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An Efficient Approach to Oligomer Screening in Extractables from Single-Use Systems

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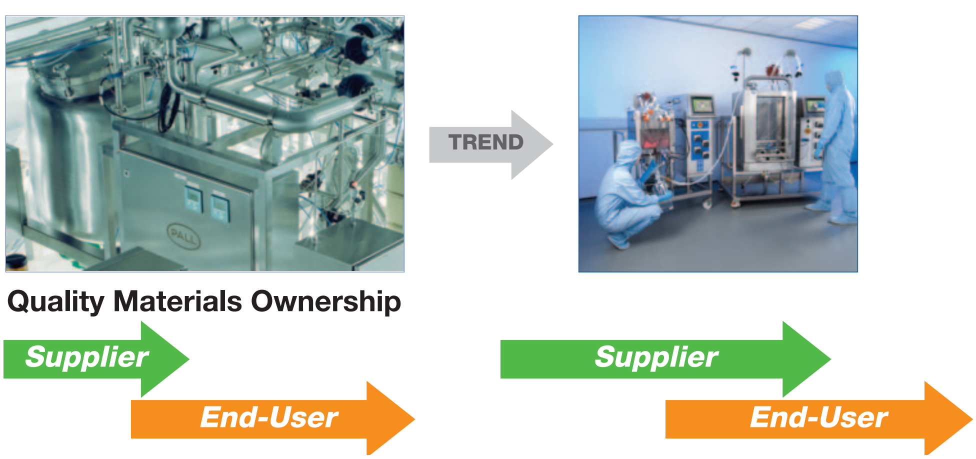
BACKGROUND

- In biopharmaceutical production, single-use systems (SUS) are now used for >85% of pre-commercial scale and is increasingly being adopted for commercial products. The industry has become to recognize SUS-based bioprocessing as generally cheaper, simpler, and preferred over use of stainless steel. On the other hand, the extractable and leachable (E/L) substances from SUS remain one of the top concerns for adopting SUS.
- Since E/L may pose health risks, thorough examination of E/L substances is essential to patient protection and regulatory documentation. However, accurate and rapid identification of unknown E/L compounds from SUS is often difficult due to the wide possibilities of additives, oligomers and degradants. Among these compounds, oligomers and degradants present a unique challenge since they are often the major component of extractables samples and will impede the identification of other extractable compounds. Therefore, we propose to screen oligomers and degradants by common fragment ions.

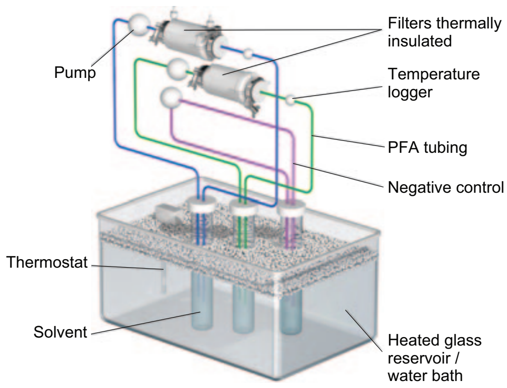
Single-Use Adoption Involves Shared Risks



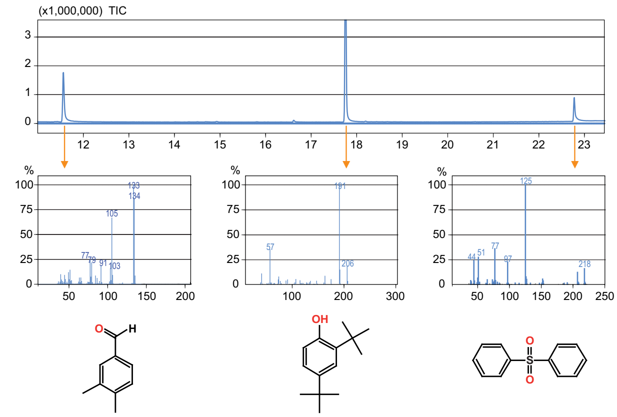
SHARED RISK = SHARED RESPONSIBILITY

Current regulatory guidance requires biopharmaceutical manufacturers to ensure the manufacturing systems do not adulterate the final drug product. The end users have used SUS extractables testing data and leachables evaluation to assess potential risks to patients of the use of these components in product manufacturing.

Preparation and Analysis of E/L Samples

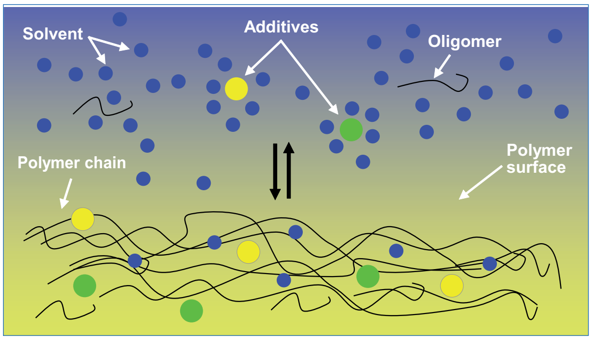


Model solvent or actual process fluid is circulated through the SUS to evaluate the extractables or leachables



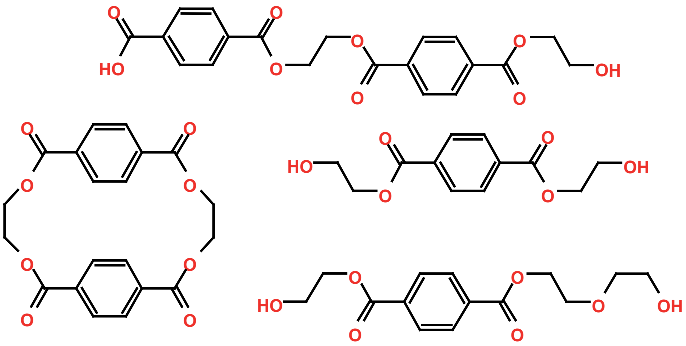
Samples are analyzed by headspace gas chromatography (GC) / MS, direct injection GC/MS, and liquid chromatography (LC) / ultraviolet (UV) / MS to cover volatile and non-volatile compounds

Sources of Extractables and Leachables

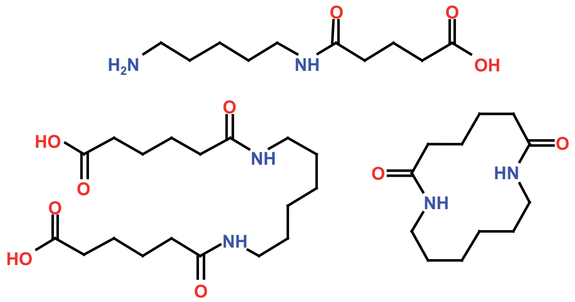


- Antioxidants
- Stabilizers
- Molding agents
- Extrusion agents
- Polymerization aids
- Pore formers
- Residual solvents
- Unreacted monomers
- Colorants
- Lubricants
- Oligomers
- Degradants

Challenge of Oligomer/Degradant



Polyethylene terephthalate (PET) oligomer
Observed 11 different compounds to date



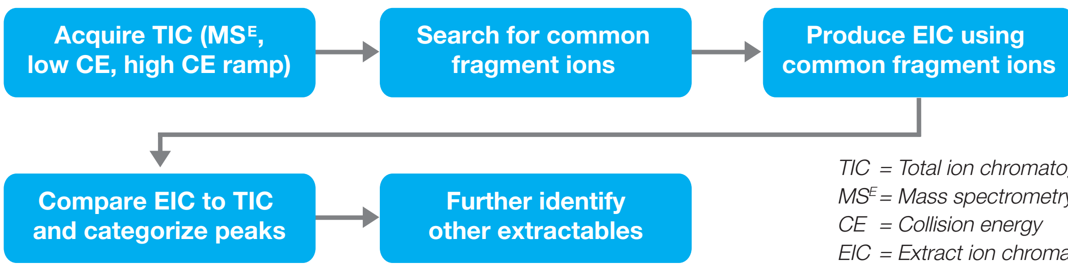
Nylon oligomer / degradant
Observed 56 different compounds to date

Challenges of oligomers and degradants:

- Many possible formulas
- Major components in extractables samples
- Lack of library
- Hard to identify by software
- Interfere identification of other extractables

METHOD & SOLUTION

- We propose to utilize common fragment ions to screen for oligomers and degradants
- Accurate mass measured by Q-TOF enables the screening of specific fragment ions with minimal interference
- We extracted gamma-irradiated Nylon 6,6 resin in 50% ethanol / 50% water at room temperature for 72 hours

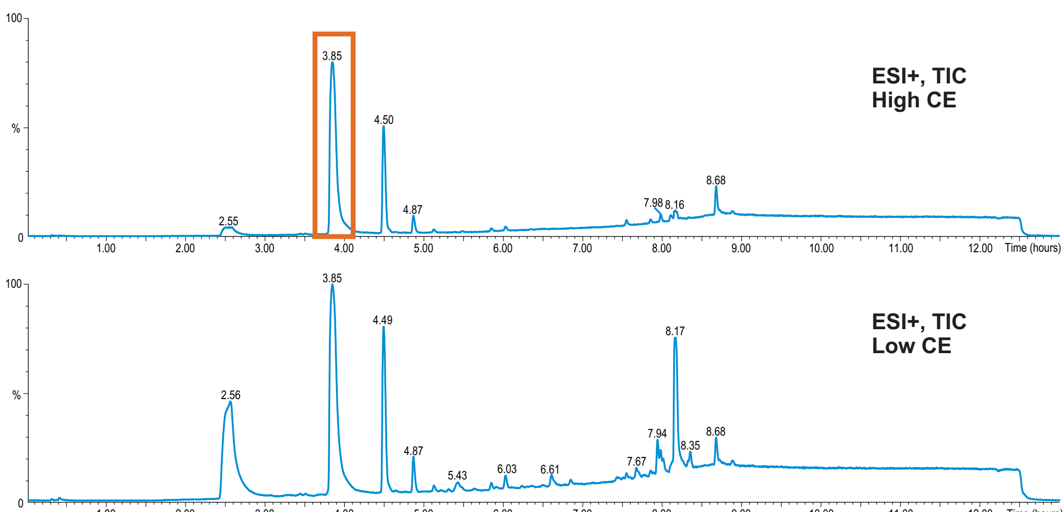


TIC = Total ion chromatogram
MS^E = Mass spectrometry elevated energy
CE = Collision energy
EIC = Extract ion chromatogram

RESULTS

Nylon 6,6 Resin Extracted with 50% EtOH / 50% Water

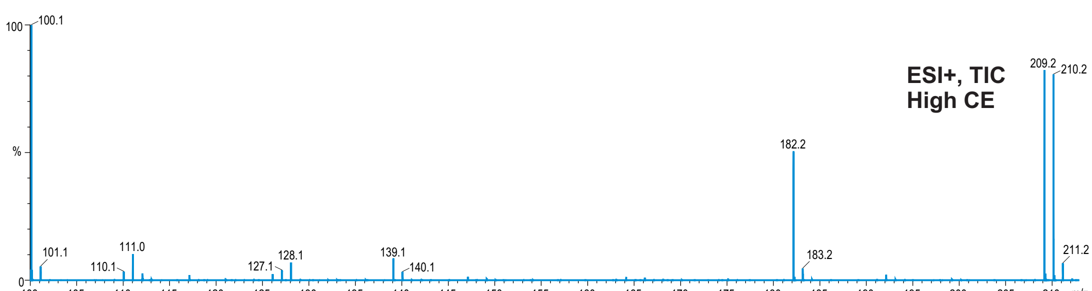
A



- Total ion chromatogram (TIC) of Nylon 6,6 extracts was acquired in ESI+ MSE High CE mode (top) and Low CE mode (bottom). Nylon 6,6 cyclic monomer, Nylon 6,6 cyclic dimer, and Nylon 6,6 cyclic trimer can be seen clearly at residence time (RT) 2.55 min, 3.85 min and 4.50 min respectively

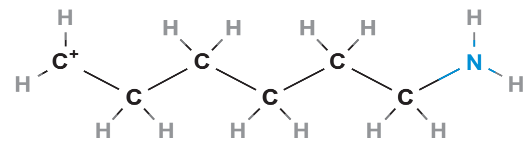
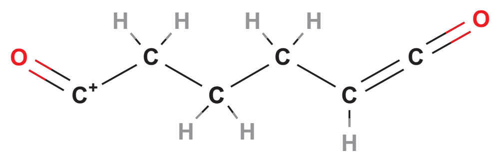
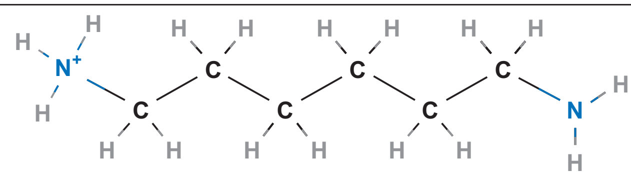
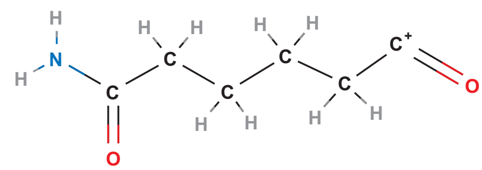
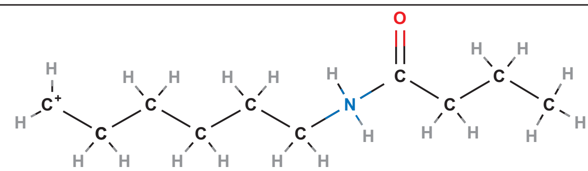
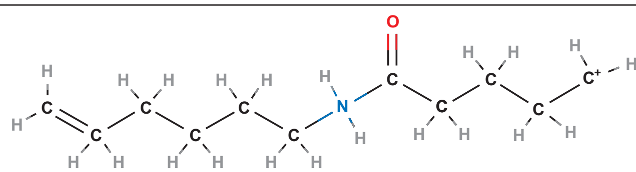
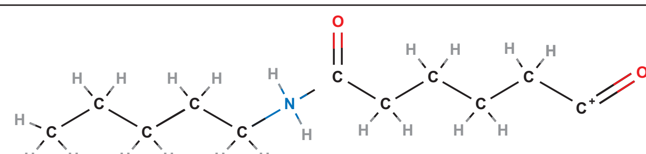
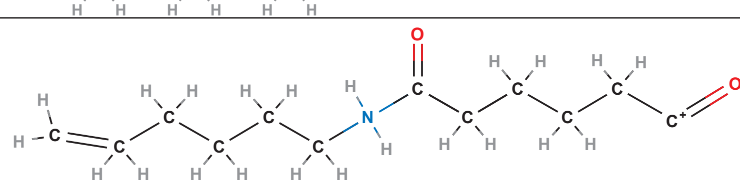
Fragments of Nylon 6,6 Cyclic Dimer at 3.85 min

B



- Fragments of Nylon 6,6 cyclic dimer (RT 3.85 min, parent ion m/z 453.344) in High CE mode are extracted and studied for structure elucidation. This compound contains most of the characteristic Nylon 6,6 fragments

Summary of Fragments of Nylon 6,6

Monoisotopic Mass	Molecular Formula	Structure
100.1126	$C_6H_{14}N$	
111.0446	$C_6H_7O_2$	
117.1392	$C_6H_{17}N_2$	
128.0712	$C_6H_{10}NO_2$	
170.1545	$C_{10}H_{20}NO$	
182.1545	$C_{11}H_{20}NO$	
198.1494	$C_{11}H_{20}NO_2$	
210.1494	$C_{12}H_{20}NO_2$	

- We utilized elucidation tools built into instrument software to help predict fragment structures based on the structure of Nylon 6,6 cyclic dimer
- The results summarized in the table include predicted structures for 8 major fragments of Nylon 6,6

CONCLUSION

- Extractables and leachables testing is increasingly significant in protecting end-user safety, especially as single-use plastics become more widespread in use. A significant challenge is the efficient and accurate identification of unknown compounds, a task made difficult by the number of additives, oligomers, and degradation products. Certain oligomers such as Nylon 6,6 can present in linear and cyclic forms with many possible degradation products, making it impractical to identify oligomer-related compounds using a general formula. We propose screening for common structure motifs and functional groups to categorize oligomers efficiently, saving processing time and improving end-user safety.
- This study has furthered our understanding of Nylon 6,6 degradation products via a systematic analysis of gamma-irradiated Nylon 6,6 resin. Targeting fragment ions which we have observed in previous projects, we were able to successfully predict reasonable structures for eight Nylon 6,6 fragments. These results will benefit our ongoing work on characterizing unknown extractables as polymer degradation products. Future work will focus on structure elucidation of Nylon 6,6 degradants.

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