Poly(β-amino ester)-Based Heat-Stable Microparticle Platform for Micronutrient Encapsulation and Delivery

The Jaklenec Group
Engineering delivery systems for global health
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1. Introduction

Background
- Globally, two billion people are suffering from micronutrient deficiencies, predominantly in low- and middle-income countries.[1]
- Dietary diversification and micronutrient supplementation are effective solutions, but these strategies are too costly to implement in the targeted regions.
- Alternatively, fortification of foods with micronutrients can be a cost-effective and globally applicable intervention to address this worldwide crisis.

Technical challenges
- Heat and moisture from common cooking and storage conditions can trigger vitamin degradation and mineral oxidation due to their intrinsic fragility.
- Protected micronutrients need to be released upon oral consumption, also bringing challenges to the delivery system design.

Solution: poly(β-amino ester)-based microparticles for micronutrient protection and delivery
- Poly(*β*-amino ester) (PAE) is used to formulate microparticles which can be mixed with common foods (e.g. bouillon) to achieve food fortification.
- This microparticle platform provides multiple hydrophobic and hydrophilic micronutrients with:
  1. individual or collective encapsulation
  2. robust protection under exposure to boiling water
  3. efficient release upon treatment of gastric fluid
- As a biodegradable polymer, PAE used as an encapsulant for microparticle formation can degrade into two natural product-based small molecules in boiling water
- Vitamin A (VA) was selected as a model micronutrient because it is thermally labile and addressing its deficiency is one of the most pressing global healthcare crises.
- Other micronutrients were also studied, including vitamin D (VD), vitamin E (VE), ferrous sulfate (Fe), and zinc sulfate (Zn).

*Microparticle is abbreviated as MP in sample labeling.

Method: Polymer

PAE synthesis
- PAE is synthesized by polymerization between isosorbide diacrylate and 4,4′-trimethylenedipiperidine (TDP).[2]

PAE degradation
- Upon boiling in water, PAE degrades into isosorbide and a *β*-amino acid.

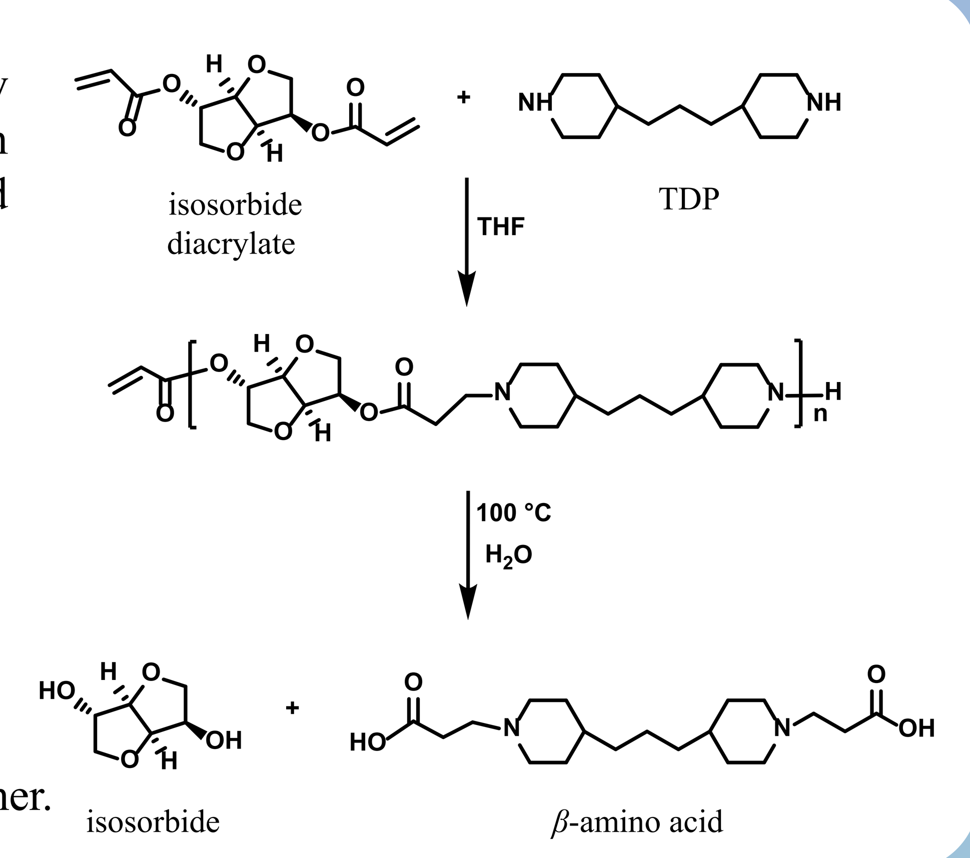

Figure 1. Synthesis and degradation of the PAE polymer.

Acknowledgment

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Method: Microparticle

Microparticle formulation
- Hydrophobic and hydrophilic micronutrients were encapsulated into microparticles by modified oil-water and water-oil-water emulsion methods, respectively.[3]
- Centrifugation and lyophilization were followed for microparticle collection.

Hydrophobic micronutrients (e.g. VA)
- VA was dissolved with PAE in dichloromethane (DCM) and then dispersed in the water phase to obtain PAE-VA MP.

Hydrophilic micronutrients (e.g. Fe)
- Fe was first dissolved with a selected excipient, dextran (Dex), in water, followed by dispersion in the mineral oil phase to form the first-step microparticle, Dex-Fe MP.
- Dex-Fe and PAE was dissolved in DCM and dispersed in the water phase, giving the second-step microparticles as the final product, PAE-Dex-Fe MP.

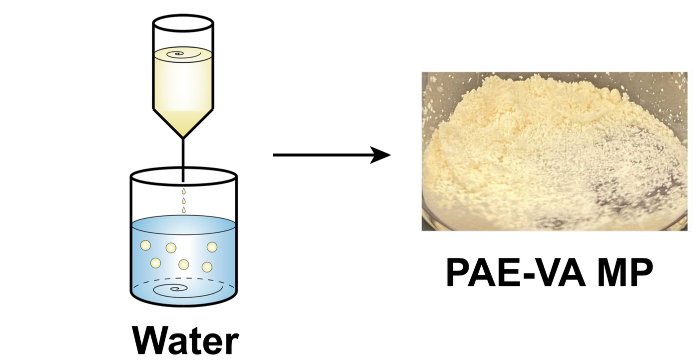
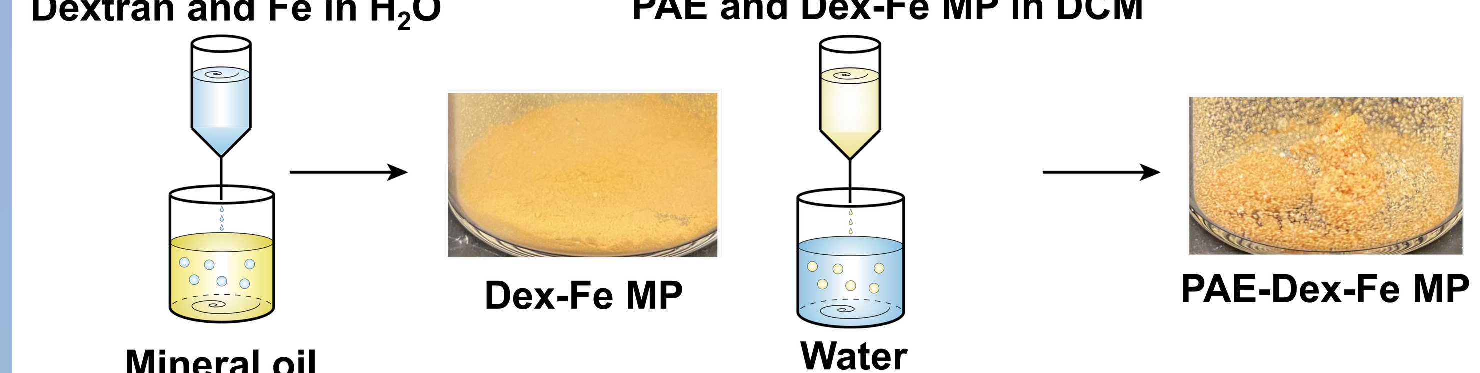


Figure 2. Encapsulation methods of hydrophobic and hydrophilic micronutrients in PAE microparticles.



Micronutrient characterization
- High-performance liquid chromatography (HPLC), inductively coupled plasma-optical emission spectrometer (ICP-OES), and colorimetric assay kits were used for micronutrient quantification.
- Two-hour boiling in water was selected as a representative stability test condition of cooking practices for its universality in target countries and adaptability to our novel microparticle platform.
- Simulated gastric fluid (SGF) was added to the microparticle samples, which were then treated under 37 °C to test micronutrient release efficiency.

Results: Microparticle

Microparticle characterization
- The final PAE microparticle products obtained from the emulsion processes were powder-like solid materials.
- The microparticles exhibited spherical, homogeneous structure with smooth surface and a size range of 100 to 200 µm, depending on encapsulated micronutrients.

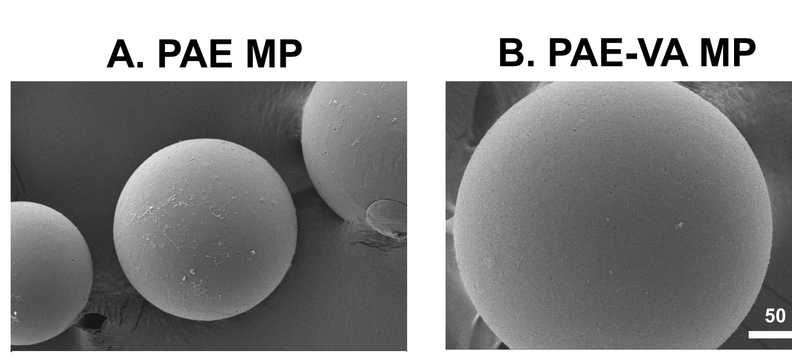


Figure 3. Scanning electron microscope (SEM) images of (A) blank PAE microparticles and (B) PAE microparticles encapsulated with VA.

Results: Micronutrient

Protection of hydrophobic micronutrients
- After exposure to boiling water for two hours, over 80% of VA and VD were recovered from individually encapsulated PAE microparticles, compared to less than 10% recovery from the free form micronutrients. The boiling stability of VE was also improved from 80% of the free from to over 90%.
- When collectively encapsulated in PAE microparticles, VD and VE showed decreased boiling stability to slightly below 80% under the same condition.
- The weakened protection, compared to the individual encapsulation, potentially resulted from increased micronutrient-to-PAE ratio.

Protection of hydrophilic micronutrients
- Two excipients, dextran (Dex) and polyvinyl alcohol (PVA) were individually used to fabricate the first-step microparticles for Fe and Zn
- Upon encapsulation by PAE to form the second-step microparticles, PAE-Dex-Fe and PAE-PVA-Zn performed the best, with a recovery of 61.26% and 101.99% after two-hour boiling in water, respectively.

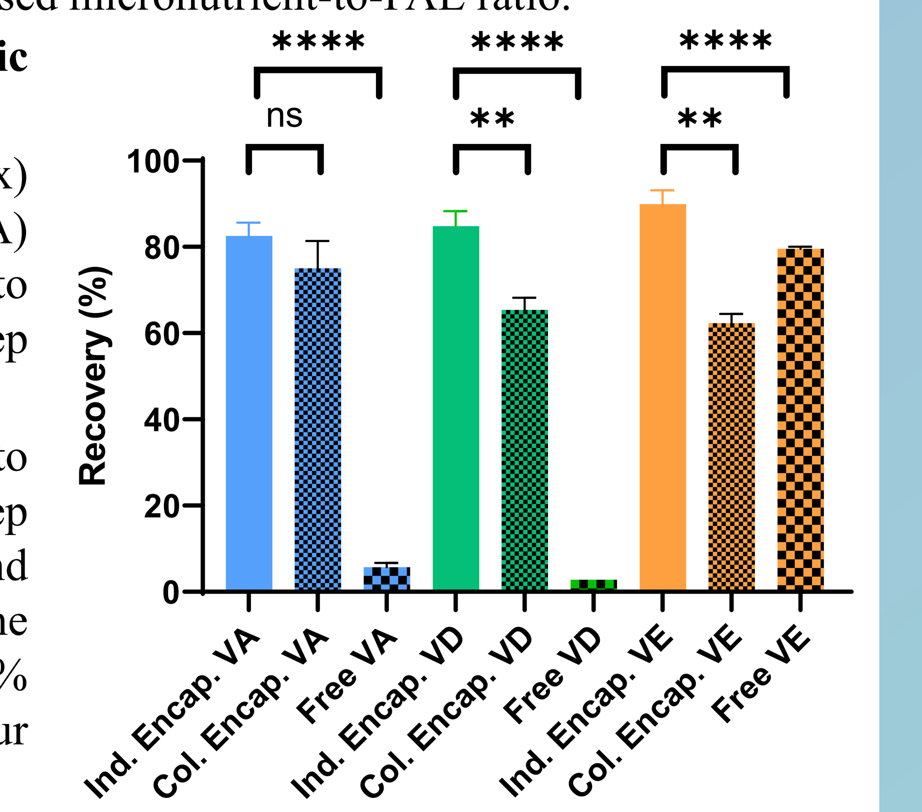


Figure 4. Boiling stability of VA, VD, and VE.

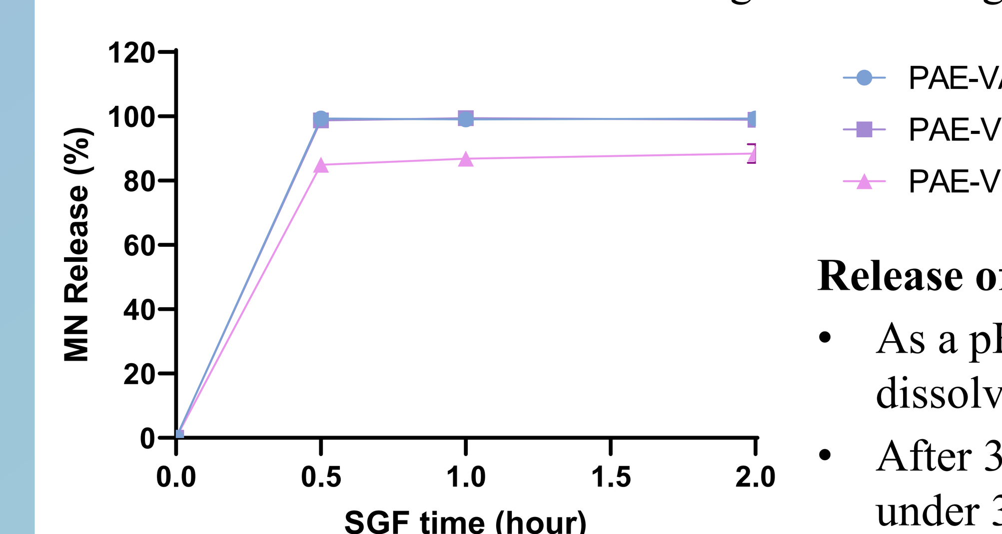


Figure 5. Release profile of VA, VD, and VE by individual encapsulation.

Release of micronutrients
- As a pH-responsive polymer, PAE dissolves in acidic aqueous solution.
- After 30-minute treatment of SGF under 37 °C, over 99% of micronutrients were efficiently released from the PAE microparticles.

Long-term stability of PAE-VA MP
- Under the accelerated storage condition at 40 °C and 75% humidity, 74.46% of VA was recovered from the PAE-VA MP sample after one month.
- Compared to a complete degradation of the free form under the same condition, PAE microparticles showed effective protection for VA.

Conclusion and Future Work

- A PAE-based MP platform was developed for micronutrient encapsulation, protection and delivery.
- Thermal stability in boiling water, accelerated storage condition, and efficient release under SGF for multiple micronutrients were achieved, presenting a promising platform for oral delivery of micronutrients.
- The next step includes (1) continue the long-term stability study, (2) expand applicability of the PAE platform to more micronutrients, and (3) conduct a mechanism study.

[1] R. L. Bailey, et al. *Ann. Nutr. Metab* (2015).
[2] D. M. Lynn, R. Langer, *J. Am. Chem. Soc.* (2000).
[3]T. Kemala, et al. *Arab. J. Chem.* (2012).