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Separation of sulfated fucose-containing isomers found in polysaccharides from brown seaweed using cyclic ion mobility mass spectrometry

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Brown seaweeds and some marine invertebrates contain bioactive polysaccharides, including fucoidan, with antimicrobial, antibacterial, and antifungal properties. Improved characterization of their structures would be valuable. To our knowledge, fucoidan extracted from the macroalga *Laminaria digitata* grown in the North Atlantic has not been structurally determined. We show that both singly and doubly sulfated fucose units, and singly sulfated galactose species present within fucoidan exist as isomers. These were identified *via* analysis of the biopolymer using Cyclic Ion Mobility Mass Spectrometry (cIM-MS), which has not been used to determine isomeric species present in fucoidan-derived structures previously. Along with using cIM, tandem MS (MS/MS) provided unique fragmentation related to differing sulfate locations and glycosidic linkages. Connectivity positions between both sulfated fucose and galactose trimers and dimers were identified using Collisional Cross Section (CCS) modelling *via* a machine-learning approach (AllCCS2 prediction software) and drift times from cIM. Previous studies determined that the linkage between the sulfated galactose dimer ($[Gal_2SO_3]^-$) was 1 → 4 due to the presence of $m/z = 361$. Herein, both fragmentation and drift time suggest glycosidic linkages between the monomer units at 1 → 4 and 1 → 3 positions. Ions of high m/z were identified, *e.g.* $[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$, along with smaller, lighter ions such as a dehydrated sulfated fucose, $[FucSO_3\text{-}H_2O]^-$. A major ion seen in this work appears at $m/z = 357$, which we hypothesize as a galactose dimer where one unit has ring-opened to yield a deprotonated glycolic acid ion. The presence of this species has not been reported in earlier structural investigations and speaks to the structural complexity of fucoidan present in *Laminaria digitata* harvested in Atlantic Canada.

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Introduction

Seaweeds, also known as macroalgae, are a common biomass found in oceans with over 10 000 species reported globally.[1] Types of macroalgae include red, brown, and green, and have been used historically in many areas from agriculture (fertilizers) to healthcare (food supplements).[2] These applications are the result of bioactive compounds being found within the biomass, including carbohydrates, minerals, lipids, and various vitamins (A, C, E, and K) which support human health.[3] Typical carbohydrate content of brown algae has been reported to range from 8–81%, with species, location (temperature), and extraction methods playing a role in composition.[4] Carbohydrates found in brown seaweeds specifically include laminarin, alginic acid, and cellulose.[5] The interesting biological properties of brown seaweeds such as antimicrobial and anti-inflammatory effects for therapeutic applications frequently result from their polysaccharide content.[6,7]

Seaweed-sourced polysaccharides, specifically alginates and laminarin, have been explored in the form of cross-linked hydrogels for drug delivery applications.[8,9] These have been successfully characterized using both spectroscopic and spectrometric methods, such as Nuclear Magnetic Resonance (NMR) and Electrospray Ionization Mass Spectrometry (ESI-MS).[10–13] However, characterization of fucoidan remains challenging for researchers due to the complexity of the biopolymer.

Fucoidan, exclusively found in brown algae, is mainly composed of sulfated L-fucose monomers with other sugars, such as galactose, glucuronic acid, mannose, and xylose present as substituents on the main fucose backbone. Its molecular weight can range significantly from low to high molecular weights (13 to 950 kDa), depending on the algal species, geographic location of its growth, and other external conditions.[14] These differences can be monitored using Gel Permeation Chromatography (GPC). The biological activity of fucoidan has been linked to its sulfate content and molecular weight, with higher values for both parameters correlating with superior biological activity compared to those that are less sulfated and lower in molecular weight. With this in mind, characterization of these biopolymers has proved challenging for researchers due to its complexity and heterogeneity. UV-vis and FT-IR spectroscopy have been able to provide some

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insights into the degree of sulfation within fucoidan samples,[15,16] but complete structural information (*e.g.* variation in position of sulfate groups) cannot be obtained using these techniques.

Mass spectrometry (MS) is a highly sensitive analytical technique, which has been used for decades for the characterization of carbohydrates and polysaccharides alike. Due to the often high molecular weights of fucoidan samples, they are normally hydrolysed under acidic conditions to produce materials more suited to MS analysis.[17–20] Previous structural research in this area has determined sulfation in the C-2, C-3, C-4, or C-6 position on the fucose monomer (Fig. 1) in fucoidan.[21] Notably, unique fragmentation of the sulfated fucose residue can be linked to its location. For example, C-2 sulfation yields a fragment at $m/z = 138.97$ only. Sulfation at the C-4 position will also yield an ion at $m/z = 138.97$ but this is accompanied by an additional ion at $m/z = 183.02$. To distinguish between the C-4 and C-6 position, MS^3 is required. Electrospray Ionization (ESI) has been commonly used by researchers in this field compared to Atmospheric Pressure Chemical Ionization (APCI) due to high m/z values and polarities of these analytes. Other techniques for identifying isomers of small organic molecules such as folic acid and lactic acid has been successful using Laser Desorption Postionization Mass Spectrometry Imaging (LDPI-MSI) and Vacuum Ultraviolet (VUV) Photoionization Reflectron Time-of-Flight Mass Spectrometry (PI-ReToF-MS), respectively.[22,23] However, these methods alone do not have the ability to both separate and characterize the isomers present in fucoidan samples.

Ion Mobility Spectrometry Mass Spectrometry (IMS-MS) has gained recent attention in literature for its ability to separate isomers of a compound based on size and charge rather than by affinity for a mobile or stationary phase.[24] Traditionally, the mobility of an ion is measured by the time taken to travel through a drift tube of fixed length. By increasing the distance ions travel through the cell, the degree by which these ions are able to separate increases. This has led to the development of cyclic ion mobility mass spectrometry (cIM-MS), wherein the ion mobility cell is configured with a cyclic geometry. The cyclic system enables the user to determine the number of passes the ion will complete before releasing to the detector.[25–27] The benefits of enhanced ion mobility separation have been demonstrated for characterizing small molecule pollutants such as PFAS,[28,29] and for biologically important analyses including lipidomics,[30,31] and carbohydrates such as glycans and oligosaccharides.[32–36] Similarly, cIM-MS has been used to differentiate anomers of both open and closed-ring forms of pentasaccharides with the assistance of heavy oxygen labelling.[37] Likewise, isomers present in disaccharide species consisting of galactose and glucose were also able to be separated based on their α or β form, and therefore determining differences in composition, connectivity, and configuration in the overall structure.[38] However, it should be noted that there are indeed limitations to the application of ion-mobility to identify isomeric species. For example, the possibility of misinterpreting the presence of multiple conformers of an ion as isomers was highlighted during analysis of glycans.[35] As far as we are aware, cIM-MS has not been employed in the characterization of isomeric sulfated fucose species and related dimers.

Characterization of fucoidans to date has typically used ESI-MS and Matrix Assisted Laser Desorption Ionization Time of Flight MS (MALDI-ToF-MS), with Collision Induced Dissociation MS (CID-MS/MS) being used to determine sulfate and glycosidic linkage location. However, spectrometric data obtained are often complex. Important studies by Anastyuk *et al.* noted the presence of sulfated fucose residues, such as $[Fuc_2\text{-}GalSO_3]^-$ without being able to determine its exact structure.[39] Other research noted needing MS^3 to determine sulfate location in oligosaccharides, with only low molecular weight fractions able to be characterized.[40]

In this work, we describe the use of cIM-MS for separation of isomers present in fucoidan residues for the determination of sulfate position and glycosidic linkage. To our knowledge, this is the first reported instance of using cIM-MS as a way of separating isomers in fucoidan characterization. The starting fucoidan material is first hydrolyzed using an acid resin to obtain oligosaccharides for analysis. Our results show the separation of isomers with sulfation occurring at differing sulfate locations (C-2, C-3, and C-4), and glycosidic linkages occurring at the (1 → 3) and (1 → 4) positions for galactose-based compounds. Transfer collision energy voltages and instrument passes were optimized for each ion of interest and cross-sectional modelling performed to correlate assignments based on fragmentation and drift time. The modelling approach herein makes use of AllCCS2 prediction software, which incorporates a machine-learning approach. Details

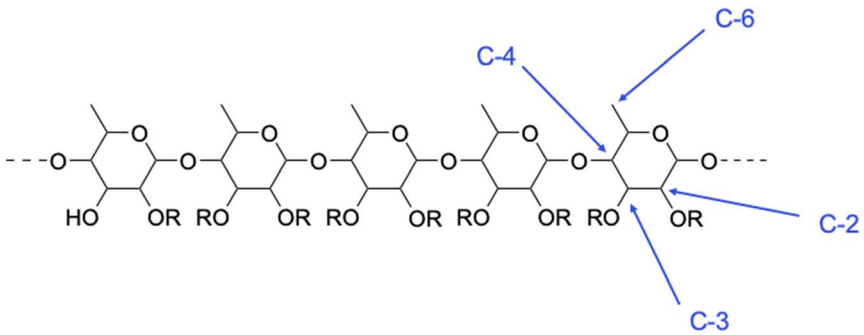

Fig. 1 General structure of fucoidan noting potential sulfation locations on the main polymeric backbone.

provided in the experimental. Therefore, despite accuracies determined by other researchers in the field using test sets incorporated within the software, it is important to use caution when interpretting the data obtained. More detailed computational modelling studies would be needed to further confirm assignments but are beyond the scope of the current study.

Experimental

Materials

Fucoidan isolated from *Laminaria digitata* was purchased from CarboSynth Chemicals. Samples were prepared *via* autohydrolysis using Amberlyst 15, wet, ion exchange resin (Alfa Aesar), along with chemicals HCl and NH_4OH from Sigma Aldrich. Optima Grade water and acetonitrile (99.999% purity, Fisher) were used throughout all procedures.

Autohydrolysis procedure

Fucoidan was hydrolyzed as previously reported by Anastyuk *et al.*[41] It was essential to use glassware due to the presence of plasticizers (containing sulfates) that could leach from plastic labware. To a burette, 15 mL of ion exchange resin was added, charged with 10 mL 0.1 M HCl and washed with 15 mL of water. A 1 mL sample from a 5 mg mL^{-1} stock solution of fucoidan was loaded onto the column and eluted using 10 mL water. The eluted sample was placed into a water bath for 48 h at 37 °C, neutralized with 2.5% (w/w) NH_4OH, and placed in the freezer for 48 h. The sample was then freeze-dried over 2 d to remove water. Blank samples were prepared using the same procedure to confirm that the sulfated fucose species were only found in the samples prepared from fucoidan. From the blank samples, we see no evidence of the ions of interest as there is an intensity of 0 at these *m*/*z* values. For ions $[FucSO_3\text{-}H_2O]^-$, $[FucSO_3]^-$, $[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$, $[Gal_2]^-$, and $[Gal_2SO_3]^-$ in samples prepared from fucoidan, we see intensities of 4.35×10^5, 8.49×10^5, 7.33×10^4, 1.01×10^6, and 2.75×10^5, respectively.

Mass spectrometric procedure

Sample concentrations of 1 mg mL^{-1} in water were used and analytes introduced using direct infusion to an Electrospray Ionization (ESI) source at a flow rate of 5.0 μL min^{-1}. Solvents used for dissolution were a working mix of 1 : 2 v/v acetonitrile to water. Sample data were collected on a Waters SELECT SERIES Cyclic Ion Mobility Mass Spectrometer (Wilmslow, UK). Samples were analyzed in negative polarity mode with a mass range of 50–1200 *m*/*z* with a cycle time of 1 s. Cone and capillary voltages were set to 40 V and 2.50 kV, respectively. Instrument source temperature was 150 °C with the cone gas flow set to 100 L h^{-1}. The desolvation gas, N_2, was set to a temperature of 250 °C at 350 L min^{-1}. Collision energy was set to 6 eV and the transfer collision energy was varied between 20 and 30 eV, depending on the ion of interest. The TOF analyzer was used in "V" mode, yielding a resolving power of 40 000 for *m*/*z* 298 ions $[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$. Values were corrected to the sulfate anion (*m*/*z* 96.9596) as direct infusion was used. For cyclic ion mobility, a single and multi-pass system were used with a separation time of 2 ms and 45 ms, respectively. Wave velocity was set to 375.0 m s^{-1} with a travelling wave height of 15.0 V. Data was analyzed using MassLynx V4.2. Collisional Cross Section modelling was performed using AllCCS2 prediction software through the Zhu Lab (http://allccs.zhulab.cn). Compounds of interest were drawn in their neutral form (no negative charge) in ChemDraw 23.1.2 to obtain SMILES data that was then inputted into AllCCS2 software. Whilst these values provide an estimate of the CCS value of each ion, it is important to acknowledge the limitations of the machine-learning model used. For example, the creators of AllCCS2 used a training set of >10 384 experimental CCS values, and reported median relative error values up to 1.64% within their testing sets.[42] With the addition of the AllCCS experimental testing set, which contains a wide range of small molecules, lipids and peptides, a total of 498 carbohydrates were incorporated.[43] The experimental dataset used for carbohydrates was obtained from McLean *et al.*,[44] and it was noted that the median relative errors for carbohydrates were 0.5% or better. We also note that the purpose of using AllCCS2 in the present study was not to predict absolute CCS values of the observed sulfated fucose ions, but rather to provide insight regarding the order of arrival times. The uncertainty associated with the predicted CCS values means that caution must be exercised when using CCS alone to distinguish between isomeric structures. However, when the CCS predictions are interpreted alongside the results of the CID experiments and MS/MS data, the confidence in both the proposed structural assignments and the observed arrival-time ordering increases. It should be noted that this machine-learning approach should be used with caution and detailed molecular modelling tools may provide more accurate structures.

Results and discussion

Fucoidan samples have been analyzed previously using ESI-MS.[39–41] In prior studies, samples were hydrolyzed to reduce the molecular weight of species being analyzed. This further increases the chance of volatilization, formation of stable gas phase ions and therefore the possibility of determining linkage and sulfate locations in fucose residues within the fucoidan. Proposed structures of key ions detected can be seen in Fig. 2. Anions with relatively high *m*/*z* values up to 517 were observed and overall spectra were similar to those reported by Anastyuk *et al.* (Fig. 3).

To get more insight into the positions of sulfation on the fucose units, tandem MS (MS/MS) was performed *via* Collision Induced Dissociation (CID). Previous studies showed the benefit of using CID to obtain structural information on fucoidan from *Sargassum siliquosum* using collision energies between 1 eV and 50 eV.[45] Herein, collision energies varying from 20 eV to 30 eV were used. In this work, similar ions of interest to those reported by Chang and co-workers such as $[FucSO_3\text{-}H_2O]^-$, $[FucSO_3]^-$, and $[Gal_2SO_3]^-$ were observed. However, ions including $[Fuc_3(SO_3)\text{-}H_2O]^-$ and $[Fuc_2SO_3\text{-}H_2O]^-$ not seen by Chang and co-workers, but previously reported by Anastyuk *et al.*[46] were seen. This confirms that fucoidan isolated

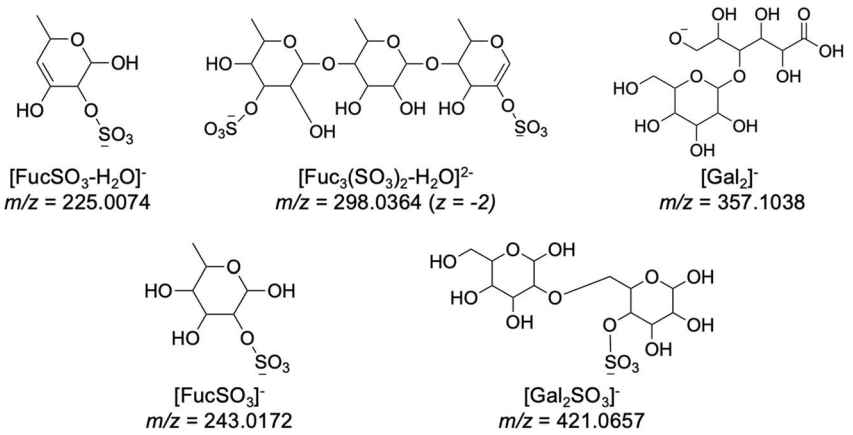

Fig. 2 Sulfated fucose residues of interest.

from *Laminaria digitata* is structurally different to that isolated from several other macroalgae such as *Sargassum siliquosum*. Although we see the same anions as other researchers, we were intrigued to see if we could obtain more data from the arrival time distributions obtained by cIM-MS regarding sulfate location within different fucose and galactose species that are related to the main fucose backbone, or as a substituent. A table of compound names, complete structures, and *m*/*z* can be found in the SI (Table S1).

Structure elucidation by MS/MS and isomer separation by cIMS

$[FucSO_3\text{-}H_2O]^-$. An ion frequently observed during mass spectrometric analysis of fucoidan is a dehydrated, sulfated fucose monomer ($[FucSO_3\text{-}H_2O]^-$) at $m/z = 225.0066$. Therefore, this was the first ion to be examined in detail within the current study. Single- and multi-pass procedures were performed to increase path length and promote separation. We could confirm that wrap-around effects were not occurring with an increased path length as we would see the faster ions overtake the slower ions and show up earlier in the arrival time distribution if this had happened.[25] However, multiple passes showed additional separation with three isomers being seen. From the MS/MS data, we can determine that the isomer seen at 16.57 ms in the one pass data is seen at 54.25 ms in the multi-pass data and the isomer seen at 19.14 ms in the one pass data is seen at 58.54 ms in the multi-pass data (Fig. 4a–c). The isomer separated at 54.25 ms does show a peak at *m*/*z* 225.0069, however the MS/MS data corresponding to this isomer yields high error when comparing to typical fragmentation of this ion. For example, cross-ring cleavage fragmentation gives a predicted *m*/*z* of 138.9706, with isomers at 51.94 and 58.87 ms showing less than 1% ppm error for this fragment. On the other hand, the isomer seen at 54.25 ms shows almost over 13% error for this fragment and therefore can be omitted from the analysis.

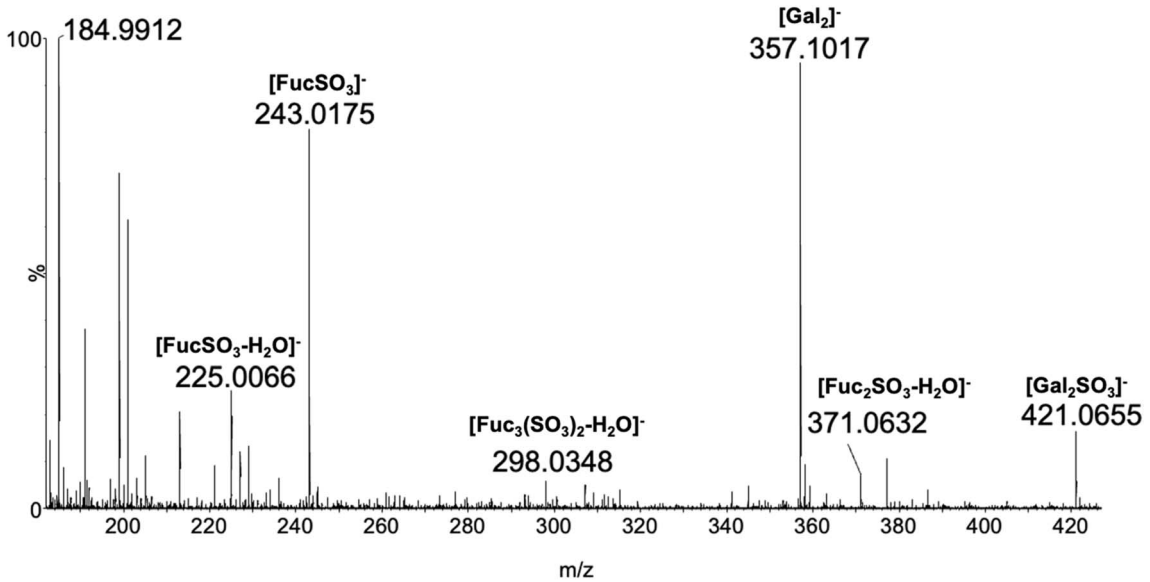

Fig. 3 Full ESI-MS (negative mode) spectrum of hydrolysed fucoidan from *Laminaria digitata*.

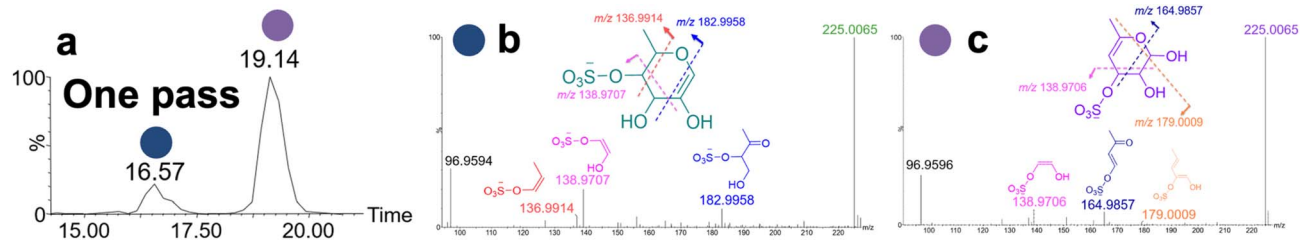

Fig. 4 (a) Arrival time distribution of $[FucSO_3\text{-}H_2O]^-$ after singular pass at (b) 16.57 ms and (c) 19.14 ms.

Between the isomers, we note similarities in the mass spectra such as cross-ring cleavage (*m*/*z* 138.970) and the presence of a sulfate anion (*m*/*z* 96.9594). The MS/MS data obtained from the isomer with the drift time 16.57 ms shows unique species at *m*/*z* 136.9914 and 182.9958, suggesting sulfation is occurring at the C-4 position (Fig. 4b). In the second isomer at a drift time of 19.14 ms, fragmentation at *m*/*z* 164.9857 and 179.0009 suggests sulfation is occurring at the C-3 position (Fig. 4c). These fragments were not observed for the isomer at 16.57 ms. The relative drift times of the two isomers are also consistent with their predicted CCS values, with the first isomer predicted to have greater mobility and a more compact CCS value (140.7 Å^2 *vs.* 142.8 Å^2).

$[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$. As the *m*/*z* of the fragment increases, it becomes more likely that more than one sugar will be present within the ion. By increasing the number of passes around the cyclic system, a total of three isomers could be resolved with drift times of 50.55, 52.93, and 54.51 ms (Fig. 5a).

Here, we note the presence of *m*/*z* = 298.0364 which is a dehydrated, doubly sulfated fucose trimer ($[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$). Doubly charged precursors typically yield singly charged fragment ions. This is why the MS/MS spectra of the *m*/*z* 298 precursor ions display peaks with *m*/*z* that are higher than their precursor. For example, the spectra from all three isomers (Fig. 5c–e) display *m*/*z* 371.0648, a dehydrated singly sulfated fucose disaccharide ($[Fuc_2SO_3\text{-}H_2O]^-$). Fragments distinguishing sulfate location can be seen at *m*/*z* 138.97, which has been previously described. Fragmentation at *m*/*z* 136.99 is due to C-4 sulfation at the non-reduced end of the compound, which has been previously described, with *m*/*z* 164.98 suggesting C-2 sulfation on the reduced end of the compound. We see no evidence of cross-ring cleavage that would support sulfate position being at other positions within the ion. For example, if cross-ring fragmentation was to occur with the sulfate at the C-3 position on the reduced end of the compound, we would see fragmentation resulting in *m*/*z* 261.0180 (as it would be $z = -2$).

One major difference between the isomer with drift time 50.55 ms and those at 52.67, and 54.51 ms is a high abundance of *m*/*z* 517.1204, which is defined as the singly sulfated trimer (loss of sulfate anion from the parent ion, $C_{18}H_{29}O_{15}S^-$). For isomers eluting at 50.55, 52.67 and 54.51 ms, we still see the singly sulfated trimer with additional fragmentation at *m*/*z* 499.11 due to additional dehydration of $[Fuc_3SO_3\text{-}H_2O]^-$. The singly sulfated trimer species is present in the remaining isomers, but in much lower abundance compared to the dehydrated species. Other than the loss of a sulfate anion and dehydration occurring, we see no differences in fragmentation between these isomers. Therefore, we can hypothesize that connectivity between the fucose

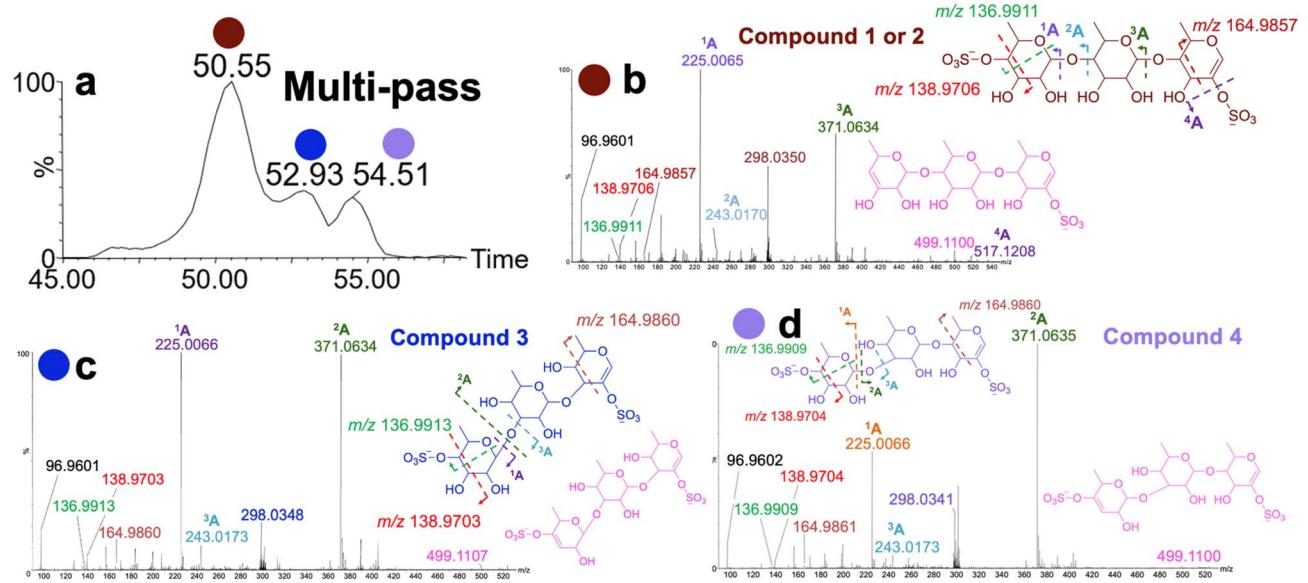

Fig. 5 (a) Arrival time distribution of $[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$ after one pass at 20 eV with corresponding MS/MS at (b) 50.55 ms (c) 52.93 ms (d) 54.51 ms.

residues within the trimer would cause this result due to steric and electronic effects taking precedence. This phenomenon has been studied previously in literature with separation of α/β glycan anomers using cIM.[47]

In $[Fuc_3((SO_3)_2)\text{-}H_2O]^{2-}$ there are two sites where glycosidic linkage can occur. Therefore, we propose that the three of ions separated by cIM, Fig. 5(b), could be alternating between (1 → 3)(1 → 3), (1 → 3)(1 → 4), (1 → 4)(1 → 3), or (1 → 4)(1 → 4) connectivity. Modelling of the CCS for these compounds with varying connectivity means we can see that a difference in shape is expected for each change in glycosidic linkage (Fig. 6). However, it is important to note that this way of processing (*i.e.*, CCS modelling) has its limitations with accuracy and therefore further studies will need to be performed to unequivocally confirm identities for each ion. The model suggests that compounds **1** and **2** are characterized by virtually identical CCS values (224.09 *vs.* 224.43 Å^2), which would require a resolving power of >600. Work reported by Giles and co-workers notes that achieving this resolving power would require >50 passes around the cIMS cell.[26,41] The arrival time distribution shown in Fig. 5b was obtained using only 5 passes. In contrast, we estimate that compounds **3** and **4** are separable under these conditions. We can hypothesize that the presence of both compounds **1** and **2** may exist at 50.55 ms due to double the intensity of the isomer compared to those separated after at 52.93 and 54.51 ms. Isotopic patterns of this compound showing a doubly sulfated precursor can be seen in the SI (Fig. S2).

$[(Gal)_2]^-$. Interestingly, one of the major ions observed in this work appears at *m*/*z* 357.1009. At this *m*/*z*, one isomer was seen at a drift time of 25.48 ms with optimized data collected using one pass around the cyclic system (Fig. 7a). Fragmentation seen in the MS/MS data suggests the precursor ion is a galactose dimer where one of the monomer units has ring opened. The ring opened ion is notably a substituent of the main fucose backbone within fucoidan. This species lacks a sulfate group and therefore fragment ions do not have sulfur-containing isotopic patterns. Fragmentation seen at *m*/*z* 75.0088 is likely the result of a deprotonated glycolic acid ion (Fig. 7b). The presence of this ion highlights once more the complexity of the overall structure, noting possible rearrangements occurring prior or during analysis taking place.

$[FucSO_3]^-$. Another ion frequently observed during mass spectrometric analysis of fucoidan is a sulfated fucose monomer ($[FucSO_3]^-$) seen at *m*/*z* 243.0172 Fig. 8. This ion showed only one isomer arriving from the system, meaning only one isomer is present. To ensure separation of any ions would occur, multiple passes around the system were used but no additional isomers were separated. Fragmentation at *m*/*z* 138.9701 and 182.9964 is indictive of C-2 sulfation, as described previously.[41,45] We can also confirm that sulfation does not occur at the C-4 position as we would see a *m*/*z* 136.9914, representing cleavage between the C-3 and C-5 position.

$[Gal_2SO_3]^-$. An ion not as frequently observed during mass spectrometric analysis of fucoidan is a sulfated galactose dimer ($[Gal_2SO_3]^-$) at *m*/*z* 421.065. Multiple passes around the system were attempted, yielding four isomer species (Fig. 9a). Little separation was seen between each isomer, suggesting that each isomer has a similar CCS value. The sulfated galactose dimer $[Gal_2SO_3]^-$ is present as a side chain (*i.e.*, not part of the fucose backbone) as seen in literature before.[45] Fragments seen in all isomers of this work include *m*/*z* 241, 361, and 138.9, which are common fragments reported representing sulfation on the non-reducing end of the isomer, a dehydrated sulfated dimer, and cross-ring cleavage, respectively.[45]

The first isomer separated at 89.89 ms shows fragmentation highlighting sulfate location. It can be confirmed that sulfation is not occurring at the C-4 position as fragmentation at *m*/*z* 152.9863 would be seen. With this in mind, we hypothesize sulfation is occurring at the C-3 position with a small peak at *m*/*z* 331.0349, representing cross-ring cleavage (Fig. 9b). This

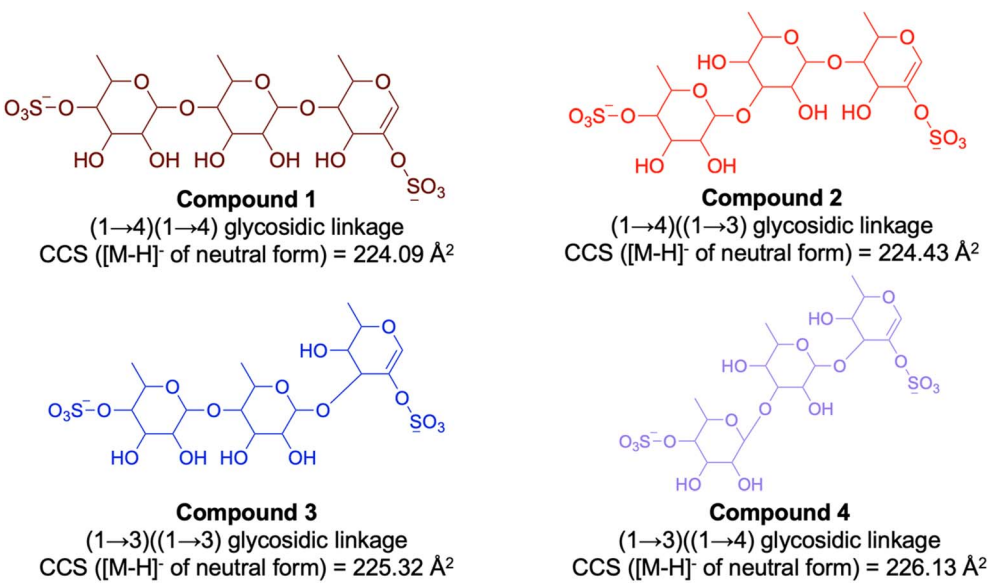

Fig. 6 Predicted CCS values of $[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$ showing (1 → 4) (1 → 4), (1 → 4)(1 → 3), (1 → 3)(1 → 3) and (1 → 3)(1 → 4) glycosidic linkage. Color of each structure aligns with colors used in Fig. 5.

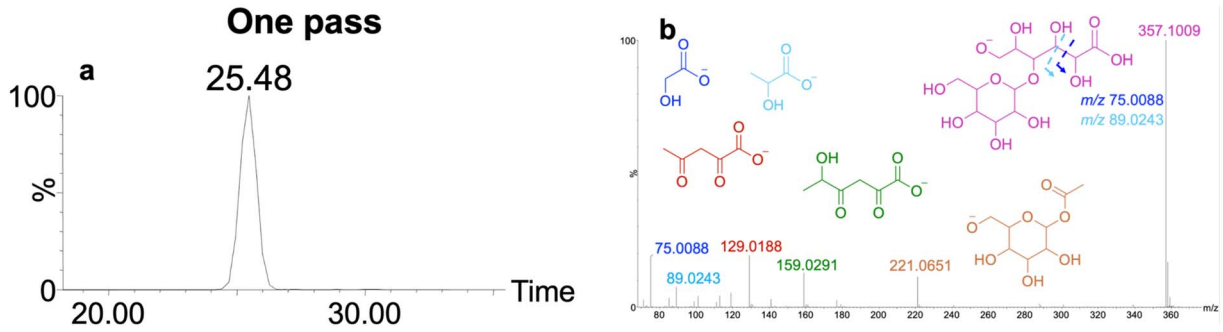

Fig. 7 (a) Arrival time distribution of $[Gal_2]^-$ after one pass at 27 eV with corresponding (b) MS/MS at 25.48 ms.

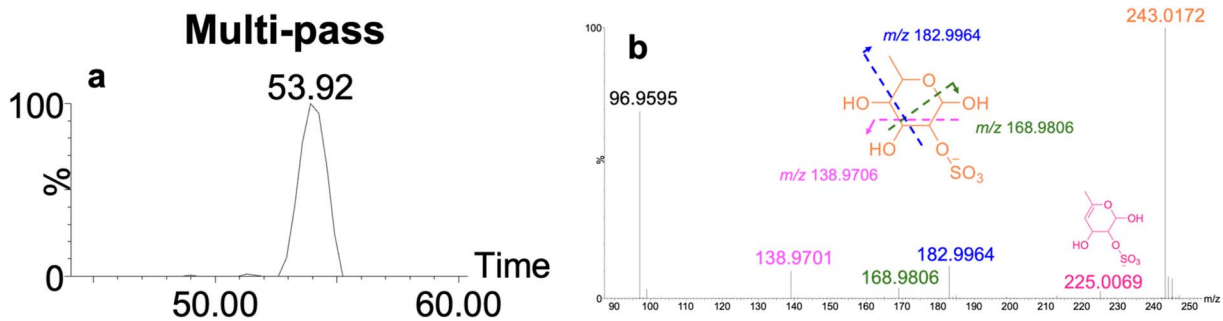

Fig. 8 (a) Arrival time distribution of $[FucSO_3]^-$ after multiple passes at 20 eV with corresponding (b) MS/MS at 53.92 ms.

fragment is also seen in the MS/MS data of the isomer separating at 95.50 ms (Fig. 9d). It is important to note that for this sulfated galactose dimer, minimal fragmentation differences are seen between isomers that would assist in determining sulfate position. With this in mind, we can hypothesize that changes in glycosidic linkages can be separated, similar to that of $[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$.

The second isomer separated at 92.53 ms has a similar fragmentation pattern to the first isomer, which is likely due to lack of complete separation between the isomers themselves within the drift tube. However, we also now see the presence of *m*/*z* 301.0234, which is representative of C-2 sulfation on the non-reduced end of the ion. We also see no evidence of *m*/*z* 331, which would suggest C-3 sulfation (Fig. 9c). Modelling of this data suggests compounds **1–4** in Fig. 10 have CCS values that can be separated using the multi-pass system used experimentally, where compounds **1** and **2** are seen at 89.89 and 92.53 ms in Fig. 9a, respectively.

Next, the third isomer separated at 95.50 ms has distinctive fragmentation at *m*/*z* 259.0124 which is the singly sulfated galactose monomer $[GalSO_3]^-$ which is not seen in the previous isomers. The presence of this ion is important as it shows a higher level of fragmentation occurring due to the larger CCS of the ion. To determine sulfate position, we see *m*/*z* 331 in this isomer noting sulfation at the C-3 position (Fig. 9d). The presence of *m*/*z* 301 is likely due to cross-ring fragmentation

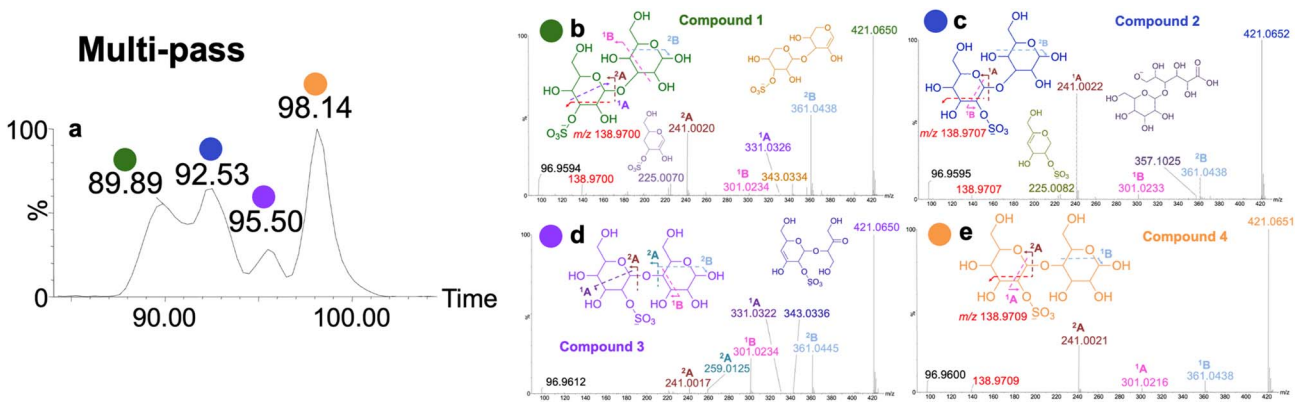

Fig. 9 (a) Arrival time distribution of $[Gal_2SO_3]^-$ after multiple passes at 30 eV with corresponding MS/MS at (b) 89.89 ms (c) 92.53 ms (d) 95.50 ms (e) 98.14 ms.

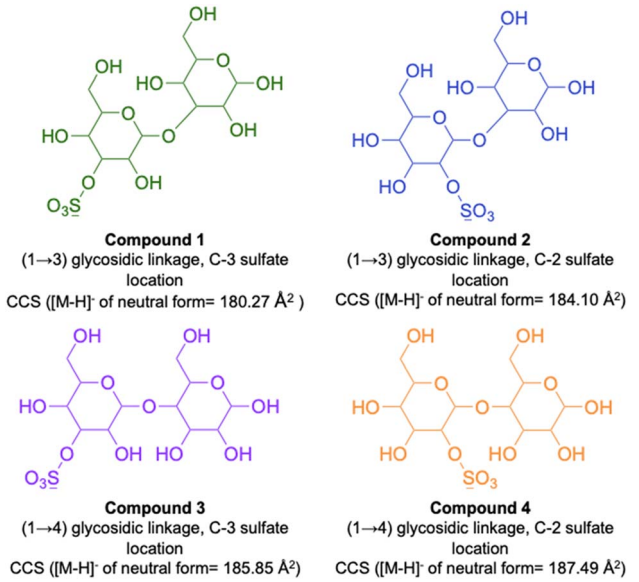

Fig. 10 Predicted CCS of $[Gal_2SO_3]^-$ showing varying glycosidic linkages with sulfate locations at the C-3 and C-2 positions. Color of each structure aligns with colors used in Fig. 9.

occurring on the galactose monomer with no sulfate. Therefore, we hypothesize this is the sulfated galactose dimer with (1 → 4) glycosidic linkage with C-3 sulfation. CCS data (Fig. 10) is also in agreement when comparing to previously tabulated values in the first and second isomers that were separated.

The final isomer separated at 98.14 ms yields similar fragmentation to the other isomers, such as *m/z* 241, 301, and 361 and no *m/z* 259 or 331, suggesting sulfation at the C-2 position (Fig. 9e). Therefore, using CCS data, we can hypothesize that this isomer contains (1 → 4) glycosidic linkage with C-2 sulfation (Fig. 10). It is worth noting that additional isomers may be present for this compound due to similar CCS values. For example, a structure featuring a (1 → 3) glycosidic linkage and sulfation at the C-2 position of the β-galactose unit yields a predicted CCS value of 183.43 Å^2. By comparing this isomer to compound **2** (with a CCS of 184.10 Å^2), the ΔCCS is 0.33 Å^2, which would require a resolving power >500 to achieve separation for the isomers.[48,49] With this in mind, work from Giles and co-workers reports that a minimum of 40 passes around the cyclic system would be needed in order to separate these isomers.[26] While increasing the number of passes (*i.e.*, path length) around the ion mobility cell can improve separation, it can also hinder the separation as much faster ions begin to overtake those moving at a slower rate, resulting in what is known as "wrap-around".[25]

Conclusions

Isomers present within hydrolyzed fucoidan oligomers that are able to be separated by cIM based on shape and size of the molecule using drift time experiments. This confirms assumptions made regarding the presence of isomers in ESI-MS data from earlier studies of fucoidan. The cIM-MS/MS data confirm the presence of sulfated fucose and galactose units in the fucoidan isolated from *Laminaria digitata* grown in the North Atlantic, and thus similar to fucoidan isolated and characterized from *Sargassum siliquosum* seaweeds.[45] To our knowledge, we also report the first instance of ring-opened and ring closed isomers separated during fucoidan analysis, along with using CCS modelling (obtained using a machine learning approach) to correlate drift times with structural differences such as sulfate position and glycosidic linkage. There are limitations based on this approach, as the test set employed during machine learning would not include these specific ions. Further confirmation of relative drift times and CCS for the isomers proposed herein would benefit from the use of state of the art CCS prediction tools (*e.g.* IMOS and MOBCAL) and may be explored in the future. Also, Gel Permeation Chromatography (GPC) will be used to understand molecular weight and dispersity of the original biopolymer. Cationizing agents will also be screened and would be useful in determining the presence of any potential neutral oligomeric species that could be present in hydrolyzed fucoidan samples.

Author contributions

F. M. K. and O. M. W. developed the idea for the project. O. M. W. drafted the manuscript and all authors edited and reviewed the manuscript. O. M. W. performed experiments and characterization, with the assistance of K. J. J. All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

Data availability

The data for supporting this article are presented within the article or in supplementary information (SI). Supplementary information: typical *m/z* values for ions of interest; and theoretical and experimental isotopic patterns for $[Fuc_3(SO_3)_2\text{-}H_2O]^{2-}$ ion. See DOI: https://doi.org/10.1039/d6ra00238b.

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