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Crosslinked Porous Starch Particles – a Promising Carrier

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation;
D – writing the article; **E** – critical revision of the article; **F** – final approval of the article

Abstract

Background. Starch is one of the most potential natural polymers used for various bio applications. Literature reports a number of modification strategies such as physical, chemical, enzymatic and genetic to enhance the positive attributes and iron out the undesired features of neat starch.

Objectives. To synthesize a crosslinked porous starch (CPS) as an efficient cargo for the delivery of calcium carbonate in an efficiently controlled manner for the treatment of hyperphosphatemia.

Material and Methods. The CPS carrier was synthesized using a natural crosslinker, malic acid. The drug delivery system was formulated, followed by the *in situ* loading of calcium carbonate during the preparation of the CPS. The developed system was characterized with respect to FTIR, DSC, SEM, moisture content, zeta potential, encapsulation efficiency, phosphate binding efficiency and dissolution studies.

Results. The developed formulation was observed to deliver calcium carbonate in an enterically controlled manner. The binding of calcium to phosphate was established to be pH dependent and efficient at pH 7. The moisture content of CPS was in the range of 0.2–0.8%. The zeta potential of the colloidal system was noted to be sufficiently high, indicating the stability. The encapsulation efficiency of CPS particles for calcium was found to be 88–96%

Conclusions. An efficient, cost-effective, facile and commercially-viable formulation was demonstrated to deliver calcium carbonate for the treatment of hyperphosphatemia (*Polim. Med.* 2015, 45, 1, 11–19).

Key words: calcium carbonate, crosslinked porous starch, hyperphosphatemia, delayed release, phosphate binding.

Starch is one the most commonly used ingredient in foods and pharmaceuticals. Its versatile role often grabs the attention of the formulator. Depending upon the source, quantity, and method of addition, its role varies, such as diluent, binder, thickener and disintegrant [1–4]. Starch is a macromolecular polysaccharide composed of amylose and amylopectin. Both of them have different characteristics [5]. Starch is abundant, economical, biocompatible, and biodegradable. To achieve great end-use quality and value addition to the material, a burgeoning number of reports are available, ranging from chemical functionalization, gelatinization, co-extrusion, and microwave treatment to supercritical fluid extraction [6–12]. One of the important methods for adding a modification to the existing properties of starch is crosslinking [8]. Crosslinking results in better mechanical properties and water resistance [13].

The literature is well stocked with the crosslinking of materials for the modification of the physical and chemical properties of materials [14–16]. The crosslinking of starch results in a more uniform particle size and hydrophobicity [17]. These properties may be positively tendered for the controlled release of nutraceuticals or drugs.

The preferred source of calcium is often calcium carbonate, which is inexpensive and readily available and often used as a food complement. The absorption site for the calcium after oral administration is the intestine. A majority of calcium accumulates in the skeleton. The route of elimination for calcium is urine and for carbonate through carbon dioxide exhalation [18]. Calcium carbonate is often indicated for hyperphosphatemia (for end stage renal disease patients) and osteoporosis [19]. A commercially available product for treatment of hyperphosphatemia is lanthanum carbon-

ate, which is available under the brand name of Fosrenol tablets and approved by the USFDA in the year 2004 [20].

In the present work, our objective was to develop and evaluate a carrier-cargo (i.e. starch-calcium carbonate) system which can deliver calcium in a controlled manner with a good phosphate binding capacity.

Materials and Methods

Materials

Malic acid and calcium carbonate were purchased from Loba Chemie, India. The starch was a kind gift from Gangwal Chemicals, India. A phosphate assay kit was obtained from Bioassay, USA. Dinitrosalicylic acid, hydrochloric acid, sodium chloride and potassium dihydrogen phosphate were acquired from Merck, India. Pancreatin and amyloglucosidase were purchased from Himedia, India. Avicel 102, sodium starch glycolate and magnesium stearate were kindly supplied by Signet Chemicals, India. Wherever required, deionized water (Millipore) was used throughout the study.

Methods

Synthesis of Calcium Carbonate Loaded CPS Particles (Ca-CPS)

2 g of starch were dispersed in 20 mL of cold water to prepare a slurry. 180 mL of water with a specified quantity of malic acid (cross-linker) was heated to 100°C in a separate beaker. The slurry was then added to the hot water under high shear mixing/homogenization using ultra-turrax (IKA-Werke, Germany). The system was steadily brought down to room temperature under stirring. While cooling, a suspension of calcium carbonate powder (2 g) was added to the gelling system. The resultant mass was then filtered and washed several times with deionized water to remove the malic acid. The material was then further dried, milled and stored under vacuum till further use. The process was redone at different starch to cross linker ratios (weight basis) of 1:2, 1:4 and 1:8, and the resulting particles were designated as T1, T2 and T3, respectively.

Determination of the Degree of Cross-Linking

Viscosity values were used to calculate the degree of cross linking (DC) of the starch particles [21]. The peak viscosity of each sample was determined by preparing a slurry of 20% w/w. The samples were subjected to a rheometer armed with the pasting cell. (AR1000,

TA instruments, USA). Following heating and cooling of the slurry, the following protocol was utilized during the testing:

- I) The sample was heated from 50 to 95°C at 11°C/min, and then held at 95°C for 2 min.
- II) Afterwards the temperature of the sample was brought down to 50°C at 11°C/min and finally maintained at 50°C for 2 min. The DC was determined using the following equation:

$$DC = (A-B)*100/A. \quad (1)$$

Where A, and B are the peak viscosities of the neat starch and cross-linked starch, respectively.

Paste Clarity

Reddy and Seib's method was used to evaluate the clarity of the starch paste [22]. In a glass stoppered test tube, 50 mg of the sample was dispersed using 5 mL of deionized water and heated to 95°C for half an hour, with intermittent shaking. The system was slowly brought down to room temperature and the clarity of the paste was determined using UV spectrophotometer (Shimadzu, Japan) at 650 nm, keeping deionized water as blank.

Swelling Factor

A test sample (0.1 g) was dispersed in 5 mL deionized water and mixed in an isothermal shaker at 70°C for half an hour [23]. The system was rapidly brought down to 20°C and mixed with 0.5 mL of Dextran blue solution (5 mg/mL). The system was centrifuged at 3000 × g for 10 min and the absorbance of the supernatant was determined at 620 nm.

Solubility

A test sample (500 mg) was added to 40 mL of warm water [24]. The temperature was slowly increased to 90°C for half an hour followed by centrifugation at 3000 × g for 10 min. The supernatant was collected, dried and weighed. The % solubility was then calculated as follows:

$$\text{solubility (\%)} = \frac{\text{weight of dried supernatant in mg}}{500} * 100 / 500. \quad (2)$$

In Vitro Digestibility

A previously published method was used for the determination of in vitro digestibility [25]. Briefly, 1 g of pancreatin was mixed with 12 mL of water using a cyclomixer (CM101, Remi, India). The supernatant was separated using an ultracentrifuge at 3000 × g for 10 min, and mixed with amyloglucosidase (0.2 mL). This system was then diluted to 10 mL using a volumetric flask. This solution (enzyme digestibility test solution) was freshly prepared for every digestibility test. A test sample (30 mg) along with 2 mL of pH 5.2 sodium acetate buffer was mixed in an eppendorf tube and incu-

bated at 37°C for 20 min. Afterwards, freshly prepared enzyme digestibility test solution (0.75 mL) was added and mixed using a cyclomixer at 500 rpm for 20 min. The system was then boiled for 10 min to cease the reaction. The dinitrosalicylic acid (DNS) method was used to determine 'rapidly digestible starch content' (RDSC). The glucose concentration was also determined at the end of 2 hours of incubation and termed as 'slowly digestible starch content' (SDSC). The resistant starch content (RSC) was determined as follows:

$$RSC = (100 - RDSC - SDSC). \quad (3)$$

Particle Size Analysis

The Ca-CPS particles were dispersed in water and subjected to analysis using a dynamic light scattering instrument (Malvern Mastersizer, UK). The measurement was done in triplicate for the samples [26].

Particle Morphology

The morphology of the particles was analyzed through a scanning electron microscope (Jeol, Japan). Briefly, the particles were first coated through a gold/platinum sputtering under vacuum process. Then the sputtered particles were mounted on aluminum stubs via double sided adhesive carbon tape. The images were taken at 15kV and 10A [27].

Zeta Potential Measurement

Zeta potential was determined by dispersing the particulate sample in purified water and analyzing the zeta potential at 25°C using a Zetasizer (Malvern, UK). The measurements were repeated in triplicate.

Moisture Uptake Studies

The particles were exposed to three different levels of moisture, specifically 31, 45 and 79.3% relative humidity, in controlled chambers for a period of one month. The corresponding increase in weight was noted.

Encapsulation Efficiency Studies

Particles equivalent to 200 mg of calcium carbonate were dissolved in 6M hydrochloric acid solution and sonicated for 10 minutes to ensure complete extraction of calcium. The calcium in the solution was then quantified with an atomic absorption spectrophotometer (Shimadzu, Japan).

Tablet Formulation

The developed particulate system was blended with the excipients, sifted through an ASTM 40 mesh and then compressed into tablets to have a final dosage form with the composition as shown in Table 1.

The tablets were compressed using a 10 mm standard concave punch with a single station manually operated machine (Cadmech, India). The crushing strength of the tablets was maintained at $8 \pm 2 \text{ kg/cm}^2$.

Table 1. Tablet composition for Ca-CPS particles

Ingredients	Quantity in mg/tab
Ca-CPS	Equivalent to 100 mg of calcium carbonate
Microcrystalline cellulose (Avicel 102)	76
Sodium starch glycolate	20
Magnesium stearate	4

In Vitro Dissolution Kinetics

A USP type 2 dissolution test apparatus was used for the study. The dissolution test apparatus was operated at 50 rpm and 37°C. The dissolution studies were carried out in two stages. The dissolution media volume was 500 mL for each stage. In the first stage of two hours the dissolution media was simulated gastric fluid without enzymes, and this was followed by the second stage of phosphate buffer of pH 7. At regular time intervals the sample was removed, suitably diluted and quantified for calcium release using an atomic absorption spectrophotometer.

Phosphate Bbinding Dependency on pH

Potassium dihydrogen phosphate was used to prepare phosphate ions in purified water with a phosphate strength of 5 mg/100mL of the solution. Particles of F3 formulation (100 mg) were added to the each phosphate solution, whose pH was adjusted to 3, 5 and 7 using hydrochloric acid. The systems were incubated in a shaker operated at 150 rpm and 37°C for 3 hours. One milliliter was removed, filtered, suitably diluted and analyzed using a phosphate assay kit, every hour. The samples were analyzed by a UV spectrophotometer (Shimadzu, Japan) at 650 nm.

Fourier Transform Infra-Red Spectroscopy

The samples were mixed with anhydrous potassium bromide at a ratio of 1:10 in a mortar and pestle. The blend was then compressed into a KBr pellet press and analyzed for FTIR spectrum by using a FTIR spectrophotometer (Perkin Elmer, USA). A total of 32 scans were performed for each sample with a resolution of 4 cm^{-1} .

Differential Scanning Calorimetry

A DSC instrument (Perkin Elmer, USA) previously calibrated using Indium, was used for the study. An empty crimped aluminum pan was used as a reference cell. 100 mg of the sample was filled into another pan, crimped and put into the test cell. Thermal scanning was performed from 40 to 900°C at a rate of 10°C/min under nitrogen purge of 20 mL/min. The heat flow within the sample was plotted as a function of the temperature and the resultant graph would give the DSC thermogram.

Results and Discussion

Malic acid was used as a crosslinker to form a cross-linked network within the starch. The use of malic acid is a well-known strategy [28]. Malic acid itself will give an acidic environment during crosslinking. Thus calcium carbonate, when incorporated during gelatinization and crosslinking, will get readily dissolved and in situ encapsulation within the network of crosslinked starch would occur [29]. The resultant calcium carbonate loaded CPS particles were water insoluble.

Degree of Cross-Linking, Paste Clarity, Swelling Factor and Solubility

The values are expressed as mean \pm RSD for triplicate. An increase in crosslinker concentration was found to be related to the degree of crosslinking in almost a directly proportional manner.

A significant decline in paste clarity was observed upon crosslinking of the starch. These reports are well in agreement with the previously reported literature [21, 30]. This change in paste clarity is a result of the change in granular structure and density through the process of crosslinking [31]. Another proposed justification for the reduced clarity of the paste is the diminished swelling capacity of crosslinked starch [21, 32].

The increased level of crosslinker resulted in decreased swelling factor. This observation was found to be consistent with an earlier published report [33]. They reported the inverse proportional relationship between the swelling factor of crosslinked oat starch and the degree of crosslinking.

Regression analysis showed that the swelling factor was related to the concentration of the crosslinking reagent in an almost linear fashion. Researchers have already demonstrated that crosslinking reinforces the bonding between starch chains, thus permitting them to oppose swelling [34, 35]. This Phenomenon contributes to the delayed release of calcium.

As expected, the solubility was also found to decrease with higher levels of crosslinker concentration. A possible reason for this observation may be the higher density of crosslinks in the starch structure which subsequently refuse to disintegrate in the media during dissolution testing [36]. Thus, this crosslinking may positively contribute to calcium release retardation.

In Vitro Digestibility

RSC was found to increase with the increase in crosslinker concentration. Almost linear curves (r^2 of more than 0.9) were obtained when the starch fraction contents were plotted against swelling factors as shown in Fig. 1. The starch fraction contents have a direct impact on the crystalline structure of the starch, the swelling power, the granule surface area and the amylose content, and these factors will influence calcium carbonate release [37, 38].

Swelling of the starch material will provide exposure of the inner core to digestive enzymes. Therefore, digestion would be hindered in the case of Ca-CPS particles, giving smaller values for RDSC and SDSC. These results were in agreement with previous studies [34, 37, 39, 40].

Particle Size Analysis

These particulate systems showed by and large a monodisperse system (Table 3). Also the particle size was in the order of $T1 < T2 < T3$. The increase in crosslinking causes an increase in viscosity and thereby larger particle size [41].

Table 3. Particle size distribution of Ca-CPS

Sample	Mean Diameter (nm)	Specific surface area (m^2/g)	Span#
T1	350	49.8	1.37
T2	525	44.2	1.29
T3	657	39.8	1.22

$$\text{Span\#} = (d_{90} - d_{10})/d_{50}.$$

Particle Morphology

The neat calcium carbonate (Fig. 2a) exhibited a glistening appearance. The particles seem to be of a crystalline nature. The crosslinked porous starch (Fig. 2b) clearly showed a spongy morphology along with the entrapped calcium carbonate particles inside the cavities. This entrapment of calcium carbonate and the interaction with the starch became the key factor for the controlled release [42].

Table 2. Values of degree of crosslinking, paste clarity, swelling factor and solubility of Ca-CPS particles

Batch Code	Degree of crosslinking	Paste clarity (%Transmittance)	Swelling factor	Solubility (%)
Neat starch	0	30.2 ± 2.12	37.4	46.5 ± 3.58
T1	39.6	2.09 ± 0.56	32	8.2 ± 1.97
T2	62.5	1.15 ± 0.22	24	5.4 ± 1.54
T3	88.7	0.86 ± 0.16	11	3.7 ± 0.87

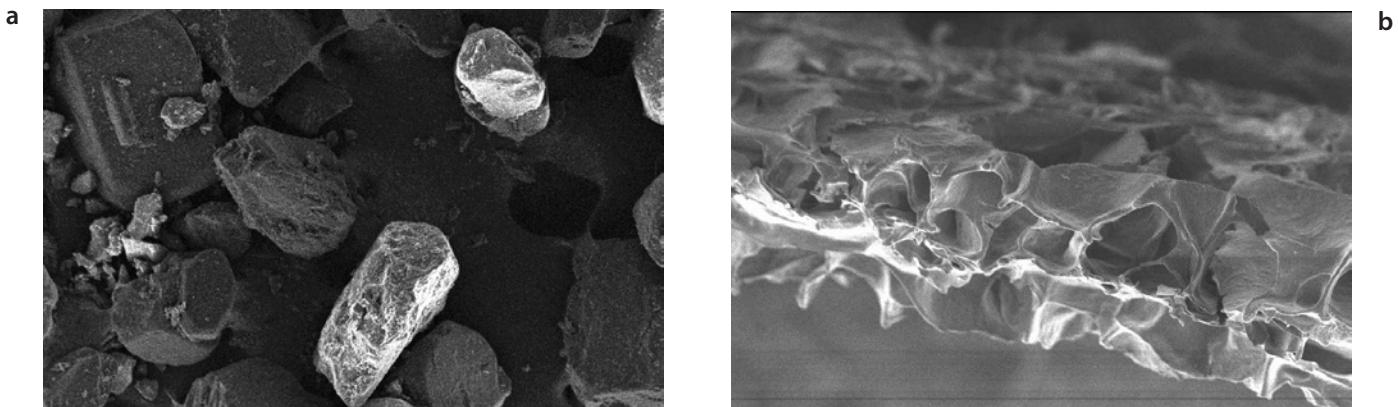
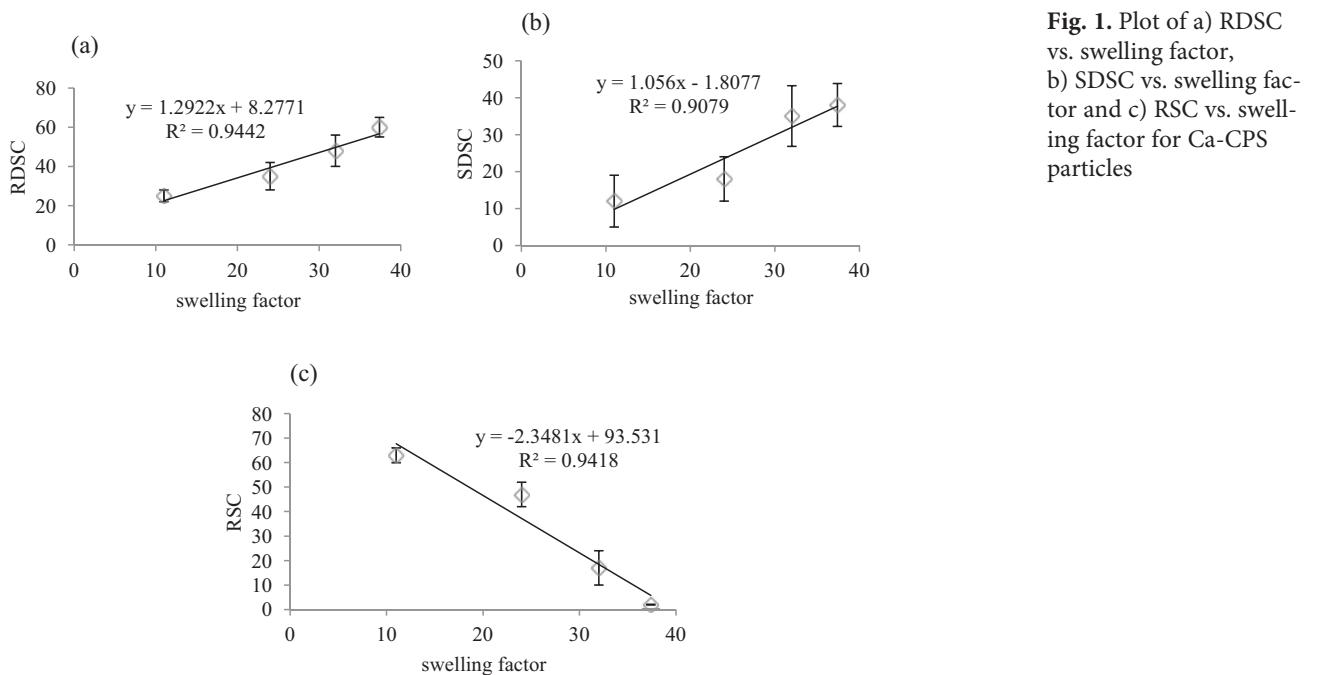


Fig. 2. Scanning electron micrograph of a) neat calcium carbonate and b) Ca-CPS particles (scale bar = 10 μ m)

Zeta Potential Measurement

The developed Ca-CPS particles were observed to have a negative charge along with a zeta potential of -7 mV to -23.7 mV. These values are sufficiently high in order to impart physical stability to the particulate system through a phenomena of electrostatic repulsion and subsequently circumventing aggregation. The existence of carbonate groups among the particulate system might have contributed to the higher values of zeta potential observed.

Moisture Uptake Studies

Usually crosslinking results in hydrophobicity, therefore its prerequisite was to study the influence of moisture on Ca-CPS particles. The data obtained is shown in Table 4.

These results showed that calcium carbonate in a Ca-CPS network heavily influence the moisture uptake. The moisture uptake for particular Ca-CPS par-

Table 4. Effect of relative humidity on moisture uptake

Sample	31% RH	45% RH	79.3% RH
T1	0.6	0.65	0.6
T2	0.4	0.4	0.4
T3	0.1	0.2	0.2

ticles was observed to be almost independent of the relative humidity environment which the system was exposed to.

Encapsulation Efficiency Studies

The highest encapsulation efficiency was observed in the case of T3 (Fig. 3). The increasing order was T3 > T2 > T1. The encapsulation efficiency for the calcium carbonate by CPS was found to be in the range of 85 to 95%. The higher encapsulation efficiency value for F3 may be the result of the use of the highest quantity of malic acid amongst all the systems, which allowed

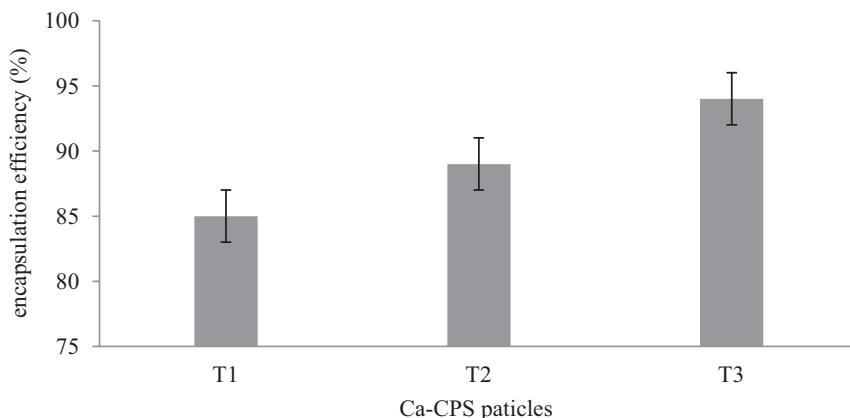


Fig. 3. Encapsulation efficiency of various particulate systems for calcium carbonate

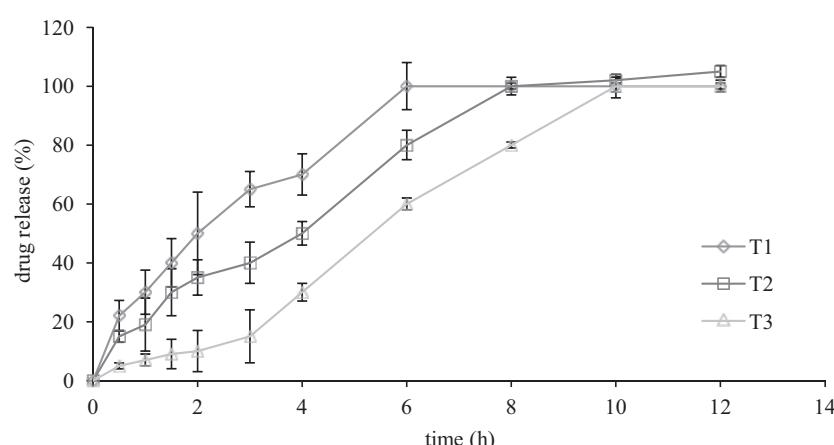


Fig. 4. Dissolution profile of calcium from different particulate systems

for the accumulation of calcium carbonate in the interior portion of the crosslinked particle. This will also safeguard them from being degraded due to moisture or acidic environment. At times, low molecular weight entities may diffuse quickly and easily and shift their encapsulation efficiencies to lower values due to their water labile nature. But the occurrence of inorganic moieties such as calcium carbonate in polysaccharide-based formulations [43]. In the crosslinked particulate systems the inorganic calcium carbonate was encapsulated to a higher extent compared to previously reported studies [44, 45]. This may be due to the formation of networks resulting from the fractional hydrolysis of calcium carbonate, thereby increasing the encapsulation efficiency with crosslinker quantity.

***In Vitro* Dissolution Study**

Fig. 4 shows the *in vitro* dissolution profile of calcium carbonate from various cross-linked starch particulate formulations. An extended release profile of calcium carbonate from the formulation was observed to be released in an extended fashion. Increase in the

crosslinker ratio for the CPS particles had an influence in terms of more retardation of the release of calcium carbonate. A burst release at the early stage of dissolution for T1 in the acid stage might be due to the adsorbed calcium carbonate on the surface of the CPS particles. Calcium carbonate, when released in acidic media will form calcium chloride, which is less efficient with respect to phosphate binding capacity as compared to calcium carbonate [46]. Therefore, to maintain the efficiency of calcium, the release should be retarded for some time, so as to deliver the calcium carbonate in the small intestine. This can easily be achieved by increasing the crosslinker concentration as seen in Fig. 4.

Phosphate Binding Dependency on pH

Calcium released from the T3 particles showed an efficient binding with phosphate ions in a pH dependent fashion as shown in Fig. 5. T3 formulation showed tremendous potential for phosphate binding at pH 7. The phosphate concentration depleted every hour, attaining the minimum value of 3.2 mg/dL in three hours, indicating a controlled release of calcium.

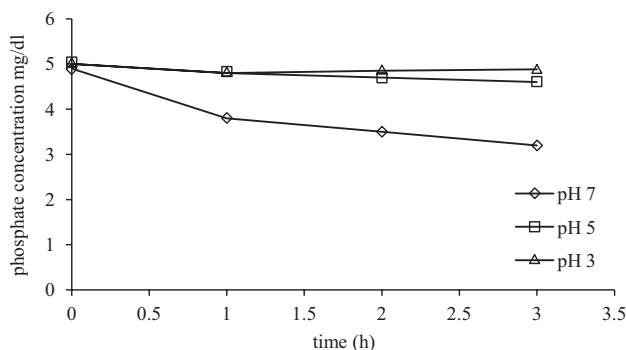


Fig. 5. Effect of pH on phosphate binding capacity of T3 formulation

Fourier Transform Infra-Red Spectroscopy

Plain calcium (Fig. 6b) carbonate showed a broad peak which is intense in nature at 1430 cm^{-1} . Other sharp peaks at 1084 cm^{-1} (symmetric stretch), 878 cm^{-1} (symmetric bend) and 713 cm^{-1} (asymmetric bend) confirm the calcite form of plain calcium carbonate [47]. The FTIR spectra of Ca-CPS showed a significant difference as compared to neat calcium carbonate. The crosslinked starch (Fig. 6a) revealed the presence of carbonyl groups through a peak at 1674 cm^{-1} , pointing to the successful crosslinking of starch. Another

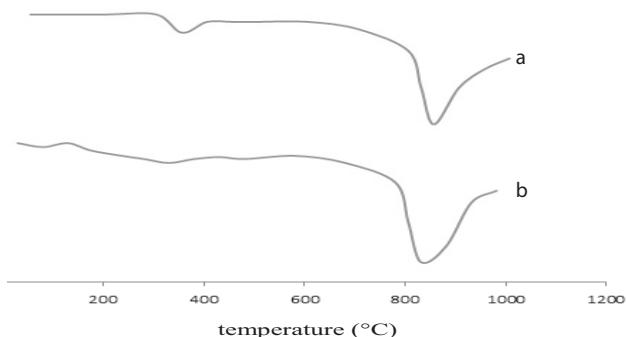


Fig. 6. FTIR spectrum of a) T3 particles and b) neat calcium carbonate particles

observation of reduced peak intensities, which were associated with calcium carbonate, suggests an interaction between calcium carbonate and crosslinked starch which consequently might have been helpful in release retardation during dissolution [48].

Differential Scanning Calorimetry

Fig. 7 shows the thermal behavior of neat calcium carbonate and T3 Ca-CPS particles. Calcium carbonate showed a phase transition around 825°C with degradation. The same peak was observed in T3 formulation, indicating that the calcium carbonate particles were successfully encapsulated within the crosslinked starch particles [44]. Peak broadening phenomena may be an outcome of the dilution effect [9].

Neat starch was modified using malic acid as a crosslinker. The crosslinked starch particles prepared using a condensation reaction showed a potential to encapsulate, retain and release the calcium carbonate for an extended period. This novel system releases calcium in delayed release fashion for the management in hyperphosphatemia. These studies, therefore, substantiate crosslinked starch particles as a promising device for calcium carbonate delivery.

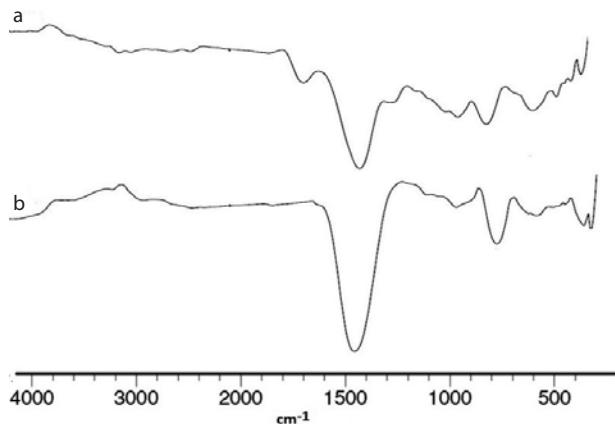


Fig. 7. DSC thermogram of a: calcium carbonate and b: T3 particles

References

- [1] Ayorinde J.O., Itiola A.O., Odeniyi M.A.: Effects of excipients and formulation types on compressional properties of diclofenac. *Acta Pol. Pharm.* 2013, 70, 557–566.
- [2] Wilson B., Babubhai P.P., Sajeev M.S., Jenita J.L., Priyadarshini SR: Sustained release enteric coated tablets of pantoprazole: formulation, in vitro and in vivo evaluation. *Acta Pharm.* 2013, 63, 131–140.
- [3] Hanson B., Cox B., Kaliviotis E., Smith C.H.: Effects of saliva on starch-thickened drinks with acidic and neutral pH. *Dysphagia.* 2012, 27, 427–435.
- [4] Odeku O.A., Akinwande B.L.: Effect of the mode of incorporation on the disintegrant properties of acid modified water and white yam starches. *Saudi Pharm. J.* 2012, 20, 171–175.
- [5] Namazi H., Dadkhah H.: Convenient method for preparation of hydrophobically modified starch nanocrystals with using fatty acids. *Carbohydr. Polym.* 2010, 79, 731–737.
- [6] Angellier H., Molina-Boisseau S., Dole P., Dufresne A.: Thermoplastic starch-waxy maize starch nanocrystals nanocomposites. *Biomacromolecules* 2006, 7, 531–539.

[7] Xu Y., Ding W., Liu J., Li Y., Kennedy J.F., Gu Q.: Preparation and characterization of organic-soluble acetylated starch nanocrystals. *Carbohydr. Polym.* 2010, 80, 1078–1084.

[8] Kim C.H., Cho K.Y., Park J.K.: Reactive blends of gelatinized starch and polycaprolactone-g-glycidyl methacrylate. *J. Appl. Polym. Sci.* 2001, 81, 1507–1516.

[9] Meer T.A., Fule R.A., Sav A.K., Amin P.D.: Porous starch: a novel carrier for solubility enhancement of carbamazepine. *AAPS PharmSciTech.* 2013, 14, 919–926.

[10] Guan J.J., Hanna M.A.: Extruding foams from corn starch acetate and native corn starch. *Biomacromolecules* 2004, 5, 2329–2339.

[11] Torres F.G., Boccaccini A.R., Troncoso O.P.: Microwave processing of starch based porous structures for tissue engineering scaffolds. *J. Appl. Polym. Sci.* 2007, 103, 1332–1339.

[12] Manoi K., Rizvi S.S.H.: Physicochemical characteristics of phosphorylated cross-linked starch produced by reactive supercritical fluid extrusion. *Carbohydr. Polym.* 2010, 81, 687–694.

[13] Antonio G.B., Pereira A.R., Fajardo S.N., Celso V.N., Adley F.R., Edvani C.M.: Starch-based microspheres for sustained-release of curcumin: Preparation and cytotoxic effect on tumor cells. *Carbohydr. Polym.* 2013, 98, 711–720.

[14] Khachatryan K., Khachatryan G., Fiedorowicz M., Para A., Tomaszik P.: Formation of nanometal particles in the dialdehyde starch matrix. *Carbohydr. Polym.* 2013, 98, 568–573.

[15] Olsson E., Hedenqvist M.S., Johansson C., Järnström L.: Influence of citric acid and curing on moisture sorption, diffusion and permeability of starch films. *Carbohydr. Polym.* 2013, 94, 765–772.

[16] Kutyła M.J., Lambert L.K., Davies N.M., McGeary R.P., Shaw P.N., Ross B.P.: Cyclodextrin-crosslinked poly(acrylic acid): Synthesis, physicochemical characterization and controlled release of diflunisal and fluconazole from hydrogels. *Int. J. Pharm.* 2013, 444, 175–184.

[17] Aschenbrenner E., Bley K., Koynov K., Makowski M., Kappl M., Landfester K., Weiss C.K.: Using the polymeric ouzo effect for the preparation of polysaccharide-based nanoparticles. *Langmuir* 2013, 29, 8845–8855.

[18] www.efsa.europa.eu accessed on 25th October 2013.

[19] Zamfirescu I., Carlson H.E.: Absorption of Levothyroxine when co-administered with various calcium formulations. *Thyroid* 2011, 21, 483–486.

[20] <http://www.fosrenol.com/clinical-trials.aspx> accessed on 25th October 2013.

[21] Kaur L., Singh J., Singh N.: Effects of cross-linking on some properties of potato starches. *J. Sci. Food Agric.* 2006, 86, 1945–1954.

[22] Reddy I., Seib P.A.: Paste properties of modified starches from partial waxy wheats. *Cereal Chem.* 1999, 76, 341–349.

[23] Tester R.F., Morrison W.R.: Swelling and gelatinization of cereal starches I. Effects of amylopectin, amylose, and lipids. *Cereal Chem.* 1990, 67, 551–557.

[24] Schöch T.J.: Swelling power and solubility of starch granules. [In:] *Methods in carbohydrate chemistry*, Ed.: R.L. Whistler, 1964, 4, 106–108. Academic Press, Orlando, Florida.

[25] Englyst H.N., Kingman S.M., Cummings J.H.: Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 1992, 46, S33–S50.

[26] Meer T.A., Sawant K.P., Amin P.D.: Liquid antisolvent precipitation process for solubility modulation of bicalutamide. *Acta Pharm.* 2011, 61, 435–445.

[27] Meer T.A., Fule R.A., Sav A.K., Amin P.D.: Fabrication of cyclodextrin-templated mesoporous silica for improved dissolution of carbamazepine. *Drug Deliv. Transl. Res.* 2013, 3, 235–242.

[28] Seidel C., Kulicke W., Heß C., Hartmann B., Lechner M.D., Lazik W.: Influence of the Cross-linking Agent on the Gel Structure of Starch Derivatives Influence of the Cross-linking Agent on the Gel Structure of Starch Derivatives. *Starch* 2001, 53, 305–310.

[29] Lin Y.H., Liang H.F., Chung C.K., Chen M.C., Sung H.W.: Physically crosslinked alginate/N,O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs. *Biomaterials* 2005, 26, 2105–2113.

[30] Lim S.T., Seib P.A.: Location of phosphate esters in a wheat starch phosphate by ³¹P-nuclear magnetic resonance spectroscopy. *Cereal Chem.* 1993, 70, 145–152.

[31] Morikawa K., Nishinari K.: Effects of concentration dependence of retrogradation behaviour of dispersions for native and chemically modified potato starch. *Food Hydrocolloid.* 2001, 14, 395–401.

[32] Reddy I., Seib P.A.: Modified waxy wheat starch compared to modified waxy corn starch. *J. Cereal Sci.* 2000, 31, 25–39.

[33] Mirmoghtadaie L., Kadivar M., Shahedi M.: Effects of cross-linking and acetylation on oat starch properties. *Food Chem.* 2009, 116, 709–713.

[34] Chung H.J., Woo K.S., Lim S.T.: Glass transition and enthalpy relaxation of cross-linked corn starches. *Carbohydr. Polym.* 2004, 55, 9–15.

[35] Janzen G.L.: Digestibility of starches and phosphatized starches by means of pancreatin. *Starch/Stärke* 1969, 38, 231–237.

[36] Jyothi A.N., Moorthy S.N., Rajasekharan V.: Effects of cross linking with epichlorohydrin on the properties of cassava (*Manihot esculenta* Crantz) starch. *Starch/Stärke* 2006, 58, 292–299.

[37] Shin S.I., Kim H.J., Ha H.J., Lee S.H., Moon T.H.: Effect of hydrothermal treatment on formation and structural characteristics of slowly digestible nonpasted granular sweet potato starch. *Starch/Stärke* 2005, 57, 421–430.

[38] Spence K.E., Jane J.: Chemical and physical properties of ginkgo (*Ginkgo biloba*) starch. *Carbohydr. Polym.* 1999, 67, 261–269.

[39] Chung H.J., Shin D.H., Lim S.T.: *In vitro* starch digestibility and estimated glycemic index of chemically modified corn starches. *Food Res. Int.* 2008, 41, 579–585.

[40] Han J.A., BeMiller J.N.: Preparation and physical characteristics of slowly digesting modified food starches. *Carbohydr Polym.* 2007, 67, 366–374.

[41] Koo S.H., Lee K.Y., Lee H.G.: Effect of crosslinking on physicochemical and physiological properties of corn starch. *Food hydrocolloids.* 2010, 24, 619–625.

[42] Kumar R., Nagarwal R.C., Dhanawat M., Pandit, J.K.: In-vitro and in-vivo study of indomethacin loaded gelatin nanoparticles. *J. Biomed. Nanotechnol.* 2011, 7, 325–333.

[43] Yu C., Cao H., Zhang X., Zhou F., Cheng S., Zhang X., Zhuo R.: Hybrid nanospheres and vesicles based on pectin as drug carriers. *Langmuir.* 2009, 25, 11720–11726.

[44] Shende P., Deshmukh K., Trotta F., Caldera F.: Novel cyclodextrin nanospikes for delivery of calcium in hyperphosphatemia. *Int. J. Pharm.* 2013, 456, 95–100.

[45] Park J.H., Ye M., Park K.: Biodegradable polymers for microencapsulation of drugs. *Molecules.* 2005, 10, 146–161.

[46] Ranganathan N.: US 8,257,750 B2, 2012.

[47] Deshmukh G.S., Pathak S.U., Peshwe D.R., Ekhe J.D.: Effect of uncoated calcium carbonate and stearic acid coated calcium carbonate on mechanical, thermal and structural properties of poly(butylene terephthalate) (PBT)/calcium carbonate composites. *Bull. Mater. Sci.* 2010, 33, 277–284.

[48] Tarique M., Ritesh F., Dipak K., Purnima A.: Solubility modulation of bicalutamide using porous silica. *J. Pharm. Investig.* 2013, 43, 279–285.

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