Characterisation of Biopolymers used in Healthcare Products

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The Use of Biopolymers in the Health Sector

Content

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Introduction

Applications

Challenges for characterisation & Technology

Case Studies
- Chitosan
- Collagen
- PEGs & PEGylated proteins
- Polymeric Antibacterial Active for wound care
- Acrylate based bone cement

Introduction

Biopolymers are used in a wide range of applications:

Drug delivery / drug formulation

Cosmetics and personal care products

Medical device such as Tissue scaffolds, Wound care, Bone putties, Dental cements

Their Functional properties are many fold:

Incorporate actives such as antibiotics, encapsulation

To aid dissolution and bioavailability of APIs

Fibres for textile and structural properties

3D structure for tissue growth

Challenges for characterisation

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Issues for Polymer Characterisation:	Analytical Technologies:
• **Molecular Weight distribution**	• MALDI-MS, Size exclusion Chromatography (SEC) with viscosity, light scattering, RI,
• **Quantification**	• Quantification of functional groups by NMR, HPLC determination of monomer
• **Residual Monomer content**	• GC-MS and LC-MS, HPLC-UV
• **Linear or Branching or Crosslinked**	• SEC with Tri-Sec detector
• **Process related impurities (residual solvents, other OVI)**	• GC-MS, GC-FID
• **Bulk Physical Properties**	• Microscopy, Thermal analysis, mechanical testing

Case Studies: Chitosan

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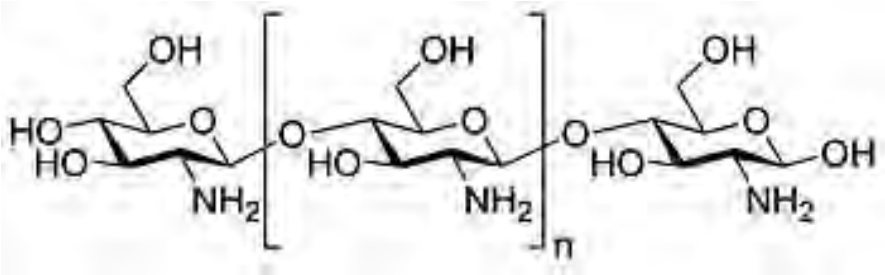
- Chitosan is a derivative of the natural polysaccharide, chitin.

- Additional treatment with NaOH removes acetyl side groups and yields a copolymer of N-acetyl-glucosamine and N-glucosamine units. When more than 50% of the acetyl groups are removed, the polymer is called chitosan.
- The ratio of glucosamine to N-acetyl glucosamine is referred to as the degree of de-acetylation, DDA and typically ranges from 50-100%.
- **Biomedical applications:**
  - Wound healing
  - Drug delivery systems
  - Ophthalmology
  - Implant coatings
  - Tissue engineering/regeneration



- Demonstrated biocompatibility, bio-degradability, nontoxic, nonacidic degradation products, ease of chemical and physical manipulation and ability to promote healing.

Case Studies: Chitosan

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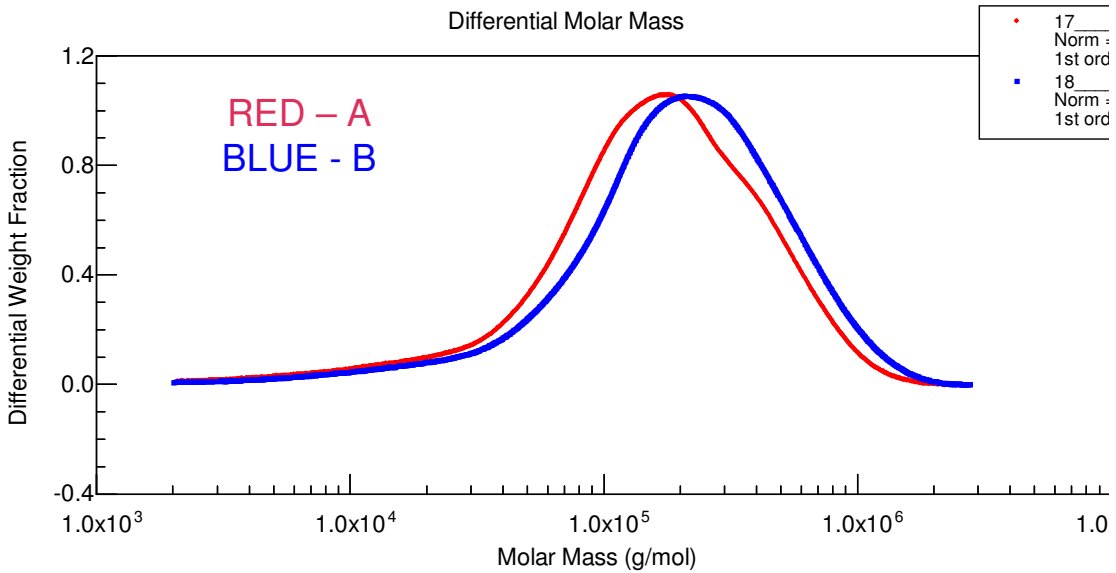
Analysis issues:
- Quantification of levels
- MW determination by SEC

Applications
- QC of raw materials
- Batch release of finished product
- Stability testing (study of potential degradation)

The following is a molecular weight distribution overlay of the sample runs;
CHITOSANS A & B



Molecular Weight Data

Sample	Run	Mn	Mw
17 Chitosan A	1	72560	231900
17 Chitosan A	2	75030	233700
18 Chitosan B	1	96090	286900
18 Chitosan B	2	90970	285900

Dn/dc used =0.185 ml/g

Case Studies: Collagen

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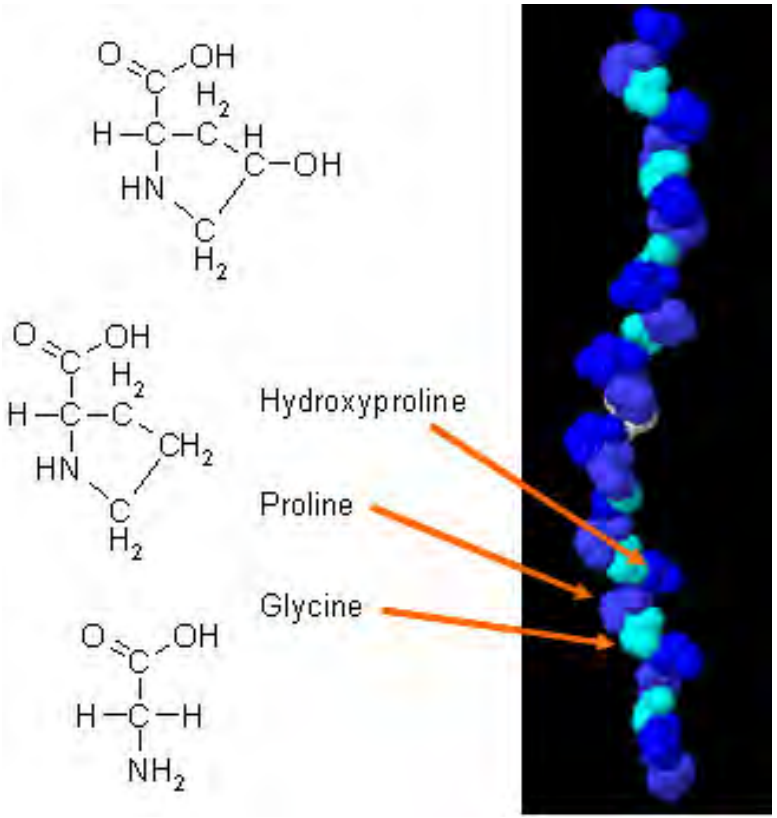
- **Collagen** is a naturally occurring protein, main component of connective tissue (25% to 35% of the whole-body protein content)
- Present in the form of elongated fibrils

Applications:
- Cosmetic surgery and beauty products
- Wound care / burn treatments
- Reconstructive surgical uses
  - Scaffold for tissue regrowth
  - Bone and dental scaffold / putties

Analytical Issues:
- Collagen content
- Collagen type
- Physical Structure
- Process residuals
- Biologic species e.g. GAGs

Sourced from Bovine, Porcine, Fish



Case Studies: Collagen

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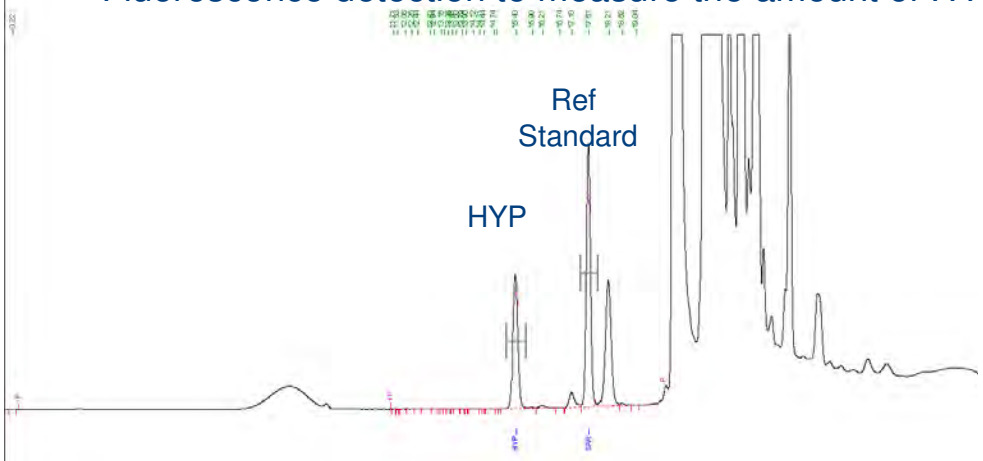
Collagen content via Hydroxyproline Content determined by HPLC

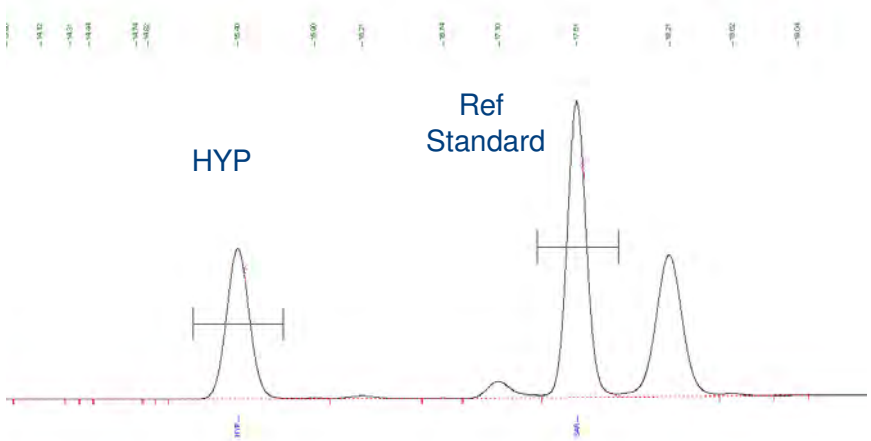
- Hydroxyproline is found in few proteins other than collagen and indeed the only other mammalian protein that includes hydroxyproline is elastin and so for this reason, hydroxyproline content can be used as an indicator to determine collagen level.

- Method adapted from the USP amino acids HPLC method – uses determined by HPLC using pre-column derivatisation with a combination of *o*-phthalialdehyde /mercaptopropionic acid (OPA) and fluorenylmethyloxycarbonyl chloride (FMOC-Cl).

- The HPLC method employed then separates the derivatised amino acids based on polarity such that the more hydrophilic OPA-derivatised amino acids elute first followed by the more hydrophobic FMOC-Cl derivatives.
- Fluorescence detection to measure the amount of HYP present



Full-scale chromatogram of hydrolysed sample solution containing 2 ppm sarcosine as internal standard.



Expanded scale chromatogram of hydrolysed sample solution containing 2 ppm sarcosine as internal standard.

Case Studies: Collagen

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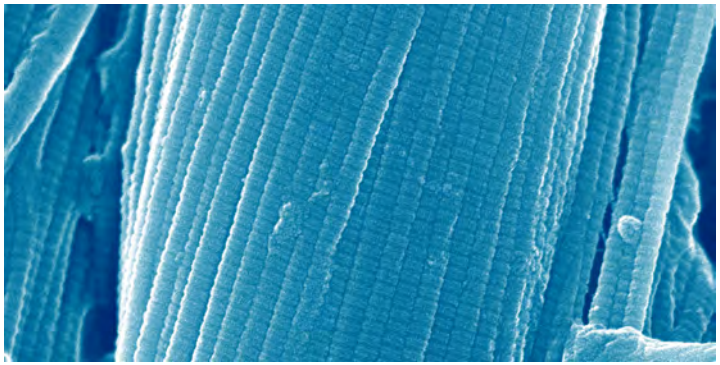
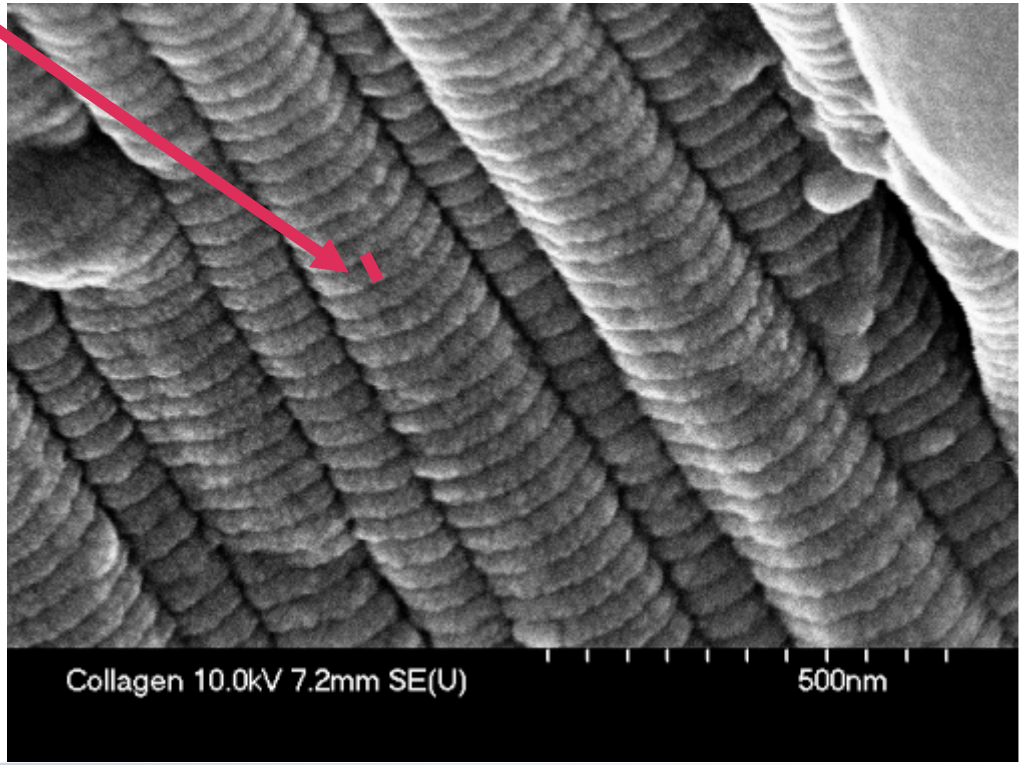
Physical characterisation

- imaging via SEM, TEM and optical microscopy (striations, porosity)

- Mechanical testing (e.g. tensile strength, etc)





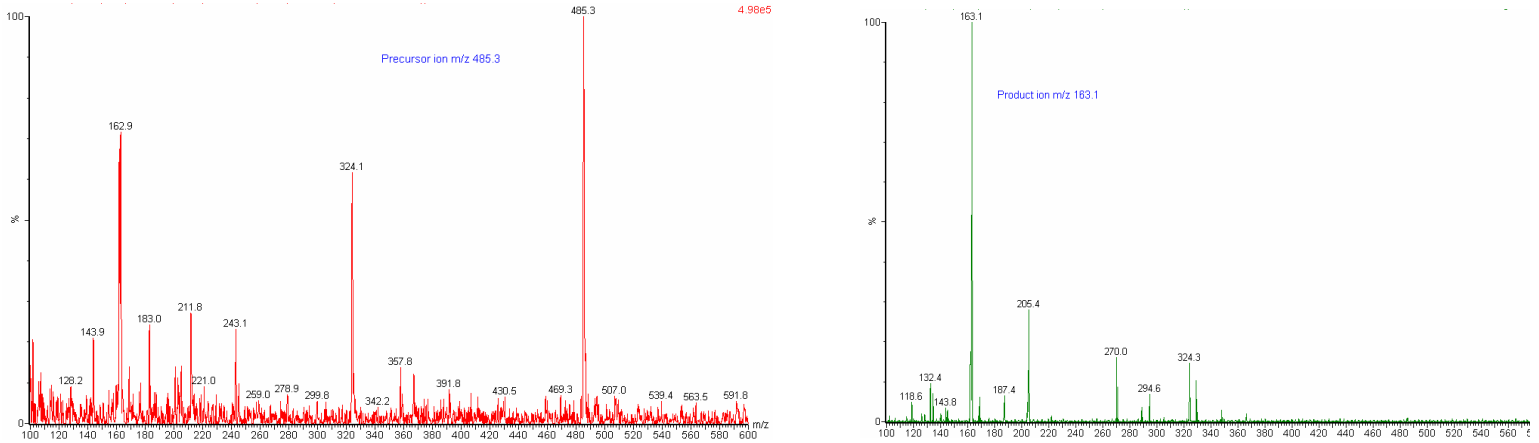
Case Studies: Collagen

- Collagens from different sources is processed in different manners to optimise their properties for the selected application.

Detection of process residuals such as antibiotics or detergents are key to ensuring a safe product.

- LC-MS/MS methods were developed to achieve quantification of antibiotics down to ppm levels in these challenging sample matrices.

- Kanamycin quantification achieved via MRM transition method (m/z 485.3 - m/z 163.1). Comparisons between spiked and unspiked product samples and standard addition allowed quantification of kanamycin at 0.2ppm in processed collagen.



LC-MS/MS precursor and product spectra for kanamycin extracted from a processed collagen scaffold

Case studies: Poly(ethylene glycol) PEGs

- Synthetic polyether that are readily available in a range of molecular weights with Mw <100,000
- Approved by the FDA for use as excipients or as a carrier in different pharmaceutical formulations, foods, and cosmetics.
- Most PEGs with Mw >1,000 are rapidly removed from the body unaltered with clearance rates inversely proportional to polymer molecular weight.

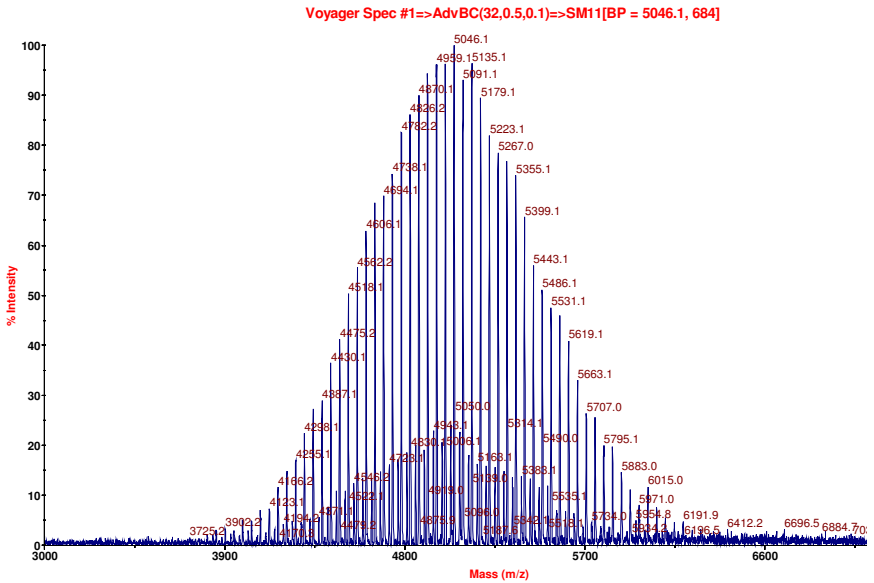
Biomedical Applications:

- Drug delivery
- Tissue engineering scaffolds
- Surface functionalization

MALDI Mass Spectrometry Analysis

MALDI-MS can be used to achieve this data as they are well behaved with respect to ionisation.

- Mn (Number average molecular weight)
- Mw (Average weight of polymer)
- Mp (Molecular weight of the peak apex) and polydispersity measurements can be made.



Characterisation of PEG by MALDI-MS.
Interek's Voyager DE-STR was used (Linear and reflectron positive ion) to study PEGs of different MW The resulting data were analysed using Polymerix software which makes an assessment of the distribution of the ions found.

Case studies: PEGylated Proteins

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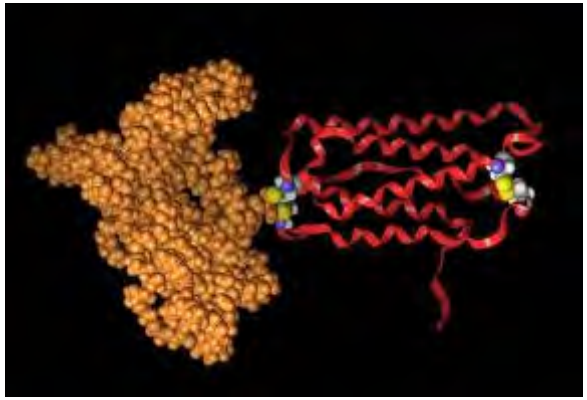
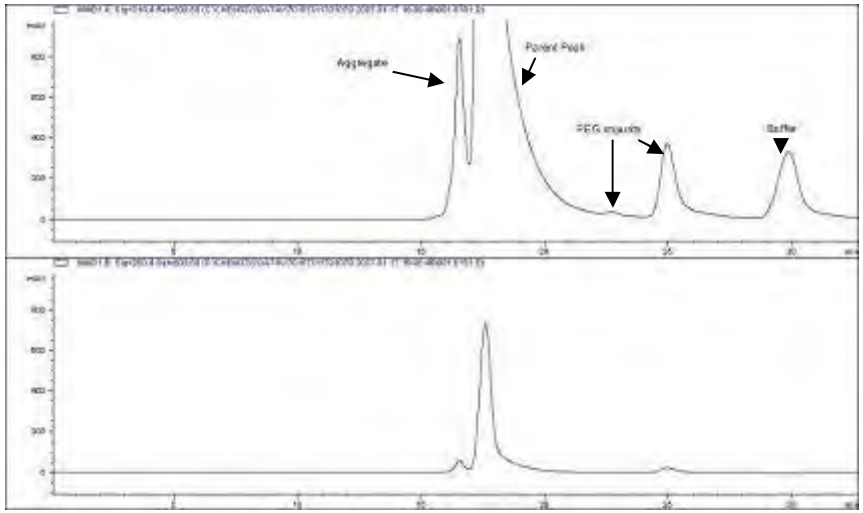
- PEGylation is a clinically proven strategy for increasing the therapeutic efficacy of protein based medicines.
- Challenge to achieve better biophysical characterization:
  - Isolation of PEGylated product related impurities
  - Characterisation of the PEGylated Protein
  - Characterisation of the PEG component
  - Understanding the biophysical behaviour of the protein



- Characterization of PEGylated proteins is difficult due to the fact that the PEG molecule is more polydisperse than the protein and imparts size heterogeneity to the conjugated protein.
- **Size-exclusion chromatography (SEC) has often been used to characterize these conjugates**.



- A limitation of SEC is that it will not detect "PEGylation site isomers" in which the protein is PEGylated at different residues.
- A wise approach would be to characterise both PEGylated proteins and the unPEGylated species.

- After cleavage of the PEG (one option is with weak alkali) it is possible to perform a range of protein characterisation tests to show no change to the protein structure or position of any other PTM such as the glycan distribution and the position of PEGylation can be confirmed. MALDI-MS and LC-MS/MS are key techniques.

Case Studies: Polymeric Antibacterial Actives

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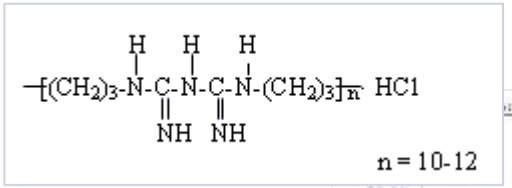


Example: PHMB

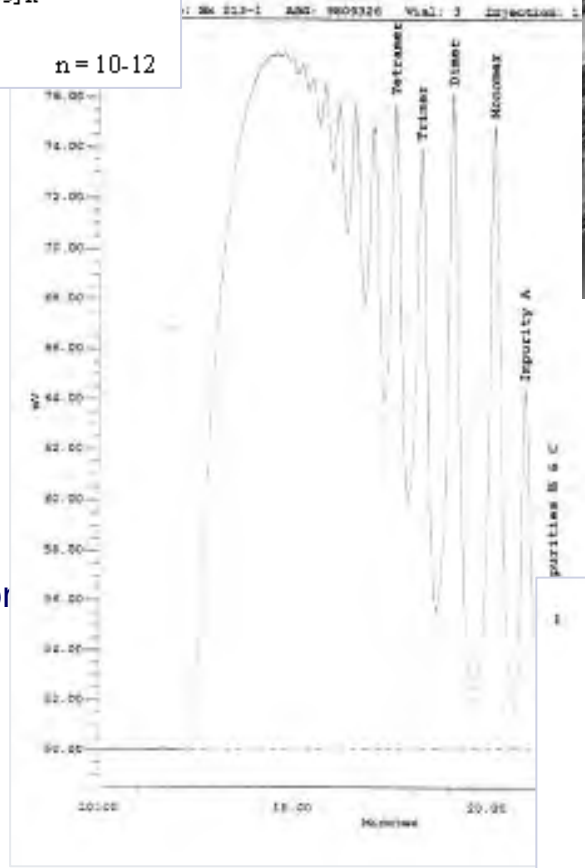
- 35 Years experience of PHMB analysis
- Characterisation Issues:

Quantification of impurities

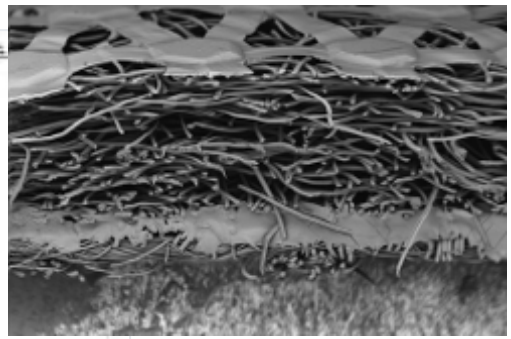
- Infra-Red Spectra
- End Group/ In-Chain Group Analysis by NMR (as percentages)
- UV Ratio of Absorbance
- Average Molecular Weight Determinatio by SEC
- Determination of Low Molecular Weight Oligomers and Impurities A, B and C by SEC

Residual Monomers:

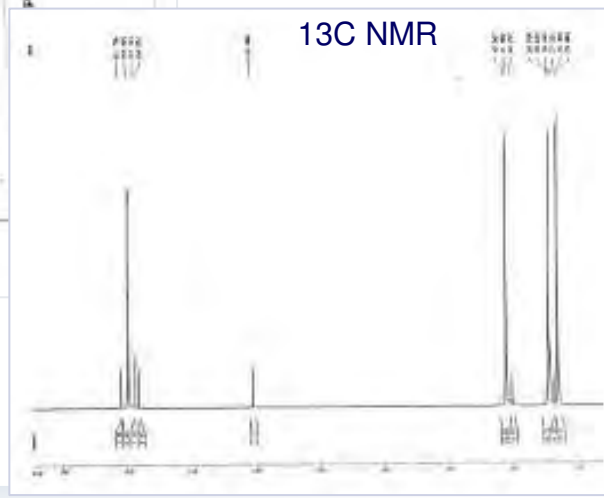
- Determination of HMBDA Content by HPLC
- Determination of HMD Content by GC



Low Molecular Weight Oligomers
Impurities A, B, C



13C NMR



Case Studies: Acrylate based bone cement

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Characterisation Issues:

Quantification of Organic volatile impurities & solvents

Residual Monomer

Degradation products

Effects of sterilisation

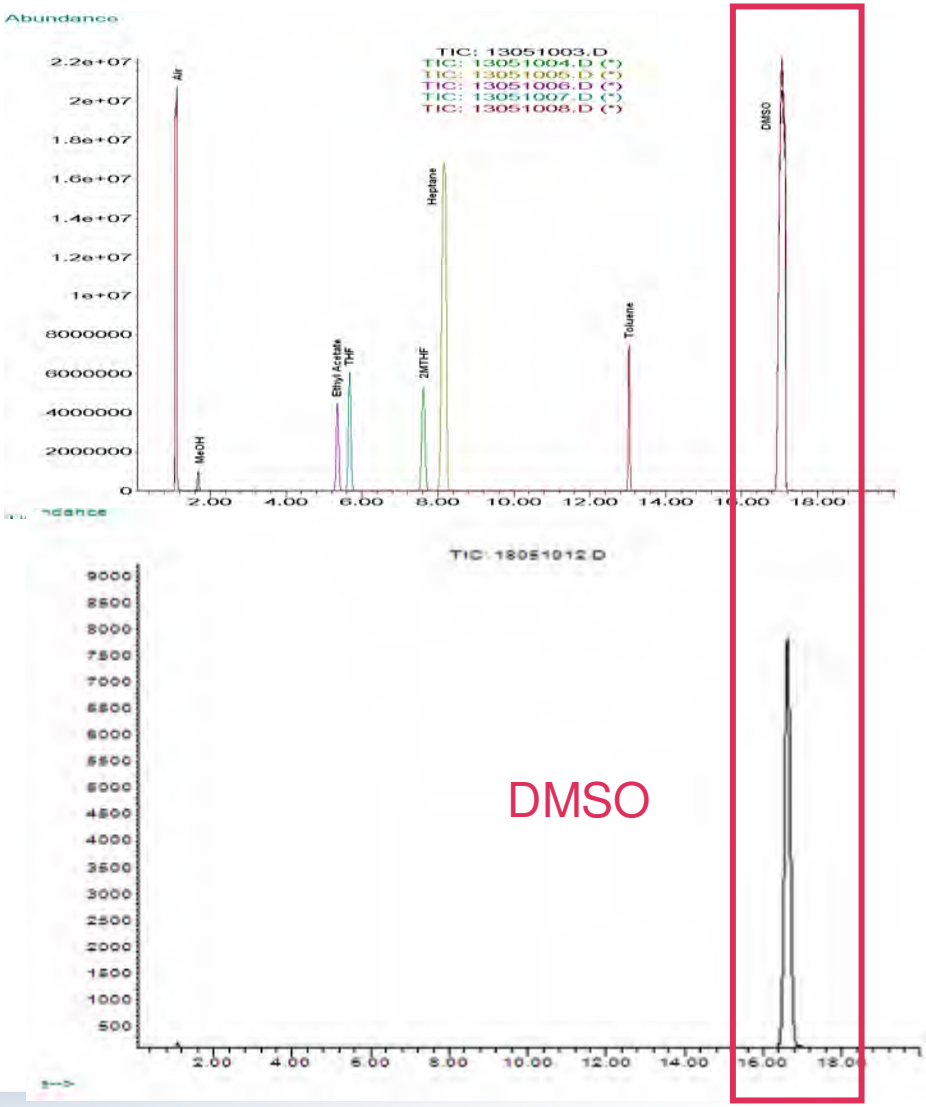
Residual Solvent Methods

USP or EP Pharmacopeia limit tests

Robust Quantification with calibration

Technology

- GC – with mass spec detection – allows for identification for cases when get a response at similar retention time as solvent (as opposed to FID detection)
- Standards are used at ICH limits



Case Studies: Encapsulated Microstructures

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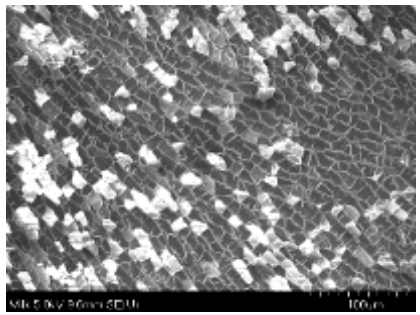
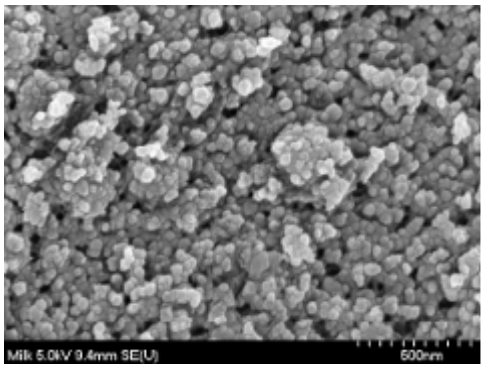
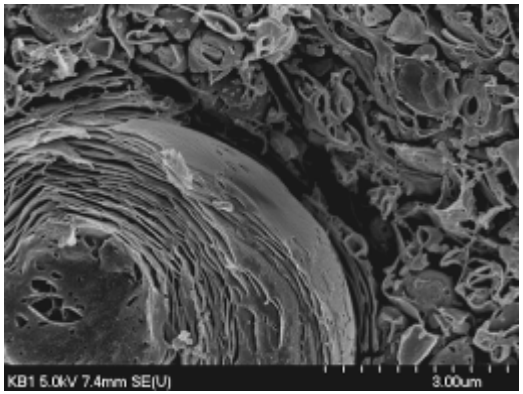
- Ability to analyse “structured system” samples using microscopy e.g.
  - polymer encapsulated systems
  - Liposomes delivery systems
- High pressure freezing to preserve “natural state” of liquid under vacuum
- Can be applied to emulsions, water based gels
- Allows visualisation and comparison of formulations for “feel” and “texture”.



Emulsion sample – conventional methodology

Same sample using high pressure freezing





Visualisation and measurement of liposomes

Conclusion

- Biopolymers present challenges to characterisation
- A wide range of technology is required to characterise the different aspects of these polymers
- Regulatory compliance must be a priority if developing these biopolymers for healthcare applications (is GLP / GMP required?)

Thank you for listening

Thanks to Biotechnology Team, Chromatography Team Microscopy Team, Polymer Experts

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