Carbohydrate Polymers 96 (2013) 253–258

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Isolation and partial characterization of delayed releasing starches of *Colocasia* species from Jharkhand, India

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ARTICLE INFO

Article history:
Received 30 December 2012
Received in revised form 2 April 2013
Accepted 4 April 2013
Available online 10 April 2013

Keywords:
Starch
In vitro release
Colocasia
Amylose

ABSTRACT

There is an increasing interest in starch manufactured from edible tubers for controlled delivery of drug. Starches of different cultivars of *Colocasia* from Jharkhand, North Eastern State of India, were isolated and their morphological, physicochemical, structural properties were studied. The yield of starches was estimated in the range of 6.46-13.75%. All the isolated starches revealed their irregular shape with a diameter of 5-10 μm. There was considerable variation in amylose content, swelling and solubility power, water hydration capacity. FTIR spectra confirmed their carbohydrate nature. Powder studies revealed that these starches possess potential for pharmaceutical industries. *In vitro* release data revealed the delayed release of all tablets made by using *Colocasia* starches at pH 6.8 and 7.4 when compared with maize starch. Delayed release of all starches showed there is a great potential to be used these starches as pharmaceutical excipient in sustained release dosage form with minimum modification.

1. Introduction

Starch is considered as important material used in both food and non food industries. Tubers are important sources of starch and are used as staple food in tropical and sub-tropical countries (Liu, Donner, Yin, Huang, & Fan, 2006) In North East India, starchy tubers such as potato (*Solanum tuberosum*) is grown in winter, taro (*Colocasia* and *Xanthosoma* spp.) and *Amorphophallus* spp. (grown widely) are produced in the rainy summer (March–August) (Saikia & Konwar, 2012). Recent advances in the research of the food properties revealed the use of food and its constituents such as starch, cellulose and gums, for the controlled delivery of drugs without any modification in their natural form. Studies on *Colocasia* starch from Jharkhand, India are not well documented. *Colocasia* species originated from the North Eastern region of the country and distributed to other parts of India (Kuruvilla & Singh, 1981). Jharkhand is one of the North Eastern regions of India in which this tuber is cultivated and is locally known as "kachalu" or "kachu" and "arbi" or "arvi". The tubers of this tropical plant belonging to *Araceae* family and has high starch content that ranges between 22% and 40% (Agbor & Rickard, 1990; Delpeuch, Favier, & Charbonniere, 1978). This tuber is less commercialized at present. Advancement in agronomic techniques and utilization of modern genetic techniques may allow this tuber to be cultured and commercialized. However, this tuber has a short shelf life because of its high moisture content. One of the best ways to preserve the tuber is by processing them to obtain flour and/or starches. As compared to other tuber starches like potato, starches obtained from *Colocasia* tuber have never been commercialized because their properties are unknown. Since the transformation into starch will decrease losses after the tubers have been harvested. A significant amount of work remains to be done on the characteristic properties of *Colocasia* (Satin, 1999). Before consideration *Colocasia* as potential source of starch to use in food and non food industries, it is necessary to characterize their chemical composition, physical, physicochemical, and functional properties. Moreover, uses of starch from *Colocasia* tuber are less explored in drug delivery as excipient. These can be used as disintegrant and binder. This paper therefore reports the results of a study undertaken to determine the characteristic properties of native starch isolated from taro tubers grown in Jharkhand, North eastern Region of India to increase its potential in food industries and also reported release characteristic of isolated starches in physiological environment to enhance their uses in pharmaceutical industries as excipient.

2. Materials and methods

2.1. Starch isolation

Different cultivars of *Colocasia esculenta* L. Schott (CA_1, CA_2, and CA_3) were purchased from the local market of North Eastern Region of India, Jharkhand. One kilogram of each tuber were thoroughly washed, peeled and sliced into pieces. Starch was isolated by

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http://dx.doi.org/10.1016/j.carbpol.2013.04.002

pre-established wet milling procedure of Flores-Gorosquera et al. (2004) with slight modification. The ratio of final weight of starch (on dry basis) to the initial weight of plant material was noted and percentage yield was calculated.

2.2. Physicochemical analysis of isolated starch

2.2.1. Moisture, ash, pH, amylose and mineral content determination

Starches isolated were analyzed for moisture and ash content following the methods described in AOAC (2000). Digital pH meter was used for determination of pH. Amylose content was determined by the method of Singh, Okadome, Toyoshima, Isobe, and Ohtsubo (2000). Mineral contents were determined by using inductive coupled plasma optical emission spectrometer (ICP-OES) (optical 2100DV, Perkin Elmer, USA). 0.2 g of each samples were first digested with HNO_3/H_2O_2 mixture in microwave digester. The clear solution obtained was filtered into a 100 ml standard flask and made up the mark with Millipore water, from which mineral contents were determined.

2.2.2. Water holding capacity (WHC)

Water holding capacity of starches isolated from *Colocasia* was analyzed by the method as described by Yamazaki (1953) with minor modification as done by Medcalf and Gilles (1965). A suspension of 1 g of starch in 15 ml of distilled water was stirred for 1 h. The free water was poured off from wet starch. After draining for 10 min, the wet starch was weighed and the result was given as percent (w/w) on dry basis.

2.2.3. Powder characteristics of isolated starch

2.2.3.1. Bulk, tap and true densities. One gram (W_i) of each starch was placed in 10 ml of measuring cylinder and the volume, V_i occupied by each of the samples was noted. After 100 taps on the table, the occupied volume V_f was read. The bulk and tap densities were calculated as the ratio of weight (W_i) of starch sample to occupied volume (V_i) and tapped volume (V_f), respectively. As per European Pharmacopoeia (2008) determinations were done in triplicate. True density (T_d) was analyzed by liquid displacement method and calculated as:

$$T_d = \frac{W_i}{(A + W_i) - B} \times S \quad (1)$$

W_i = weight of sample, S = specific gravity of solvent, A = weight of bottle + solvent, B = weight of bottle + solvent + sample.

2.2.3.2. Powder flowability. By using the Hausner's ratio (HR) and Carr's Index (CI) (Carr, 1965) powder flowability was calculated. HR was determined as the ratio of tapped density (D_T) to bulk density (B_D) of the samples and CI was calculated using the equation:

$$CI = \frac{D_T - B_D}{D_T} \times 100 \quad (2)$$

2.2.3.3. Angle of repose. According to the fixed funnel and free standing cone method, the static angle of repose (θ) was determined. A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The starch powders were poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameters (d) of the base and height of starch heap (h) were determined and the tangent of the angle of repose calculated using the equation:

$$\tan\theta = \frac{2h}{d} \quad (3)$$

2.2.3.4. Porosity. Porosity of starches was calculated as:

$$\varepsilon = \frac{1 - B_D}{T_d} \times 100 \quad (4)$$

here, B_D is bulk density and T_d is true density.

For all the analysis of starch powder characteristics, the results were given as g/ml and % (Banker & Anderson, 1986).

2.2.4. Swelling and solubility power

Swelling and solubility power of *Colocasia* starches were determined by dispersing the each isolated starch (1%, w/v, db, 5.0 ml) in distilled water and resulting slurries were heated in thermostatically controlled water bath at 60 °C, 70 °C, 80 °C, and 90 °C for 1 min with continuous stirring. Slurries were then cooled to room temperature and each solution was centrifuged at 3000 rpm for 15 min. Supernatants of each starch were carefully decanted in pre-weighed petridish for subsequent analysis of solubility pattern. Weight of swollen starches was noted. Supernatant transferred in per-weighed petridish was evaporated overnight at 110 °C. The analysis was carried out in triplicates (Gomand, Lamberts, Visser, & Delcour, 2010; Hasjim, Srichuwong, Scott, & Jane, 2009). Weight of the residue obtained after drying of supernatant of each sample represented the amount of starch solubilized in water (Raina, Singh, Bawa, & Saxena, 2006; Subramanian, Hoseney, & Bramel-Cox, 1994). Swelling and solubility power were estimated as (Siqun, Xiaoxin, Weiqi, Hengxu, & Zhiyan, 2012).

$$\text{Swelling power}\,(\%) = \frac{W_S \times 100}{S \times (100 - \%\,\text{solubility})} \quad (5)$$

$$\text{Solubility}\,(\%) = \frac{\text{Weight of soluble starch} \times 100}{S} \quad (6)$$

here, W_S is weight of wet starch (g) and S weight of starch on dry weight basis (g).

2.2.5. Paste clarity

Paste clarity was measured according to the method given by Craig, Maningat, Seib, and Hoseney (1989) and Perera and Hoover (1999). Two percentage of aqueous solution of each starch was prepared and heated in boiling water bath for 30 min thoroughly stirring every 5 min. Samples were then stored at 4 °C for 5 days and the percentage transmittance was measured after 24 h for the period of storage using water as blank.

2.3. Microscopical examination of starch granules

Starch granule morphology was studied by using scanning electron microscope (JEOL – Japan, JSM 6390 LV). For scanning electron microscopy, samples were sprinkled on the double sided adhesive tape mounted on metal stub and it was then coated with platinum to make the sample conductive and images were examined at an accelerating potential of 10 kV.

2.4. Infrared spectroscopy of starches

Fourier transform infrared (FTIR-8400 S, Shimadzu, Japan) spectroscopy was used to evaluate the structure of starch samples.

2.5. In vitro release study

In vitro release study was done for all three starches isolated from tubers of *Colocasia* and compared with maize starch. Paracetamol tablets were prepared using each type of starch by wet granulation method. Formula for tablet formulation is given in Table 1 and release study of tablets was carried out using USP dissolution type 2 test apparatus by rotating at 50 rpm in 900 ml of

Table 1
Composition of formulation used for *in vitro* release study.

Ingredients	Quantity per each tablet (%)
Drug	46.8%
Lactose	28.8%
Starch	4.7%
Sodium CMC	7.2%
Talc	8.4%
Magnesium stearate	4.2%

0.1 N HCl buffer (pH 1.2) for first 2 h, then 900 ml of phosphate buffer (pH 6.8) for another 2 h, and 900 ml of phosphate buffer (pH 7.4) for 4 h by maintaining the temperature at 37 ± 0.5 °C for each dissolution medium. Samples (5 ml) were withdrawn at specified intervals, and replaced with equal amount of fresh dissolution medium. After withdrawing, samples were assayed spectrophotometrically at 243 nm (Paola Mura, Francesca Maestrelli, Marzia Cirri, Luisa González Rodríguez, & Antonio, 2003).

2.6. Statistical analysis

All analysis reported, are averages of triplicate observations and data is expressed as means ± standard deviation.

3. Results and discussion

3.1. Physicochemical properties of isolated starches

3.1.1. WHC, amylose, ash, moisture, pH and mineral content

The ash value, amylose content, moisture content, pH, water holding capacity and mineral contents of *Colocasia* starches are presented in Table 2. Amylose content of isolated starches was 27.66%, 13.5% and 14.1% for CA_1, CA_2, and CA_3 respectively. The amylose content of CA_1 starch was higher than that of CA_2 and CA_3 starch. The higher amylose content of starch from CA_1 was may be due to smaller granule size (Sandhu, Singh, & Kaur, 2004). pH of all three starches isolated from *Colocasia* was near to neutral (Table 2). Moisture content of isolated starches was 4.5%, 9% and 12%. Level of calcium was higher in the starch isolated from all three tubers of *Colocasia* but less than that was reported earlier by Ndabikunze et al. (2011). Ash value was found to be 2.5%, 3.5% and 3.1% for CA_1, CA_2, and CA_3 respectively. Little amount of ash value can be correlated with small level of calcium (Sira, 2000). WHC of *Colocasia* starches CA_1, CA_2, CA_3 was found to be 453%, 328% and 506% respectively. Starch from *Colocasia* CA_2 has low water binding capacity; the reason for this may be due to the formation of a larger proportion of hydroxyl groups of hydrogen and covalent bonds between starch chains than the water (Hoover & Ratnayake, 2002).

3.1.2. Powder characteristics

There were less significant differences in true density, bulk density and tap density of isolated *Colocasia* starches (Table 3). Particle density of powders is known to influence mixing and segregation potential while particle shape, size and size distribution would, in addition, influence the packing down behavior of materials during the various unit operations of capsule/die filling and compression (Wray, 1992). The particle size and shape of the starches may be responsible for the differences in the density values. The fluidity of all starch materials (given as Carr index), was found as 14.01, 23.03 and 13.73 for all three starches isolated from *Colocasia* CA_1, CA_2, and CA_3 respectively (Table 3). Low Carr's index revealed better powder flowability (Carr, 1965). The Carr index provides an indirect measure of material fluidity and the higher its value, the more cohesive the substance. Generally, materials with CI up to 16% indicate good flow behavior while those above 28% indicate cohesive or poor powder flow (Riley et al., 2004). All type of starches studied had CI value below 28% thus indicating good flow behavior and low cohesiveness. Angle of repose (Table 3) of starches also shows good to fair flow property of powder. The density of the all starches showed no direct influence on the rate of paracetamol dissolution in this study; however, it gave an indication of the starch granule porosity. CA_3 was found to be more porous as compared to other two starches. Porosity has been shown to have a direct effect on the rate of drug dissolution (Riley, Adebayo, Wheatley, & Asemota, 2008).

3.1.3. Solubility and swelling power

Swelling power and solubility of all *Colocasia* starches at temperature ranging between 60 °C and 90 °C are shown in Table 4. As the heating temperature increased, swelling and solubility power among the starches was also increased. Swelling power and solubility power of all three starches varied from 1.40 g/g to 5.60 g/g and 8.10–49.03%. It has been reported, swelling power is depend upon amylose content, water holding capacity (Lee & Osman, 1991) and crystalline properties (Tester & Karkalas, 1996) of starches. Solubility and swelling power of starches, gives the evidence to the presence of interaction between starch chains within amorphous and crystalline domains (Hughes et al., 2009; Wang et al., 2006).

3.1.4. Paste clarity

Paste clarity (% transmittance) (Table 5) was found to increase in the cases of isolated starches when observed for 5 days. On fifth day % transmittances were 92.21%, 95.67%, 93.59% for *Colocasia* CA_1, CA_2, and CA_3 starches respectively. Increase in paste clarity may be related to smaller granule size (Xia et al., 2012). Starch paste clarity is also related to the state of dispersion and the retrogradation tendency of the starch, and hence will affect other important properties of starch. It is influenced by many factors such as paste concentration, pH, granule structure and other granule properties (Craig et al., 1989).

3.2. Morphology of starch granules

All three types of starches isolated from *Colocasia*, varied in the size of granule and the shape when observed under scanning electron microscope (Fig. 1). Shape of granules was found to be irregular, with the diameter ranging between 5 μm and 10 μm. The morphology and size of starch granules depends on the

Table 2
Proximate analysis of *Colocasia* starches CA_1, CA_2 and CA_3.

Samples	Yield (%)	Moisture (%)	Ash (%)	pH	Amylose (%)	WHC (%)	Mineral content (ppm)						
							Ca	Fe	Mg	Mn	Zn	Ni	Cu
CA_1	13.75	4.5 ± 0.01	2.5 ± 0.20	6.8 ± 0.01	27.66 ± 0.30	453 ± 0.01	19.07	3.166	1.889	0.078	15.96	0.012	0.045
CA_2	8.94	9 ± 0.05	3.5 ± 0.09	6.8 ± 0.06	13.5 ± 0.21	328 ± 0.04	23.00	0.467	1.647	0.079	23.69	0.016	0.183
CA_3	6.46	12 ± 0.01	3.1 ± 0.07	6.7 ± 0.03	14.1 ± 0.17	506 ± 0.01	17.31	0.55	1.61	0.057	22.08	0.016	0.12

All values are means of triplicate analysis ± standard error mean.

Table 3
Powder characteristics of isolated *Colocasia* starches.

Samples	Bulk density (gm/ml)	Tapped density (gm/ml)	Hausner's ratio	Carr' index (%)	True density (gm/ml)	Angle of repose (°)	Porosity (%)
CA_1	0.36 ± 0.80	0.421 ± 0.03	1.16 ± 0.02	14 .01 ± 0.03	1.31 ± 0.02	21.5 ± 0.33	73.28 ± 0.22
CA_2	0.35 ± 0.73	0.50 ± 0.63	1.31 ± 0.02	24.02 ± 0.23	1.39 ± 0.01	30.1 ± 0.20	70.50 ± 0.11
CA_3	0.35 ± 0.23	0.41 ± 0.30	1.17 ± 0.05	14.63 ± 0.10	1.41 ± 0.01	27.5 ± 0.10	69.29 ± 0.02

All values are means of triplicate analysis ± standard deviation.

Table 4
The swelling and solubility power of starches isolated from tubers of *Colocasia*.

Samples	Swelling power (g/g)				Solubility power (%)			
	60 °C	70 °C	80 °C	90 °C	60 °C	70 °C	80 °C	90 °C
CA_1	1.40 ± 0.01	1.60 ± 0.02	2.01 ± 0.10	5.16 ± 0.15	12.12 ± 0.19	16.21 ± 0.11	40.01 ± 0.02	48.01 ± 0.11
CA_2	1.22 ± 0.24	1.10 ± 0.02	2.25 ± 0.13	4.23 ± 0.01	8.10 ± 0.13	12.51 ± 0.09	44.11 ± 0.01	49.03 ± 0.09
CA_3	0.89 ± 0.19	1.18 ± 0.04	3.24 ± 0.01	5.60 ± 0.04	10.11 ± 0.08	14.40 ± 0.01	38.02 ± 0.01	42.04 ± 0.05

All values are means of triplicate analysis ± standard deviation.

Table 5
Paste clarity of *Colocasia* starches CA_1, CA_2 and CA_3.

Samples	% Transmittance				
	Day 1	Day 2	Day 3	Day 4	Day 5
CA_1	40.08 ± 0.19	67.87 ± 0.22	78.65 ± 0.01	85.34 ± 0.13	92.21 ± 0.11
CA_2	37.51 ± 0.10	57.62 ± 0.02	75.21 ± 0.25	86.13 ± 0.23	95.67 ± 0.10
CA_3	42.10 ± 0.21	62.31 ± 0.02	77.12 ± 0.14	81.24 ± 0.21	93.59 ± 0.03

All values are means of triplicate analysis ± standard deviation.

biosynthesis of starch, as well as physiology of the plant involving percent light transmittance, amylose content, water holding capacity and swelling power (Badenhuizen, 1969). The variation in the size of granules depends on the biological origin (Svegmark & Hermansson, 1993).

3.3. Fourier transformed infrared spectroscopy

Starches isolated from all three *Colocasia* were assessed using FT-IR to determine the presence of functional groups leading to structure elucidation. The obtained data are graphically shown

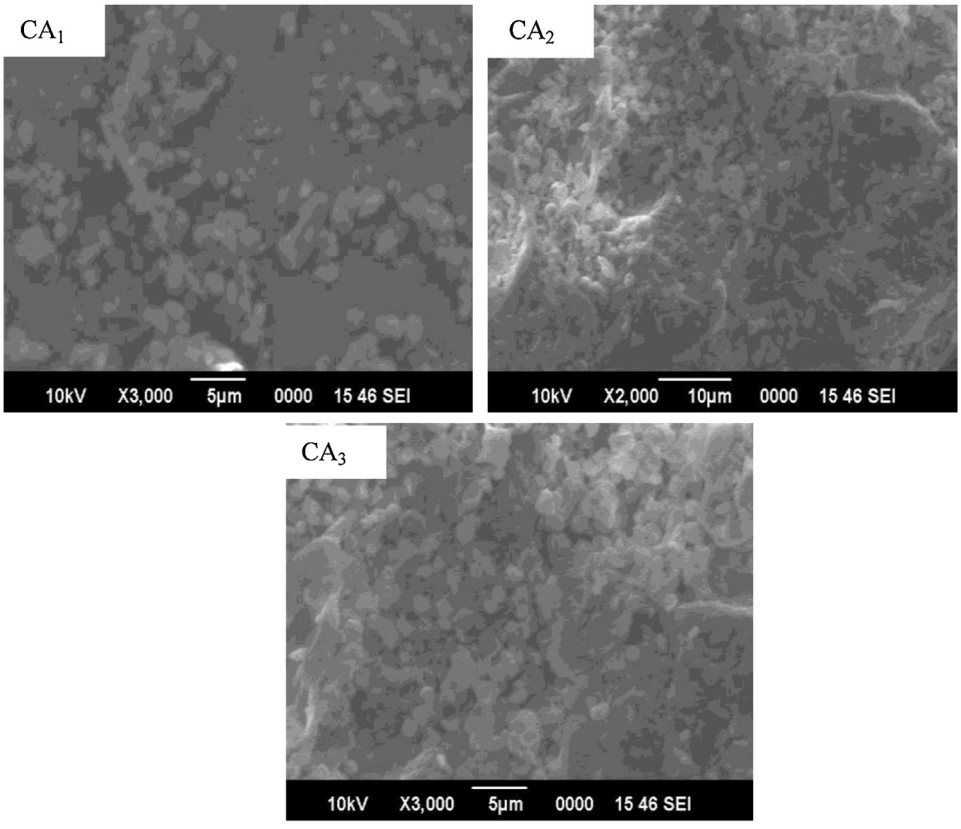

Fig. 1. Scanning electron micrograph of starches isolated from *Colocasia*.

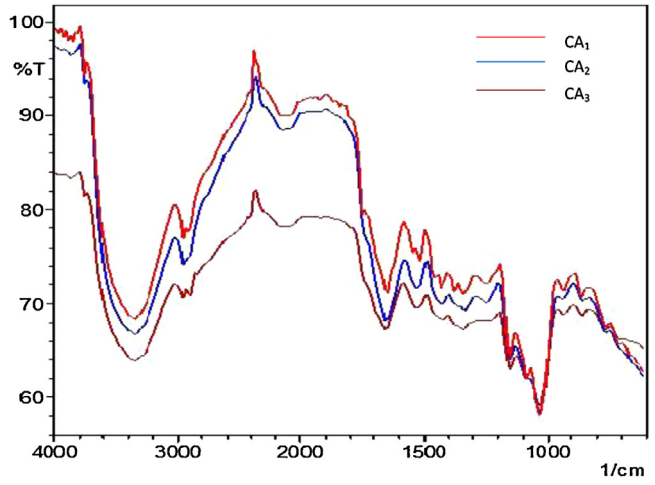

Fig. 2. FTIR patterns of *Colocasia* starches.

in Fig. 2. The wide band at 3344.68 cm^{-1} in case of CA_1 and CA_2 and at 3336.96 cm^{-1} in case of CA_3 might be attributed to O—H bond stretching and its width ascribed to the formation of inter and intra molecular hydrogen bonds (Saikia & Konwar, 2012) which is almost same for all three starches. Spectra of starches showed three characteristics peaks between or near 900 cm^{-1} and 1149.61 cm^{-1}, indicating the presence of C—O stretching (Lopez-Rubio, Clarke, Scherer, Topping, & Gilbert, 2009). Peaks at 1028.09 cm^{-1}, 1030.02 cm^{-1}, and 1031.95 cm^{-1} and 1149.61 cm^{-1} may refer to anhydroglucose ring C—O stretch of C—O—H in starch. Peak at 1149.61 was not prominent in CA_2 and CA_3. The bands near 1647 cm^{-1} were attributed to deformation vibrations of hydroxyl groups. The peak near 2930 cm^{-1} was ascribed to asymmetric stretching of C—H (Saikia & Konwar, 2012).

3.4. *In vitro* release study

In vitro release study revealed that at pH 1.2 percent release in all cases of *Colocasia* starch was found to be negligible but at pH 6.8 and pH 7.4 there was maximum release of 60.75–75.31%. In case of the formulation prepared by using commercial maize starch, it showed 99.02% release in 5 h when compared to other formulation prepared by using *Colocasia* starch. There is only 6-10% release of drug up to 3 h in case of *Colocasia* starches (Fig. 3) and the starch from *Colocasia* CA_3 showed maximum release of 75.31% in 8 h as compared to other two starches. Therefore, it could be attributed that all three type of

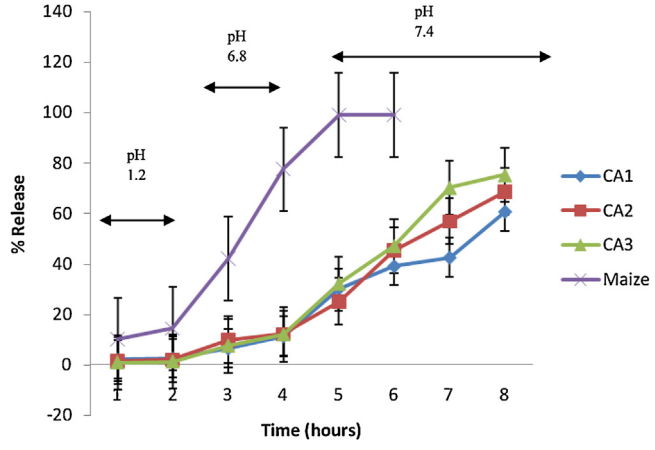

Fig. 3. *In vitro* release of starches isolated from *Colocasia* along with commercial maize starch.

starches are capable of protecting the drug releasing completely in physiological environment of the stomach and small intestine (Bharaniraja, Jayaram Kumar, Prasad, & Sen, 2011) when compared with maize starch. Maize starch showed complete release of drug in 4 h.

4. Conclusions

The results obtained from the starches isolated from all the tubers of *Colocasia* showed some differences in physicochemical, structural and thermal properties. From the studies of different physicochemical properties of starches it can be concluded the starch could be used in food industries. Water holding capacity, swelling power and solubility power of starches showed significant differences. FTIR spectra of two varieties of *Colocasia* starches confirmed their carbohydrate nature. Shape of all three types of starch granules was found to be small and small sized could be used in food with minimum modification. Due to small sized granule it could also be used as a binder with orally active ingredients (Saikia & Konwar, 2012). Studies of powder properties of starches revealed that these can be used as pharmaceutical excipient. Depending upon the powder characteristics of all the starches *in vitro* release study was done by using *Colocasia* starch as binder in the formulation. *In vitro* release data shows that *Colocasia* starches are promising source which could be used in sustained release dosage forms.

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