Liquid-core micro and nano-capsules for the extraction of drugs and pesticides/herbicides

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Liquid-core micro and nano-capsules

• Can be defined as miniature sized particles, which consist of a liquid-core completely enveloped within a defined membrane
  • Different structural forms exist
  • Many exploitable characteristics for applications in biotechnological or chemical processes

• Liquid-core micro and nano-capsules can occur naturally in nature i.e. ova and/or can be manufactured from a wide range of natural and/or synthetic materials
Characteristics of liquid-core microcapsules

- Structurally, 4 different types
  1. Mononuclear (simple/single core)
  2. Double/multi-shell (membrane)
  3. Polynuclear (multi-core)
  4. Irregular or non-spherical

- Size
  - Microcapsules  1-1000 μm
  - Macrocapsules  > 1000 μm
  - Nanocapsules  < 1 μm

- Membrane/core materials
  - Natural
  - Semi-synthetic
  - Synthetic
  - Membrane – porous/non-porous

- Applications
  - Protection, controlled release, extraction aids, drug targeting etc
Manufacturing techniques

Manufactured by various processes (lab-scale to industrial-scale)

1. Chemical processes
   • Interfacial polycondensation
   • In-situ polymerization

2. Physico-chemical processes
   • Coacervation
     • Simple or complex (phase separation)

3. Mechanical processes
   • Droplet forming devices
   • Jet- cutting devices
   • Rotating devices
   • Prilling devices
   • Vibrating Nozzle technique
Vibrating nozzle technique

- Based on the theory devised in the 19th century
  - Lord Rayleigh
  - Weber
- A liquid extruded through a nozzle (orifice) results in laminar flow drop generation

Continuous jet of liquid

Axial symmetrical vibrations results in jet break-up

Formation of spherical drops due to surface tension

\[ d = \text{monodisperse} \]

Vibrating the nozzle at defined frequencies

Applies a superimposed force to the extruded liquid jet resulting in the formation of equally sized droplets
Vibrating nozzle technique

- Characteristics (size) of the droplets formed is dependent on
  - Nozzle (orifice) diameter
  - Flow rate of the extruded jet
  - Viscosity
  - Frequency applied

\[ f' = \frac{v}{\lambda} \]
\[ \lambda_{opt} = \pi \sqrt{2D} \sqrt{1 + \frac{3\eta}{\sqrt{\rho \sigma D}}} \]
\[ d = 3\sqrt{1.5D^2 \lambda_{opt}} \]

Mathematical models

Empirical approach
Schematic of vibrating nozzle encapsulator

Size range 150μm-2mm

Standard size deviation ± 1%
Vibrating nozzle technique for manufacturing of liquid-core microcapsules

- Extruding 2 liquids through the nozzle (liquid-core inner flow) will result in the formation of liquid-core microcapsules when a vibration is applied to the jet
- Co-extrusion laminar jet break-up technique
  - Inotech encapsulator

Size deviation of ± 2.5%
• 300 μm – 2 mm, outer diameter of capsule
• Core volume 10-90% of total microcapsule volume

Porous hydrogel
• Pore size 5-200 nm
• Dextran standards (HPLC)

Mass transfer characteristics
• Stirring speed
• Membrane characteristics
• Liquid-core characteristics

Mechanical (strength) resistance
• Texture analyzer
• Very stable microcapsules
Case 1:

Pesticide/ drug recovery from environment
Presence and fate of pesticides and pharmaceuticals in the aqueous environment

- For many decades the presence of pesticides in the aqueous environment has being of major concern
  - Toxic nature of these compounds
    - Carcinogenic
    - Mutagenic, etc

- In recent years the release of pharmaceuticals has also emerged as a concerning issue
  - Exert biological effects
  - Recent studies have shown the toxic effects of these compounds in the environment

- There exists a multitude of potential input pathways for the release of these organic pollutants into the aquatic environment
  - Inadequate disposal, production spills etc

- Point sources of entry
  - Effluent water from sewage and water treatment plants
- Sewage and water treatment plants
  - Activated sludge
  - Adsorption
  - Naturally biodegradation
    - Bioremediation
    - Photodegradation
  - Tertiary treatment
    - Ozonation
    - Advanced oxidation
    - Membrane filtration
    - Liquid-liquid extraction
    - Activated carbon

- Little or no removal/degradation

- Very expensive
  - Dilute streams
  - Not always possible to re-cycle
• **Liquid-liquid extraction**
  • Cheap, simplistic and robust methodology
    • High affinity for extractants
  • Used for over a century
    • Chemical industry
  • Recent times
    • Water treatment
  • Several problems for use in water treatment
    • Unfeasible for large volumes of dilute streams
      • High contact area
    • Increase agitation speed and/or volume of solvent required
      • Increased costs
      • Formation of stable emulsions (see in a later slide)
        • Prevent re-cycling
    • Solvent may also contaminate the water being treated due to the direct contact
  • Overcome problems by encapsulating solvent within a hydrogel membrane
Removal of pharmaceuticals and pesticides from water using liquid-core microcapsules

- Novel approach termed ‘Capsular Perstration’

Capsular Perstration → Derived from permeation and extraction

- Alginate hydrogel membrane
  - Chitosan, cellulose sulphate etc

- Liquid-core
  - Hydrophobic material
  - Oleic acid, vegetable oils etc
Selected Pesticides/Herbicides

- **Ethylparathion (EP) and Methylparathion (MP)**

- **Atrazine**

- **2,4-dichloro-phenoxyacetic acid (2,4 D)**

<table>
<thead>
<tr>
<th></th>
<th>EP 3.73</th>
<th>MP 2.82</th>
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<tbody>
<tr>
<td>LogP&lt;sub&gt;oct&lt;/sub&gt;</td>
<td></td>
<td></td>
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<tr>
<td>S&lt;sub&gt;w&lt;/sub&gt; (mg/L)</td>
<td>EP 24</td>
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<table>
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<tr>
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<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;w&lt;/sub&gt; (mg/L)</td>
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<th></th>
<th>2.62</th>
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<td></td>
</tr>
<tr>
<td>S&lt;sub&gt;w&lt;/sub&gt; (mg/L)</td>
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</table>

- **Banned in many EU countries**
  - Highly toxic carcinogens
  - Classified as persistent organic pollutants (POPs)
  - Detection in ground, surface and drinking water
  - Inability to be removed by conventional sewage and water treatment plants
Recovery of pollutants using liquid-core microcapsules
- Dibutyl sebacate (core material)
  - High encapsulation affinity
  - High affinity for selected compounds

- 3.5% (v/v) liquid volume ratio
- 25-75% extracted
  - <100 min
- Amount extracted based on the hydrophobicity of the extracted species
  - ↑ hydrophobicity, ↑ extraction
Selected pharmaceuticals

• Pre-selected on their ability to match the following criteria
  • Varying LogP\textsubscript{oct} values
  • In the top 100 pharmaceuticals sold in Ireland
  • Specific mode of action
  • Inability to be adequately/fully removed by STPs
  • Presence in the aquatic environment
  • Toxicity towards the aquatic environment

• Recovery of pollutants using liquid-core microcapsules
  • Core material either
    • Dibutyl sebacate
    • Oleic acid
<table>
<thead>
<tr>
<th>Compound</th>
<th>Therapeutic Class</th>
<th>Chemical Structure</th>
<th>LogP&lt;sub&gt;oct&lt;/sub&gt;</th>
<th>% Removal in STPs</th>
<th>Conc. in environmental waters (ng/L)</th>
<th>Sphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfamethoxazole</td>
<td>Antibiotic (bacteriostatic)</td>
<td><img src="image1" alt="Image" /></td>
<td>0.89</td>
<td>17-71</td>
<td>410 1000 13-45</td>
<td>Ground water, Surface water, Drinking water</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>Beta-blocker (receptor)</td>
<td><img src="image2" alt="Image" /></td>
<td>1.9</td>
<td>0-83</td>
<td>2200</td>
<td>Surface water</td>
</tr>
<tr>
<td>Furosemide</td>
<td>Loop diuretic</td>
<td><img src="image3" alt="Image" /></td>
<td>2.03</td>
<td>8-54</td>
<td>1.72-25</td>
<td>Surface water</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anticonvulsant (antiepileptic)</td>
<td><img src="image4" alt="Image" /></td>
<td>2.45</td>
<td>0-7</td>
<td>1100 1100 30</td>
<td>Ground water, Surface water, Drinking water</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>Metabolite of three lipid regulators</td>
<td><img src="image5" alt="Image" /></td>
<td>2.57</td>
<td>34-51 50 0-91</td>
<td>4000 175-185, 1075 270</td>
<td>Ground water, Surface water, Drinking water</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Anticoagulant</td>
<td><img src="image6" alt="Image" /></td>
<td>3.0</td>
<td>-</td>
<td>1.0</td>
<td>Surface water</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Analgesic/Anti-inflammatory</td>
<td><img src="image7" alt="Image" /></td>
<td>4.50</td>
<td>0-75</td>
<td>15-489</td>
<td>Surface water</td>
</tr>
</tbody>
</table>
• Recovery of selected pharmaceuticals using dibutyl sebacate and oleic acid liquid-core microcapsules simultaneously
  • Different selectivity's
  • Achieve effective recovery

- 4% (v/v) liquid volume ratio
- 15-100% of the initial drug concentration withdrawn within 50 min
- % recovery higher compared to conventional STPs
  • Exceptional differences in some cases
  • Increase extraction by increasing capsule number
  • Extraction not based on hydrophobicity of extracted species
Liquid-core microcapsules as a reservoir for biodegradation of organic pollutants

- Disposal: Incineration, questionable!!
- Natural (bio)-degradation
- Atrazine biodegraded naturally by *Pseudomonas* strains
  - Use atrazine as a sole nitrogen source for growth
- Atrazine is toxic at low concentrations
  - $\geq 100$ mg/l
- Overcome problem
  - Using liquid-core microcapsules to deliver the atrazine
    - Delivered below toxicity levels
      - Based on partitioning effect
• Mineralization of the atrazine to water, CO₂ and biomass
  • Total initial concentration of atrazine in the capsules
    • 4800, 9600 and 19000 mg/L
    • 12 ml of organic phase within the microcapsules

Growth (OD) curve of *Pseudomonas* sp., in which atrazine contained within microcapsules was used as the sole nitrogen source

![Growth rates linear due to being limited by rate of atrazine exo-diffusion from capsules (atrazine is sole N-source)]
Biomass is autoclaved for disposal
Capsules can be recycled for future use
  • Extraction/disposal experiments

<table>
<thead>
<tr>
<th>Initial conc of atrazine in the culture (mg/L)</th>
<th>Final conc of atrazine in the culture (mg/L)</th>
<th>Degradation (mg/L)</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>4</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>200</td>
<td>19</td>
<td>181</td>
<td>90.5</td>
</tr>
<tr>
<td>400</td>
<td>104</td>
<td>296</td>
<td>74</td>
</tr>
</tbody>
</table>

≥ 100 mg/L
Particles 2009 Berlin

**Sewage Treatment Plants**

- **Sewage influent**
- **Primary Treatment**
  - Mechanical Treatment
- **Secondary Treatment**
  - Aerobic biological process
- **Tertiary Treatment**
  - Chemical process

Effluent

Little agitation due to high demand on energy
Improved production and recovery of geldanamycin: Using liquid-core microcapsules as a novel approach
Geldanamycin (GA)

- Benzoquinone polyketide antibiotic
  - Ansamycin family
- *Streptomyces hygroscopicus var geldanus var nova*
  - Filamentous/pellet growth
- First described in 1970
  - Weak antimicrobial

Pelleted growth
(magnification 40X)
Why GA?

- **Novel antitumor antibiotic**
  - Targeted cell-therapy
- **Heat shock protein 90 (Hsp90) is its principal cellular target**
  - Abundant chaperone in eukaryotes
  - Necessary to maintain signalling proteins involved in cancer progression growth and survival
- **GA binds to the ATP binding site and inhibits the chaperone**
  - Results in tumour destruction

The interaction of GA (yellow) with Hsp90 side chains (pink). GA occupies the ATP-binding site on the protein
GA Analogues

- Severe heptatoxicity in animals
  - Failure in clinical trials

- Over 500 derivatives have being reported
  - Mainly substitution at the 17-position

- Two excellent derivates in clinical trials
  - 17-DMAG (first phase)
  - 17-AGG (second phase)

- Similar molecular activities
  - Inhibit Hsp90
  - Improved solubility (DMAG)
    - Formulation

- Large amounts of naturally GA required if drug is to be a clinical success
Batch culture of *S. hygroscopicus*

- Why are net GA levels decreasing after maximum at around 12h?
- Inhibitory fermentation environment, pH, temperature, molecular oxygen etc?
- Feed-back (product) inhibition
- Product stability?
Decreasing Conc. of GA

- GA shown to be stable in fermentation environment
  - Temp, pH, agitation speed etc
- No product inhibition at these production levels
- Stability of product in fermentation environment?
- Micro-organism: possible release of hydrolytic enzymes into cultivation media
GA degradation

- Fermentation was carried-out
  - GA production
  - No GA present

- Fermentation until 20h, cells removed (filtration) and broth was tested:
  - 100 mg/L of pure GA was added to the broth
    1. Untreated
    2. Treated (70 degrees for 90 min)
ISPR of GA

• To increase product titers
  • In-situ product recovery (ISPR)
    • Removal of a product from the vicinity of its production environment as soon as it is formed
      • Numerous techniques available
      • Physiochemical profile of the GA

• Reduce the number of downstream processing steps

Table 1: Physico-chemical properties of GA used to assign a suitable ISPR methodology.

<table>
<thead>
<tr>
<th>Physicochemical Property</th>
<th>Limiting Value for property</th>
<th>GA Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical Properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volatility (boiling point)</td>
<td>Volatile &lt; 80 °C &lt; Non-volatile</td>
<td>&gt; 80 °C</td>
</tr>
<tr>
<td>Molecular weight (MW)</td>
<td>Low MW &lt; 1000 Da &lt; High MW</td>
<td>560.64 Da</td>
</tr>
<tr>
<td><strong>Chemical Properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charge</td>
<td>Negative, neutral and positive&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Neutral</td>
</tr>
<tr>
<td>Hydrophobicity</td>
<td>hydrophilic &lt; log P&lt;sub&gt;oct&lt;/sub&gt; = 0.8 &lt; hydrophobic</td>
<td>logP&lt;sub&gt;oct&lt;/sub&gt; = 1.71 ± 0.03</td>
</tr>
<tr>
<td>Specific elements</td>
<td>Binding constant &gt; 10&lt;sup&gt;3&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

<sup>a</sup>Under operating conditions; <sup>b</sup>Binding based on biorecognition (e.g. by antibody or receptor). N.A., Not available.
Solvent Extraction

- Physiochemical profile of GA
  - Recovery using solvent extraction (liquid-liquid extraction)
- To obtain the optimal organic phase extractant, liquid-liquid extraction experiments were carried-out and the partition co-efficient was determined
- The partition co-efficient (K) defined as the ratio of the concentrations of the dissolved GM between two immiscible phases at equilibrium

<table>
<thead>
<tr>
<th>Organic solvent</th>
<th>LogP\text{oct} of solvent</th>
<th>Partition Coefficient (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miglyol</td>
<td>N.A.</td>
<td>54.88 ± 0.911%</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>1.25</td>
<td>179 ± 1.25%</td>
</tr>
<tr>
<td>Octanol</td>
<td>3.07</td>
<td>51 ± 5.68%</td>
</tr>
<tr>
<td>Hexane</td>
<td>3.90</td>
<td>15.85 ± 1.95%</td>
</tr>
<tr>
<td>Dibutyl sebacate</td>
<td>6.2</td>
<td>192.20 ± 2.03%</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>7.7</td>
<td>212.33 ± 7.08%</td>
</tr>
</tbody>
</table>

- Oleic acid and dibutyl sebacate
  - Could be due to their considerable higher hydrophobicity
  - Chosen for further work
Solvent addition

Fermentation

Pelleted growth

Dibutyl sebacate addition
Fermentation
Suppressed growth and GM production

Oleic acid addition
Fermentation
Suppressed growth and GM production

1. Direct addition of solvents was toxic towards the cells.
2. Stable emulsions were formed which can prevent product and solvent recovery
Capsular Perstraction

- To overcome drawbacks of solvent extraction
  - Encapsulation of organic solvents (either dibutyl sebacate or oleic acid) within an alginate hydrogel
- Recovery of GA from fermentation media/broth

Recovery of GA from fermentation media using dibutyl sebacate liquid-core microcapsules

Rapid extraction of the GA from the media
Microcapsules to alleviate GM degradation

- Fermentation was carried-out
  - GA production
  - No GA present

- Fermentation till 20h, cells removed (filtration) and broth tested:
  - 100 mg/L of pure GA was added to the broth
    1. Untreated
    2. Oleic acid liquid-core microcapsules were added

All GA was absorbed by capsules and was protected from the action of the degradation compounds
Back-extraction of GA from Microcapsules

Dibutyl sebacate liquid-core microcapsules

- Acetonitrile and methanol
- Removal of core material

Capsules before acetonitrile wash

Capsules after acetonitrile wash

Capsules before methanol wash

Capsules after methanol wash

Recovered greater than 95% of GA
Oleic acid liquid-core microcapsules

- Methanol
  - Removal of core material
- Acetonitrile
  - Limited solubility between both
  - Core will be slowly removed
- Capsules washed with acetonitrile saturated with oleic acid do not remove the core material

Capsules before acetonitrile wash

Capsules after acetonitrile wash

- Partition coefficient of GA between acetonitrile and oleic acid is 3.3
  - Small quantities of acetonitrile needed
Re-crystallization of GA back-extracted from microcapsules

- Distillation
  - B.P. acetonitrile 82 °C

Crystalline structure of GA, which was back-extracted from microcapsules
Magnification 40 X
Conclusions

• Liquid-core microcapsules are possible ISPR method for GM recovery from cultures
  • Alleviate the problem of product degradation
  • Rapid extraction of the GM

• Provide a means to reduce the number of downstream processing steps

• Final stage of this study involves the addition of capsules to fermentations to determine max levels of GM produced (requires medium and condition optimisation to now obtain higher biomass and product titres)

• Testing of liquid-core nanocapsules dia 2-200 nm- improved extraction kinetics, less easy to recover, but may be used as sensor for detection of low levels HOPs.
Summary

✓ Enables the removal of a large range of compounds
  ✓ Simplistic methodology (single step)
✓ No direct contact between liquid-core and aqueous phase water – no contamination
✓ No stable emulsions
✓ High interfacial area –
  ✓ Requires little agitation for mass transfer to occur
  ✓ Nano-capsules
✓ Simply recovery
  ✓ Flotation
  ✓ Sedimentation
  ✓ Magnetic membrane
✓ Controlling characteristics of capsules during production
Summary

✓ Recovery of organic pollutant from microcapsules
  ✓ Highly stable microcapsules
  ✓ Biodegradation of the organic pollutant
  ✓ Organic solvents (back-extraction)
  ✓ Re-cycling of microcapsules

✓ Concentration to enable detection

✓ Microencapsulation technique allows encapsulation of most organic solvents or oils
  ✓ Encapsulation of many different organic solvents to recovery a larger range of compounds
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