
EXTRA-GRANULAR	F1	500	5	-	25 (5)	-	125	650
	F2	500	-	5	25 (5)	-	125	650
	G1	500	5	-	50 (10)	-	100	650
	G2	500	-	5	50 (10)	-	100	650
	H1	500	5	-	75 (15)	-	75	650
	H2	500	-	5	75 (15)	-	75	650
	I1	500	5	-	125 (25)	-	25	650
	I2	500	-	5	125 (25)	-	25	650
INTRA-GRANULAR	J1	500	5	-	-	25 (5)	125	650
	J2	500	-	5	-	25 (5)	125	650
EXTRA-GRANULAR	K1	500	5	-	-	25 (5)	125	650
	K2	500	-	5	-	25 (5)	125	650

Compression of granules

Batches of the granules were compressed into tablets using a single punch tableting machine (Manesty Machines, UK) at a compression pressure of 40 tonnes. The die volume was adjusted to compress tablets of uniform weight by using granules weighing 650 mg. The tablets made were then kept in air tight containers and stored in a desiccator until evaluation.

Evaluation of tablets

The following tests were carried out on the compressed tablets using standard procedures: weight uniformity, hardness, friability and disintegration time [16].

Uniformity of weight

The weight of each of 20 tablets was determined from each batch using an electronic balance (Mettler Toledo, Switzerland) and the mean weights and standard deviations computed.

Hardness test

The hardness of each of ten tablets per batch was determined by diametral compression using a tablet hardness tester

(Campbell Electronics, Model HT-30/50, India) and the mean hardness calculated.

Friability test

The weight of ten tablets was determined on the electronic balance. The tablets were then placed in the drum of a Roche friabilator (Erweka Apparatebau GmbH, Gemany) revolving at 25 rpm. After 4 min, the tablets were de-dusted and reweighed. The weight was then recorded and friability calculated as percentage loss in weight.

Disintegration time

The BP tablet disintegration unit apparatus (Type MK IV, Manesty Machines Ltd, Liverpool, England) was used. The disintegration times of six tablets per batch of the tablets were determined in distilled water at 37 ± 0.5 °C. The time taken for the tablets to break into its primary particles, which passes through the mesh of the apparatus was recorded and their average times computed.

Dissolution test

The dissolution profiles of the different batches of acetaminophen tablets were determined using the BP paddle method (Caleva ST7, UK). A dissolution medium of 900 ml of 0.1 N HCl solution maintained at 37 ± 0.5 °C with a revolution of 100 rpm was used. A 5 ml volume of the dissolution fluid were withdrawn at various time intervals up to 360 min and replaced with an equivalent volume maintained at same temperature (37 ± 0.5 °C). The samples were filtered and diluted appropriately with 0.1 N HCl solution and their absorbances measured at λ_{max} of 244 nm (T70, PG Instruments Ltd). Triplicate determinations were carried out and mean values with standard deviations were reported. The concentration and the percentage of acetaminophen released at each time interval was calculated using the equation derived from the standard calibration plot obtained from the pure drug.

Statistical analysis

Statistical difference in the tablet parameters of the batches were subjected to student's t-test at 5 % level of significance using GraphPad InStat 3.10.

RESULTS AND DISCUSSION

Physical properties of *P. tuber-regium* powder

The physical properties of *P. tuber-regium* powder are shown in Table 2. The powder was tasteless, creamy in colour with a faint odour and rough in texture. It was insoluble in water at room temperature. The percentage yield and the swelling index of the powder was 71.4 and 50 % respectively.

Table 2: Some physical properties of *P. tuber-regium* powder

Properties	*P. tuber-regium* powder
Appearance (colour)	Cream
Odour	Faint
Texture	Rough
Solubility (30 °C)	Insoluble
Yield (%)	71.4
Swelling index (%)	50

Drug-excipient compatibility

Thermal analysis: Figure 1 shows the DSC thermograms of pure acetaminophen powder (a) and the acetaminophen granules prepared with Eudragit RL-100 (b) and Eudragit RS-PO (c). Acetaminophen thermogram shows a sharp endothermic peak, corresponding to its melting point (169 °C). This sharp peak which appears as a spike is indicative of its purity and crystallinity. On the other hand,

the thermogram of the granules containing Eudragit RL-100 (b) and Eudragit RS-PO (c) and acetaminophen together showed two sharp endothermic peaks with the characteristic peak of pure acetaminophen.

FTIR: The FTIR spectrum of pure acetaminophen powder in Figure 2 (a) showed characteristic peaks at 1227.00 cm^{-1}, 1636.42 cm^{-1} and 3171.00 cm^{-1}. These peaks observed for acetaminophen remained unchanged when compared with the spectral data of the granules with Eudragit RL-100 (Figure 2 (b)) and Eudragit RS-PO (Figure 2 (c)). This observation ruled out the possibility of chemical interaction and complex formation between acetaminophen and Eudragit RL-100 or Eudragit RS-PO during the mixing process.

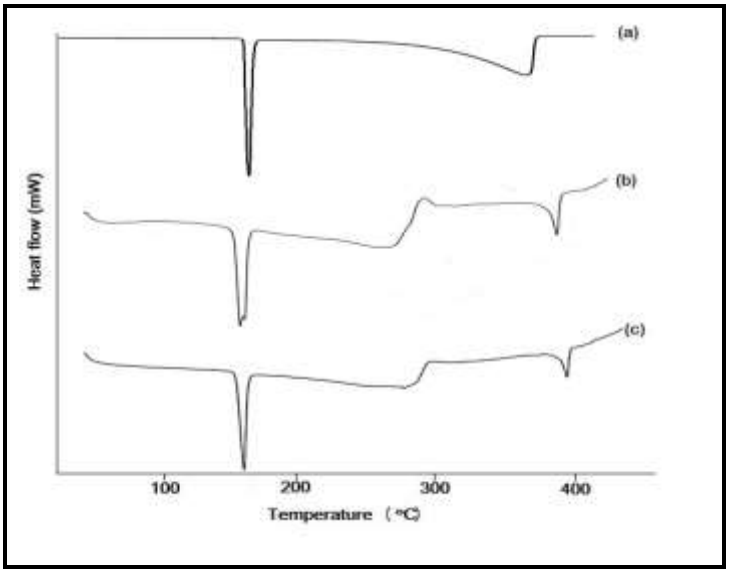

Figure 1: DSC of acetaminophen (a), acetaminophen granules prepared with Eudragit RL-100 (b) and Eudragit RS-PO (c).

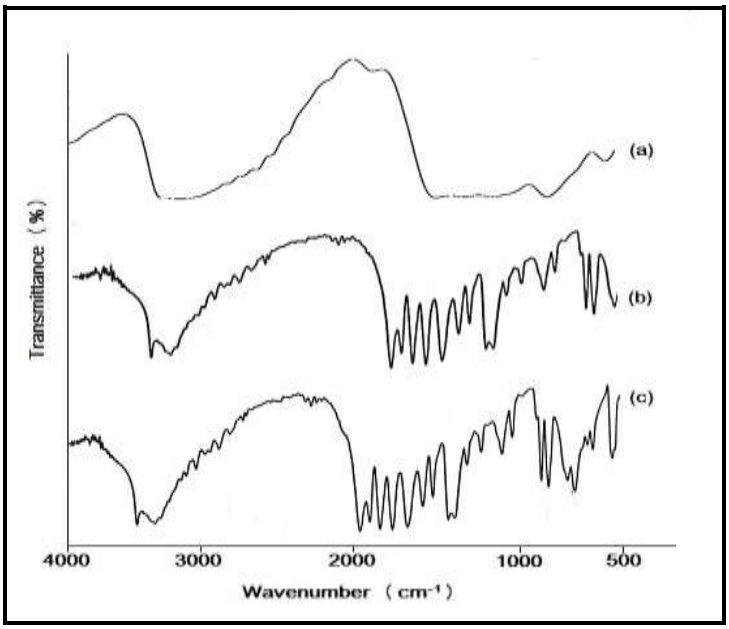

Figure 2: FTIR of acetaminophen (a), acetaminophen granules prepared with Eudragit RL-100 (b) and Eudragit RS-PO (c).

Tablet properties

Tablet weight

The physicochemical properties of the acetaminophen tablets formulated are shown in Table 3. The weight uniformity test on the tablets indicated no significant differences ($p > 0.05$) in weights of tablets made from Eudragit RL-100 or Eudragit RS-PO at varying concentrations of *P. tuber-regium*. All the tablets conformed to the BP specification, i.e., that not more than two of the individual weights should deviate from the average weight by more than ± 5 % and none should deviate by more than ± 10 % [17]. The adjustments of the tablet weights carried out with the filler lactose may account for the uniformity seen in the weights of the tablets.

Hardness

There were significant differences ($p < 0.05$) in the tablet hardness amongst the tablets prepared with the two polymers (Eudragit RL-100 and Eudragit RS-PO). The tablets prepared by extra-granular incorporation of *P. tuber-regium* powder

showed a more marked difference. Generally there was a decrease in tablet hardness with an increase in the concentration of the *P. tuber-regium* powder incorporated intra-granularly and extra-granularly with hardness values ≤ 9.70 ± 0.55 kp. Tablets prepared without disintegrants (A1 and A2) and those with 5 %w/w maize starch both intra-granularly (J1 and J2) and extra-granularly (K1 and K2) gave harder compacts and higher values of 8.62, 8.71, 8.40, 9.60, 8.50 and 9.70 kp, respectively. Incorporation of *P. tuber-regium* powder as disintegrant in the tablets is thought to bring about a distortion in the number and strength of the interparticulate bonds formed within the tablet's matrix system with a corresponding increase in concentration of the *P. tuber-regium* powder and this may explain the decrease in the tablet hardness with increase in the concentration of the disintegrant.

Friability

Results of the friability test (Table 3) showed that friability values of the tablets increased slightly with increasing concentrations of *P. tuber-regium* powder incorporated both intra-granularly and extra-granularly. However, all the tablets did not meet the BP specification of a maximum loss of 1 % of the weight of tablets tested [17] except for tablets of batches A1 and A2. There was increase in the friability values of the tablets with increasing concentrations of *P. tuber-regium* powder, with the increase more pronounced with the extra-granularly incorporated tablets for Eudragit RL-100 and RS-PO. This increase with increasing amount of *P. tuber-regium* powder incorporation may be attributed to a weakening of the inter-particulate bonds resulting from the incorporation of the disintegrant into the compact, thus giving soft and highly friable tablets.

Disintegration time

Only batches of tablets with intra-granular incorporation of *P. tuber-regium* (B1-E1) and (B2-E2) using both polymers disintegrated within 15 min as specified by BP for uncoated tablets [17]. The intra-granular incorporated 5 % maize starch batches (J1 and J2) did not disintegrate until after 1 h. All the batches of tablets with extra-granular incorporation of *P. tuber-regium* or maize starch powders irrespective of the polymer type had variable disintegration times but none of them disintegrated with 15 min. Batches A1 and A2 without *P. tuber-regium* or maize starch powders as disintegrant did not disintegrate after 6 h of testing. Eudragit polymers usually form matrix granules and so they are not expected to disintegrate except where very low concentrations of the polymers are used which may result in formation of crumbling tablets. Results from the study revealed significant differences in disintegration times of the tablets with the mode of incorporation of *P. tuber-regium* powder as disintegrant. This may be attributed to the swelling properties of the powder when in contact with aqueous medium and hence, an enhanced breakup of the compacts into its primary particles.

Dissolution

Results from the dissolution studies (Figure 3 and 4) showed that dissolution increased with increase in concentration of *P. tuber-regium* powder regardless of the mode of incorporation or the type of polymer, which is probably due to the fact that dissolution correlates with or is directly proportional to disintegration. However, all the tablets formulated showed some measure of delayed release as none of the batches passed the BP dissolution test for conventional tablets which specifies that at least 75 % of the

drug should be in solution after 45 min [17].

Generally, the inclusion of *P. tuber-regium* powder enhanced the release of drug from the matrix tablet system irrespective of the mode of incorporation or polymer type. Batches A1 and A2 without any disintegrant markedly retarded acetaminophen release with 60 and 51 % drug release in 6 h, respectively. The enhanced drug release observed with the inclusion of *P. tuber-regium* powder was higher in the intra-granular incorporated batches of tablet as seen from their higher drug release profiles when compared with those of the extra-granular batches.

Table 3: Some physicochemical characteristics of the acetaminophen tablets

Polymer	Mode	Batch Code	Mean Weight (mg)	Hardness (kp)	Friability (%)	Disintegration Time (min)
EUDRAGIT LS-100	INTRA-GRANULAR	A1	650 ± 0.025	8.62 ± 0.42	0.55	469.5
		B1	653 ± 0.005	6.85 ± 0.15	1.37	11.76
		C1	652 ± 0.025	6.60 ± 0.27	1.39	8.31
		D1	654 ± 0.021	6.00 ± 0.21	1.50	2.55
		E1	655 ± 0.026	5.58 ± 0.33	1.63	0.55
		J1	650 ± 0.054	8.40 ± 0.42	1.15	72.47
	EXRA-GRANULAR	F1	652 ± 0.042	8.05 ± 0.22	1.72	60.12
		G1	650 ± 0.035	8.35 ± 0.36	1.81	41.24
		H1	650 ± 0.023	7.30 ± 0.17	2.39	37.32
		I1	650 ± 0.017	6.60 ± 0.14	2.97	17.65
		K1	649 ± 0.033	8.50 ± 0.55	1.23	81.62
		A2	656 ± 0.051	8.71 ± 0.32	0.69	474.4
EUDRAGIT RS-PO	INTRA-GRANULAR	B2	651 ± 0.023	6.10 ± 0.82	1.82	18.44
		C2	652 ± 0.044	5.00 ± 0.79	1.93	14.09
		D2	650 ± 0.022	4.95 ± 0.68	1.25	1.10
		E2	649 ± 0.033	4.60 ± 0.55	1.80	0.15
		J2	655 ± 0.016	9.60 ± 0.82	1.25	94.86
	EXRA-GRANULAR	F2	650 ± 0.050	9.60 ± 0.55	1.12	77.71
		G2	653 ± 0.018	9.50 ± 0.71	1.18	62.25
		H2	655 ± 0.016	9.50 ± 0.82	2.67	38.93
		I2	650 ± 0.015	6.20 ± 0.35	3.01	19.31
		K2	650 ± 0.052	9.70 ± 0.31	1.27	85.09

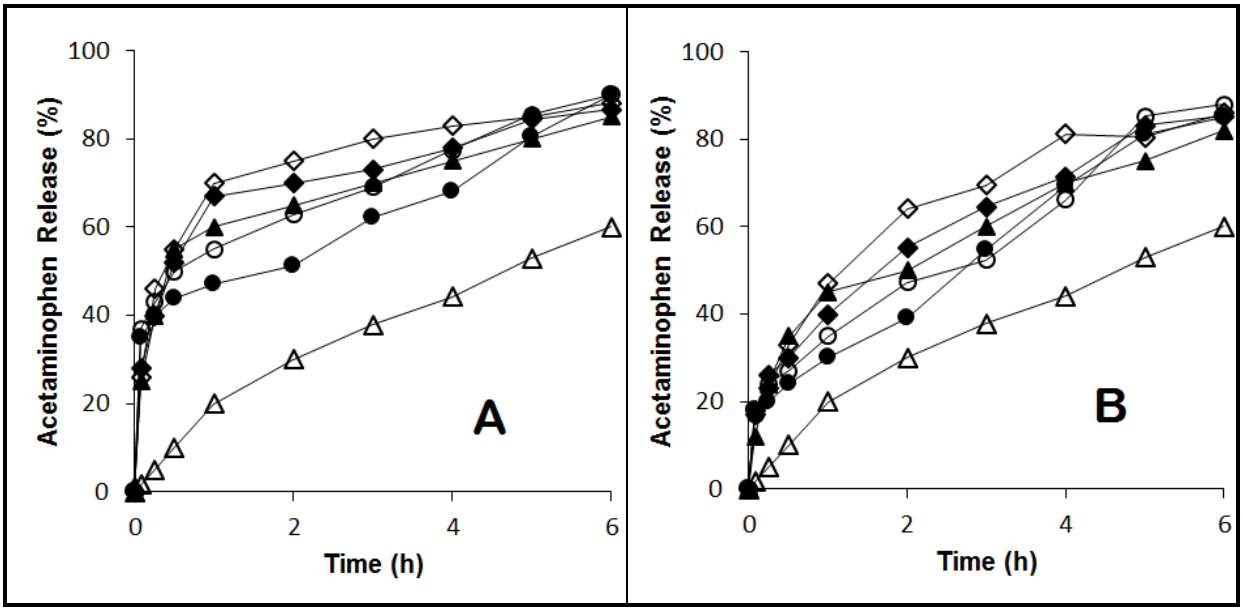

Figure 3: Dissolution profiles of acetaminophen tablets formulated with Eudragit RL-100 and intra-granular (A) and extra-granular (B) incorporation of disintegrant.
P. tuber-regium: 0 % (A1,△), 5 % (B1,●), 10 % (C1, O), 15 % (D1,◆), 25 % (E1,◇)
Maize starch: 5 % (J1,▲)

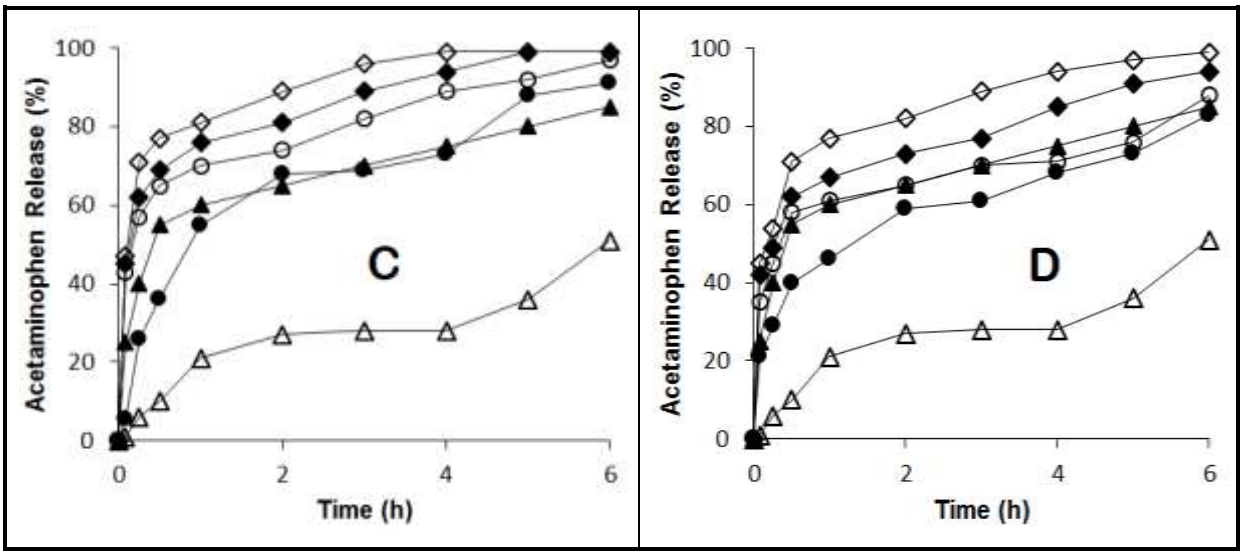

Figure 4: Dissolution profiles of acetaminophen tablets formulated with Eudragit RL-PO and intra-granular (C) and extra-granular (D) incorporation of disintegrant.
P. tuber-regium: 0 % (A1,△), 5 % (B1,●), 10 % (C1, O), 15 % (D1,◆), 25 % (E1,◇)
Maize starch: 5 % (J1,▲).

CONCLUSION

Pleurotus tuber-regium powder as a pharmaceutical excipient can serve as a swelling agent with a potential for enhancing drug release from matrix tablet systems at appropriate concentrations. Incorporation of the *Pleurotus tuber-regium* powder intra-granularly resulted in optimal drug release from the acetaminophen matrix tablets.

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