

## RESEARCH ARTICLE

**ARTIFICIAL CELL MICROCAPSULE FOR ORAL DELIVERY OF THALIDOMIDE: DESIGN, PREPARATION, AND IN-VITRO CHARACTERIZATION FOR CROHN'S DISEASE APPLICATION****Marc Fakhoury\***, Michael Charley-Coussa, Hani SalehFadhl Al-Salami and Satya Prakash

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\*Corresponding author's email: [marc.fakhoury@mail.mcgill.ca](mailto:marc.fakhoury@mail.mcgill.ca), Phone : +1 (514) 710-7060**ABSTRACT**

Crohn's disease is a chronic inflammatory disorder of the gut and is classified as a type of inflammatory bowel disease. The anti-inflammatory drug thalidomide has shown to be very effective against Crohn's disease, but presents several limitations such as drowsiness, skin rash and hypertension. Therefore, development of novel delivery system is urgent and necessary. The aim of this paper is to present the formulation of Alginate-Poly-L-Lysine-Alginate (APA) microcapsules for the delivery of thalidomide to desired locations of the gastrointestinal tract. APA microcapsules were designed, prepared and characterized in-vitro for thalidomide release. Mechanical stability of capsules and the drug release profile were monitored in a simulated gastrointestinal model. Data suggest that APA microcapsules enable a slow release of thalidomide in a pH and time-dependent manner. Indeed, the characteristics of APA microcapsules make it a suitable carrier for the targeted delivery of thalidomide to specific areas of the gastrointestinal tract.

**Keywords:** Alginate-Poly-L-Lysine-Alginate (APA); Simulated gastric fluid (SGF); Simulated Intestine fluid (SIF); Crohn's disease

**INTRODUCTION**

Crohn's disease is a chronic inflammatory disorder of the gut and is classified as one type of inflammatory bowel disease. More than 400 000 people are affected by Crohn's disease in North America<sup>1</sup>. Epidemiological studies suggest that this disease occurs in genetically susceptible individuals as a result of defects in mucosal barrier function and dysregulated Th1-type mucosal inflammation<sup>2,3</sup>. In Crohn's disease, inflammation can affect any part of the gastrointestinal tract, from the mouth to the anus. Most common symptoms include abdominal pain, diarrhea, vomiting, and weight loss<sup>3</sup>. There is no cure for Crohn's disease and surgery cannot be used to treat this disease<sup>4</sup>. Medications used to treat Crohn's disease include anti-inflammatory drugs such as 5-aminosalicylic acid (5-ASA) and immunomodulators such as azathioprine, mercaptopurine, methotrexate, infliximab, adalimumab, certolizumab and natalizumab<sup>5,6</sup>. Although these pharmaceutical compounds have shown to be effective against Crohn's disease, they show limited clinical efficiency and several side effects. The anti-inflammatory drug thalidomide has shown to be very successful in inhibiting the inflammation associated with Crohn's disease but presents side effects due to his high dose requirements<sup>7</sup>. Appropriate delivery systems must be developed in order to overcome the limitations and issues of the currently available treatment for Crohn's disease. Artificial cell microencapsulation is a promising tool in scientific research that allows for the delivery of pharmaceutical compounds to specific tissues in the body, in a time dependent fashion<sup>8,9</sup>. Creating an artificial cell involves the preparation of artificial structure of cellular dimension using different types of polymers and proteins. Although several encapsulation techniques are being used, the most promising formulation is the encapsulation of calcium alginate beads with poly-L-lysine (PLL), forming alginate-poly-L-lysine-alginate (APA) microcapsules<sup>10</sup>. APA microcapsules are formed by the ionic interaction between negatively charged alginate molecules and positively charged calcium ions. This allows the entrapped

material to be protected from the external environment. This paper proposes the use of APA microcapsules for the specific delivery of thalidomide to treat Crohn's disease. The goal of this study is to evaluate the drug release characteristics in different intestinal segments and to determine the effect of thalidomide on a simulated intestinal inflammation by using RAW 264.7 macrophage cells.

**MATERIAL AND METHODS****Chemicals and laboratory equipment**

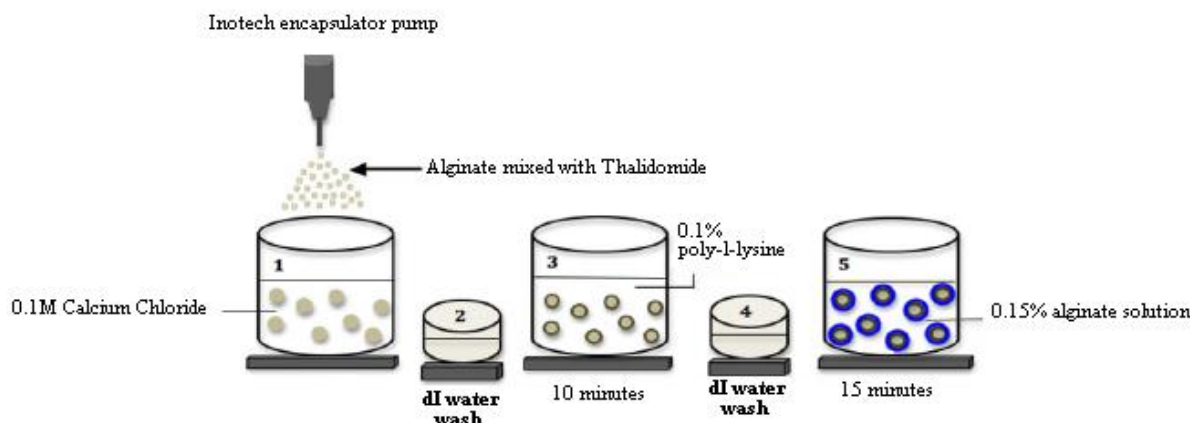
The Research IER-20 cell encapsulator was supplied by InotechBiosystems International. The Lab-Line Environ Shaker 3527 was supplied by Lab-Line Designers and Manufacturers and the Varian Cary 100 Bio Spectrophotometer was supplied by Varian. The chemicals thalidomide, alginic acid, poly-L-lysine (Hydrobromide) and dimethyl sulfoxide were supplied by Sigma-Aldrich Canada.

**Preparation of APA microcapsules containing thalidomide**

Figure 1 illustrates the preparation of APA microcapsules containing Thalidomide. Alginic acid was added to deionized water to make a 1.5% alginate solution. (±)-Thalidomide ((±)-2-(2,6-Dioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione) was dissolved in deionized water at a concentration of 0.035 mg/ml by stirring and heating for 24 hours and added to the alginate solution. Alginic acid was additionally added to maintain a 1.5% concentration after the thalidomide and water solution were included. APA beads were then formed by running the above solution through an Inotechencapsulator pump using a 300µm nozzle. Frequency was set to 528 Hz, flow rate to 20.8 ml/min and voltage to 1.48 kV. Formed beads were collected in a prepared 0.1M calcium chloride solution to avoid cell aggregation. The beads were then washed with deionized water and soaked in a 0.1% poly-L-lysine bath for 10 minutes. Beads were washed again and

soaked in 0.15% alginate solution for 15 minutes. Final washing was done with water and beads were transferred into calcium chloride for storage. The capsules were

visually evaluated for uniformity and integrity through a Lomo light microscope with 250X magnification.



**Figure 1:** Schematic diagram of the preparation of thalidomide loaded APA microcapsules

### Characterization of thalidomide

A standard curve for thalidomide in deionized water was prepared. Thalidomide was dissolved in deionized water at a concentration of 0.035 mg/ml by stirring and heating for 24 hours. The absorbance of different dilution factors was measured at 220 nm and plotted against the concentration of thalidomide in mM (Figure 2).

### Physical integrity of APA microcapsules

In-vitro experiments were conducted to study the physical integrity of APA microcapsules containing thalidomide. In order to simulate *in vivo* shear stress, microcapsules were incubated for 48 h in saline solution and shaken at 150 rpm<sup>11</sup>. The percentage of damaged and undamaged microcapsules were visually determined using a light microscope from supernatant samples taken at 1h, 9h, 12h, 24h and 48h after incubation. Pictures were taken before and after incubation to analyze the morphology of the microcapsule. Moreover, the release of thalidomide was measured spectrophotometrically over time as a marker of membrane permeability (Figure 4).

### Evaluating thalidomide release in simulated gastric and intestine fluid

Simulated gastric fluid (SGF) and simulated intestine fluid (SIF) were prepared in order to mimic the external environment encountered in the stomach and small intestine. SGF was prepared by dissolving Sodium Chloride (NaCl) and pepsin in deionized water. Hydrochloric acid was added to acidify the solution. The final pH of the solution was 1.5. Then, 250 mg of dried APA microcapsules containing thalidomide was added to 1 ml of SGF and shaken at 125 rpm for 30 min. The optical density at 220 nm (OD 220) was measured from the supernatant every 10 min and the results are shown in figure 5. SIF was prepared by dissolving potassium phosphate monobasic, sodium Hydroxide and pancreatin in deionized water. The pH of the solution was 6.5. In this experiment, 305 mg of APA microcapsules containing thalidomide were added to 1 ml of SIF and shaken at 125 rpm from 60 min. The absorbance was measured from the supernatant every 10 min and plotted on Figure 6.

### Evaluating thalidomide release in full simulated gastrointestinal model

In order to evaluate the survival and stability of APA microcapsules containing thalidomide in the gastrointestinal environment, different solutions were prepared. Such solutions were designed in order to mimic the stomach, the small intestine, the colon ascendans, the colon transversum and the colon descendans with respect to their pH. Table 1 describes the retention time and pH of each solution. 15.65 g of dry capsules was initially added to the first solution and shaken at 250 rpm for 46h. The desired pH was maintained by the continuous addition of HCl (0.102 M) and NaOH (0.2 M). OD 220 of supernatant was measured at several time points and the concentration of thalidomide was calculated in g/ml as shown in figure 7.

**Table 1:** Retention time and pH of gastrointestinal solutions. Solutions 1 represent the stomach and small intestine respectively, while vessels 3, 4 and 5 represent the ascending colon, the transverse colon and the descending colon respectively. Fixed amount of APA microcapsules were added into these gastrointestinal solution and the concentration of thalidomide released was measured over 46 hrs incubation.

SOLUTION	INTESTINAL SEGMENT	Retention Time(h)	pH
1	stomack	2	2.32
2	small intestine	6	5.31
3	colon ascendans	9	5.85
4	colon transversum	18	6.20
5	colon descendans	11	6.74

### Statistical analysis

Values are expressed as mean  $\pm$  SD. Study was considered a randomized balanced design. Statistical comparisons between various biomarkers were carried out by repeated measures analysis of variance (ANOVA). Statistical comparisons between various treatment groups were

carried out by using the general linear model (GLM).

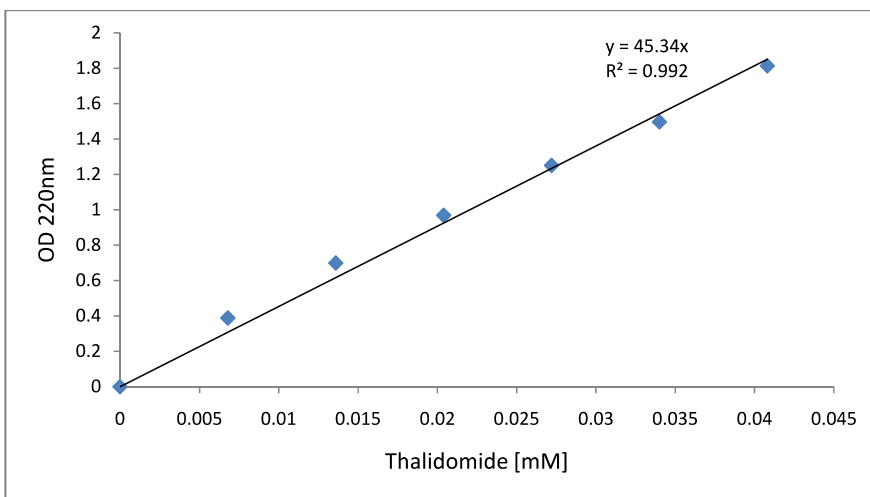
Statistical significance was set at  $p < 0.05$ .

**RESULTS AND DISCUSSION:**

**Thalidomide standard curve**

The optical density at 220 nm was measured from the supernatant and plotted in Figure 2. Data suggest a direct proportionality between the optical density and

thalidomide concentration. The mathematical equation  $y=45.464x$ , where  $y$  represents the absorbance at 220nm and  $x$  the concentration in mM, will be used for calculating the amount thalidomide in samples.



**Figure 2:** Standard curve for calculating thalidomide concentration. All data are done in triplicate and the equation displayed on the chart was used for calculating the concentration of thalidomide in supernatant samples.

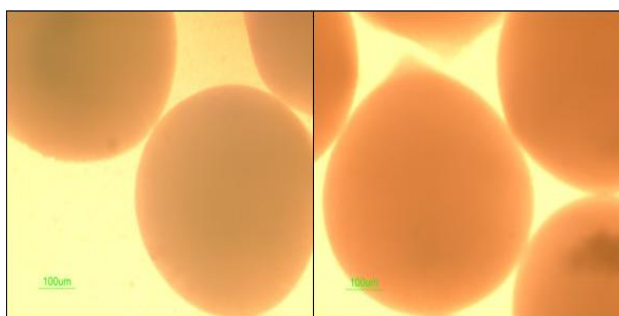
**Physical integrity of APA microcapsules**

In order to mimic in vivo shear stress, the APA microcapsules were incubated in saline solution for 48h and shaken at 150 rpm. The percentage of intact APA microcapsule was  $96\% \pm 1$  after incubation in saline for 48h. APA microcapsules displayed a consistent spherical shape with a diameter of 350–400  $\mu\text{m}$  (Figure 3). The majority of APA membrane is found intact after 48h hours

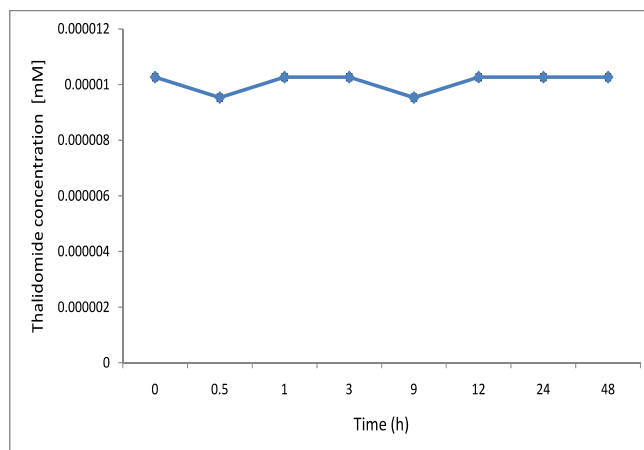
of incubation in saline solution. The intact ratio of APA microcapsules is illustrated in table 2. Although the capsules were shaken at 150 rpm for 48h in saline solution, thalidomide was largely maintained inside the APA microcapsules. Data illustrated in Figure 4 suggests a minimal release of thalidomide after 48h ( $10.26 \text{ mM} \pm 1.27 \times 10^{-6} \text{ mM}$ ).

**Table 2:** Percentage of intact APA microcapsules after incubation in saline for 48h and vigorous shaking at 150 rpm. Values are expressed as mean + S.D. of three independent experiments

Incubation time	1h	9h	12h	24h	48h
% intact APA capsules	100	$99.7 \pm 0.58$	$98 \pm 1.0$	$96.7 \pm 0.58$	$96 \pm 1.0$



**Figure 3:** Photomicrographs of APA microcapsules containing thalidomide before (left) and after (right) incubation in saline solution at 150 rpm under 250X magnification. Size ranges from 300–350  $\mu\text{m}$ .



**Figure 4:** Thalidomide retention efficacy profile of APA microcapsules in saline. After 48h of incubation, the concentration of thalidomide measured from the supernatant was  $12.29 \times 10^{-6} \text{ mM}$ .

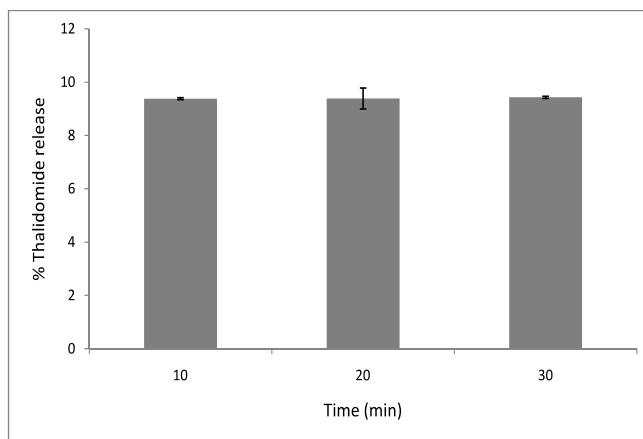
Despite the large size of formed beads, this study showed that thalidomide was mainly maintained within the microcapsules after 48h of incubation in saline solution at 150 rpm. This was largely due to the presence of electrostatic forces between the positively charged drug and the negatively charged alginate molecule.

#### ***Evaluating thalidomide release in simulated gastric and intestine fluid***

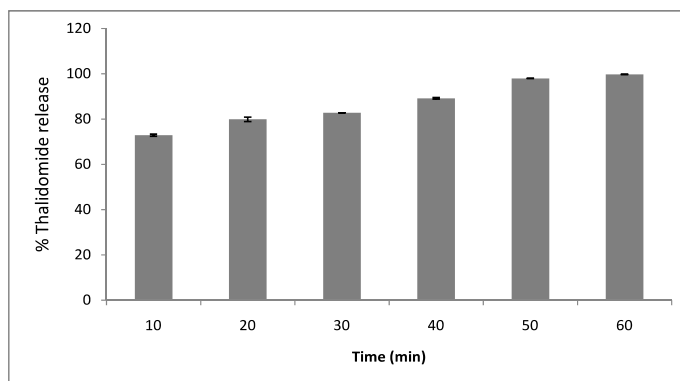
The release of thalidomide was kept relatively constant following incubation of APA microcapsules in simulated gastric solution for 30 min. (Figure 5). Results show that after 30 min of incubation and shaking in simulated gastric fluid, thalidomide release was minimal (9.43%). However, after incubation in simulated intestine fluid, the percentage release of thalidomide significantly increased (Figure 6). After 10 min incubation in SIF, the percentage release of thalidomide was 72.89%, and reached a value of 82.79% following 30 min incubation. Maximal release of thalidomide was achieved after 60 min incubation in SIF. These results demonstrated the fact that APA microcapsules are specifically designed to protect the entrapped material from the harsh acidic conditions normally found in the stomach, while allowing the encapsulated material to be slowly released in the intestines.

#### ***Evaluating thalidomide release in full simulated gastrointestinal model***

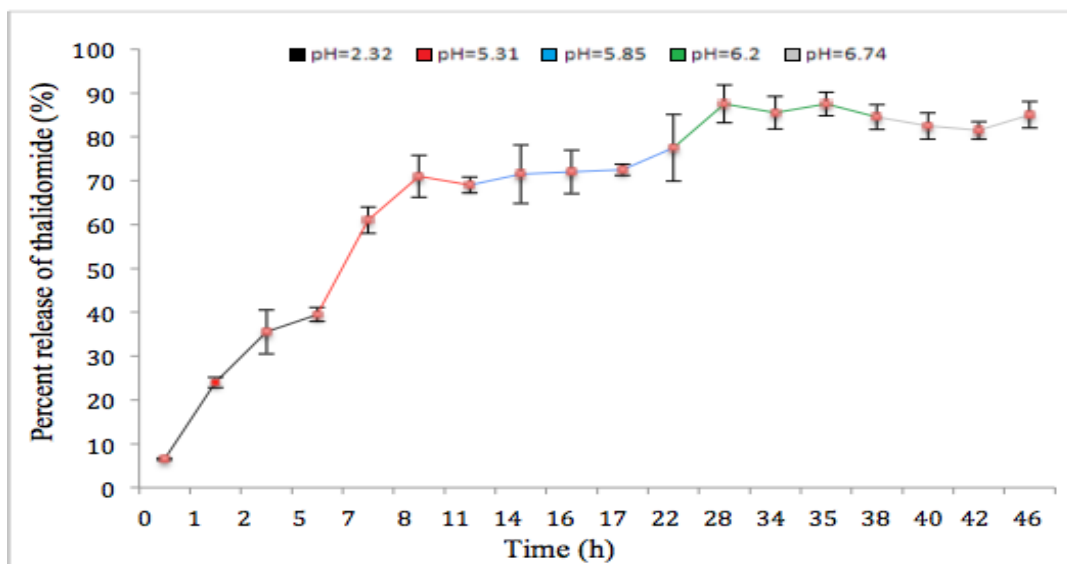
Figure 7 illustrates the release profile of thalidomide in solutions simulating the acidic and basic conditions normally encountered in the stomach, the small intestine, the colon ascendans, the colon transversum and the colon descendans. Data suggest that thalidomide release from APA microcapsules is pH-dependent. Thalidomide release is minimal at pH 2.32 ( $6.25 \times 10^{-7}$  g/ml) but dramatically increases when the pH is above 5.31 to finally reach a peak of ( $18.43 \times 10^{-7}$  g/ml).



**Figure 5:** Thalidomide release from APA microcapsules in simulated gastric fluid. The percentage release of thalidomide is  $9.38 \pm 0.04$  %,  $9.39 \pm 0.39$  % and  $9.43 \pm 0.04$  % at time 10, 20 and 30 min respectively. Values are expressed as mean + S.D. of three independent experiments.



**Figure 6:** Thalidomide delivery profile of APA microcapsules in simulated intestine fluid. The average percentage release of thalidomide is  $99.72 \pm 0.06$  % after 60 min of incubation. Values are expressed as mean + S.D. of three independent experiments.



**Figure 7:** Thalidomide delivery by APA microcapsules in simulated gastrointestinal fluids. Results suggest a burst release of thalidomide when the APA microcapsules are transferred from low pH (2.32) to a high pH (5.31) environment. Maximal drug release is observed when the microcapsules are transferred in a pH=6.2 and pH=6.74 environments.

This figure shows that an increase in pH triggers the release of thalidomide from APA microcapsules. This can be explained by the fact that the membrane of APA microcapsule is composed of two layers of alginate and one layer of poly-L-lysine that degrades when the pH is high.

#### CONCLUSION:

This experiment was aimed at determining the mechanical strength of APA microcapsules in vitro and analyse the drug release profile with respect to change in pH and external environment. APA microcapsule containing thalidomide has shown to be stable and the membrane retained its original shape and integrity. This characteristic of APA microcapsules makes it an appropriate carrier for drug delivery by oral administration. Moreover, the present study showed that the release of thalidomide from APA microcapsules is significantly increased when the capsules are transferred from a simulated gastric fluid into a simulated intestine fluid, demonstrating the ability of the

microcapsules to prevent the delivery of thalidomide in the stomach while allowing its slow release in the intestines. Moreover, results demonstrated a burst release of thalidomide, which strongly suggests that the pH of the external solution plays a crucial role on the mechanical strength and stability of the APA membrane. This important characteristic of APA microcapsule could be useful in the treatment of Crohn's disease where local delivery of the encapsulated drug to affected sites of the gastrointestinal tract is required. However, further in-vivo studies are needed to evaluate the full capacity of thalidomide loaded APA microcapsules in treating Crohn's disease.

#### ACKNOWLEDGMENTS

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