Yeast Biocapsules: More than just a carrier for food flavours

Craig Duckham

CD R&D Consultancy Services Ltd

www.cdrnd.co.uk

Innovations in Encapsulation

John McIntyre Conference Centre, Edinburgh 10th June 2016

INTRODUCTION

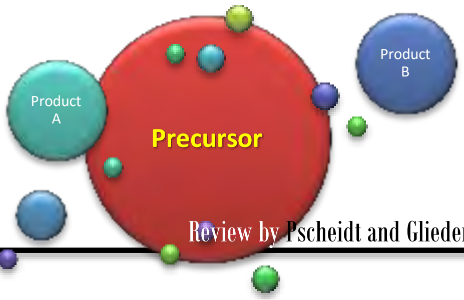
- What has yeast ever done for us?
- Properties of yeast
- Target molecules – process improvements
- Future prospects

What Has Yeast Ever Done For Us?

- Over 5000 years ago yeast was used in fermentation for wine and bread-making

- Pure yeast used in brewing from 1883 by Carlsberg's Emil Hansen

- Yeast cell factories



Review by Pscheidt and Glieder 2008

Good Yeast vs Bad Yeast

- Baker's and Brewer's yeast
Saccharomyces cerevisiae or
S. pastorianus (aka *S. carlsbergensis*)

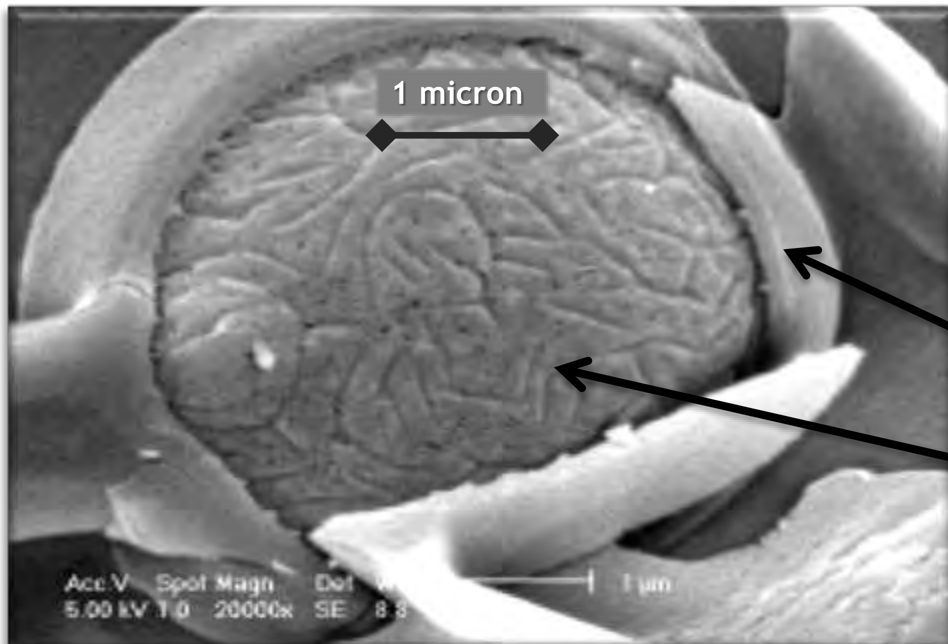
- Food and drink spoilage caused by "wild yeast" and moulds.

- Yeast Infections (Candidiasis)

James Heilman, MD

Biocapsules

- Yeast cells as natural capsules for functional ingredients

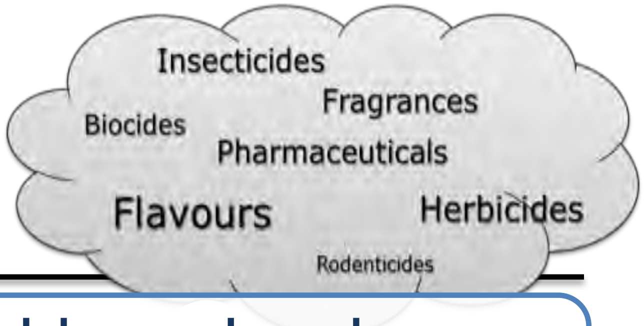


Freeze-fracture cryo-SEM of active baker's yeast *(Saccharomyces cerevisiae)*

Why Use Yeast As An Inert Carrier?

- Pre-formed microcapsules
- Produces readily dispersible dry powders and granules
  - Spray drying or spray agglomeration
- Contents are protected by a robust cell wall
  - Products are amenable to blending, extrusion and high heat processing
- Ideal for small fat-soluble molecules (hydrophobes)
- Yeast is a readily available natural raw material, a commodity product with a consistent supply

History – Process NPD



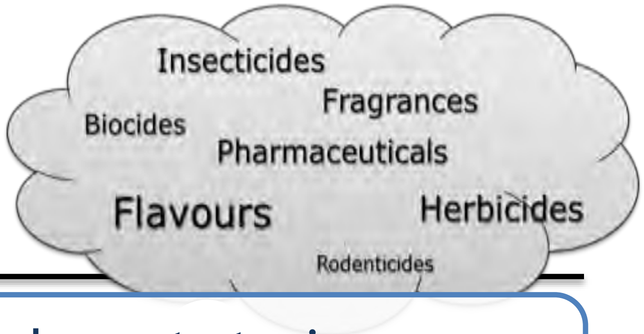
1973
- Yeast used to absorb water soluble molecules
- Serozym Laboratories French patent 2179528

1977
- High lipid strains used for trapping hydrophobes
- Swift and Company US patent 40001480

1983
- Use of co-solvents to aid uptake
- Dunlop Ltd Patent EP 0085805

1986
- Encapsulation of pesticides
- Dunlop Ltd Patent GB 2162147

History – Process NPD



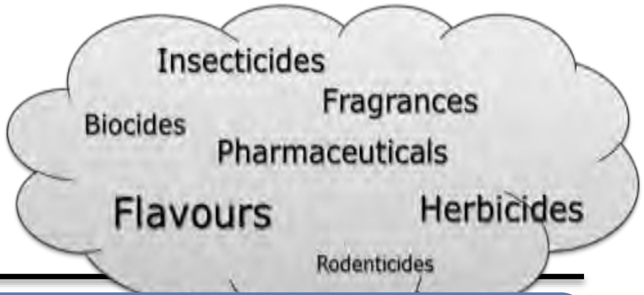
1987
- Aqueous mixing process using standard yeast strains
- AD2 Patent EP 0242135

1991
- Yeast biocapsules applied to textiles
- BTTG Patent EP 0511258

1991
- Process improvements in Japan
- Mitsubishi Paper Mills/Kirin Brewery EP0453316/EP0460945

1991
- Yeast biocapsules used in pesticide formulations
- Welcome Foundation Ltd Patent WO2005030383

History – NPD



1993
- Process for de-odourising yeast biocapsules for laundry use
- Procter & Gamble Patent WO1993011869

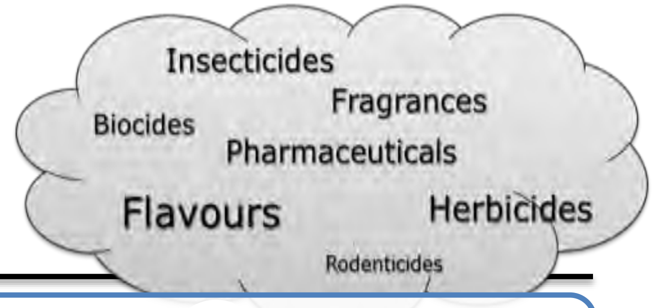
1994
- Flavour containing microbes for tobacco materials
- Quest International Patent WO9409653

1998
- Passive diffusive uptake process characterised
- Bishop, *et al*. J. Microencapsulation 15, 761

2000
- Use in cosmetics
- Ciba Speciality Chemicals Patent EP 00810021

2000
- Use as a nicotine carrier for smoking cessation products
- Micap Ltd Patent EP 1176961

History – NPD



2004
- Use as a food flavour carrier adopted by Firmenich and launched as Thermarome® | Various patents followed

2005
- Baker's yeast cell walls for agricultural use of terpenes
- Eden Research Plc Patent WO 2005113128

2005
- Herbicidal and antimicrobial compositions
- Micap plc Patents WO 2005102045 and WO 2005104842

2005
- Impact of water in release mechanism
- Normand, et al., J. Agric Food Chem. 53, 7532

2005
- Aerosol based cleaning products
- Reckitt Benkiser (UK) Ltd Patent WO2005030383

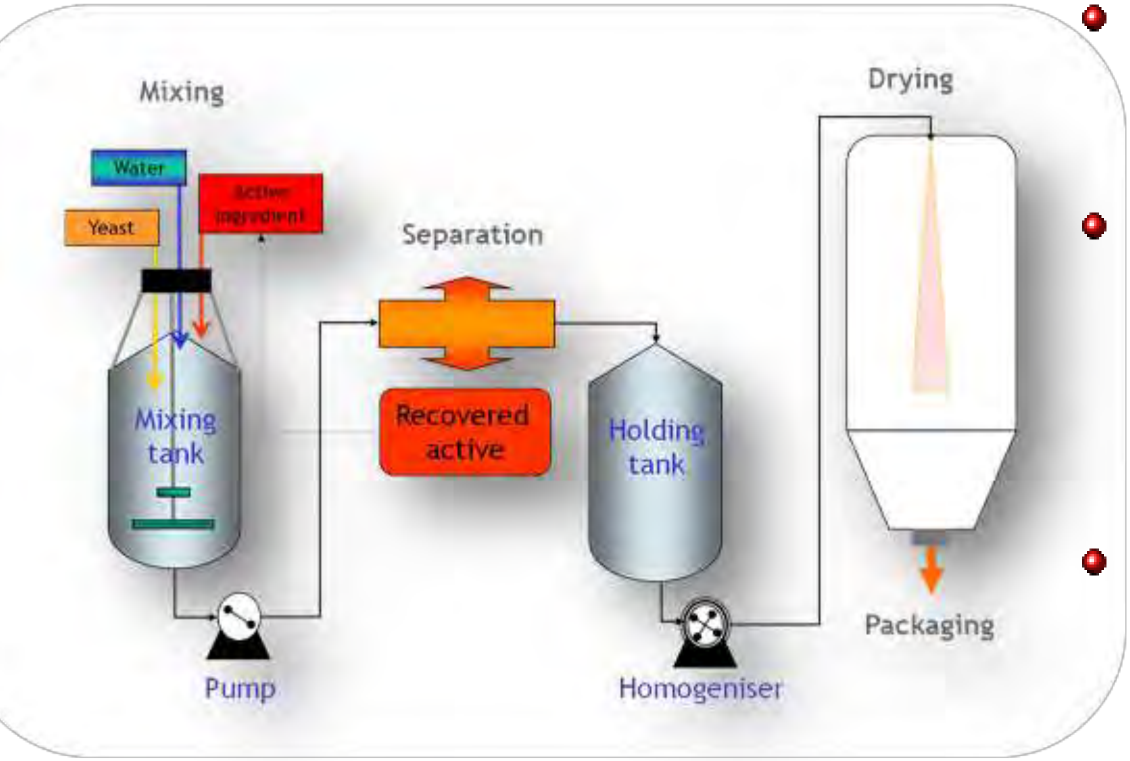
Target Ingredients

- Flavours and tastes
  - Flavour and aroma
  - Cooling agents
  - Health and wellness ingredients
- Agriculture
  - Fungicides
  - Insecticides and herbicides
  - Semiochemicals/attractants
- Healthcare
  - Pharmaceutical – APIs & Paracellular drug delivery
  - Repellents
  - Wound care, sanitizers, cleaning products

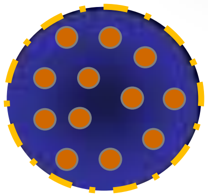
Same Old Challenges

- Convert liquids to solids – Improve handling
- Targeted delivery – Improve impact or bioavailability
- Controlled & delayed release – Process stability
- Masking taste and odour – bitter plant extracts and volatiles
- Protection for sensitive ingredients against
  - UV
  - Heat
  - Moisture
  - Oxidation
- Isolation of reactive components
- Stabilisation of volatile ingredients to improve shelf life
  - Flavours and fragrances

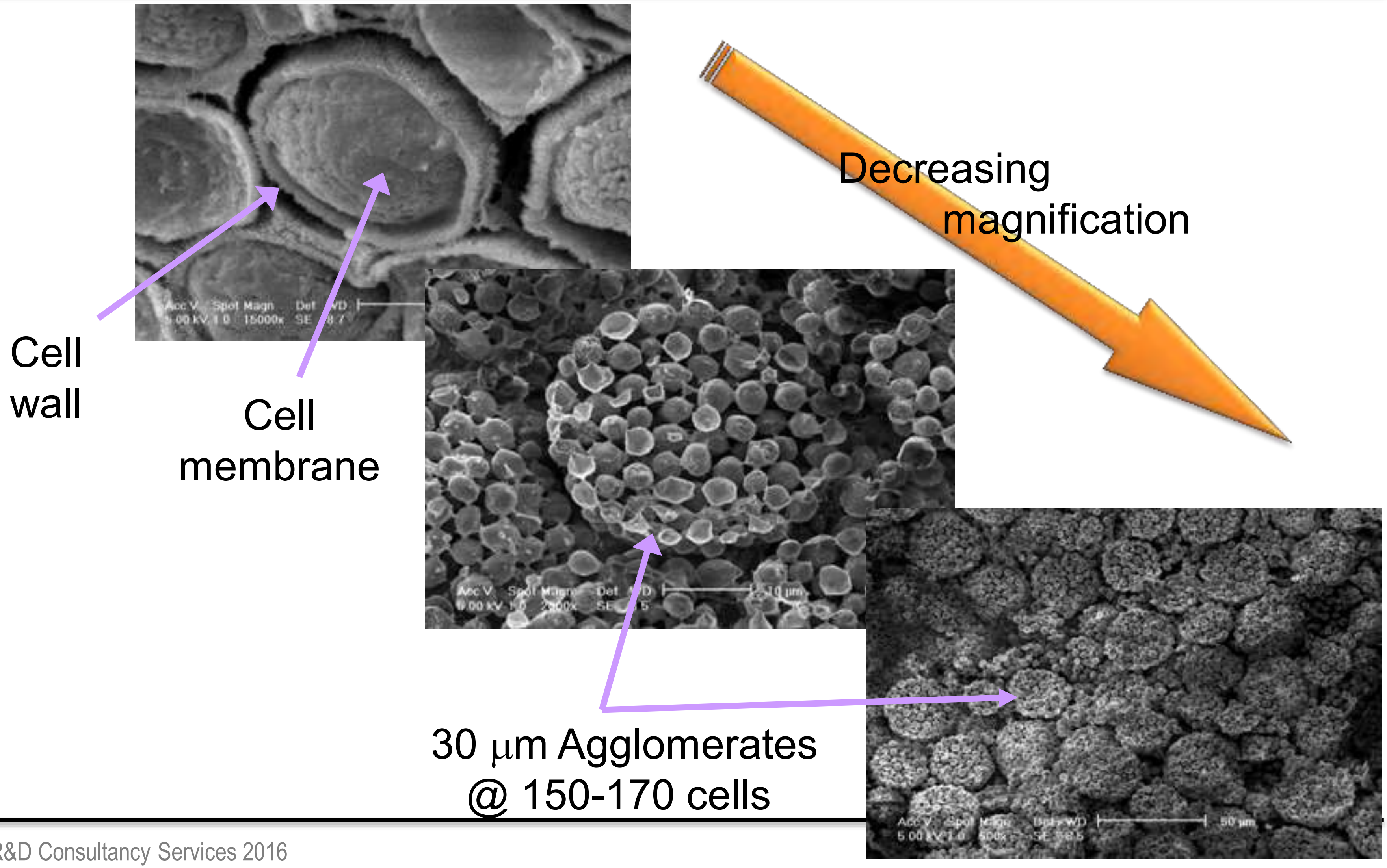
Production - Spray Drying



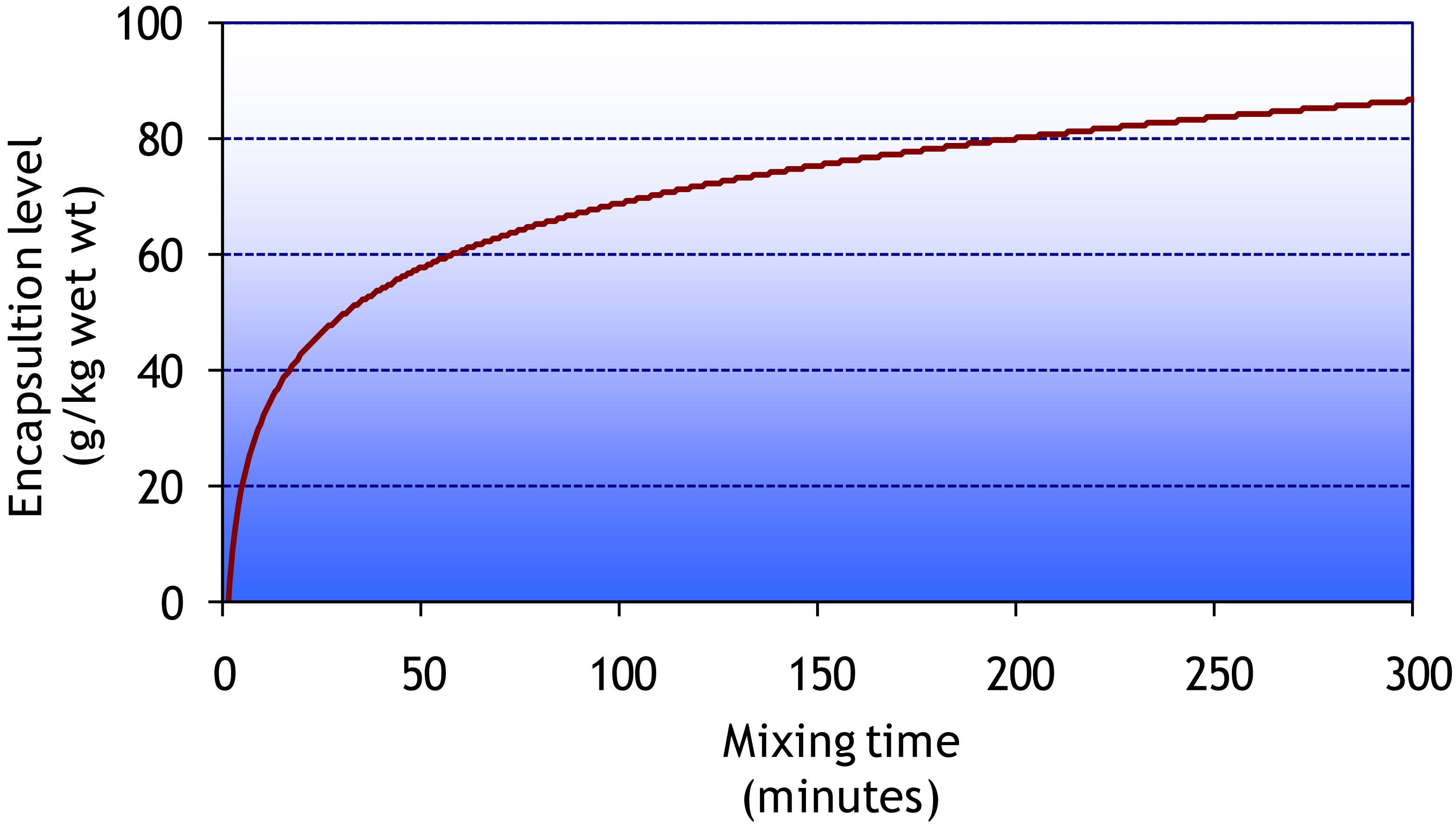
- Emulsifiable liquids such as essential oils and liquid flavours
- Typically water or oil soluble principal components are typically mixed with a matrix material e.g. maltodextrin and gums or **<u>yeast</u>**
- A two-fluid nozzle disperses the liquid into fine droplets, water evaporates in the drying chamber forming dry particles



Production - Spray Drying



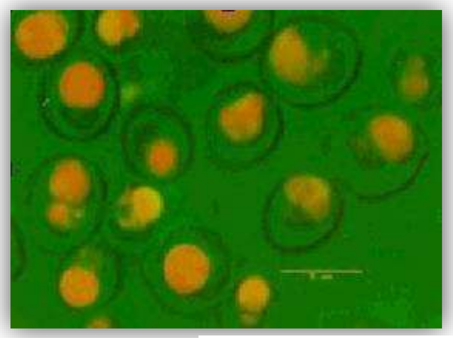
Uptake Profile Of Tea Tree Oil



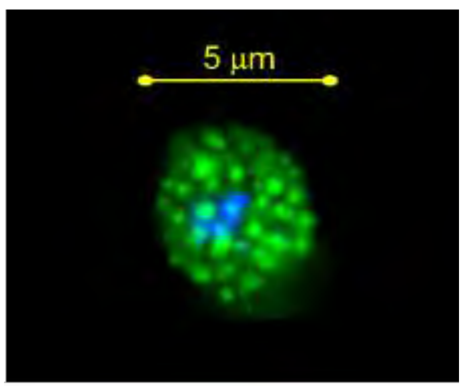
When yeast was used to encapsulate antimicrobial Tea Tree oil the initial rate of uptake was rapid over the first 30 minutes and approached a plateau after 5 hours

Duckham *et al*. Proc. of 14th International Symposium on Microencapsulation, September 4-6 2003.

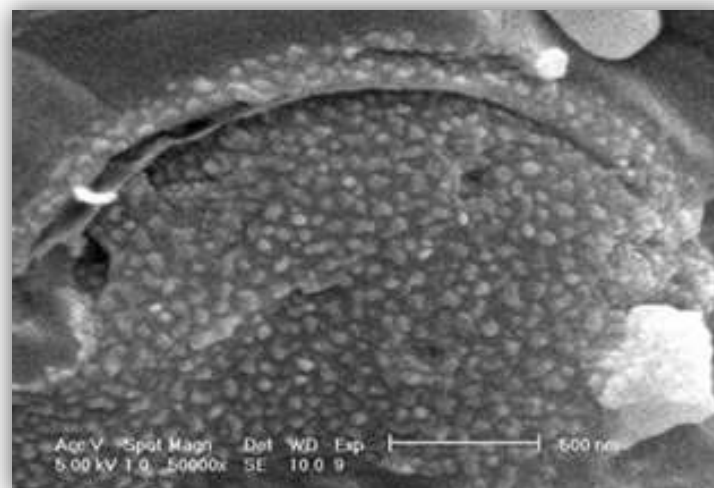
Localisation Within Cells



Fluorescence microscopy

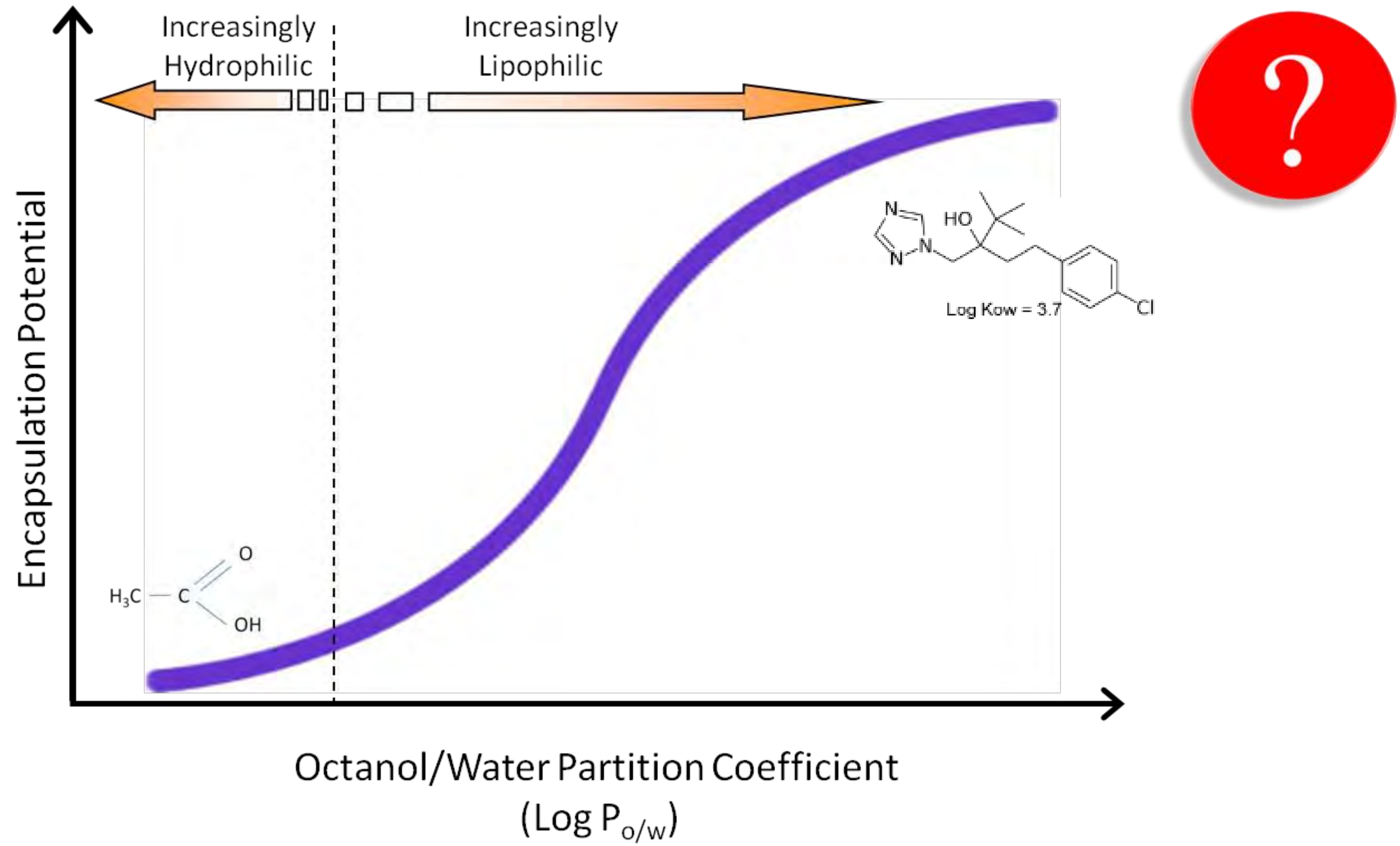


Freeze fracture scanning electron microscopy



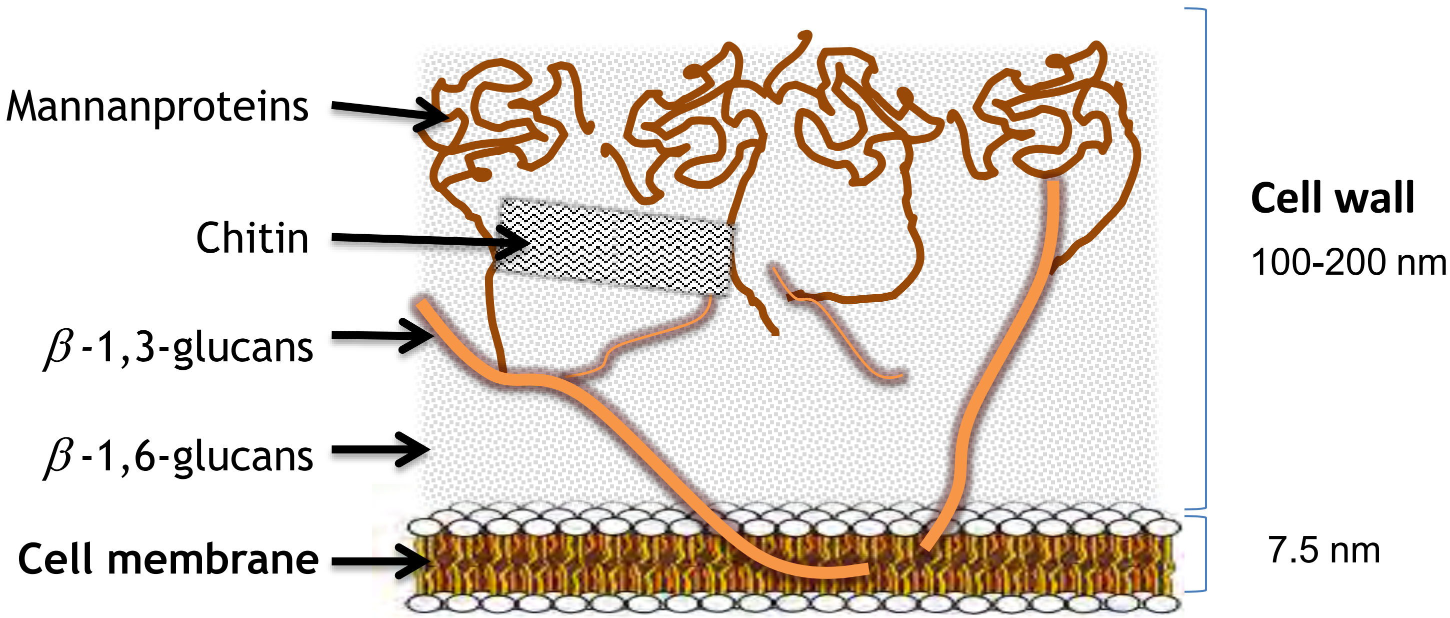
Confocal-fluorescence and scanning electron microscopy used to visualise encapsulation
Nile red (in green) to indicate lipid droplets and DAPI stain to show the nucleus (in blue)

Mechanism Of Encapsulation



After Dardelle *et al*. (2007). Food Hydrocolloids. 21, 953-960

Unique Barrier Protection Of Cell Contents



The structure of the yeast protective envelope comprises the cell wall and the lipid membrane, the key selective barriers in the uptake of fat soluble flavours

after Kilcher et al.(2008) Faraday Discuss. 139, 199-212

Staining With Nile Red

- Nile Red is non-ionic and emission does not depend on local pH, or on the presence of specific chemical compounds
- It has poor water solubility and exhibits bright red-yellow fluorescence in a hydrophobic environment
- Exhibits solvatochromic behaviour

Nile Red

Log Kow = 4.4

NR ideal for targeting intracellular lipidic droplets

Greenspan *et al*. 1985

Staining With Nile Red

NR exhibits negligible fluorescence in water and fluoresces according to the medium it is in. Due to these useful spectral properties it has been extensively used for microscopic imaging purposes; in particular for intracellular lipidic droplets

1:1 CH_3OH:H2O | CH_3OH | CH_3CN | THF | Toluene | Cyclohexane | Hexane

after Ciamponi, *et al.* (2012) *Applied Microbiol Biotechnol. 95*, (6) pp 1445-1456

Visualisation Of Absorbed Fat Soluble Flavour

Appl Microbiol Biotechnol
DOI 10.1007/s00253-012-4127-8

BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Yeast cells as microcapsules. Analytical tools and process variables in the encapsulation of hydrophobes in *S. cerevisiae*

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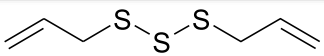
Received: 24 January 2012 / Revised: 20 April 2012 / Accepted: 20 April 2012

Abstract Yeast cells can be used as biocompatible and biodegradable containers for the microencapsulation of a variety of actives. Despite the wide application of this process, e.g. in the food industry, mechanism and controlling factors are yet poorly known. In this study we have studied kinetics and mechanistic aspects of the spontaneous internalization of terpenes (as model hydrophobic compounds) in *Saccharomyces cerevisiae*, quantifying their encapsulation through HPLC analysis and fluorescent staining of lipidic bodies with Nile Red, while in parallel monitoring cell viability. Our results showed that this encapsulation process is essentially a phenomenon of passive diffusion with negligible relevance of active transport. Further, our evidence shows that the major determinant of the encapsulation kinetics is the solubility of the hydrophobe in the cell wall, which is inversely related to partition coefficient (log *P*).

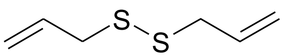
Keywords Encapsulation · Yeast · Cell wall · Flavours · Diffusion

Electronic supplementary material The online version of this article (doi:10.1007/s00253-012-4127-8) contains supplementary material, which is available to authorized users.

Hydrophobic Probes



Diallyl trisulfide



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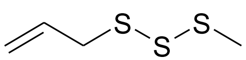
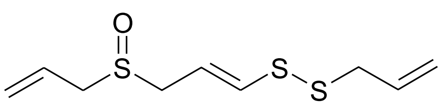


Allyl methyl trisulfide



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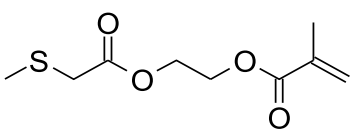
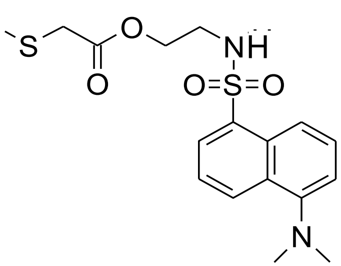


Polymerizable



Fluorescent probe

Ad hoc synthesized

- Early studies showed that components of garlic oil were encapsulated with high efficiency

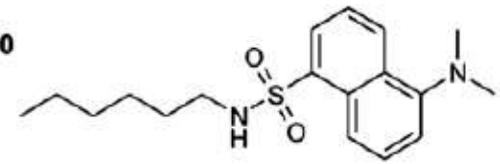
- Polymer probes were created to investigate compartmentalisation and test the molecular weight cut off characteristics of yeast biocapsules

Kilcher *et.al.* (2007) *Langmuir*, 23 12309-12317

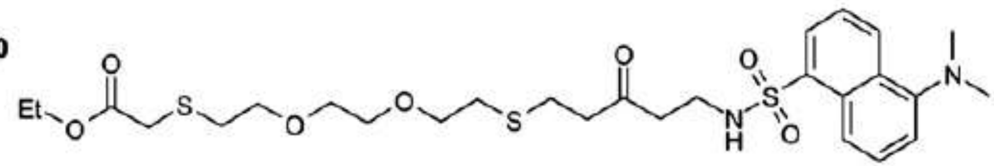
Fluorescent Probes Synthesised For The Study

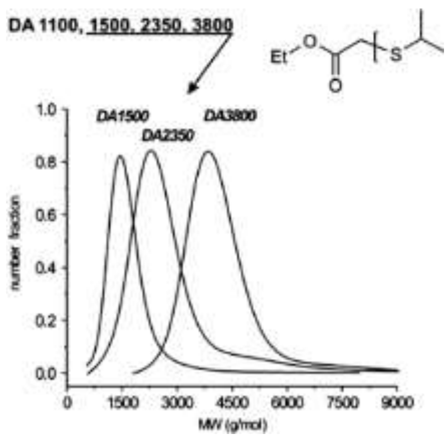
The introduction of dansyl or 2-acetoxyethyl groups was accomplished by endcapping thiolate groups (for DA1100 to DA3800 at the termini of polysulfide chains) with dansyl acrylate and ethyl 2-bromoacetate, respectively

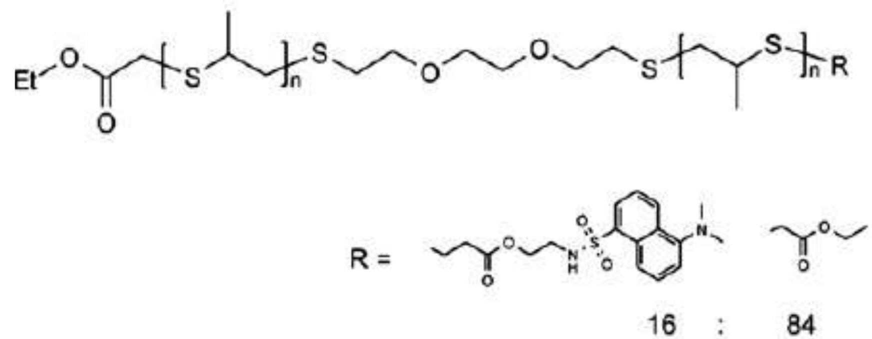
DA 320



DA 620







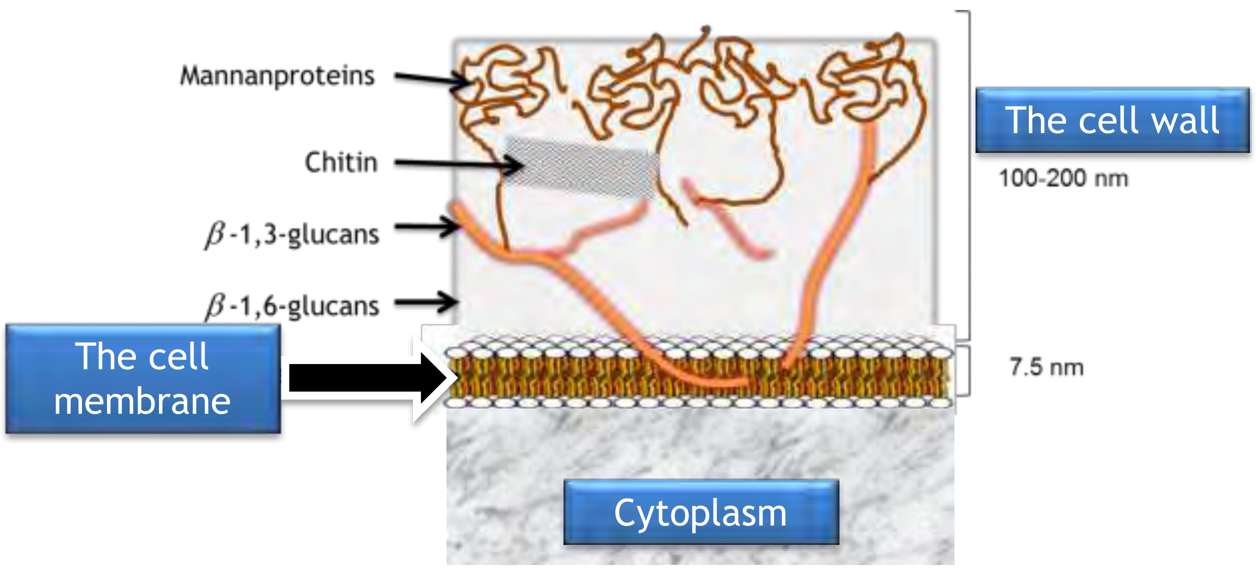
The dansyl group was chosen for its high stability both to chemical agents and to photo-bleaching (λ_{exc} = 343 nm; λ_{em} = 494 nm)

Kilcher *et al.* (2008) *Faraday Discuss.* 139, 199-212

Cell Membrane Removal

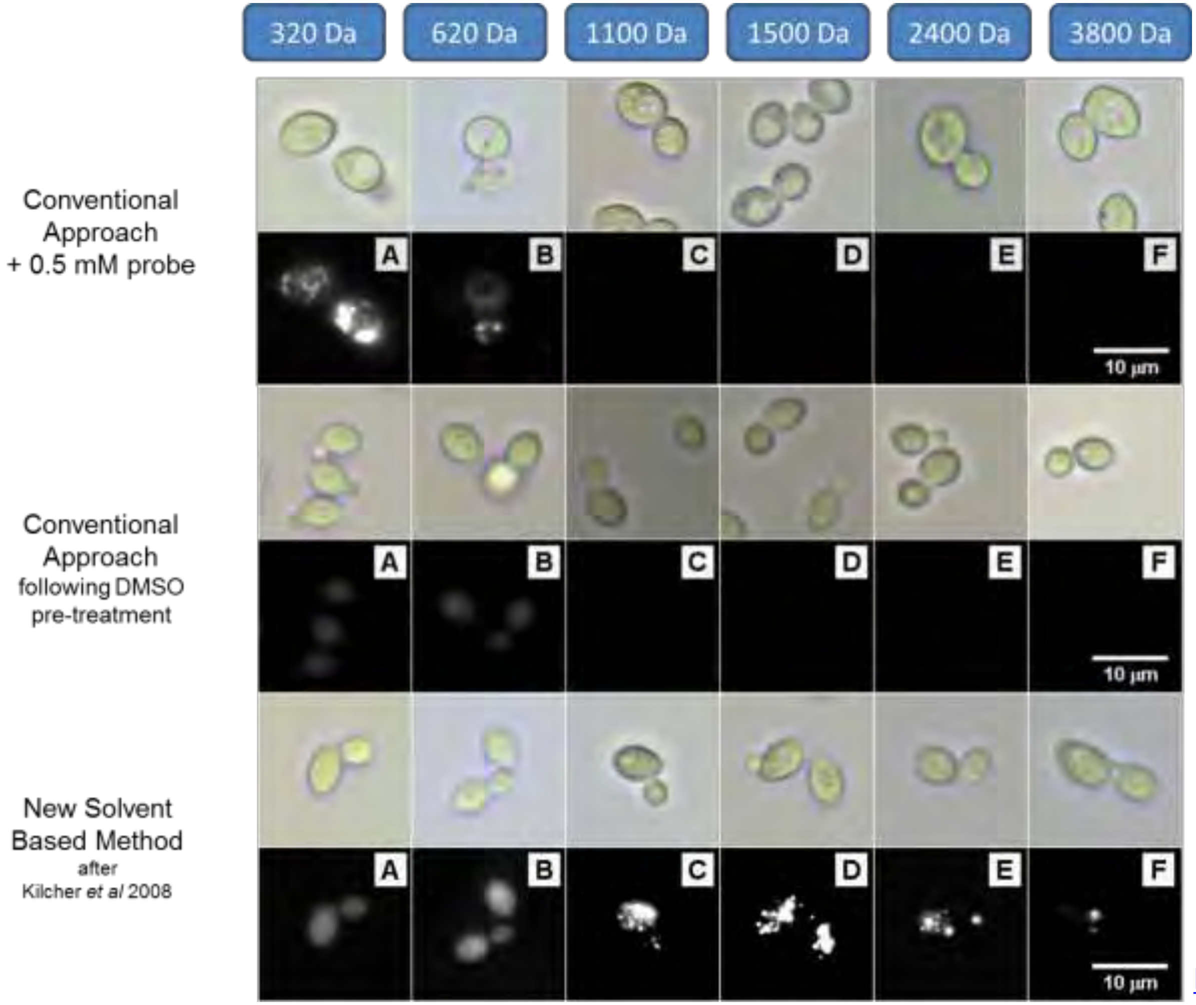
S. cerevisiae yeast cells initially in PBS, are transferred into DMSO for 2hr and then spun down and returned to PBS

A 20 nm thick gap appears where the lipid bilayer resided between the cytoplasm and cell wall, initially clearly visible, seems to disappear, as do the membranes of internal cell organelles. These membranes are not restored when cells are transferred back to water milieu after exposure to DMSO.



Kilcher *et.al.* (2008) *Faraday Discuss.* 139, 199-212

Improvements In The Uptake Process



This shows how droplets of fluorescent polysulfides above 600 daltons could not pass across the cell wall using conventional aqueous mixing. Selected solvents in a water free system facilitated the process for very hydrophobic molecules to almost 4000 daltons.

Kilcher *et.al.* (2008) *Faraday Discuss.* 139, 199-212

Images courtesy of N. Tirelli, University of Manchester

Testing Yeast Viability During Encapsulation

Appl Microbiol Biotechnol
DOI 10.1007/s00253-012-4127-8

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Images of the localization and fluorescence of FUN-1
Cells were also stained 25 μM Calcofluor white to highlight the cell wall.

Ciamponi, *et al.* (2012) *Applied Microbiol Biotechnol. 95*, (6) pp 1445-1456

Raw Materials – Dead Yeast

- Yeast

Overview

Trigger:

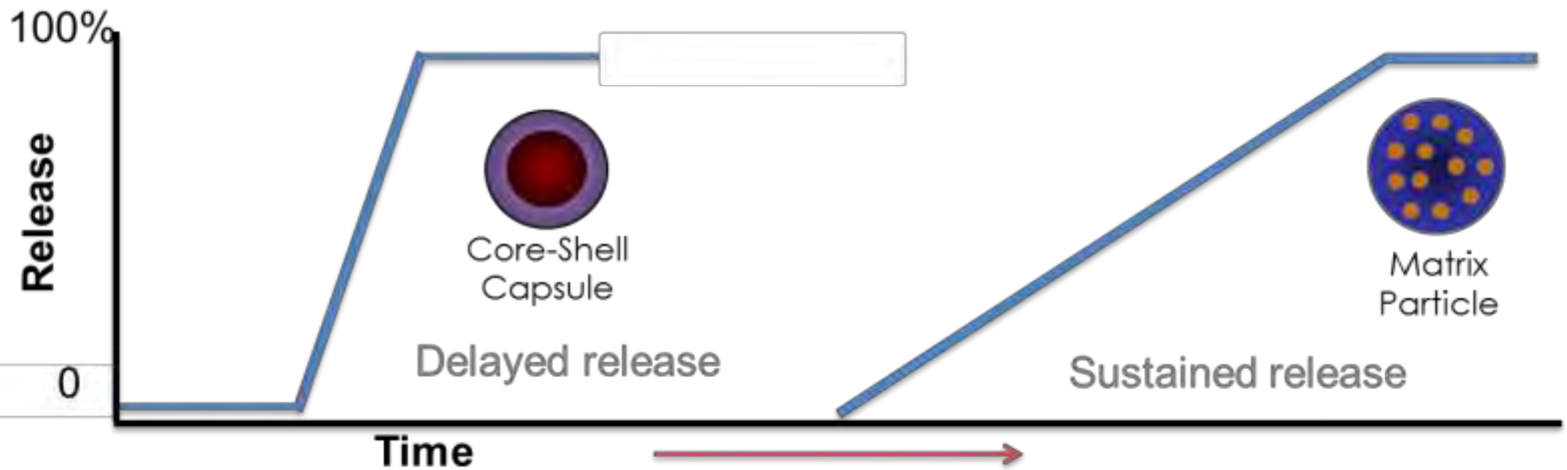
- Moisture
- pH
- Temperature
- Pressure
- Shear

Release:

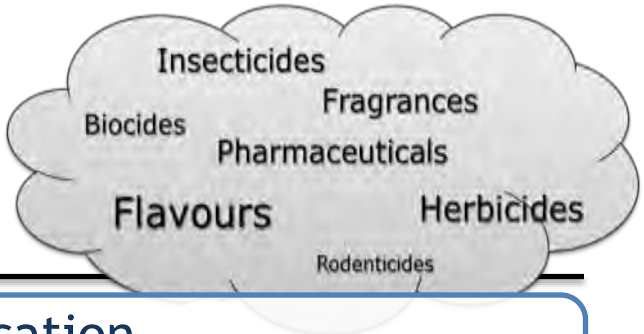
- Sustained
  Diffusion
- Burst
  Shear rupture

Target:

- Crop protection
  Fungicide/Herbicide/Insecticide
- APIs
  Oral/Topical/Paracellular DD
- Flavours



Recent Developments



2007
- Modelling of uptake and release in application
- Dardelle, *et al.*, Food Hydrocolloids 21, 953-960

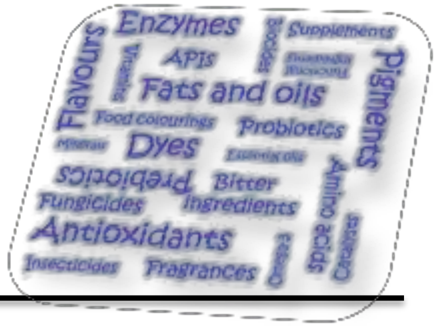
2007
- Flavoured tobacco products
- Philip Morris Products S.A. Patent WO/2008/023271

2008
- Probing transport through cell walls
- Kilcher et al. Faraday Discuss. 139, 199

2009
- New Method of Encapsulation for larger hydrophobic molecules
- University of Manchester Patent WO 2009053711

2015
- Approved use for vine fungal pathogen control in Europe
- Eden Research Plc

BENEFITS: Improved Delivery



- Effective delivery
  - Modified flavour profiles
  - Improved bioavailability
  - A sustained release platform delivery system
- Protection from
  - Evaporation
  - High temperatures
  - High shear processes
  - Structurally - from pH changes and hydrolysis
- Prospects for new product concepts
- Stable aqueous dispersions for fat soluble ingredients
- Easy-to-handle: fine powder and liquid spray coatings
- Natural – retain ingredient labelling advantages

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  Department of Biological Sciences, Lancaster University
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  Division of Food Sciences, University of Nottingham
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and

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