Electronic Supplementary Material (ESI) for Analyst.

High pH instability of quaternary ammonium surfactant coatings in capillary electrophoresis

Lisa Shulman, Lei Pei, Mahmoud F. Bahnasy, and Charles A. Lucy*

Department of Chemistry, Gunning/Lemieux Chemistry Centre, University of Alberta, Edmonton T6G 2G2, Alberta, Canada.

Supplementary Materials

* **Corresponding author:** Professor Charles A. Lucy, Department of Chemistry, University of Alberta, Gunning / Lemieux Chemistry Centre, Edmonton, Alberta, Canada T6G 2G2
Tel.: +01 780 492 0315; **Fax:** +01 780 492 8231.

E-mail address: charles.lucy@ualberta.ca

Experimental

Mass Spectrometric Characterization of DODAB Degradation

Chemicals

Silica particles were 5 μm HPLC grade particles (spherical, 100 Å, 290 m^2/g) from Restek (Bellefonte, PA, USA). All liquid-liquid extractions were carried out using dichloromethane (DCM) (Sigma) as the extraction solvent. The resulting organic layers were dried over Na_2SO_4 (Aldrich).

NALDI-MS

The chemical stability of DODAB and CTAB in the presence of silica was studied via Nanostructured Laser Desorption Ionisation (NALDI) MS and MS/MS analysis. SiO_2 particles were added to 5 mM surfactant in Nanopure water (buffer components suppressed MS ionisation), such that the mass ratio of surfactant to silica particles was 1:10 for each solution. All silica-surfactant solutions were sonicated for 30 min at 60°C, and then stirred for 30 min at room temperature. The sonicate/stir cycles were repeated 15 times for each solution over 48 h. Surfactant solutions were allowed to cool to room temperature, and filtered through a 0.45 μm nylon filter prior to NALDI-MS analysis.

NALDI was performed on an ultraflexXtremeTM MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA) in positive MS mode. One μL of sample was spotted onto a Bruker NALDI™ target (Bruker Daltonics), and allowed to dry.

Accurate molecular weight analysis was performed with the same NALDI target on a Bruker 9.4T Apex-Qe FTICR mass spectrometer (Bruker Daltonics) in positive MS mode.

MALDI-MS

Mechanistic studies of the decomposition of DODAB on silica were carried out using Matrix-Assisted Laser Desorption Ionisation (MALDI) MS. Solutions of DODAB which had been exposed to silica (above) were adjusted to pH 10 with 1 M NaOH, and extracted with 4 ×100 mL DCM. The organic phases were collected, washed with H_2O twice (30 mL each), washed twice with 30 mL of brine, dried over Na_2SO_4, and evaporated under reduced pressure to yield a clear colorless liquid. The extract was dissolved in 1 mL DCM and mixed with the same volume of 10 mg/mL 2[(2E)-3-(4-tert-butylphenyl)-2-methylpropy-2-enylidene]-malononitrile (DCTB) in DCM. 0.8 μL of sample/matrix solution was spotted

onto a stainless steel MALDI target. Analyses were performed on an ultraflexXtreme™ MALDI-TOF/TOF (Bruker Daltonics) mass spectrometer in positive MS mode.

RP-HPLC-MS

The mechanism of DODAB degradation in the presence of silica was further probed by HPLC-MS analysis. Surfactant was exposed to SiO_2 as outlined above. RP-HPLC-MS analysis was performed by injecting 5 µL of sample into an Agilent 1200 SL HPLC System (Santa Clara, CA, USA) with a Jupiter C_{18} column (5 µm, 300 Å, 50 × 2.0 mm i.d., Phenomenex, Torrance, CA, USA) at 30°C, using a 0.50 mL/min gradient of 2% ACN for 0.5 min, then linear gradient to 98% ACN over 15 min, followed by a 4.5 min hold at 98% ACN. The mobile phase contained a constant 0.1% formic acid.

ESI mass spectra were acquired in positive mode using an Agilent 6220 Accurate-Mass TOF equipped with a second sprayer providing a reference mass solution. Conditions were: drying gas 9 L/min at 300°C; nebulizer 30 psi; mass range 100-1000 Da; acquisition rate of ~1.03 spectra/s; fragmentor 150 V; skimmer 65 V; capillary 3200V; instrument state 4 GHz High Resolution. Mass correction was performed for every individual spectrum using peaks at m/z 121.0509 and 922.0098 from the reference solution. Data acquisition and analysis used the Mass Hunter software package (ver. B.04.00) and Agilent Mass Hunter Qualitative Analysis software (ver. B.03.01 SP3), respectively.

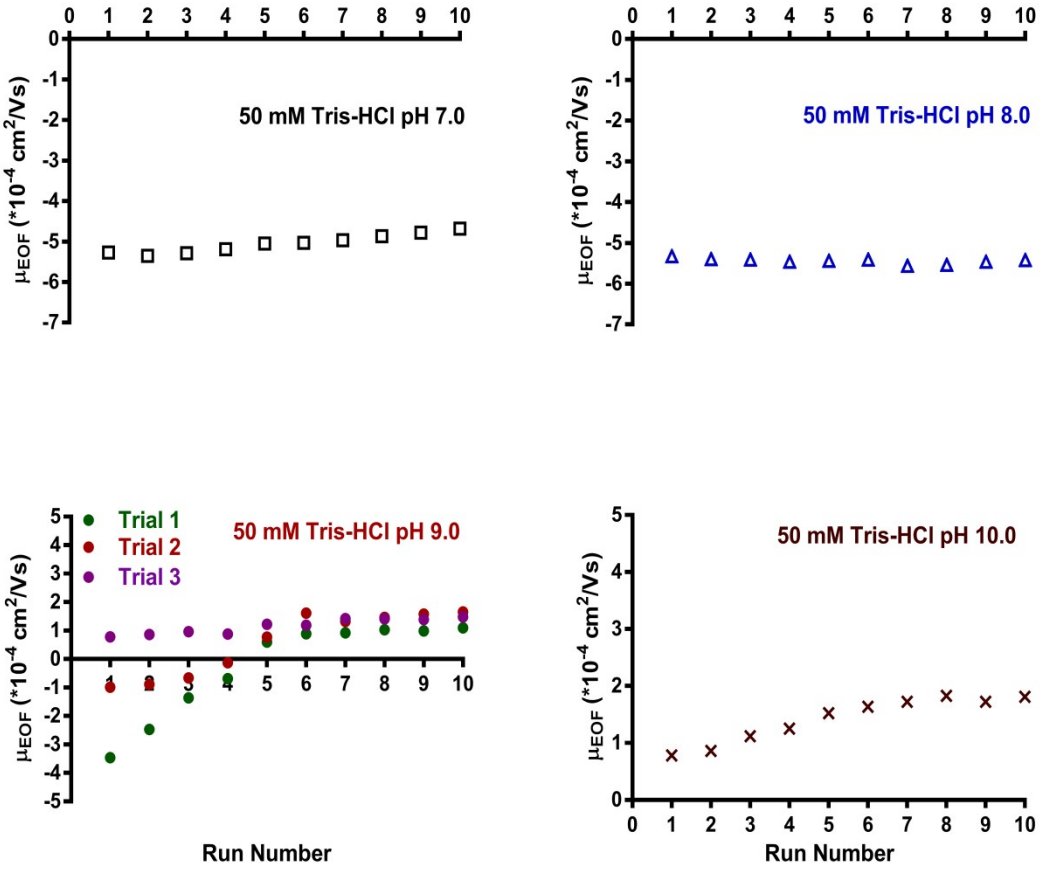

Fig. S1 Stability of 0.1 mM DODAB coated capillaries in Tris-HCl buffers at pH ≥7.0: 50 mM Tris-HCl pH 7.0 (□), 50 mM Tris-HCl pH 8.0 (Δ), 50 mM Tris-HCl pH 9.0 (● ● ●) and 50 mM Tris-HCl pH 10.0 (×). Conditions: 32 cm × 50 μm i.d. capillary (23.5 cm to detector); 2 mM benzyl alcohol (λ, 214 nm) or 2 mM mesityl oxide (λ, 254 nm) injected for 3 s at 50 mbar; temperature, 25 °C.

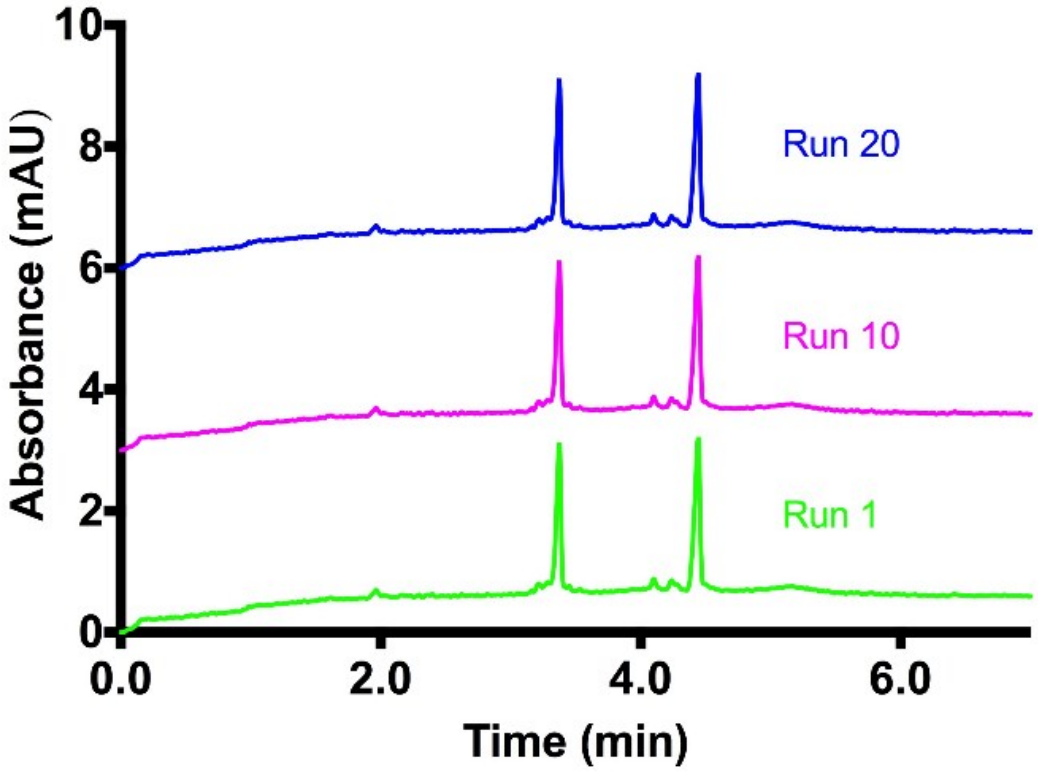

Fig. S2 Separation of cationic proteins RNase A and cytochrome *c* at pH 3.5 on a 50 µm i.d. capillary coated with 0.1 mM DODAB. Proteins: (1) RNase A, and (2) cytochrome *c*. Conditions: voltage, -20 kV; capillary, 30.2 cm (20 cm to detector); separation buffer, 22.4 mM sodium phosphate pH 3.5; λ, 214 nm; 0.2 mg/mL protein sample dissolved in water and injected hydrodynamically at 5 mbar for 5 s.

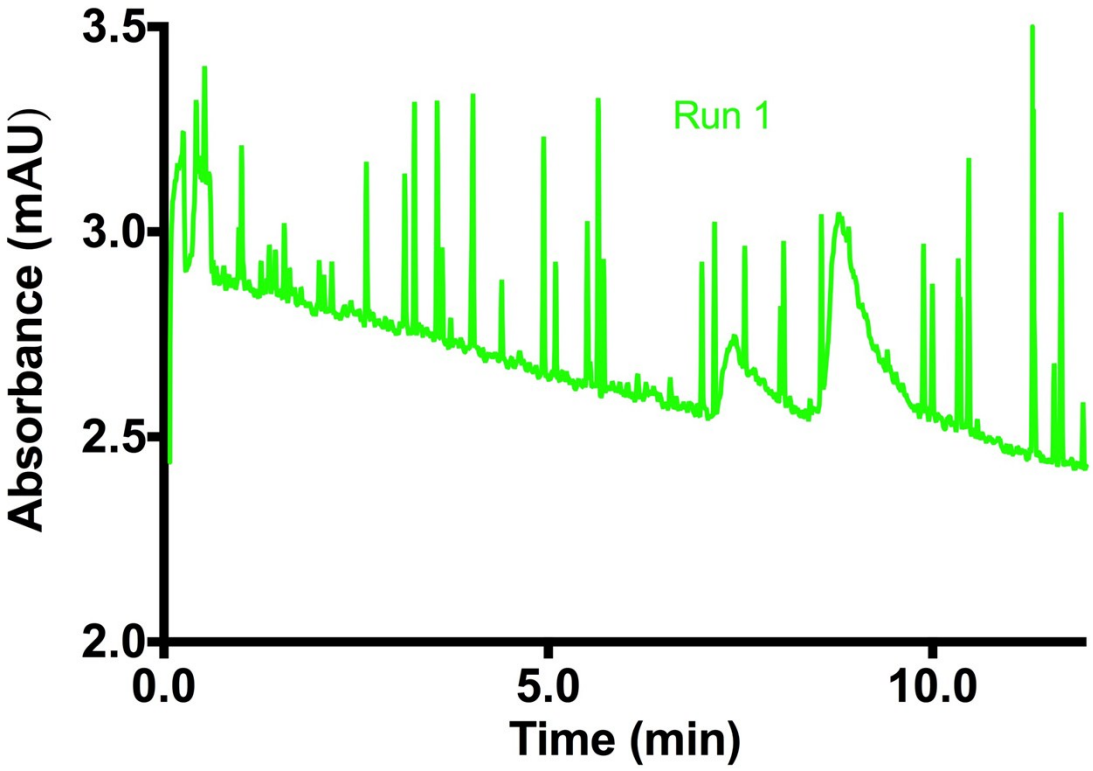

Fig. S3 Separation of cationic proteins RNase A and cytochrome *c* at pH 9.0 on a 50 µm i.d. capillary coated with 0.1 mM DODAB (Run 1). Proteins: (1) RNase A and (2) cytochrome *c*. Conditions: applied voltage, -20 kV; capillary, 30.2 cm (20 cm to detector); separation buffer, 6.7 mM sodium phosphate pH 9.0; λ (nm), 214; 0.2 mg/mL protein sample dissolved in water and injected hydrodynamically at 5 mbar for 5 s.

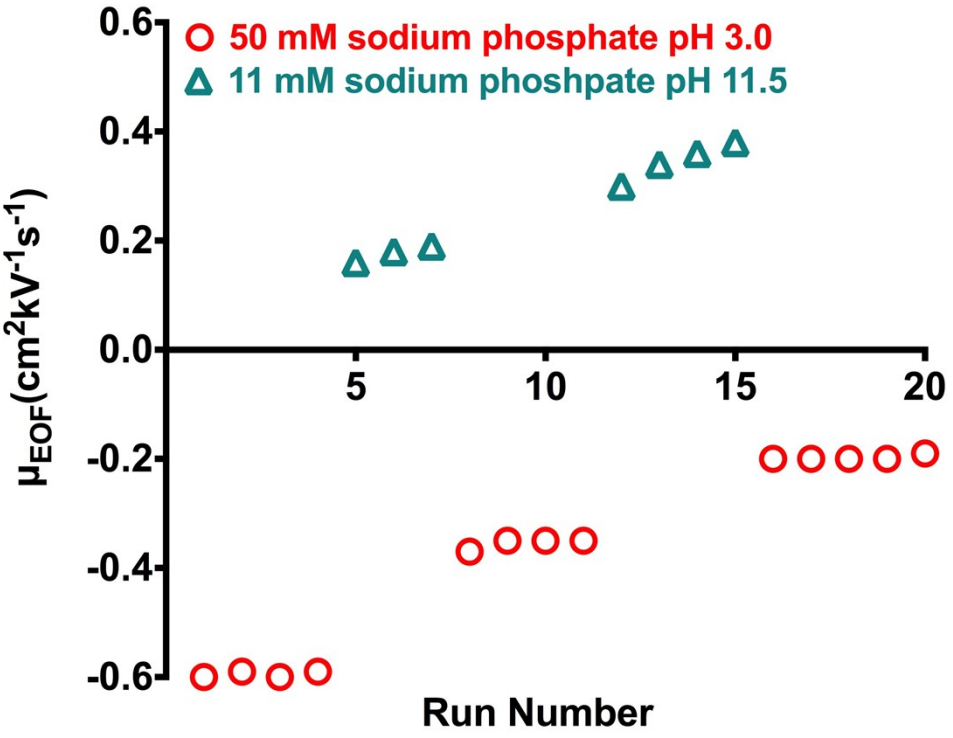

Fig. S4 EOF DODAB coated capillary switched between pH 3.0 and pH 11.5. Before each switch, the capillary was rinsed with water for 1 min, then with buffer for 1 min, and finally equilibrated with buffer for 15 min. Conditions: capillary coated with 0.1 mM DODAB; ± 15 kV; 32 cm × 50 μm i.d. (23.5 cm to detector); 2 mM benzyl alcohol (λ, 214 nm) injected for 3 s at 50 mbar; temperature, 25 °C.

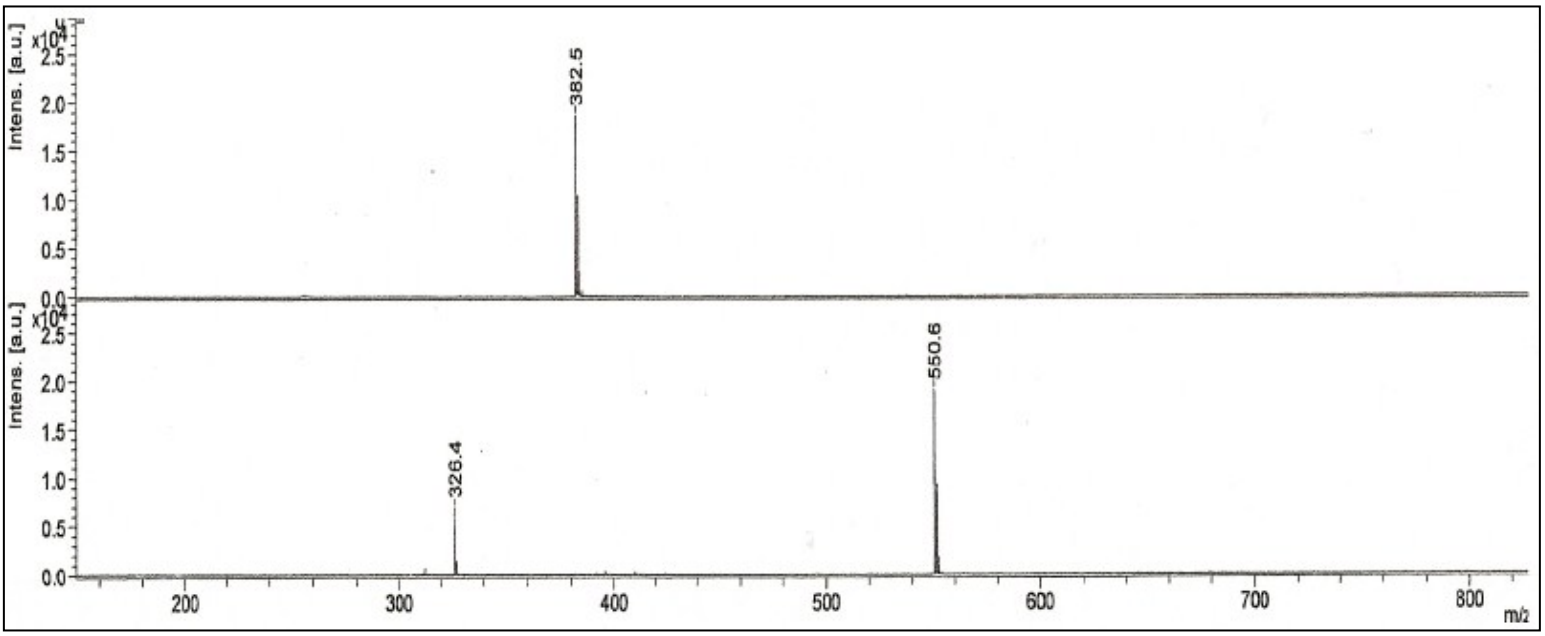

Fig. S5 NALDI-MS spectra of DODAB in water in the presence of SiO_2. Five mM DODAB was prepared in deionised water, sonicated, cooled to room temperature, and filtered, as described in the Experimental Section of this Supplementary Information. Top spectrum: DDAB/SiO_2/H_2O; Bottom spectrum: DODAB/SiO_2/H_2O. DODAB degraded under these conditions. The peaks at *m/z* 550.6 and 326.4 correspond to the molecular ion ($DODA^+$) and degradation peaks, respectively. The signal at *m/z* 382.5 corresponds to the DDA^+ molecular ion peak

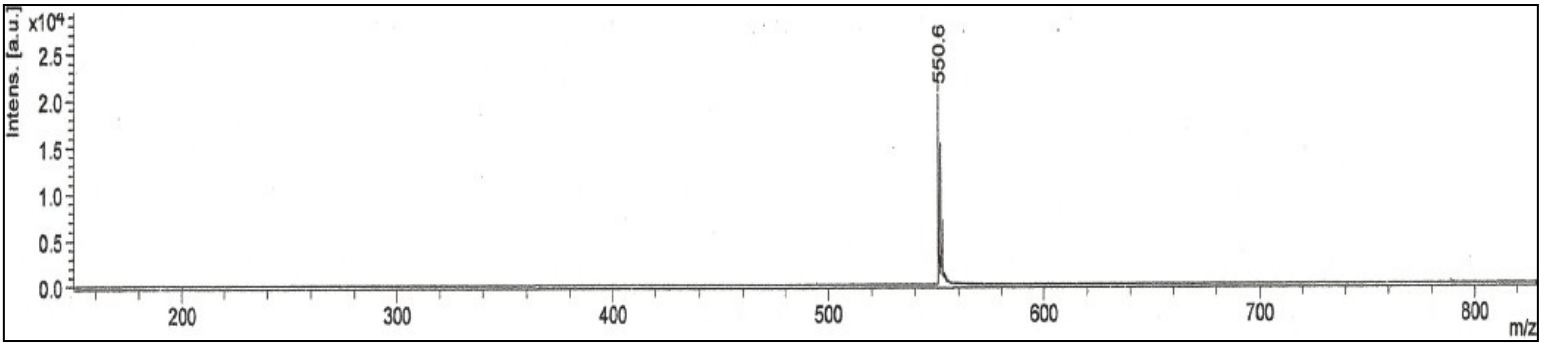

Fig. S6 NALDI-MS spectrum of DODAB in water. Five mM DODAB was prepared in deionised water, sonicated, cooled to room temperature, and filtered, as described in the Experimental Section. DODAB did not degrade under these conditions. The peak at *m/z* 550.6 corresponds to the molecular ion peak ($DODA^+$).

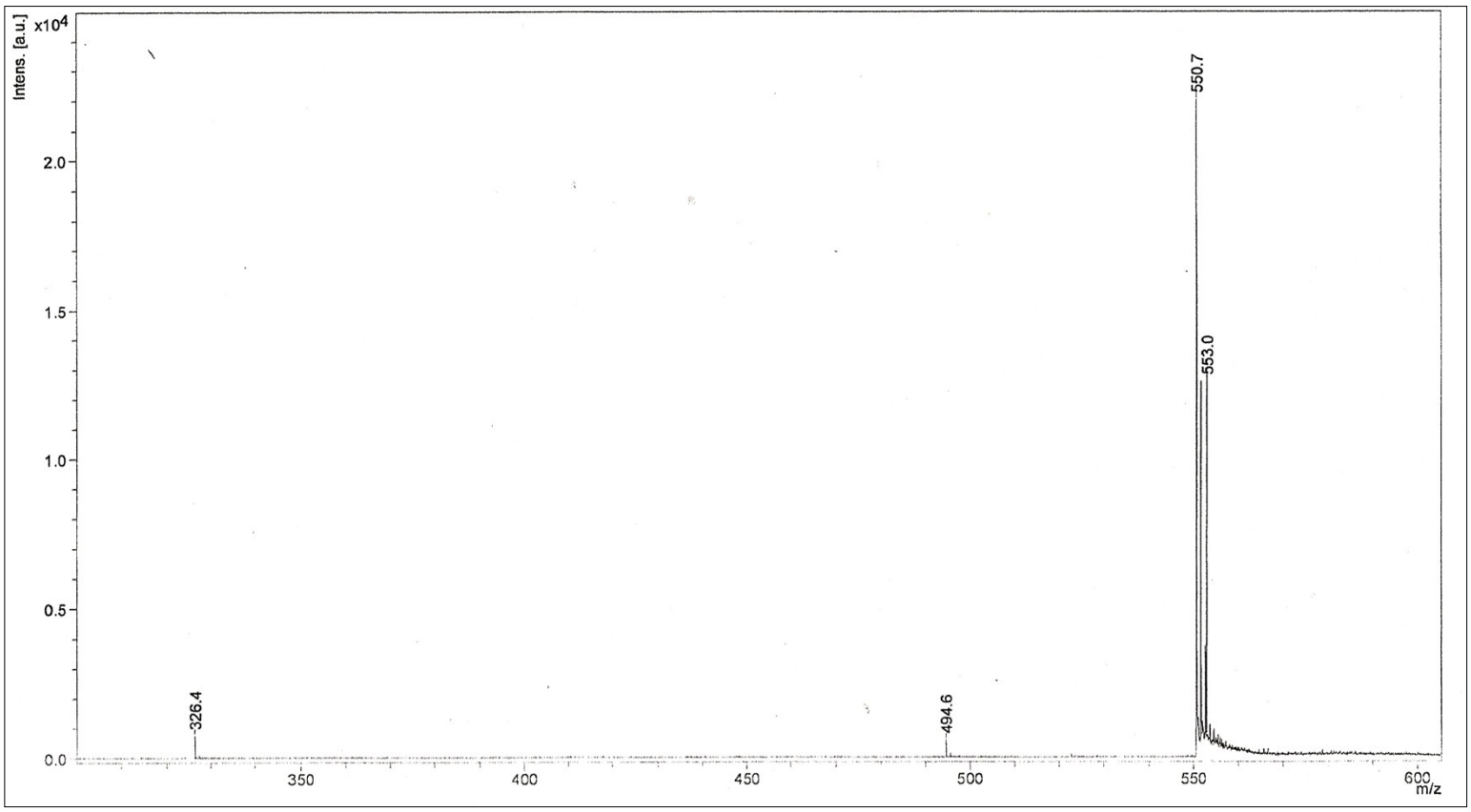

Fig. S7 MALDI-MS spectrum of the isolated organic layer, following liquid-liquid extraction on a previously decomposed solution of DODAB in silica. The spectrum shows the presence of a new decomposition peak at *m/z* 494.6. MS/MS analysis further confirmed that the signal corresponds to another quaternary ammonium species.