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Note

Model for the structure–retention relationship for positional isomers of unsaturated fatty acid methyl esters on polar capillary columns

BRUNO D. BEAUMELLE* and HENRI J. VIAL
UA 530 CNRS, INSERM U 58, 60 Rue de Navacelles, 34100 Montpellier (France)
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Fatty acid methyl esters (FAMEs) are nowadays routinely analysed by gas–liquid chromatography (GLC) on capillary columns[1–8]. Capillary columns coated with polar stationary phases make the analysis of either positional or geometric FAME isomers possible and readily accessible[1–5], and thus reveal the great diversity of fatty acids (FAs) present in biological materials. Precise studies of FA composition often suffer from a lack of routinely available standards for most of the minor FAs. When dealing with a standard FA and trying to identify one of its isomers, it is therefore helpful to be able to predict at least the direction in which the retention time will evolve. According to data in the literature, it appears that the order of elution of geometric isomers (the separation of which is more difficult) on polar capillary columns varies from one column to another[3,9].

On the other hand, it is well known that in GLC on polar columns, whether capillary[1–10] or packed[11,12], the retention times of positional isomers of unsaturated FAMEs are longer when a double bond is closer to the methyl end[4]. Semilogarithmic plots of retention time *versus* carbon number can be used to identify isomers in the same omega series (omega is the number of carbon atoms from the methyl end of the molecule to the middle of the nearest double bond)[11,13]. This behaviour has been verified on our capillary column[14], on columns coated with the same stationary phase[2,9] and on other widely used polar stationary phases[1,3–8], regardless of the method of fixing the phase to the column wall[10]. Nevertheless, the phenomenon has never been explained at the molecular level. In this paper, we propose a simple model, based on charge interactions, to describe this structure–retention relationship for unsaturated species on polar capillary columns. The model is based on the demonstration of the assumption that when an unsaturated FAME is in a polar capillary column, the probability of a point on the FAME molecule touching the column wall increases as the point considered on the molecule moves closer to its extremity.

As a FAME molecule is fairly long and, especially in the case of unsaturated species, has limited flexibility, it can, to a first approximation, be represented as a flexible match of length l. The capillary column diameter is nl and we locate the FAME molecule in the column by an X,Y coordinate system as shown in Fig. 1. In Fig. 1 the FAME is located with respect to the nearest wall (the argument can be expanded by symmetry). M is the middle of the FAME molecule, chosen as the origin as it involves considering only one kind of figure. Similar results are obtained with

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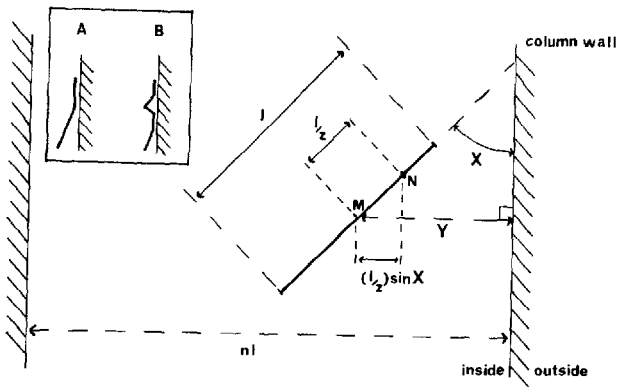

Fig. 1. System for locating the FAME molecule with respect to the capillary column wall. l = FAME length; X = angle, in radians; Y in the same units as l (length); the position of N, taken at random on the FAME (or dimethyl acetal) molecule, is defined by its distance from the middle (M) of the molecule ($MN = l/z$ where $z \geqslant 2$); M is taken as the origin because of the particular properties of the molecule's centre (see text). Insert shows possible conformations of a FAME molecule of limited flexibility in contact with the capillary column wall. (A) Considered here; (B) highly improbable.

other origins (data not shown). N is a point taken at random on the molecule; there is contact between N and the column wall if $Y \leqslant (l/z)\sin X$ (see Fig. 1 for details). In this argument, only the kinds of conformations depicted in Fig. 1A are considered, namely, if a point N on the molecule is in contact with the coating, every point between N and the nearest end of the molecule is itself in contact with the coat.

In the gas stream inside the column, the FAMEs must still have a certain rigidity (like the flexible match taken as a model) because, if it did not, the probability of a point on the molecule coming into contact with the phase would not depend on the location of the point on the molecule (data not shown). Each double bond would therefore have the same probability of coming into contact with the column wall. In this instance, positional isomers of FAME would be similarly retained by a double

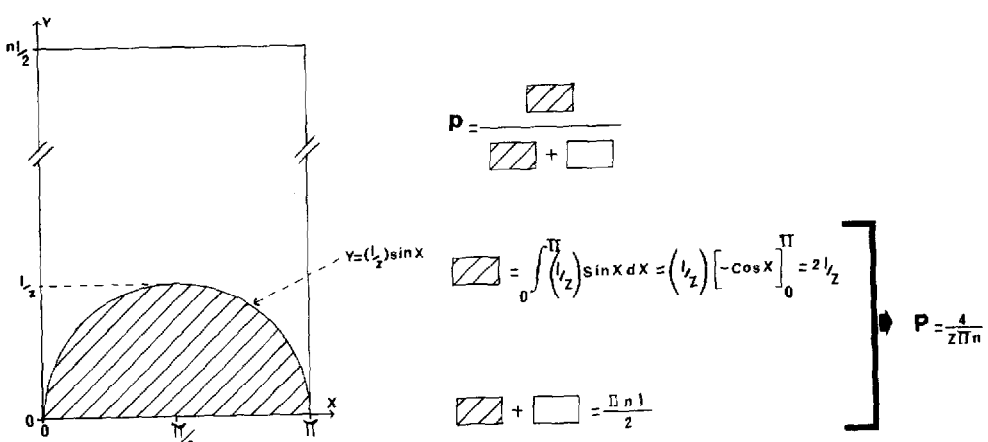

Fig. 2. Diagram of the probability (P) of contact between a point on the FAME and the capillary column coat; X, Y, l, n and z are defined in Fig. 1; P is determined by the ratio of two areas as indicated.

bond, regardless of the location of the unsaturation, which is ruled out by experiment (see oleate/vaccenate isomers in refs. 3, 5–8 and 14).

Because of the limited flexibility of unsaturated FAME molecules, the conformations depicted in Fig. 1B are highly improbable and we did not take account of them in our calculations.

As $0 \leqslant Y \leqslant nl/2$, $0 \leqslant X \leqslant \pi$ and $z \geqslant 2$, the possible positions of a FAME molecule that is in the gas stream of the column, and is not touching the coating, are represented by the open area in Fig. 2. We assume that the gas stream homogenizes the population so that equiprobability in this area is verified. The "positive" cases in which there is contact between the FAME and the column wall are under (or on) the curve $Y = (l/z)\sin x$ in the hatched area of Fig. 2.

The probability (P) of contact between point N and the stationary phase is the ratio of the hatched area to the sum of the open area plus the hatched area. The calculations in Fig. 2 give $P = 4/z\pi n$. Thus, P increases as z decreases, *i.e.*, as N moves further away from M. In conclusion, the probability of a point on the FAME molecule coming into contact with the column wall increases the closer the point is to the end of the molecule.

We are aware that the relationship between contact probability and distance from the centre of the FAME is more complex than a simple direct proportion. This is especially true as the probability density is not homogeneous in the hatched area ("positive cases") in Fig. 2, because of the limited FAME flexibility. This is represented in Fig. 3, where conformation A is more probable than conformation B because there is less flexing ($X_a < X_b$). Nevertheless, these refinements do not cast doubt on the conclusion that the probability of a point on a FAME molecule coming into contact with the column wall increases with increasing distance of the point from the centre of the FAME molecule.

Hence the different chromatographic behaviours of two monounsaturated positional isomers such as oleate (ω9) and vaccenate (ω7) can be explained by the higher probability (and thus the greater frequency) of interaction of one isomer's (vaccenate's) double bond with the polar wall of the capillary column [nitro groups with positive charges on the nitrogen for free fatty acid phase (FFAP)] as it is closer to the methyl end than the double bond of the other isomer (oleate). As the contribution of the other charged part of the molecule, *i.e.*, the ester linkage [or acetal linkage in dimethyl acetals (DMAs)] is the same in both FAME (or DMA) isomers, vaccenate elutes after oleate[3,5–8,14]. This has also been verified with other octadecenoic FAME positional isomers (see Fig. 6 in ref. 4).

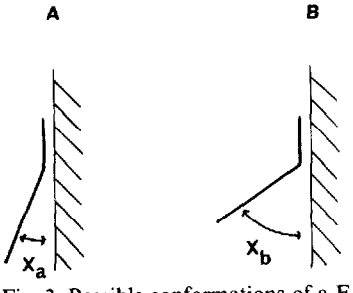

Fig. 3. Possible conformations of a FAME molecule in the capillary column, when the same length is in contact with the wall. X is defined in Fig. 1.

This model can also be expanded to positional isomers of polyunsaturated FAMEs (PUFAMEs). All mammalian PUFAs have methylene-interrupted double bonds[15], which can thus be considered as one polar group whose location shifts along the molecule with the ω value. Hence the closer this polar group is to the methyl end of the FAME backbone, the more easily the double bonds can interact with the polar wall and the longer is the retention time. This was verified on our capillary column for all isomers of identified PUFAMEs (*i.e.*, 20:3 and 22:5 isomers, in ref. 14), and also on other capillary columns coated with polar stationary phases[2,3,5–7,10].

It must be noted that the proximity of the first double bond to the methyl end of the PUFAME molecule has a greater effect on chromatographic properties than the number of double bonds itself, when it is greater than 2. For example, on FFAP-coated capillary columns, FAME 20:3ω3 emerges after 20:4ω6, close to 20:5ω3, whereas 20:3ω9 and 20:3ω6 emerge before 20:4ω6 (see refs. 2 and 14).

It has also been shown that the proximity of double bonds to the methyl end plays an important role in enzyme specificity for FAs[16], in silver thin-layer chromatography of phospholipids[13] and FAMEs[17,18] and in the physical properties of FAs (see ref. 1 for a review).

This mathematical model, which is based on simple and readily comprehensible probability calculations, can be used to describe the behaviour of isomers of mono- and polyunsaturated FAMEs (or DMAs) on polar capillary columns.

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