應用手冊

Investigating Solvent-Induced Ionic Interaction in Water-Soluble Biopolymers Using Advanced Polymer Chromatography™

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Abstract

Sodium carboxymethylcellulose (NaCMC) as water-soluble and non-toxic biopolymer finds use across a lot of industries in various products such as food and biopharmaceutical. Its thickening as well as its mechanical and viscoelastic properties are governed by molecular weight and degree of substitution which in turn affects its solubility. It is important to suitably characterize NaCMC prior to its incorporation into a product to ensure optimal performance. Due to the ionic nature, finding an appropriate mobile phase for characterization is challenging. Moving away from a lengthy traditional size exclusion chromatography method, this study focuses on method development using an Advanced Polymer Chromatography™ System to rapidly screen mobile phases and determine the molecular weights of five NaCMC samples of two different viscosity grades. Significantly different chromatographic responses were observed as a result of ionic interactions between the mobile phases tested and the polymer. Neutral pH and elevated salinity exhibited minimal ionic interactions and yielded best chromatographic performance.

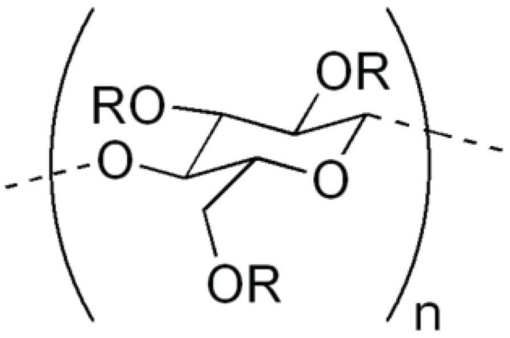
Benefits

- APC proved a valuable tool for efficient and fast method development

- BEH AQ columns yielded reproducible results with low RSD over a broad pH range

Introduction

Over the years, industries have seen a steady increase in bio-based polymers such as cellulose and its derivates in their products and processes.[1] Since native cellulose is challenging to use, it is often modified to affect its physicochemical properties such as solubility and viscosity. One example of modified cellulose is NaCMC. Up to three modifications per glucose unit are possible for a mono sodium carboxymethyl substitution (Figure 1).



$R = H$ or CH_2CO_2H

Figure 1. Possible sites for substitution.[2]

Different modification processes provide varying degree of substitution (DS) —*i.e.*, the average number of sodium carboxymethyl groups substituted per anhydro glucose unit. NaCMC is increasingly used in many sectors such as foods, pharmaceuticals, paints, ceramics, and oil industry. Aqueous NaCMC solutions can form highly viscous solutions that find use for their thickening, suspending, and stabilizing properties in especially foods and cosmetics. NaCMC can have a heterogeneous chemical composition and a wide range of molecular weight (MW). In order to determine the impact of the molecular structure of NaCMC on application properties, average molecular weight information is important.[2] The increasing industrial importance of NaCMC has created interest in different techniques and methods for its characterization. This cellulose-based polyelectrolyte, like many other polysaccharide derivatives, is very difficult to characterize due to its heterogeneity not only in molecular weight (MW) but also in chemical composition.[3] In order to improve the functionality within existing applications, or to develop new applications, it is essential to determine the physicochemical characteristics, e.g., DS, MW, and rheological properties. The DS has a pronounced effect on the solubility of this compound amongst other properties. Otherwise, NaCMC is readily soluble in water. For NaCMC, MW heterogeneity and its distribution determination, conventional size exclusion chromatography (SEC) based methods have been mostly reported.[4,5] However, conventional SEC measurements typically requires longer run times and are often less sensitive to minor MW heterogeneity due to low resolution as an effect of the larger sorbent particle size and instrument related band-broadening.[6] UPLC-like SEC separation overcomes these deficiencies and has been utilized successfully for characterization bio-based and semi-synthetic polymers in the past.[7]

When establishing a robust method for a size-based chromatography type technique, a few important factors must be considered. The main factors are stationary phase selection, mobile phase selection, and sample preparation. An in-house method was utilized for sample preparation and is not further investigated for this study. The focus was centered on mobile phase selection for a given stationary phase: the BEH AQ column.

The focus of this work is to screen multiple mobile phases within a short time frame to overcome ionic interaction featuring Aadvanced Polymer Chromatography™ (APC™) to reproducibly determine MW of NaCMC.

Experimental

Mobile phases for APC, ***i.e.***, acetate buffer at pH = 5; diluted NaOH at pH = 8.8; 0.2M $NaNO_3$ + 0.01M NaH_2PO_4 at pH=7 were prepared by dissolving the respective amount of salt in deionized water. pH was adjusted with acetic acid, hydrochloric acid, and/or sodium hydroxide.

Sample Description

The sample set comprises five NaCMC samples of two different viscosity grades. NaCMC 1 and 2 are high viscosity grade samples and NaCMC 3 through 5 are of medium viscosity grade. 100 mg NaCMC was dissolved in 10 mL deionized water on a linear shaking water bath at 80 °C (70 rpm) for 1 hour. The solutions were cooled to room temperature and diluted with eluent to make a 1 mg/mL sample solution.

LC Conditions

LC System:	ACQUITY APC™
Detection:	Refractive Index (RI)
Column(s):	AQ900 Å + AQ 450 Å + AQ 125 Å/AQ 45 Å
Column temp.:	40 °C
Sample temp.:	25 °C
Injection volume.:	10 µL
Flow rate:	1 mL/min and 0.5 mL/min

Data Management

Chromatography Software:	Empower™ 3 with GPC option

Results and Discussion

De-ionized water as mobile phase was chosen as a starting point to assess polymer-polymer as well as polymer-surface interaction. The five NaCMC samples eluted within the same retention time range at the approximate void volume (V_0) as narrow peaks with spikes and shoulders as shown in Figure 2. The early elution with little to no separation is an apparent result of the combined action of coil expansion by repulsion and ion exclusion. This elution behavior of NaCMC in water is typical for polyelectrolytes in polar solvents of low ionic strength.[9] These charge effects are independent of the stationary phase type as this elution behavior of NaCMC with water as eluent is identical to that observed on gel-based columns.[9]

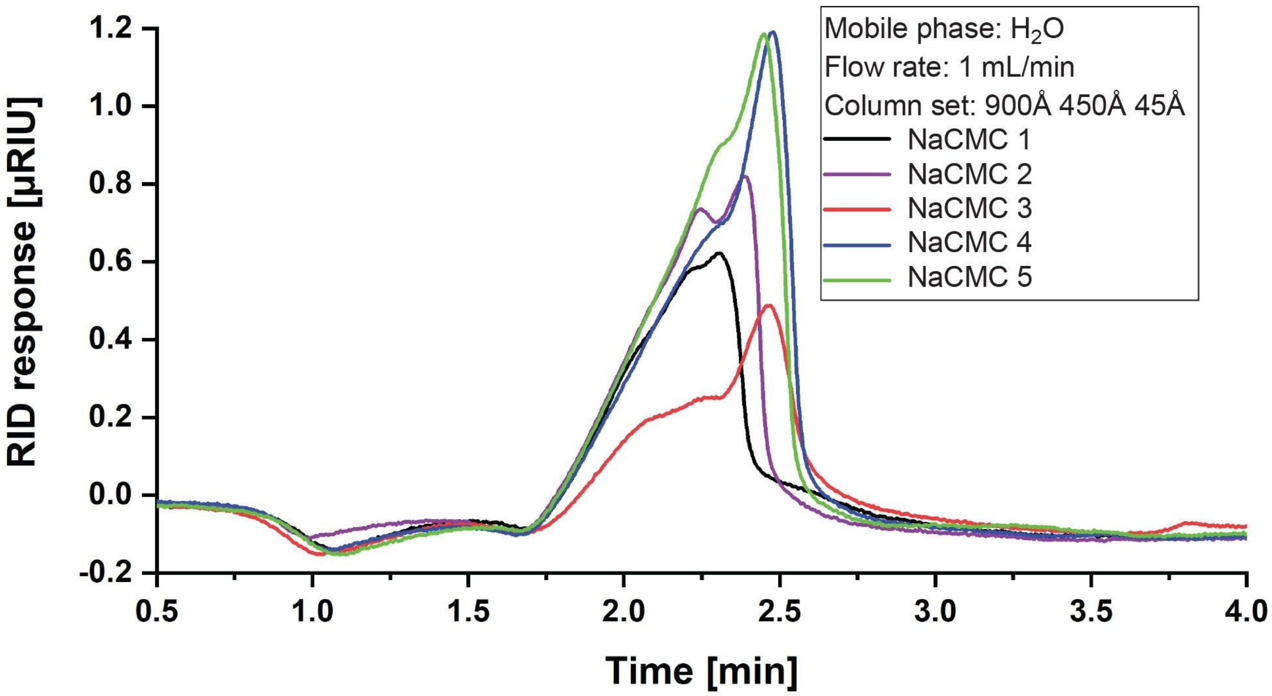


Figure 2. Overlay of RI chromatograms of NaCMC 1–5 analyzed in water as mobile phase.

In order to mitigate the polyelectrolyte effects of NaCMC, subsequent steps in a method development workflow consist of changing the pH along with the addition of salts to investigate which combination minimizes the ionically induced repulsion and other effects most efficiently. With an acetate buffer at pH 5, the retention times of the sample peaks increased slightly, and the chromatograms displayed two distinctive peaks as seen in Figure 3. The first peak corresponded to NaCMC while the second peak between 5–6 minutes was identified as an eluent peak by superimposing a blank run on the sample chromatogram. This fact rules out salt rejection, *i.e.* salt excluded from the pores as result of the Donnan equilibrium.[3] The sudden sharp rise of the main peak indicated a large sized fraction of the sample was excluded from the largest column pores and eluted unseparated. Based on prior work, NaCMC forms associates in buffer solutions.[10,11] These associates tend to elute at the void volume of the column and could have been responsible for the steep rise in the chromatograms. The preparation of associate-free solutions of water-soluble polysaccharides, especially charged ones is a generally known problem.[11] It was difficult to discern, if the tailing of the sample peak evolved from secondary interaction or representing low MW fractions that are part of the sample. Because silica is a polar surface, it can be concluded that polymer-surface interaction is minimal. However, a closer look at the chromatograms revealed a possible second population between 3.5- and 5-minute retention time in sample NaCMC 2, 4, and 5. Column artifacts can be ruled out, because NaCMC 1 and 3 do not exhibit this characteristic shoulder. The fractions that make up the shoulder can be considered sample intrinsic. Overall, this mobile phase condition was considered insufficient and not discriminatory enough.

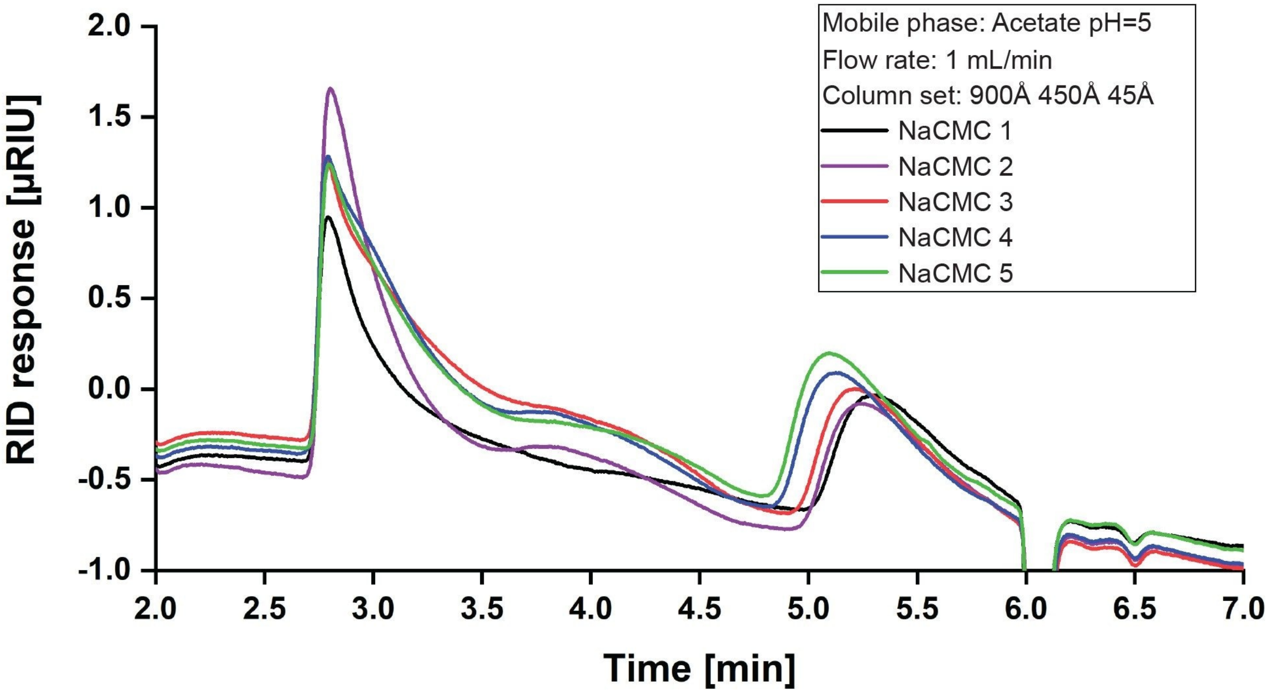


Figure 3. RI overlay of all NaCMC samples analyzed in acetate buffer at pH 5.

Since low pH improved the separation only slightly, a diluted NaOH solution at pH 8.8 was investigated as displayed in Figure 3. The intention of increasing the pH was to see, if the in Figure 3 observed polyelectrolyte effects can be further reduced along with lowering the flow rate. The peak shape improved over the previously tested mobile phases. Interaction could still have occurred, but of a different kind as the high pH impacted the shape of the coiled polymer chain differently. Despite the seemingly more uniform peak shape, a high pH mobile phase did not significantly discriminate between the five samples of different viscosity grades. Moreover, the second population observed with the low pH acetate buffer did not get separated at high pH. Thus, the high pH mobile phase did not yield significantly enhance separation. There is a chance that the sole improvement is rested upon the reduction of flow rate from 1 mL/min to 0.5 mL/min.

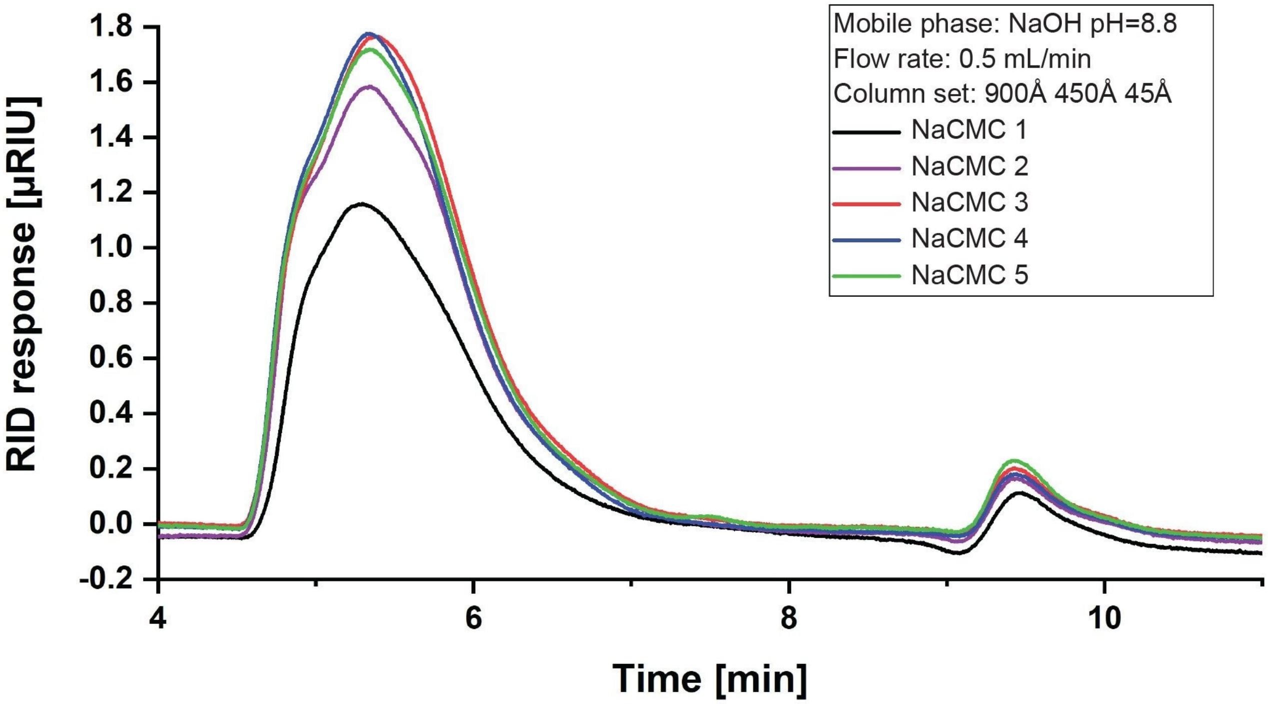


Figure 4. RI overlay of all NaCMC samples analyzed in diluted NaOH at pH 8.8.

After high and low pH mobile phases did not sufficiently suppress polyelectrolyte typical behavior, the impact of a neutral pH phosphate-nitrate mobile phase with an elevated amount of salt was investigated.[12] Additionally, the AQ 45 Å that has been used in the previous trials was exchanged with an AQ 125 Å column. Separation was achieved in less than 11 minutes. As can be seen in Figure 5, the samples separated into two populations except for NaCMC 1, as it was already observed in the low pH trial. It indicates, the abundance of low molecular weight fractions in NaCMC 1 was not as pronounced as with the other samples. It appeared those undesirable interactions between NaCMC and column packing material were reduced by running in a neutral pH mobile phase with salt.

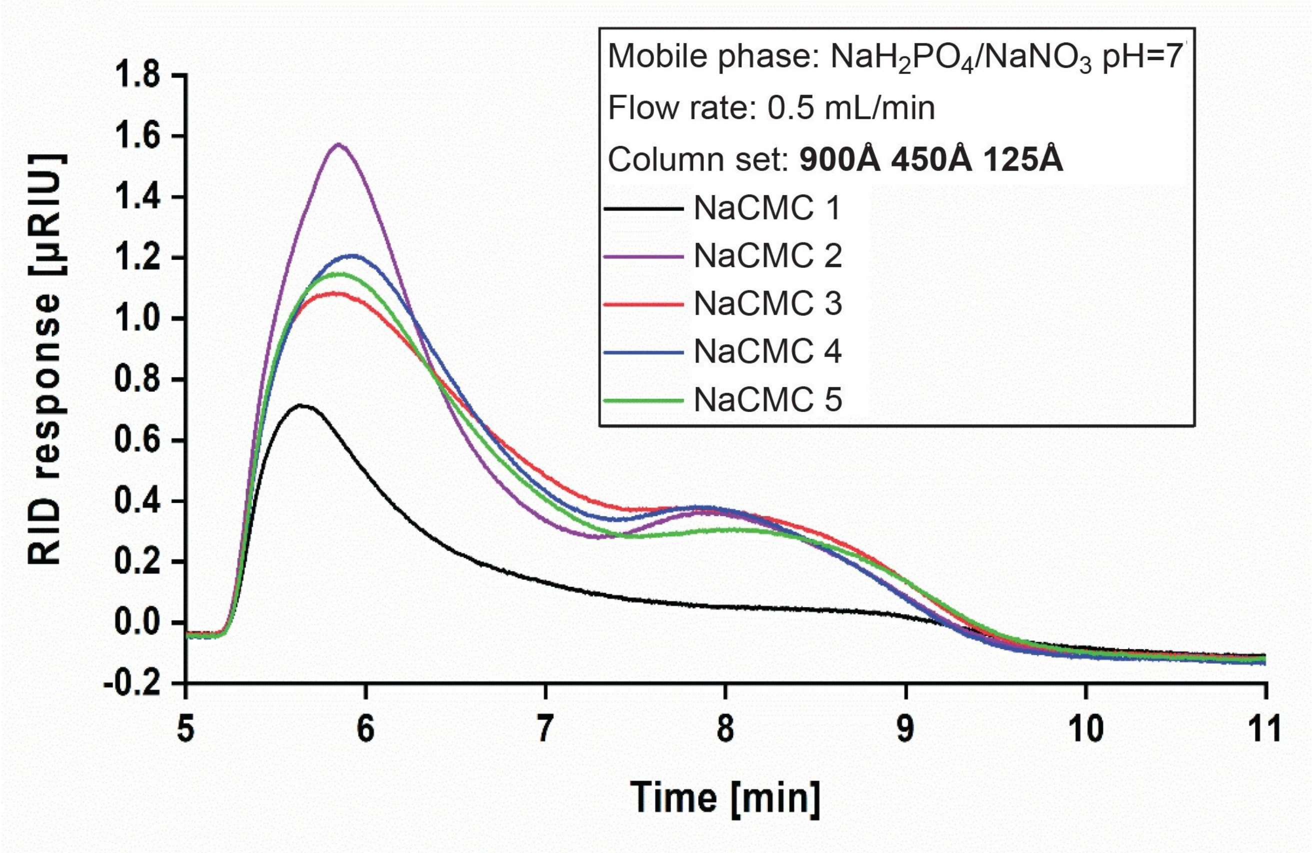


Figure 5. RI overlay of all samples in 0.01 M NaH_2PO_4/0.2 M $NaNO_3$ at pH 7 of all NaCMC samples.

The determined molecular weights of all the NaCMCs are reported in Table 1. The calculated relative standard deviations (RSD) for the samples are <4.5 indicating the excellent repeatability of the measurements. Moreover, the low RSD values also suggest the absence of potential degradation of the polymer chains under shear as this would lead to low repeatability of the signal (chromatograms), hence high RSD values.

Sample	Mn, kDa	%RDS	Mw, kDa	%RDS	Đ
NaCMC 1 (high viscosity)	201.5	3.2	1051.9	1.8	5.23
NaCMC 2 (high viscosity)	190.2	1.2	905.6	3.3	4.76
NaCMC 3 (medium viscosity)	151.6	2.8	698.2	3.1	4.61
NaCMC 4 (medium viscosity)	175.7	4.4	718.1	4.5	4.09
NaCMC 5 (medium viscosity)	154.0	0.6	750.0	2.6	4.87

Table 1. Molecular weights and standard deviations of all NaCMC samples in 0.01 M NaH2PO4 /0.2 M NaNO3 pH = 7.

As expected, the high viscosity grade samples NaCMC 1 and 2 exhibited the highest weight-average molecular weight (MW) in the range of 900–1000 kDa while the medium viscosity grades exhibited MW in the range 700–750 kDa. Moreover, NaCMC 1 is more polydisperse with Đ of 5.23 compared to NaCMC 2 with Đ of 4.76. The marked differences can clearly be traced to the chromatograms (Figure 4) which show NaCMC 1 comprised mostly high MW fraction and some amount of low MW fractions which appeared as broad tailing. Comparatively, NaCMC 2 had a high MW fraction as well as a second well-separated distinct low MW fraction which eluted later at a higher retention time. On the other hand, the chromatograms of all the medium viscosity grades had both high and low MW fractions. The high MW fraction of the medium grades, however, eluted at a relatively higher retention time compared to the high MW fraction of the high viscosity grades. It becomes obvious from these plots that within each viscosity grade set no two lots were identical, especially with the high viscosity grade set (NaCMC 1 and NaCMC 2). In fact, it was qualitatively observed during sample filtration prior to injection that the solution viscosity of NaCMC 1 was much higher than NaCMC 2 and the rest of the samples. Comparing all the four mobile phases tested, the neutral pH mobile phase in combination with AQ 900 Å + 450 Å + 125 Å columns achieved the best separation of the NaCMC samples.

Conclusion

Comparing the chromatographic results of all samples in ascending salinity amount and low and high pH values revealed that:

1. Polyelectrolyte effects decreased with increasing amount of salt in the mobile phase
2. The pH level played a lesser role in mitigating polymer-polymer interaction
3. The combination of elevated salinity and neutral pH as in 0.01M NaH_2PO_4/0.2M $NaNO_3$ at pH 7 yielded reproducible MW numbers as well distinguishable chromatograms for all samples. This mobile phases suppressed the ionic effects most efficiently
4. The fast runtimes achieved with an APC System even with a three-column bank helped to screen the mobile phases eliminating the necessity for lengthy solvent exchanges

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Disclosure

Ekaterina Sobich, Esther Bochmann, and Samuel Kyeremateng are employees of AbbVie and may own AbbVie stock. Claudia Lohmann is an employee of Waters Corporation and has no additional conflicts of interest to disclose. AbbVie sponsored and funded the study; contributed to the design; participated in the collection, analysis, and interpretation of data, and in writing, reviewing, and approval of the final publication.

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