

CHROM. 16,386

## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON DYNAMICAL- LY MODIFIED SILICA

### V\*. INFLUENCE OF NATURE AND CONCENTRATION OF ORGANIC MODIFIER IN ELUENTS CONTAINING CETYLTRIMETHYLAMMONIUM BROMIDE

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(Received October 11th, 1983 )

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#### SUMMARY

The influence of the nature and concentration of organic modifiers on retention in high-performance liquid chromatography on dynamically modified silica has been investigated.

Adsorption isotherms were determined on bare silica (LiChrosorb Si 60) using eluents containing various concentrations of methanol or acetonitrile and with the addition of phosphate buffer (pH 7.5) and cetyltrimethylammonium bromide. With decreasing modifier concentration in the eluent, the amount of cetyltrimethylammonium adsorbed on to the silica surface rises faster as its concentration increases and to a higher end-value. The maximum amount adsorbable for eluents containing either methanol or acetonitrile was found to be *ca.* 1.2 mmol/g (*ca.* 2.5  $\mu\text{mol}/\text{m}^2$ ).

As is also the case in reversed-phase chromatography on chemically bonded alkylsilica, great variations in selectivity were seen when methanol, acetonitrile, or tetrahydrofuran were substituted for one another as the organic modifier in the eluent. The optimization of a separation by the use of ternary eluent mixtures was demonstrated.

The possible use of gradient elution in high-performance liquid chromatography on dynamically modified silica is discussed.

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\* For Part IV, see ref. 5.

## INTRODUCTION

High-performance liquid chromatographic (HPLC) separations performed on bare silica dynamically modified by the addition of quaternary ammonium salts to the eluent have been discussed in recent publications<sup>1-5</sup>. The aim of the present work was to investigate the variations in the amount of cetyltrimethylammonium (CTMA) ions adsorbed on to the silica at different methanol concentrations in the eluent, and the corresponding changes in retention of various test compounds. Furthermore, the effect of changing the nature of the organic modifier was to be examined, *e.g.* by substituting acetonitrile or tetrahydrofuran for methanol. This was done especially with the intent of investigating whether it is possible to optimize a given separation by the use of ternary eluent mixtures, as is often done in chromatography on bonded-phase materials.

## EXPERIMENTAL

### *Apparatus*

Chromatographic testing of the individual systems was performed on a Waters liquid chromatograph consisting of a 6000 A pump, a 710 A WISP autoinjector, a 440 ultraviolet absorbance detector (operated at 254 nm), a 730 data module and a 720 system controller, or on a liquid chromatograph consisting of a Kontron Model 410 LC pump, a Pye-Unicam LC-UV detector (operated at 254 nm) and a Rheodyne Model 7125 injection valve. Chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder. Retention data were collected on a Waters 730 data module or on a Hewlett-Packard Model 3353 A laboratory data system.

### *Procedures*

Determination of the amounts of CTMA adsorbed on to the column material was performed by the elution method as previously described<sup>6</sup>.

### *Chromatography*

All experiments were performed on 120 × 4.6 mm I.D. columns from Knauer (Berlin, F.R.G.), packed by the dilute slurry technique with LiChrosorb Si 60 (5 μm) (E. Merck, Darmstadt, F.R.G.). The eluent was various concentrations of methanol, acetonitrile, or tetrahydrofuran in water, with the addition of 5% of 0.2 M phosphate buffer (pH 7.5) and various amounts of CTMA bromide. The buffer pH stated is that measured in the undiluted buffer and not in the final eluent. The buffer was prepared from 0.2 mol of potassium dihydrogen phosphate dissolved in 930 ml of water, titrated with 5 M potassium hydroxide to pH 7.5 and finally diluted to 1000 ml. During chromatography the column was guarded by a silica precolumn (120 × 4.6 mm I.D.) situated between the pump and the injection device. The chromatographic system was equilibrated by overnight elution. Following each adsorption experiment the column was brought to its initial state by elution with methanol-0.05 M phosphoric acid (1:1) and finally with methanol.

### *Chemicals*

Acetonitrile HPLC grade S and tetrahydrofuran HPLC grade were obtained

from Rathburn (Walkerburn, U.K.) CTMA bromide and all other reagents were of analytical grade from E. Merck. All chemicals were used as received from the manufacturers.

## RESULTS AND DISCUSSION

### *Effects of changes in modifier concentration*

To investigate the effects of variations in modifier concentration on the adsorbed amount of CTMA, adsorption isotherms were determined for four different methanol concentrations and for three acetonitrile concentrations ranging from 10 to 75% and from 10 to 50%, respectively. Fig. 1 shows that the end-value of adsorbed CTMA increases with decreasing modifier concentration. From 30 to 10% methanol, however, the increase is very small, indicating that *ca.* 1.2 mmol is the maximum amount of CTMA adsorbable per gram of the silica used; this points to the formation of no more than a monomolecular layer. In neat aqueous solvents it has been shown<sup>7</sup> that a double layer is formed. The maximum amount adsorbed in the said monomolecular layer corresponds to  $2.5 \mu\text{mol}/\text{m}^2$ , which represents a coverage of *ca.* 30% of the silanol groups on the silica surface (*cf.* ref. 8). Another effect of decreasing the modifier concentration is that the rise of the isotherm becomes more steep.

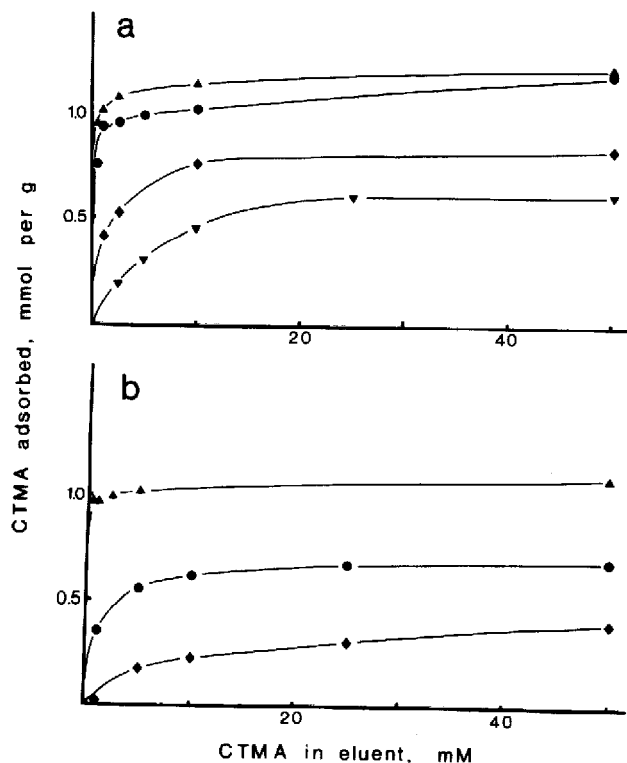


Fig. 1. Relationship between the concentration of CTMA in the eluent and the amount of CTMA adsorbed per gram of silica using various concentrations of methanol (a) or acetonitrile (b). Symbols: ▲ = 10%; ● = 30%; ◆ = 50%; ▼ = 75%.

Fig. 2 shows the relationship between the retention of benzene and the concentration of CTMA in the eluent. All curves except that for 75% methanol exhibit a maximum. It has been shown previously<sup>3</sup> that these maxima for retention of benzene correspond to the critical micellar concentrations (CMC) of CTMA. A prerequisite for the use of common rules for variations in the retention of actual solutes<sup>3,5</sup> when changing the composition of the eluent, for example, is to use CTMA concentrations below the CMC. On the other hand, it is not convenient to use CTMA concentrations far below 2.5 mM owing to the resulting prolonged equilibration times. Thus in practical HPLC on dynamically modified silica it is advisable to avoid methanol concentrations below 30% and acetonitrile concentrations below 20% unless the special effects from micellar formation are wanted.

Fig. 3A shows the amounts of CTMA adsorbed at a concentration of 2.5 mM in the eluent using various concentrations of the three organic modifiers. Fig. 3B shows the corresponding relationship using methanol only and various CTMA concentrations. From Fig. 3A it appears that the result of decreasing the modifier concentration is an increase in the amount of CTMA adsorbed, this increase being greatest for the weaker eluting methanol and least for the stronger eluting tetrahydrofuran. The methanol curve deflects at the lowest methanol concentration as the adsorbed amount approaches its absolute maximum of *ca.* 1.2 mmol CTMA per gram of silica.

### Selectivity

The curves in Fig. 3A may be used to indicate the modifier concentration when

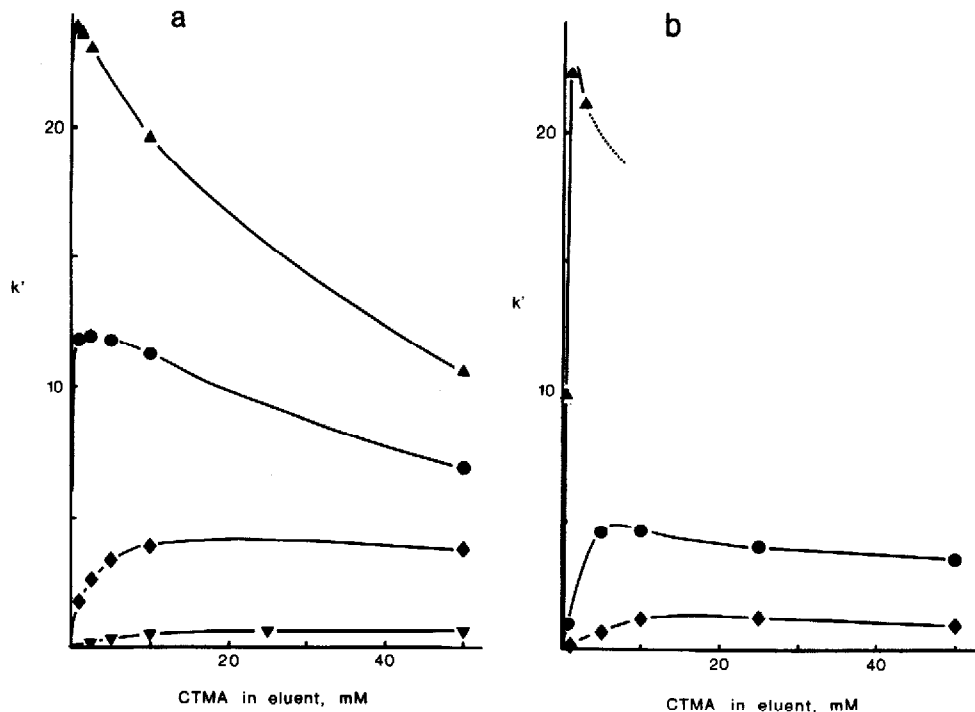


Fig. 2. Relationship between the concentration of CTMA in the eluent and the retention of benzene using various concentrations of methanol (a) or acetonitrile (b). Symbols as in Fig. 1.

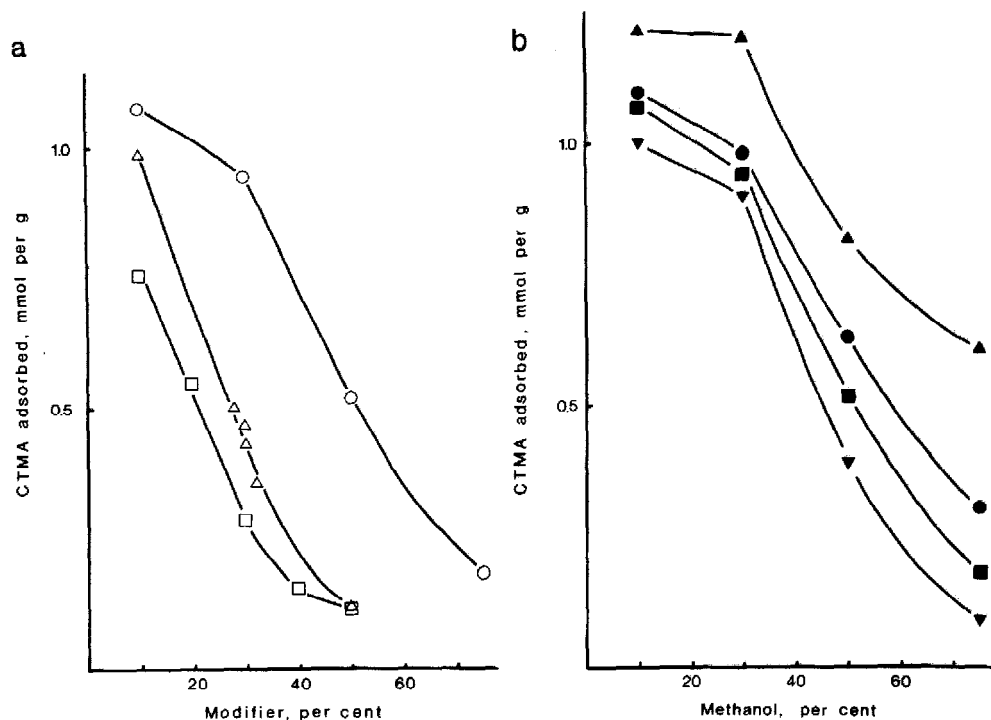


Fig. 3. Influence of the nature and concentration of organic modifier on the adsorbed amount of CTMA at a concentration of 2.5 mM (a) or at various concentrations using methanol as the modifier (b). (a) Modifier:  $\circ$  = methanol;  $\triangle$  = acetonitrile;  $\square$  = tetrahydrofuran. (b) CTMA concentrations:  $\blacktriangle$  = 50 mM;  $\bullet$  = 5 mM;  $\blacksquare$  = 2.5 mM;  $\blacktriangledown$  = 1 mM.

a change is wanted from, for example, a methanol-containing eluent to an acetonitrile-containing eluent of the same eluting strength. When using a CTMA concentration of 2.5 mM and an eluent containing 50% of methanol, an adsorbed amount of CTMA of *ca.* 0.5 mmol per gram of silica is the result. The acetonitrile concentration needed to achieve the same concentration of stationary phase can be calculated to be *ca.* 28%, and similarly the concentration of tetrahydrofuran to be *ca.* 21%. In practice it was found that to achieve the same eluting strength as the methanol-containing eluent, slightly higher concentrations should be used, namely 30% acetonitrile and 28% tetrahydrofuran.

Table I lists the retentions of sixteen solutes observed with the three organic modifiers in the said isoelutotropic concentrations. It appears that considerable variations in selectivity occur when the organic modifier is changed, in accordance with the well-known solvent selectivity effects in reversed-phase chromatography on bonded-phase materials<sup>9</sup>. Within bonded-phase chromatography it has been demonstrated by several authors (*e.g.* refs. 10 and 11) that the use of ternary eluent mixtures, *i.e.* two different organic modifiers, affords further possibilities in solvent selectivity. The resulting selectivity towards a given mixture of solutes may differ from those obtained when using each of the two modifiers singly. To examine whether the selectivity in reversed-phase chromatography on dynamically modified silica can be

TABLE I

RETENTION (CAPACITY FACTOR,  $k'$ ) OF SIXTEEN TEST SUBSTANCES USING THREE DIFFERENT ORGANIC MODIFIERS IN THE ELUENT AT ISOELUOTROPIC CONCENTRATIONS

Column, LiChrosorb Si 60 (120 × 4.6 mm I.D.); eluent, 2.5 mM CTMA and 10 mM phosphate (pH 7.5) in the stated concentrations of organic modifier.

Solute	Organic modifier		
	Methanol 50%	Acetonitrile 30%	Tetrahydrofuran 28%
Benzene	2.78	3.41	4.53
Toluene	5.07	6.17	7.78
Benzyl alcohol	1.06	1.07	1.18
2-Phenylethanol	1.45	1.52	1.63
Acetophenone	1.13	1.68	1.63
Propiophenone	2.01	3.25	3.19
Anisole	2.67	3.27	4.08
Acetanilide	0.94	1.02	1.00
Phenol	3.67	3.42	3.87
<i>p</i> -Cresol	6.35	5.45	5.09
Benzoic acid	7.77	8.19	4.69
Sulphanilic acid	1.65	1.32	1.92
Pyridine	0.23	0.42	0.27
Salbutamol	0.35	0.19	0.30
Terbutaline	0.91	0.42	0.76
Phenylethylamine	1.04	0.44	0.87
Mean $k'$ for sixteen solutes	2.47	2.57	2.50

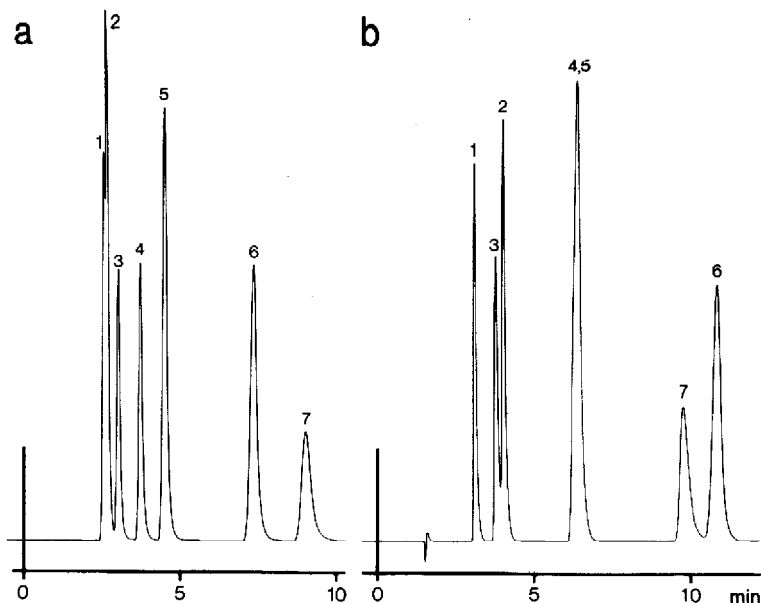


Fig. 4. Chromatograms of a mixture of aromatic compounds. Column: LiChrosorb Si 60 (120 × 4.6 mm I.D.). Eluents: 2.5 mM CTMA in (a) methanol-water-0.2 M phosphate buffer (pH 7.5) (50:45:5), (b) acetonitrile-water-0.2 M phosphate buffer (pH 7.5) (30:65:5). Flow-rate, 1 ml/min; detection wavelength, 254 nm. Peaks: 1 = benzyl alcohol; 2 = acetophenone; 3 = 2-phenylethanol; 4 = propiophenone; 5 = anisole; 6 = toluene; 7 = *p*-cresol.

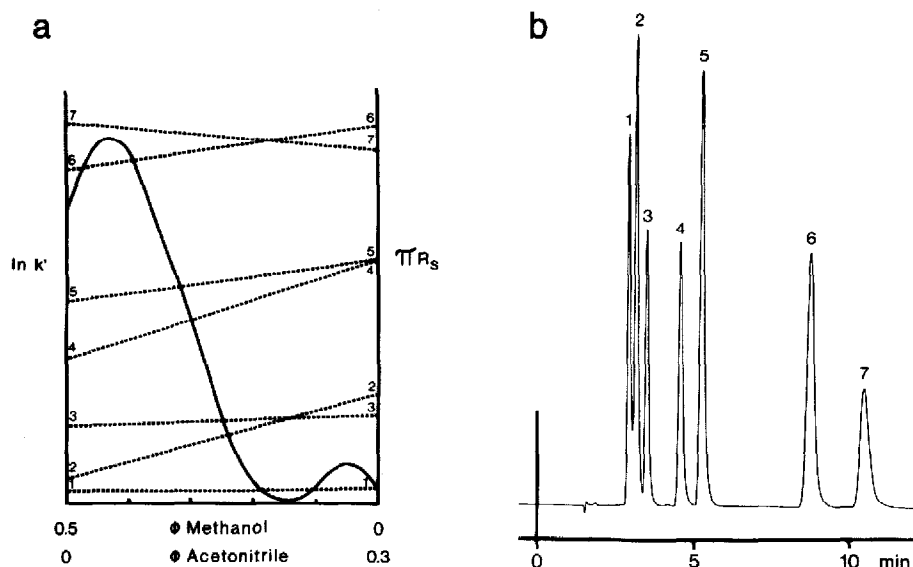


Fig. 5. Optimization of the separation from Fig. 4. (a) Optimization diagram according to Schoenmakers

*et al.*<sup>11</sup>.  $\varphi$  = Volume fraction of modifier;  $\Pi R_s = \prod_{i=1}^{n-1} \frac{k_{i+1} - k_i}{k_{i+1} + k_i + 2}$  (b) The resulting chromatogram.

Eluent, 2.5 mM CTMA in methanol-acetonitrile-water-0.2 M phosphate buffer (pH 7.5) (40:6:49:5). Other chromatographic conditions and peak identification as in Fig. 4.

similarly varied, the separation of seven aromatic compounds was investigated. Fig. 4 shows the result of two separations using 50% methanol and 30% acetonitrile, respectively, as the modifier. Neither of the two separations is satisfactory, owing to partly or totally overlapping peaks. In Fig. 5A is shown the optimization diagram for the use of a ternary eluent mixture in accordance with the approach used for bonded-phase chromatography by Schoenmakers *et al.*<sup>11</sup>. Maximizing the product

TABLE II

RETENTION TIMES FOR SEVEN AROMATIC TEST COMPOUNDS UNDER GRADIENT ELUTION CONDITIONS

Eluents: (A) 0.25 mM CTMA in acetonitrile-water-0.2 M phosphate buffer (pH 7.5) (10:85:5); (B) 40 mM CTMA in acetonitrile-water-0.2 M phosphate buffer (pH 7.5) (50:45:5). Gradient from 0% to 100% B in 15 min. Delay time, 10 min.

	Gradient run No.							
	1	2	3	4	5	6	7	9
Benzyl alcohol	6.85	8.14	7.75	7.67	7.62	7.71	7.70	7.75
2-Phenylethanol	8.76	10.61	9.99	9.96	9.74	9.83	9.85	9.90
Acetophenone	10.65	12.86	11.89	11.74	11.46	11.51	11.55	11.59
Anisole	14.35	17.50	15.88	15.90	15.04	15.08	15.10	15.27
Propiophenone	15.06	18.04	16.38	15.80	15.48	15.50	15.50	15.69
Toluene	17.53	20.45	18.94	18.50	18.30	18.25	18.25	18.50
<i>p</i> -Cresol	17.88	20.54	19.12	18.68	18.44	18.55	18.34	18.67

$\Pi R_s$  corresponds to an even spreading of the peaks over the chromatogram, and hence the best separation should be achieved by using a modifier containing 40% methanol and 6% acetonitrile. Fig. 5B depicts the resulting chromatogram.

#### *Gradient elution*

We investigated whether gradient elution in HPLC on dynamically modified silica can be used in the separation of very complex samples containing solutes of widely differing polarities. Relative to chromatography on bonded-phase materials, however, matters are more complicated here because a change in modifier concentration will also affect the concentration of CTMA on the silica surface. It would be rather difficult to design a gradient profile in which this effect is compensated for by altering the CTMA concentration. From Fig. 3B it appears that in order to use a gradient from *e.g.* 35 to 70% methanol at a coverage of 0.65 mmol CTMA per gram of silica, the CTMA concentration has also to be varied as a gradient from 0.5 mM to 50 mM. To ensure total equilibration before each run a rather extended pre-equilibration time is required.

The difficulties mentioned do not prevent the practical application of gradient elution in the dynamically modified silica approach. The gradient profiles can, however, be chosen only on a rather empirical basis, and total equilibration with the eluent composition in use at any given time cannot be achieved. The effects of the gradient are even retarded by the precolumn. A fixed delay time between each gradient run is therefore needed. Table II shows the results of a series of gradient runs on the test mixture of aromatics mentioned above. The gradient profile was designed to take into account the fact that both the modifier and the CTMA concentrations should be varied through the run. It appears from the table that retention times become stable after relatively few runs.

#### CONCLUSION

It has been demonstrated that the nature and the concentration of the organic modifier greatly influence retention and selectivity in HPLC on dynamically modified silica. When using the standard concentration of 2.5 mM CTMA in the eluent the concentration of methanol or acetonitrile should exceed 30% and 20%, respectively, to avoid micelle formation.

It has been found possible to optimize reversed-phase separations on dynamically modified silica by the use of ternary eluent mixtures. This is in accordance with what is known for bonded-phase chromatography.

Gradient elution at constant concentration of CTMA on the silica surface is not readily possible. However, when carrying out gradient elution by changing the concentrations of both modifier and CTMA in the eluent, a steady state condition is reached after a few runs, thus making practical application of gradient elution possible.

#### ACKNOWLEDGEMENT

This work was supported by the Danish Medical Research Council, grant No. 12-0649.



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