Development of inhalable carriers for in-situ production of short-lived natural antibiotics

Ondřej Kašpar*, Lucie Mašková, Mária Kalina, Karolína Slonková, Denisa Lizoňová, and Viola Tokárová

University of Chemistry and Technology, Prague, Department of Chemical Engineering
Technická 3, Prague 6, 166 28, Czech Republic,
Laboratory of Biomimetic Engineering: biomimetic-lab.vscht.cz
*e-mail: kasparo@vscht.cz

Laboratory of Biomimetic Engineering

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Introduction

Garlic (*Allium sativum*) is well-known for its antibacterial, antiviral, and antifungal properties, making it one of the most extensively researched plants.

Today, a wide variety of garlic-based products are available that claim to offer numerous health benefits, such as lowering the risk of cardiovascular disease, reducing blood pressure, and combating multidrug-resistant bacteria. However, the effectiveness of these products is often debated, primarily because the most active compound, allicin, is highly unstable and unsuitable for long-term storage.

Thus, the next logical step is to emulate the internal architecture of garlic cells and keep the precursors- that is, alliin and the enzyme alliinase- separated until highly effective allicin is needed. With this in mind, we aim to present some of the strategies we have developed over the years that focus on the encapsulation of stabilised alliinase and alliin. Our work has resulted in a diverse range of materials suitable for inhalation or topical application, including planar films[1], microparticles[2,3], and three-dimensional printed porous hydrogels. Both the antibacterial potential and cytotoxicity of the generated allicin were tested on relevant cell lines.

The main concept

Enzyme - E (Alliinase)

- presented in exceptionally high amounts
- more than 10% of all soluble proteins
- extraction from crude extract by fractional precipitation

Extraction → Encapsulation

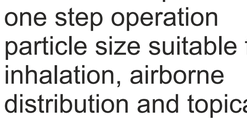
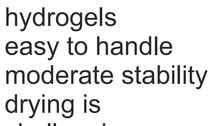
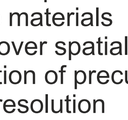
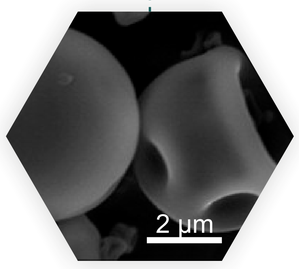
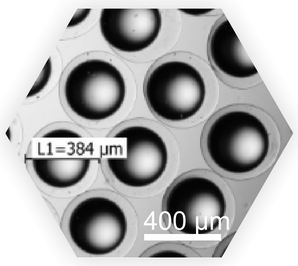
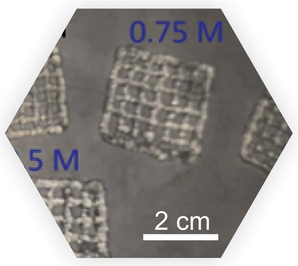
Substrate - S (Alliin)

- stable, crystalline substance
- synthesis by two-step oxidation from L-cysteine and allyl chloride

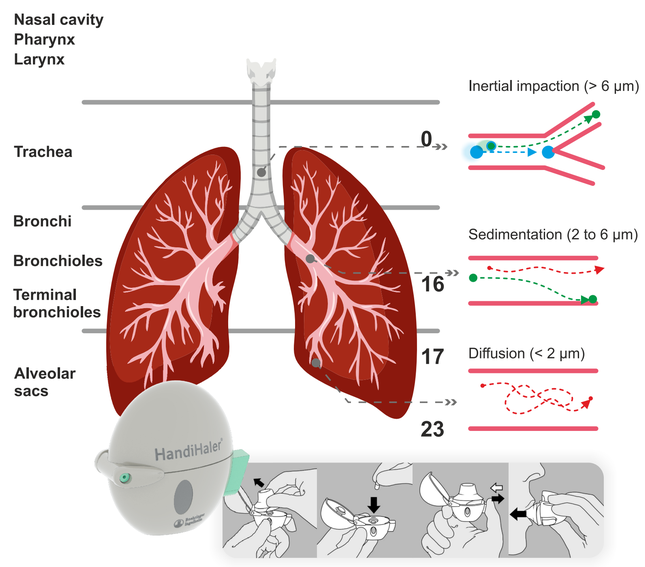
Various strategies

Multi-component carriers

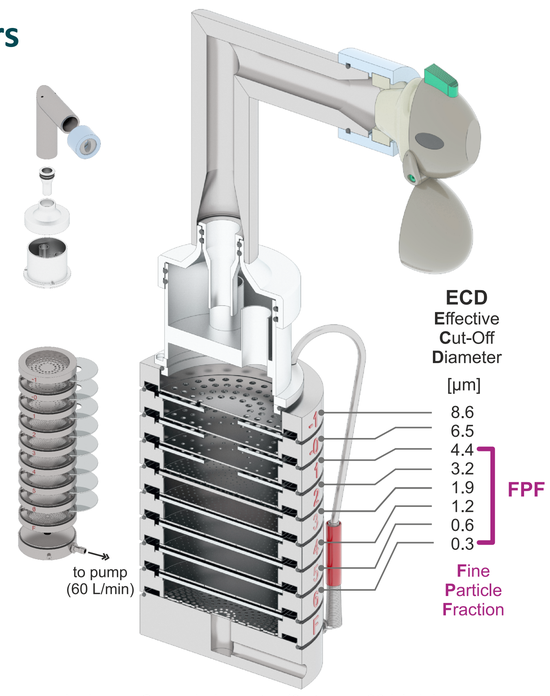
Particles (microparticles/beads) | **Macroscopic structures**

	Dry powder	Hydrogel beads	Layered material
structure			
	size 1 - 10 μm	100 - 1000 μm	> 1 mm
technology	Spray drying	Encapsulation	3D bioprinting
			
product			
	• very quick process • suitable for heat-sensitive compounds • one step operation • particle size suitable for inhalation, airborne distribution and topical use	• matrix or core-shell structure • limited to ionic hydrogels • easy to handle • moderate stability • drying is challenging	• well-defined structure based on CAD model • possible to print from multiple materials • control over spatial distribution of precursors • limited resolution

Inhalable powders

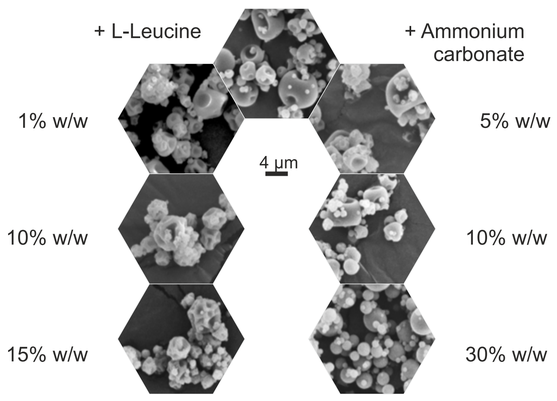


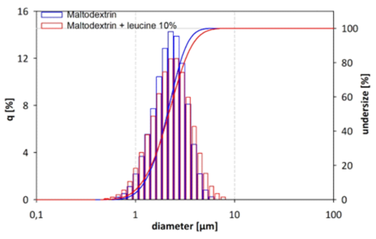
DPI - dry powder inhaler HandiHaler® [4]



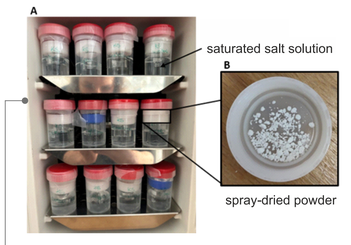
Andersen Cascade Impactor (8 stages, 60 L/min)

Maltodextrin (carrier material)



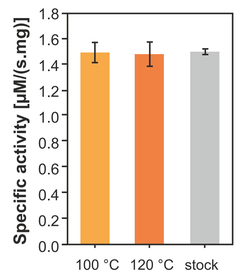


Typical PSD of spray-dried particles



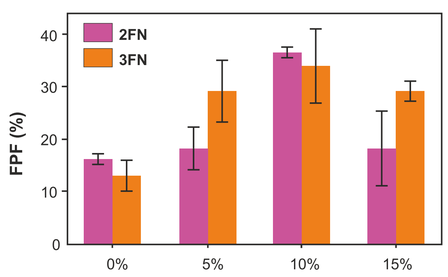
Effect of relative humidity

Effect of drying temperature

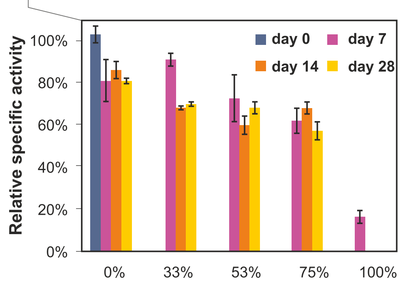


Drying temperature

FPF vs L-Leucine content



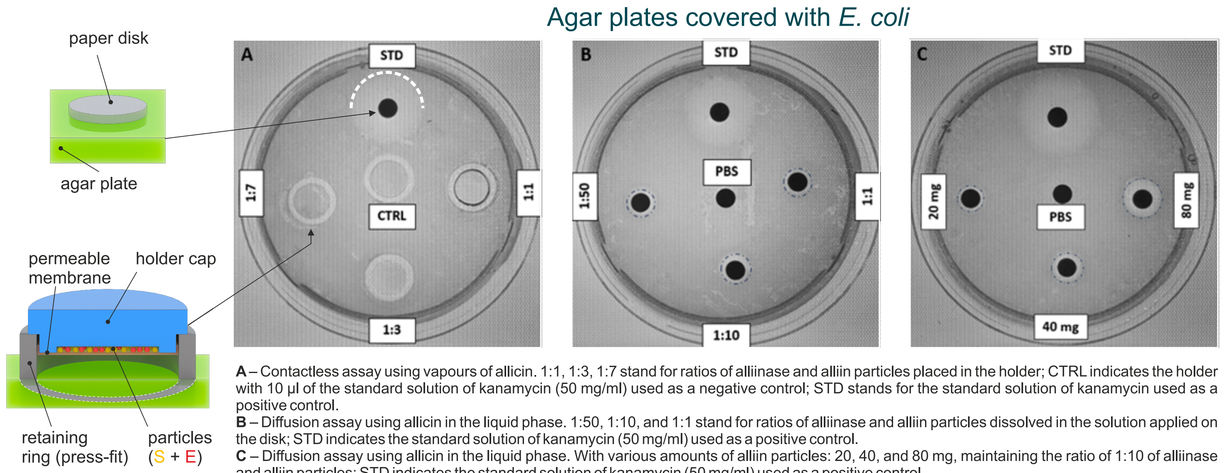
L-Leucine content % (w/w)



Relative humidity

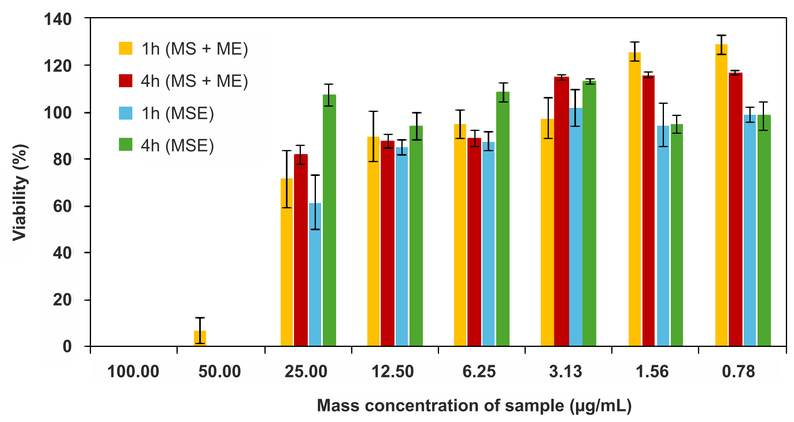
Antibacterial activity and cytotoxicity

Agar plates covered with *E. coli*



A – Contactless assay using vapours of allicin. 1:1, 1:3, 1:7 stand for ratios of alliinase and alliin particles placed in the holder; CTRL indicates the holder with 10 μl of the standard solution of kanamycin (50 mg/ml) used as a negative control; STD stands for the standard solution of kanamycin used as a positive control.
B – Diffusion assay using allicin in the liquid phase. 1:50, 1:10, and 1:1 stand for ratios of alliinase and alliin particles dissolved in the solution applied on the disk; STD indicates the standard solution of kanamycin (50 mg/ml) used as a positive control.
C – Diffusion assay using allicin in the liquid phase. With various amounts of alliin particles: 20, 40, and 80 mg, maintaining the ratio of 1:10 of alliinase and alliin particles; STD indicates the standard solution of kanamycin (50 mg/ml) used as a positive control.

A549 - adenocarcinomic human alveolar basal epithelial cells



Viability of A549 cells after exposure to in situ-formed allicin generated from spray-dried powders prepared using either a two-phase or a three-phase nozzle.

The powders were dissolved in culture medium and applied at estimated allicin concentrations ranging from 0.78 to 100 μg/mL.

Exposure times were either 1 hour or 4 hours, followed by incubation until 24 hours from sample addition.

Yellow and red bars represent the two-phase nozzle samples (MS + ME) after 1 hour and 4 hours of exposure, respectively. Blue and green bars represent the three-phase nozzle samples (MSE) after 1 hour and 4 hours of exposure, respectively.

The x-axis shows the estimated allicin concentration, calculated assuming complete enzymatic conversion of alliin.

The y-axis represents cell viability, expressed as a percentage of the untreated control. Data are presented as mean ± standard deviation based on six replicates per condition.

Acknowledgement and references

[1] Mašková, L., Závišová, L., Kašpar, O., Knejzlík, Z., Rimpelová, S. Tokárová, V. - Design and evaluation of composite films for in situ synthesis and antibacterial activity of allicin vapour. Journal of Materials Science (2024). doi:10.1007/s10853-024-09990-x
[2] Večerková, L., Mašková, L., Knejzlík, Z., Kašpar, O., Tokárová, V. - Development of spray-dried powder hand sanitiser with prolonged effectivity. Scientific Reports 14, 4827 (2024). doi:10.1038/s41598-024-55503-w.
[3] Mašková, L., Janská, P., Klimša, V., Knejzlík, Z., Tokárová, V., & Kašpar, O. - Development of compartmentalized antibacterial systems based on encapsulated alliinase. Advanced Powder Technology (2021). doi:10.1016/j.apt.2021.05.045.
[4] https://www.medsinfo.com.au/media/byispihh

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The artwork was inspired by Kaurna, Narungga, Ngarrindjeri artist Lawson 'Wukawe' Dodd for the MicroTAS conference 2025.