

A vegan-friendly nanotechnology approach to counteract vitamin B12 deficiency in plant-based diets

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Plant-based foods

Rapidly growing industry



\$43.77 billion (2023)

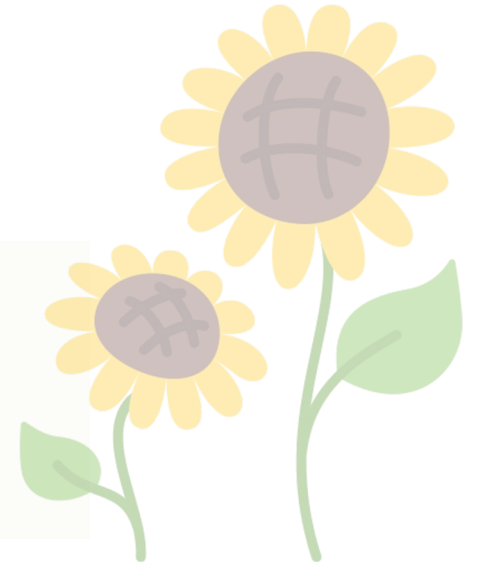
projected to reach **\$162 billion** by 2030

- ↑ Awareness of sustainability, animal welfare, and health
- ↑ Innovation in food technology

Introduction

Aims

Results





Plant-based diets

Introduction

Aims

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Significant impact on global health and sustainability



- ↓ Risk of chronic diseases, such as heart disease and diabetes
- ↑ Gut health
- ↓ Inflammation



- ↓ Greenhouse gas emissions
- ↓ Cropland, irrigation water and fertilizer



Plant-based diets

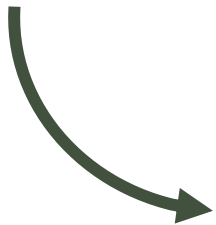
Introduction

Aims

Results



Nutritional deficiencies



Need for supplementation



Plant-based diets

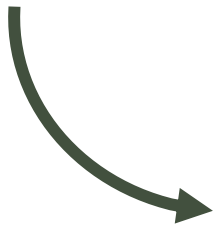
Introduction

Aims

Results



Nutritional deficiencies



Need for supplementation



Vitamin B12



Vitamin B12

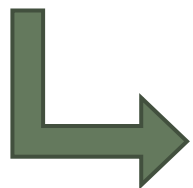
Introduction

Aims

Results

- Essential for neurologic function, red blood cell production, and DNA synthesis
- Absent in most of plant-derived foods

Deficiencies increase the risk of several neuro, vascular, immune, and inflammatory disorders



Need for supplementation or consume of fortified foods

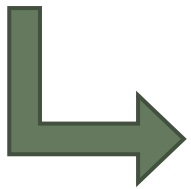
Vitamin B12-fortified foods

Introduction

Aims

Results

- Limited bioavailability
- Low stability under different pH and temperature conditions
- Insufficient dosage to guarantee daily needs
- Food processing may lead to vitamin B12 losses



Need to produce vitamin B12-fortified foods with improved stability and nutritional value



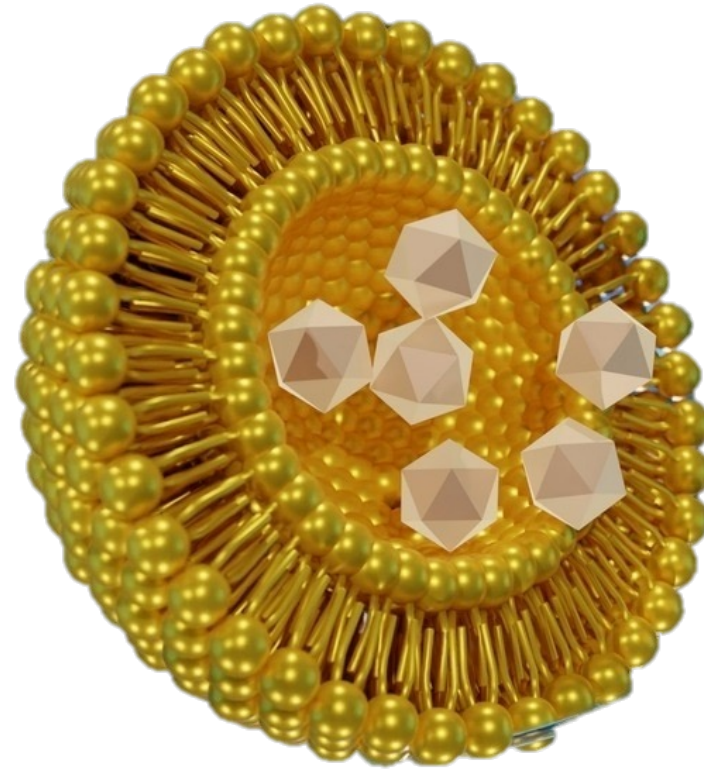
Nanoencapsulation

Introduction

Aims

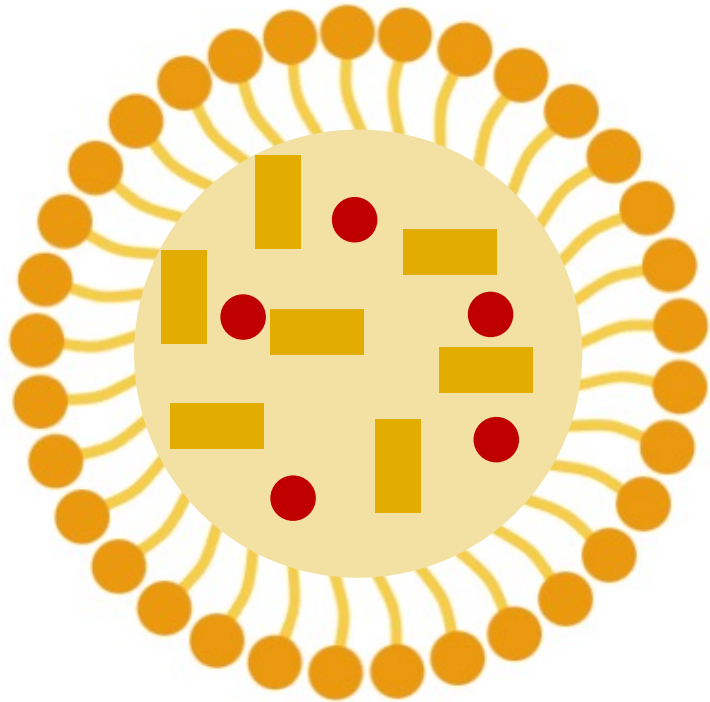
Results

- Improved stability and shelf-life
- Enhanced oral bioavailability
- Controlled and targeted delivery
- Reduced side effects
- Improved sensory properties





Strategy



**Design of a vegan-friendly
nanoformulation based on
nanostructured lipid carriers
(NLC) for vitamin B12
encapsulation**

Introduction

Aims

Results

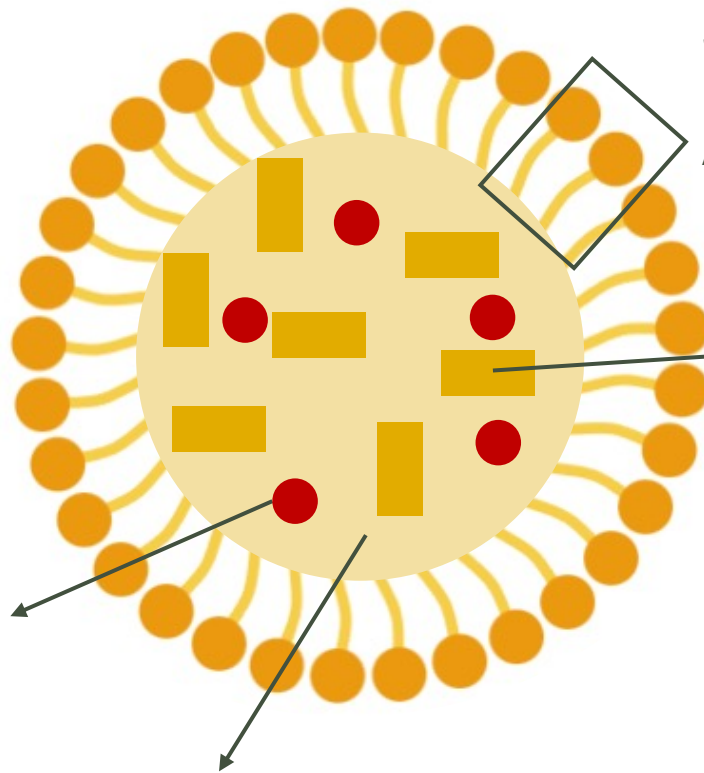


Strategy

Introduction

Aims

Results



Surfactant: Soy lecithin
Plant-based
Adequate emulsifying properties

Solid lipid: Compritol
High melting point and stability
Adequate for oral administration

Vitamin B12

Liquid lipid: extracted from *Opuntia ficus-indica* seed oil
High antioxidant activity
Nutritional value: rich in linoleic and palmitic acids
Valorization of a natural resource waste



universidade
de aveiro

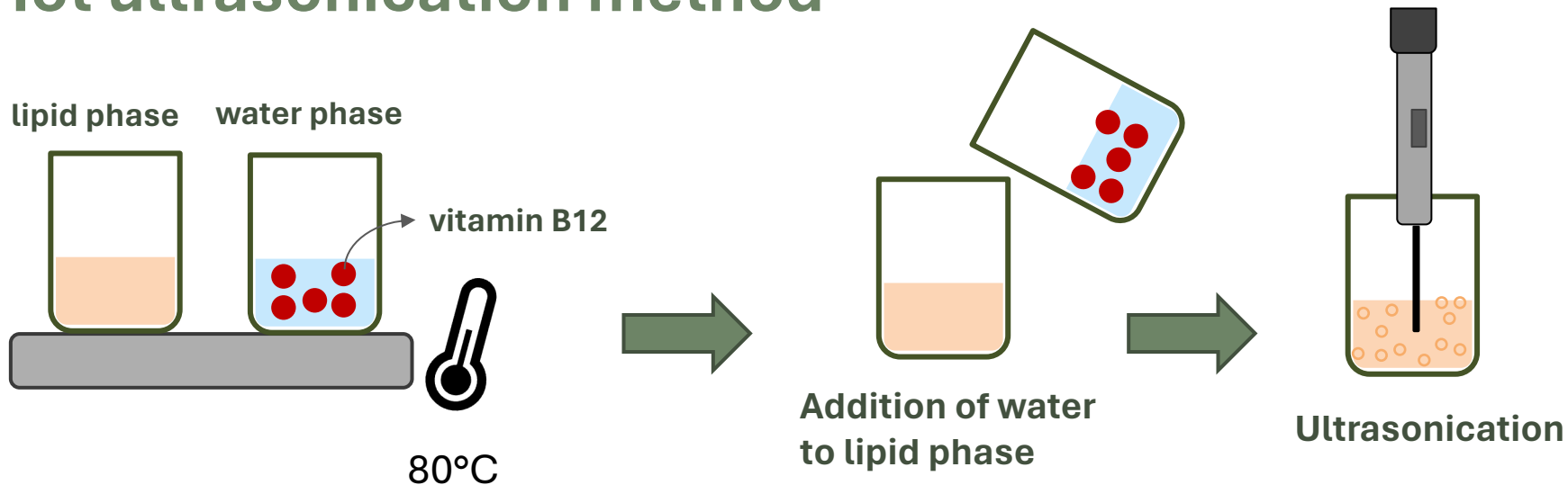
Nanoparticles production

Introduction

Aims

Results

Hot ultrasonication method



- ✓ Simple method
- ✓ Low cost manufacture
- ✓ Potential for scale-up



Physicochemical characterization

Introduction

Aims

Results

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Encapsulation efficiency (%)
SLN (w/o oil)	1330 ± 84	0.379 ± 0.03	-53 ± 2	-
NLC	212 ± 3	0.137 ± 0.00	-44 ± 1	-
NLC Vit B12	332 ± 7	0.206 ± 0.00	-41 ± 1	53 ± 2



Physicochemical characterization

Introduction

Aims

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✓ Opuntia ficus-indica seed oil incorporation significantly improves nanoparticle size and PDI



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Introduction

Aims

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✓ Suitable size for oral administration and intestinal absorption



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Aims

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- ✓ Highly negative zeta potential, suggesting high particle stability
- ✓ More than 50% of encapsulation efficiency



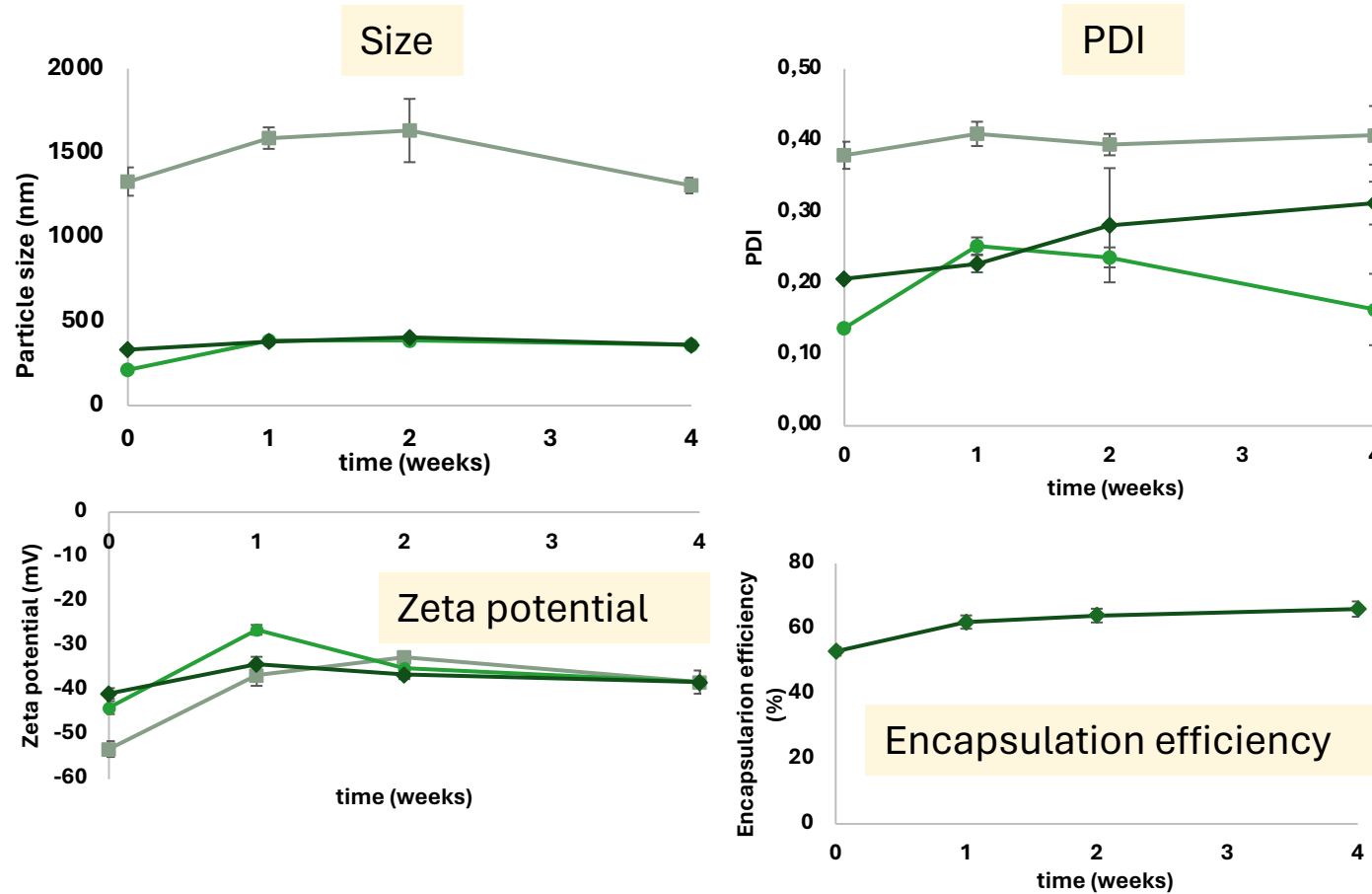
Storage stability

Introduction

Aims

Results

- SLN
- NLC
- NLC Vit B12



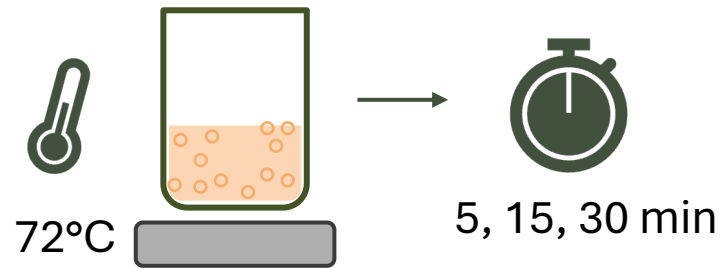
Nanoparticles remained stable over 4 weeks

Resistance to thermal treatments

Introduction

Aims

Results



Treatment time (min)	Particle Size (nm)	PDI	Zeta Potential (mV)	Encapsulation efficiency (%)
5	357 ± 19	0.180 ± 0.04	-38 ± 1	61 ± 2
15	369 ± 4	0.188 ± 0.05	-40 ± 4	60 ± 1
30	374 ± 13	0.191 ± 0.05	-40 ± 3	60 ± 1

Nanoparticles exhibited high thermal stability upon exposure to thermal treatments typically used in the food industry

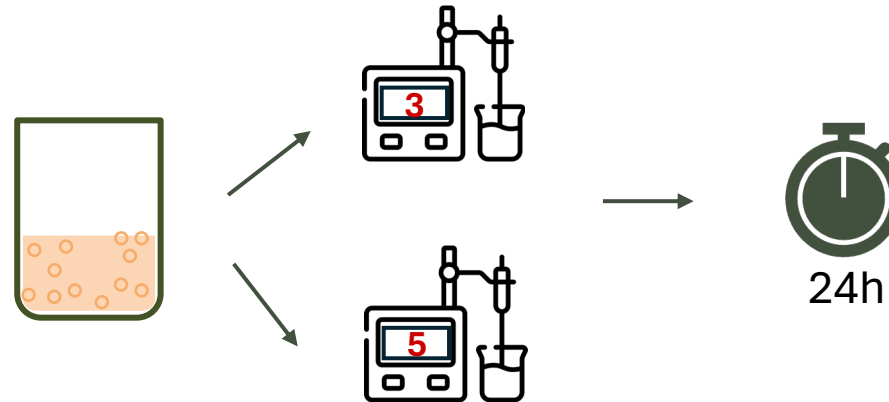


Resistance to acidic pH

Introduction

Aims

Results



pH	Particle Size (nm)	Polydispersity	Zeta Potential (mV)	Encapsulation efficiency (%)
3	346 ± 21	0.203 ± 0.004	-30.04 ± 9	51 ± 2
5	358 ± 9	0.191 ± 0.003	-38.04 ± 3	57 ± 1

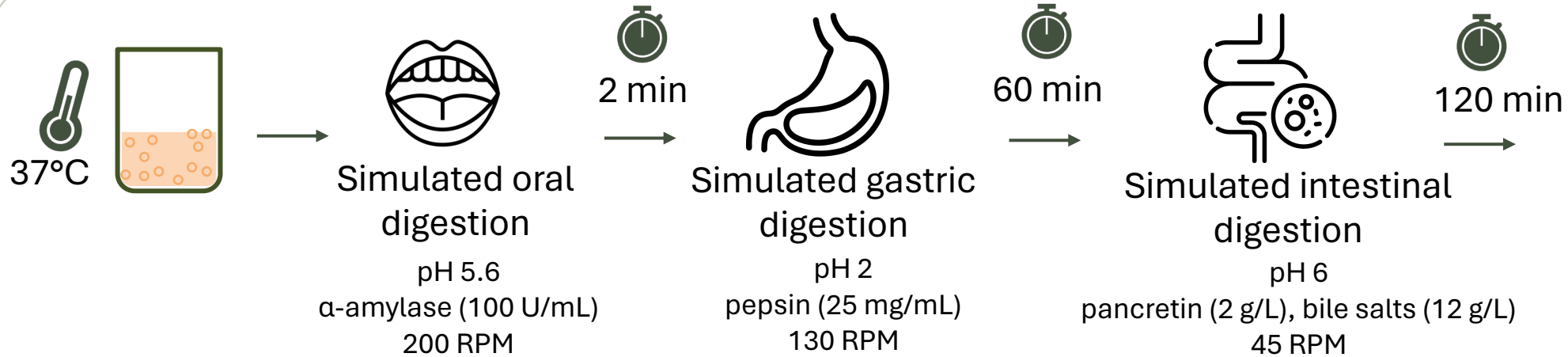
NLC display robust stability profile under acidic pH conditions, indicating their suitability for food product applications

Resistance to simulated gastrointestinal digestion

Introduction

Aims

Results

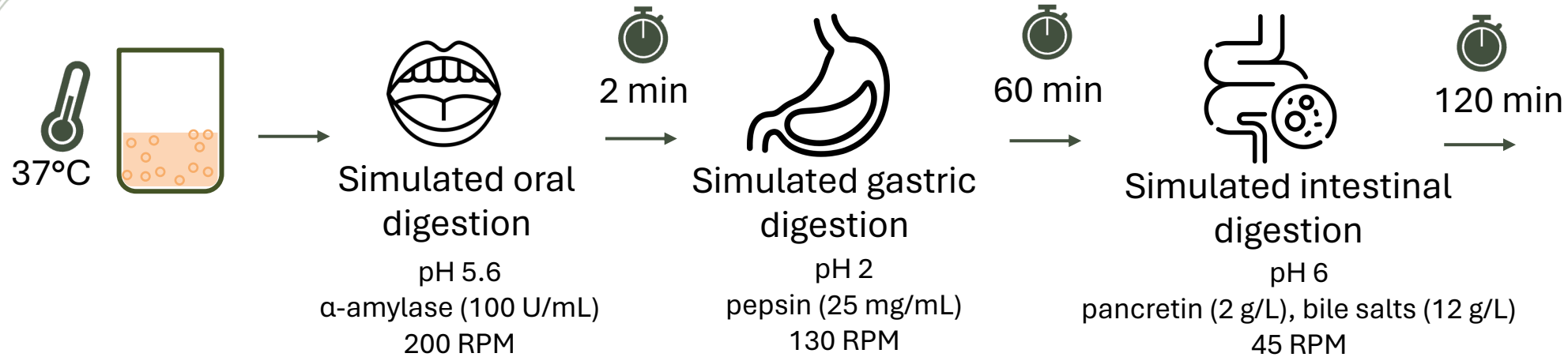


Resistance to simulated gastrointestinal digestion

Introduction

Aims

Results



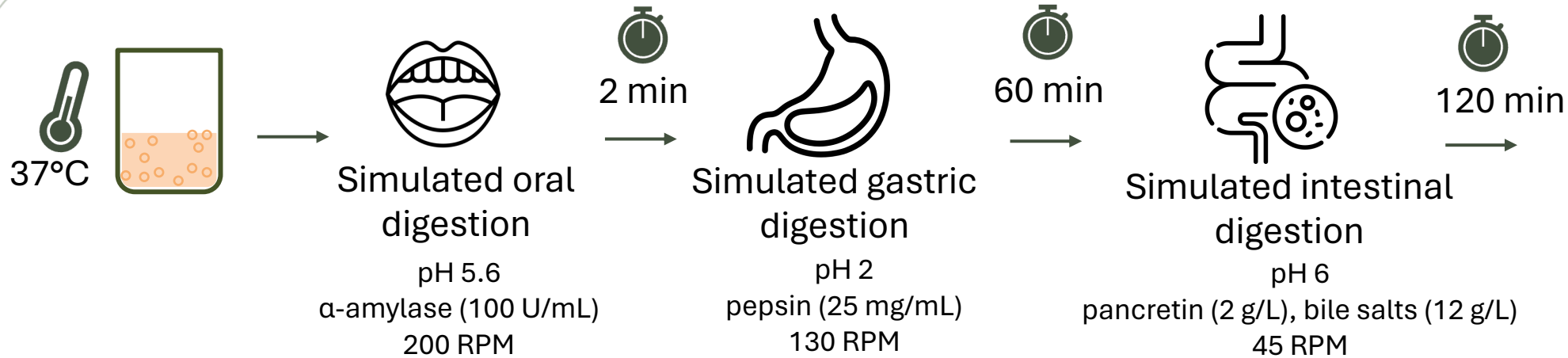
	Particle Size (nm)	PDI	Zeta Potential (mV)	Free Vitamin B12 (%)
Initial solution	389 ± 7	0.174 ± 0.032	-38 ± 1	48 ± 3
During gastric digestion	397 ± 4	0.193 ± 0.061	-37 ± 3	62 ± 1
During intestinal digestion	461 ± 2	0.211 ± 0.052	-30 ± 4	73 ± 2
After digestion	502 ± 17	0.371 ± 0.039	-21 ± 2	82 ± 2

Resistance to simulated gastrointestinal digestion

Introduction

Aims

Results



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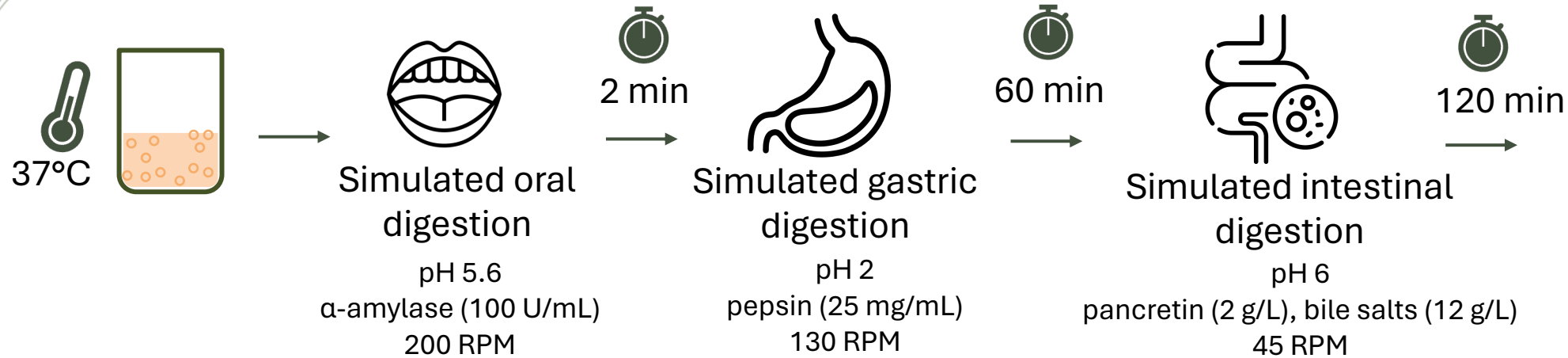
Nanoparticles were able to resist the harsh conditions of the oral and gastric digestion with only a limited amount of vitamin B12 release

Resistance to simulated gastrointestinal digestion

Introduction

Aims

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Nanoparticle aggregation occurred under intestinal digestion with partial release of vitamin B12

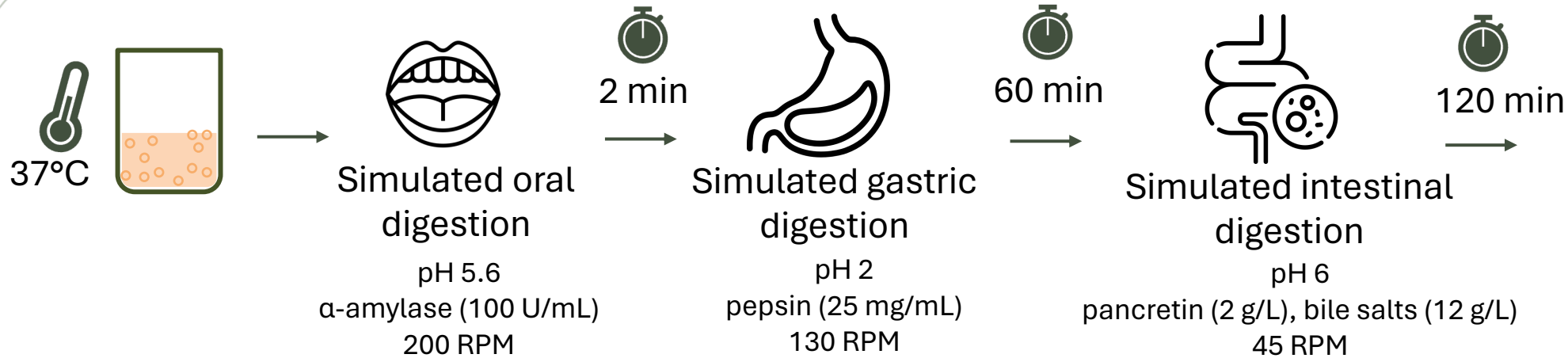
Free vitamin B12 can be absorbed in the intestine

Resistance to simulated gastrointestinal digestion

Introduction

Aims

Results



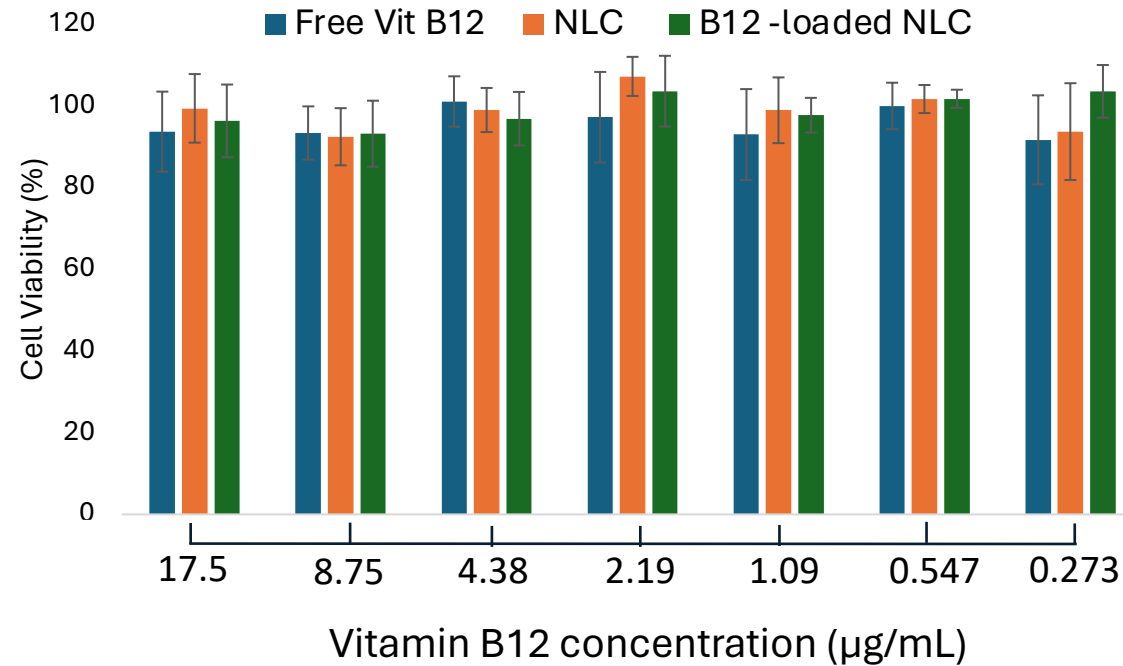
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~20 % of the vitamin B12 remained in the NLC after digestion, which can be absorbed in the intestine



Cytocompatibility

L929 cell line
(ISO 10993-5)



Nanoparticles were cytocompatible up to **17.5 µg/mL** of Vitamin B12

Above the recommended daily dose of **2.4 µg** per day

Introduction

Aims

Results



Conclusions



Vegan-friendly vitamin B12- loaded NLC were rationally designed:

- ✓ Adequate characteristics for oral administration
- ✓ Resistance to thermal treatments and acidic pH conditions
- ✓ Resistance to simulated oral and gastric digestion
- ✓ After gastrointestinal digestion, 20% of vit B12 remained entrapped
- ✓ Cytocompatibility up to 17.5 $\mu\text{g}/\text{mL}$ of Vitamin B12

Conclusions



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 - ✓ Cytocompatibility up to 17.5 $\mu\text{g}/\text{mL}$ of Vitamin B12

Potential for the production of improved vitamin B12-fortified foods

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10.54499/UIDP/ 50006/2020 and UIDB/50006/2020 DOI
10.54499/UIDB/50006/ 2020)

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**Thank you
for listening!**

