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Eco-Friendly Alginate Films embedding Zinc Oxide Nanostructures: Assessing the Oral Bioaccessibility of Zinc in a Dynamic Mode using a fully automatic flow-through system

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INTRODUCTION & AIM

The problem of pollution caused by petroleum-based plastics has boosted the research on alternative food packaging based on biopolymers. In this study, films based on sodium alginate (Alg), which is extracted from brown algae, with embedded antimicrobial zinc oxide nanostructures (ZnO NSs) at 0.3%$_{w/w}$ are proposed [1]. The NSs were prepared using an electrochemical-thermal method [2] and added directly to the alginate solution. Alg/ZnO NSs films were made by dry casting followed by cross-linking with Ca^{2+} ions. To assess the non-hazardous nature of the films, a study on the bioaccessibility of zinc in Alg/ZnO NS films was performed by realizing a dedicated flow system that exploits sequential injection analysis (SIA) to simulate human digestion by gastric fluid (GF). In this way, a dynamic and automatic SIA system was designed to profile the temporary release of zinc ions from the nanocomposites [3], combined with an in-line spectrophotometric detection using Zincon [4]. The SIA method was thus fully validated by mass balance, comparing the sum of bioaccessible and residual Zn content with the total Zn in the original film, the latter detected by ICP-OES analysis, after the Alg/ZnO NS films had been microwave-digested in an acidic medium. Our findings demonstrate the non-hazardous nature of the Alg/ZnO NS films since the zinc release is below the daily limit of mg of zinc intake.

METHODS

Film preparation

Sodium alginate (SA) powder 1%$_{w/v}$ and 0.3%$_{w/w}$ ZnO NSs were mixed as solids before water was added for solubilization for 4 h at 40°C, films were formed by pouring in Petri dishes and drying at 37°C overnight (Fig. 1). Similarly, SA films without NSs were formed. Crosslinked films (Alg and Alg/ZnO NSs) were made by immersion in a 0.45 M $CaCl_2$*$2H_2O$ hydroalcoholic solution (50 mL) for 30 min at RT, the crosslinked films were washed to remove any unbound surface cations. The films were then dried for 24 h at room temperature and stored in a desiccator for at least 48 h before use (Fig. 1). Alg and Alg/ZnO NSs are shown in Figure 2. The presence of ZnO NSs in the modified film is confirmed by optical microscopy (Figure 3).

Figure 1

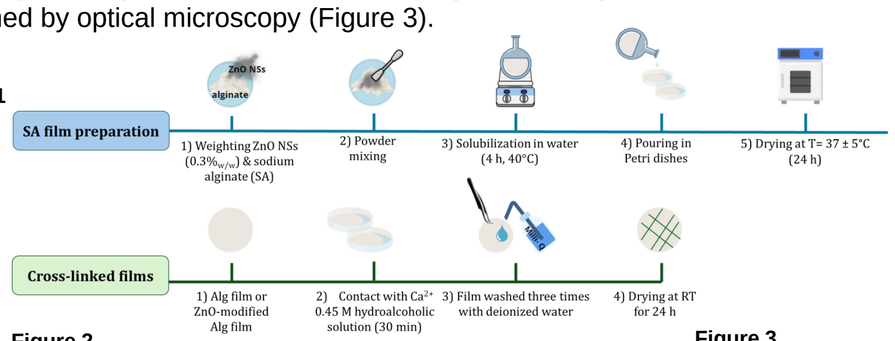


Figure 2

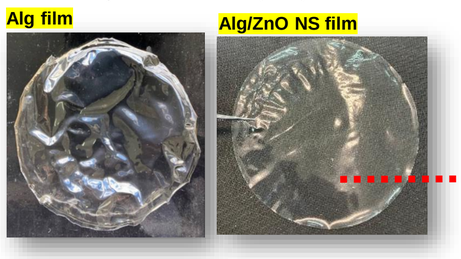
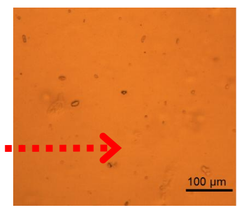


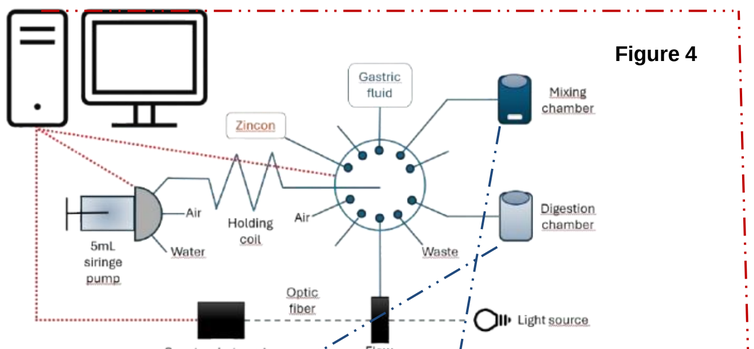
Figure 3



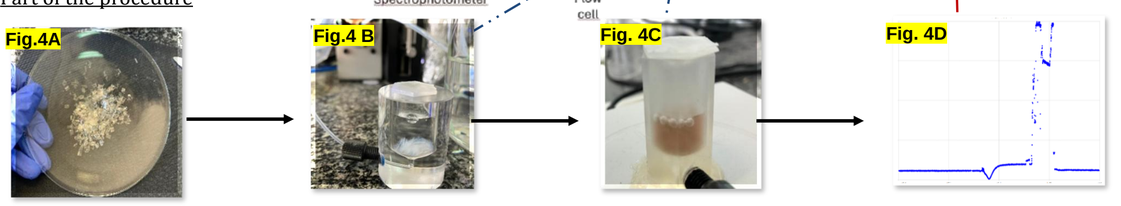
SIA Procedure

In the SIA sequential flow injection system method (Figure 4), Alg/ZnO NS films were first cut and weighed (Fig. 4a) and then placed into a digestion chamber (Figure 4b). GF at pH 1.4 was prepared with 0.26%$_{v/v}$ HCl and 0.2%$_{w/v}$ NaCl. The release of each sample was automatically evaluated 6 times (every 5 minutes over a total period of 30 minutes) by immersing approximately 50 mg of film pieces in fresh GF. To this end, 2 mL of GF was channeled into the digestion chamber and kept in contact with the film pieces for 5 minutes (Figure 4b). After that, the sample was weakly shaken using the bubbling method, at a speed of 2000 µL/min. Subsequently, a portion of this contact GF, i.e. 50 µL, was directed into a mixing chamber and mixed with 950 µL of Zincon and borate buffer (Figure 4c). This mixed solution was then aspirated from the mixing chamber and channeled into the flow cuvette, using an in-line UV-Visible spectrophotometer. At this point, it was possible to take a reading of the spectrum at 620 nm. Figure 4D shows a fiagram obtained in the first 15 minutes of contact of the film pieces with the GF.

Figure 4



Part of the procedure



1) Cutting film at 0.3% $_{w/w}$ of ZnO NSs

2) Contact of film pieces with gastric fluid in digestion chamber

3) Mixing contact GF with films and Zincon + buffer borate, in mixing chamber

4) Fiagram obtained at 620 nm

RESULTS & DISCUSSION

Online Bioaccessibility vs Offline method to evaluate the bioaccessibility of zinc

In order to evaluate the effectiveness and robustness of the SIA automation system in assessing the bioaccessibility of zinc from modified films, two methods were compared: the abovementioned online spectrophotometric method (shown in green) and an offline method (shown in orange). In the latter, the modified cut films were manually put in contact with GF and Zincon-buffer mixed solution. Figure 5 shows the average cumulative values of Zn^{2+} release (expressed as µg of Zn per g of film) calculated over the time interval between 5 and 30 minutes for both methods. The results showed that the amounts of released Zn^{2+} ions were similar for both offline and online methods. However, more dispersed values were found for the offline method, indicating a greater reliability and precision of the automation of the online method.

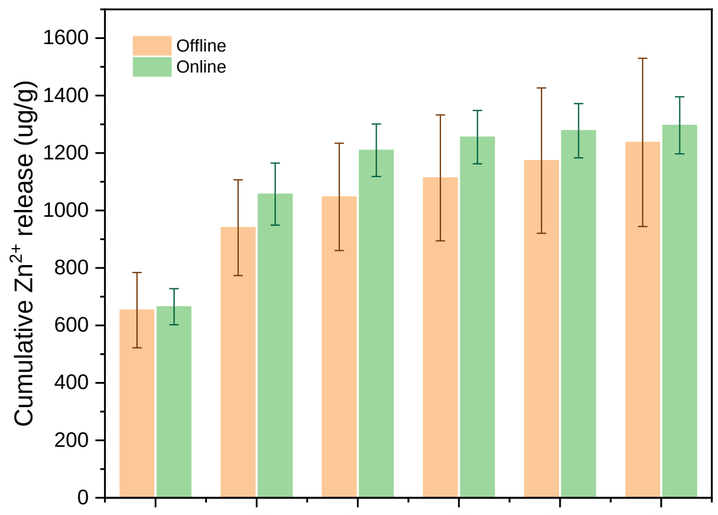


Fig.5 Comparison offline vs online spectrophotometric methods - Cumulative release of Zn^{2+} ions (µg/g).

Mass balance validation

The SIA analysis method was validated by mass balance, evaluating the total zinc content in films by ICP-OES technique. ICP-OES analyses were performed on digested samples of both fresh Alg/ZnO NSs films and films previously used in the SIA method. In the case of fresh films, the total zinc content was determined, while for the used films, the analysis provided information on the residual zinc (i.e., the amount remaining in the film and thus not released into the GF). It was shown that the total zinc determined by ICP-OES is not statistically different from the sum of the bioaccessible zinc (determined with the SIA online spectrophotometric method) and the residual zinc (Table 1, $p>0.05$).

Tab.1 Zinc concentration (µg/g) extracted/released by Alg/ZnO NSs (0.3%$_{w/w}$) and determined by different methods.

Zinc concentration (µg/g)	Total digestion (µg/g)	Online Bioaccessibility (µg/g)	Residue (µg/g)	Bioaccessibility (µg/g)+ Residue (µg/g)
Average	1828	1308	255	1563
SD	311	89	147	154

Maximum Daily Limit of Zinc

Though zinc is not considered particularly toxic, the US National Institute of Health has established a tolerable upper intake level (UL) of 40 mg per day for adults [5]. In our study, if we hypothesized a total assumption of 400 mg of Alg/ZnO NSs 0.3%$_{w/w}$ in a day, the zinc daily intake (DI), as calculated below, is 37 mg, which is less than the reported limit. However, UL is lower for children (4-12 mg, depending on age), raising additional concerns.

$$DI = \frac{Maximum\ Amount\ (mg) film\ ingested}{day} \times \frac{mg\ total\ Zn\ film}{mg\ film} \times f$$

$$f = bioaccessibility\ fraction = \frac{\frac{mg}{g} bioaccessible\ Zn\ (SIA\ method)}{\frac{mg}{g} total\ Zn (ICP - OES\ method)} \times 100$$

CONCLUSION / FUTURE WORK

The proposed dynamic extraction method, based on an automatic SIA system, provides interesting solution for the high-throughput assessment of the bioaccessibility of metal ions without the need for in vivo or in vitro cellular assays. Furthermore, it could be applied to other targets (e.g., plasticizers and antioxidant compounds) by simply modifying the analytical method.

References

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[5] Institute of Medicine. Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc Washington, DC: National Academy Press; 2001

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