

Reaction products of halobutyl rubber oligomers with excipients (glycine, histidine, Polysorbate) and solvents (alcohol, water)

The reactivity of chloro- and bromobutyl rubber oligomers C13H23Cl/Br and C21H39Cl/Br towards thiol and amino groups is well known. But what is with other functional groups like carboxyl and hydroxy?

Therefore, two common excipients in biopharmceutical formulations, glycine and histidine, were treated with rubber oligomer C13H23Br (CAS 2514965-51-4). Glycine and histidine show a carboxyl and an amino group, histidine an imidazole ring in addition. The reaction products were analysed by LC-MS (pos and neg) and MS/MS.

In both cases, two reaction products occurred, a major and a minor one. Based on the MS/MS data of the major product (loss of HCOOH and/or CO2) and the response in neg mode, there is a free carboxyl group present. So the main side of attack (at least under the given reaction conditions) is the amino/imidazol group.





Top MS/MS spectrum of peak 6.80 min, insert zoomed region around m/z 208 (loss of HCOOH) Bottom LC-MS ESI neg EIC m/z 252 [M-H⁻] and m/z 298 [M+HCOO⁻]



Top LC-MS extracted ion chromatogram (EIC) m/z 334, bottom ESI pos spectrum $[C_{19}H_{31}O_2N_3 + H^+]$, Histidine adduct

Top MS/MS spectrum of peak 6.37 min, Bottom LC-MS ESI neg EIC m/z 332 [M-H⁻]

The minor product might be the carboxyl adduct. However, heptanoic acid, treated with the rubber oligomer, did not show a reaction product, even under harsh conditions (60 °C, alkaline solution with NaOH). So, these findings are somehow in contrast to the ones given by Piet Christiaens from Nelson Labs [1]. In this presentation, the reaction product of glycine with rubber oligomer is given as an ester (reaction with the carboxyl group).

Solvents like water and alcohols seems to be indifferent towards halo rubber oligomers. You can boil them in water/2-PrOH without formation of any adduct. However, sometimes, the hydroxy or alkylether products can be observed. I left an old C13H23Br stock solution in 2-propanol on the workbench near the window in the sunlight. After several weeks, the propylether product was visible (light induced radical reaction?).



Top GC-MS chromatogram, bottom EI spectrum of peak RT 9.75 min, M^{+.} m/z 238 (C13 isopropyl ether)

I was also able to generate the hydroxy and ethylether derivatives from C21H39Br (CAS 2518227-14-8). The E-and Z-vinyl isomers of C21H39Br were not reactive. Chromatograms and spectra of the compounds are given below. The spectra will soon be added to my EI spectra database of rubber oligomers and made public. Please note, the structures were not confirmed by NMR yet, but the elemental compositions were confirmed by HR-LC-MS.



Top GC-MS chromatogram, bottom EI spectrum of peak RT 12.50 min, M^{+.} m/z 308 (C21 alcohol)



Top GC-MS chromatogram, bottom EI spectrum of peak RT 12.30 min, M^{+.} m/z 336 (C21 ethyl ether)



Top GC-MS chromatogram C21H39Br E-isomer, bottom GC-MS chromatogram C21H39Br Z-isomer, no reaction products.

Furthermore, Polyethylenglycol (PEG) and Polysorbate 20 (PS20) were treated with rubber oligomer C13H23Br (CAS 2514965-51-4). In both cases, reaction products were observed. Analysis was performed with high resolution LC-MS (ESI positive).

In the case of PEG, the evaluation was quite simple. In the total ion chromatogram of treated PEG (bottom), an additional peak was visible (10.36 min), compared to untreated PEG (top). The mass spectra are shown in the insert (top 4-6.6 min, bottom 10.3-10.5 min).



Based on the elemental composition, the reaction products are monoethers with the general formula:



An example of PEG H-[OCH2CH2]₁₃-OH (top) and its reaction product H-[OCH2CH2]₁₃-O-C13H23 (bottom) is given below:



Extracted ion chromatogram m/z 608.3839 and mass spectrum [M+NH4+] and [M+Na+], corresponding to sum formula C26H54O14.



Extracted ion chromatogram m/z 786.5568 and mass spectrum [M+NH4+] and [M+Na+], corresponding to sum formula C39H76O14.

For PS20, the peak pattern is much more complex. PS20 is a mixture of PEG, PEG-sorbitan and PEG-isosorbid, either esterified (full or partial esters) with fatty acids (40-60% lauric acid) or as free alcohols. Also the number of PEG-chains is variable [2]. An LC-MS chromatogram (TIC) is given below:



In the TIC of treated (bottom) and untreated PS20 (top), no differences are visible.



A target search on predicted products was performed. The theoretical masses of NH4+ und Na+ adducts of PEG, PEG-sorbitan, PEG-isosorbid, PEG-monolaurate, PEG-sorbitan-monolaurate and PEG-isosorbid monolaurate, modified by C13H23Br (delta C13H22), were calculated and the corresponding ion traces were extracted. Reaction products (C13H23-ether) were found for all free alcohols and PEG-isosorbid monolaurate. The EIC's and mass spectra are given below:



Extracted ion chromatogram m/z 786.5568 and mass spectrum [M+NH4+], corresponding to sum formula C39H76O14 (PEG-C13 ether).



Extracted ion chromatogram m/z 875.5330 and mass spectrum [M+Na+], corresponding to sum formula C43H80O16 (PEG-sorbitan-C13 ether).



Extracted ion chromatogram m/z 738.5002 and mass spectrum [M+NH4+], corresponding to sum formula C37H68O13 (PEG-isosorbid-C13 ether).



Extracted ion chromatogram m/z 1057.6987 and mass spectrum [M+Na+], corresponding to sum formula C55H102O17 (PEG-isosorbid-monolaurate-C13 ether).

Whether these compounds can be generated during pharmaceutical product storage is unknown. Just simple application of heat and time is not sufficient to convert alcohol groups with halobutyl rubber oligomers to ethers. For this reason, simulation studies will most likely fail.

As described above, light induced reaction is one possible reaction pathway. Other conditions to generate radicals like the presence of oxygen and Fe or Cu ions or other radical initiators might promote ether formation. The oleate in PS 80 is known to undergo degradation at the double bond, induced by radicals, so radical formation in stored Polysorbate formulations is not uncommon:

"Polysorbates are known to undergo degradation in solutions via autoxidation, which is often catalyzed by metal ions and ultraviolet (UV) radiation as a seed for free radicals ..."[3].

- Rubber Oligomers SVP Symposium, March 31, 2022 Presenter: Dr. Piet Christiaens, Scientific Director Nelson Labs Europe <u>https://www.nelsonlabs.com/wp-content/uploads/2022/03/2022-03-31_5_Presentation-Piet-Christiaens_Rubber-Oligomers.pdf</u>
- [2] Oleg Borisov, molecular heterogeneity of Polysorbates and its Implications studies with LC-MS, 9th symposium on the practical applications of mass spectrometry in the biotechnology industry, Sep 09, 2012
- [3] Kishore RSK, Kiese S, Fischer S, Pappenberger A, Grauschopf U, Mahler HC. 2011. The degradation of polysorbates 20 and 80 and its potential impact on the stability of biotherapeutics. Pharm Res 28: 1194–1210.