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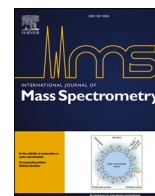
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The use of salts, buffers and surfactants in LAP-MALDI MS

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ABSTRACT

Biological samples such as tissue extracts and enzymatic assays typically have a complex composition, which can interfere with analyte ionisation and detection in mass spectrometry (MS). Ionisation techniques such as electrospray ionisation (ESI) are often coupled online to an upfront chromatographic separation, whereas sample preparations for techniques such as conventional matrix-assisted laser desorption/ionisation (MALDI) are performed offline and, in the case of MALDI, rely on sample clean-up owing to different crystallisation behaviour. Liquid atmospheric pressure matrix-assisted laser desorption/ionisation (LAP-MALDI) MS is a hybrid ionisation technique that has been previously used to analyse a wide range of biological samples at fast acquisition rates. Here we report data from a systematic investigation of the influence of various buffer compounds, salts, surfactants, and other compounds necessary for biological sample preparation reflected in the signal intensity of a standard peptide mixture. Tricine showed the least signal reduction from the buffer compounds tested as did octyl- β -D-glucopyranoside for the surfactants. It can be concluded that LAP-MALDI MS can be used to analyse biological samples directly without major sample clean-up if their content of additives is not too high.

1. Introduction

Many compounds that are naturally present in biological samples or are often added to such samples to provide favourable conditions for biological processes can have a significant impact on analyte detection in mass spectrometric analyses [1]. For example, salts are ubiquitous in biological matrices and can be essential for protein folding [2], enzyme activity [3,4] and cell viability [5–7]. However, their adverse effects on mass spectrometric analysis are well known [8]. Non-volatile salts typically contaminate the mass spectrometer's inlet, and hence can cause significant down-time in larger studies. They also provide different ionisation pathways in addition to protonation, reducing the analyte signal-to-noise ratio [1]. Beyond salts, substantial loss in analyte ion signal due to the presence of other compounds can be the result of a competition for charge either in the post-desorption gas phase or, for ionisation techniques like electrospray ionisation (ESI), already in the pre-desorption liquid phase. This competition for charge can lead to analyte suppression and is influenced by the compounds' spatial distribution in the pre-desorption solid or liquid phase of the sample [8] as well as the setup-specific desorption/ablation characteristics [1], and adds to the intrinsic differences in ionisation efficiency between analytes.

Sample clean-up prior to analysis is therefore often needed but, due to time and cost implications, its avoidance and mitigation strategies involving instrumental and chemical modifications are preferred. In ESI,

substances are commonly added to the spraying solution [9] or in the gas phase [10,11], and spraying configurations are modified to yield smaller initial droplets [12]. Matrix-assisted laser desorption/ionisation (MALDI) also frequently employs additives [13–17], specially tailored matrix compounds [18,19] and specific sample preparation techniques [20,21] to minimise ion suppression and adduct formation.

Liquid atmospheric pressure matrix-assisted laser desorption/ionisation (LAP-MALDI) mass spectrometry (MS) is suitable for a variety of analytes [22–24] and complex biological matrices [25,26] even at high analysis speeds [27]. For conventional solid-state MALDI the suitability of screening assay buffers was investigated [28]. However, a systematic study of the suitability of different compounds typically encountered in biological mass spectrometry for LAP-MALDI MS is missing to date. In this work, we show the influence of different additives on the signal intensity of a peptide mixture which can act as a guideline for future studies and experimental design.

2. Materials and methods

2.1. Materials

α -Cyano-4-hydroxycinnamic acid (CHCA), propylene glycol (PG), bradykinin acetate salt (Brdk), angiotensin I human acetate salt hydrate (Ang), leucine enkephalin acetate salt hydrate (LeuEnk), melittin from honey bee venom and synthetic melittin (Mel), substance P acetate salt

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hydrate (SubP), bovine serum albumin (BSA), 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate (CHAPS), triton™ X-100, triton X-114, tween® 20, tris(hydroxymethyl)aminomethane (tris), amidosulfobetaine-14 (ASB-14), sodium deoxycholate (SDC), octyl-β-D-glucopyranoside (OGP), sodium dodecyl sulphate (SDS), sodium chloride, potassium chloride, ammonium chloride, magnesium chloride hexahydrate, magnesium acetate, 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino)ethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), L-serine, ammonium tartrate dibasic, ammonium hydroxide solution, ethylenediaminetetraacetic acid (EDTA), urea, and LC-MS-grade formic acid (FA) were purchased from Sigma-Aldrich (Gillingham, UK). HPLC-grade water, calcium chloride and phosphate buffered saline (PBS) 10X, ammonium sulphate, LC-MS-grade trifluoroacetic acid (TFA), HPLC-grade acetonitrile (Chromasolv™; Honeywell Riedel-de-Haën™) and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific (Loughborough, UK). NP-40 alternative was obtained from Calbio-Chem® (Nottingham, UK) and ammonium acetate was bought from BDH (Poole, UK). Sodium acetate, ammonium dihydrogenphosphate, ammonium oxalate monohydrate and ammonium citrate dibasic were purchased from Fluka (Dorset, UK). Tricine was bought from Acros Organics (Geel, Belgium).

2.2. Methods

2.2.1. Sample preparation

The MALDI matrix was prepared by dissolving CHCA in acetonitrile and water (1:1; v/v) to a concentration of 5 mg/mL. After sonication, 60% PG (v/v) was added. The analyte mixture was prepared by mixing aqueous peptide stock solutions to give concentrations of 16 pmol/μL for Ang, 10 pmol/μL for Brdk, 20 pmol/μL for LeuEnk, 20 pmol/μL for SubP and 12.5 pmol/μL for Mel (see SI Table 1 for further information). The MALDI matrix and analyte mixture were mixed in a ratio of 3:1 (v/v). The additives (or water) were added to this matrix/analyte solution in a ratio of 1:1 (v/v) and thoroughly mixed. Samples were prepared in replicates and from each replicate one LAP-MALDI sample with a volume of 0.3 μL was spotted on a Waters™ 384-well MALDI sample plate unless otherwise stated. Surfactant-containing samples were mixed and spotted using reverse pipetting to limit foaming.

EDTA was prepared using ammonium hydroxide solution for dissolution. For evaluating the capability of certain compounds to reduce salt adduct formation, additives were mixed with water or aqueous 10 mM NaCl solutions before being mixed with the matrix/analyte solution.

2.2.2. LAP-MALDI MS

LAP-MALDI MS analysis was performed on a modified SYNAPT™ G2-Si (Waters Corp., Wilmslow, UK) Q-ToF instrument as described before [29] using a 343-nm diode-pumped solid-state Yb:YAG laser. For automated sample acquisition, custom WREnS (Waters Research Enabled Software) scripts controlled the sample plate movement and labelled mass spectral scans with the sample position for post-processing using a modified MassLynx™ (Waters) version. Each LAP-MALDI sample was irradiated by the laser with a pulse repetition rate of 500 Hz for 5 s with acquisition scan times of 0.25 s and interscan delays of 10 ms.

2.2.3. Data processing

Separate data files were created for each sample's ion signal according to the labelled sample position on the sample plate using a custom slicing script, similar to previously reported scripts [30]. Batch processing of these files, including the summation of scans, smoothing, and extracting signal intensities, was performed with specproc (<https://sourceforge.net/projects/specproc/>). For analyte ion intensity values, peaks were selected using an m/z tolerance window of ± 25 ppm and the requirement to be present in at least 2 out of 3 replicates using a Python script. Peaks were assigned according to their m/z value. Please note that for substance P non-oxidised potassiumated and oxidised sodiated ion

species might in some cases be undistinguishable as their mass difference is approximately 0.021 Da. Similarly, for ion signals with imperfect peak shape, e.g. due to low signal-to-noise, the m/z assignment might in some cases fall outside the Python script's m/z tolerance window of ± 25 ppm. On the other hand, background noise might contribute to the ion signal if it falls within the above window.

Chemical structures were drawn with ChemDraw® 17.1 (Perkin Elmer, Waltham, US).

3. Results and discussion

3.1. Buffers

Buffers are widely used in sample preparations for biological samples [31] to mimic physiological conditions and allow enzyme activity. A list of useful buffer compounds was compiled by Good et al. [32] and was amended over time [33,34]. Here, buffers with a morpholinic ring (MES, MOPS), piperazinic ring (HEPES) and tris-derived buffers (tris, tricine) were tested against a still widely used phosphate-based buffer (PBS). Structures and pK_a values can be found in SI Table 2.

When mixing 100 mM tris with the matrix/analyte mixture, a colour change from clear to bright yellow was observed, which is an indicator of a chemical reaction occurring. The same colour change can be observed when only the matrix is mixed with tris (see SI Fig. 1). Accidents for the reactivity of the tris amine can be found in the literature [31,35,36] and present a strong argument against the use of tris as a buffer.

For HEPES, MES and MOPS, clusters of $[nM+H]^+$ (where M is the buffer compound) are observed in the LAP-MALDI mass spectra. Furthermore, MES yields similar clusters by sodiation $[nM+Na]^+$. In contrast, tris and tricine only show intense protonated monomer molecules $[M+H]^+$. Some buffer compound adducts were observed with peptides. Singly charged SubP showed adducts with CHCA, MES, MOPS, HEPES and tris (as well as CHCA-tris, see SI Fig. 2). Doubly protonated Brdk was detected with HEPES as an adduct.

General trends for the influence of the buffers on peptide ion signal intensity were similar for all peptides analysed (see Fig. 1). Although structurally similar, tris and tricine performed differently, especially at higher concentrations. At ≥ 5 mM, tris severely suppressed analyte ion signal, whereas tricine led to increased protonated and overall signal intensities at 5 mM for most of the peptides compared to all other solutions at 5 mM as well as the water control. PBS showed substantial ion suppression for concentrations of $\geq 5\%$, for LeuEnk even at 0.5%. MES, MOPS and HEPES generally reduced the peptides' ion signals at 5 mM or more although the degree of suppression varied between analytes. Compared to water (with the caveat of a few larger error bars for some ion signals), all peptides showed overall higher ion signal intensities at 0.05 mM HEPES, and at 0.005 mM, 0.05 mM, and 0.5 mM concentrations for MES and MOPS, apart from 0.5 mM MOPS for SubP and Mel.

For proteins, spectral degradation above 50 mM tris or 20 mM phosphate buffer was reported for solid MALDI MS in an earlier study [37]. In general, phosphate buffers are not recommended for solid MALDI MS analysis owing to interfering background signals [38] and impeded crystallisation for many MALDI matrices [39]. PBS severely suppressed the ion signal intensity of small molecules using the ESI-based ECHO® MS system despite large dilutions [40]. As phosphate is not considered inert in biological systems, its use as a buffer is inherently limited. However, as the data for LAP-MALDI MS shows, the use of PBS of up to 0.5% or higher results in no significant analyte ion signal loss and therefore presents a comparative advantage to solid MALDI and ESI MS.

Another study analysing proteins with solid MALDI found that up to 50 mM tris had no impact on signal intensity but yielded broader peaks due to a less homogeneous crystallisation [39] whereas HEPES and MOPS gave greater signal intensities than in pure water [39]. These detrimental effects on resolution are not expected on a Q-ToF instrument

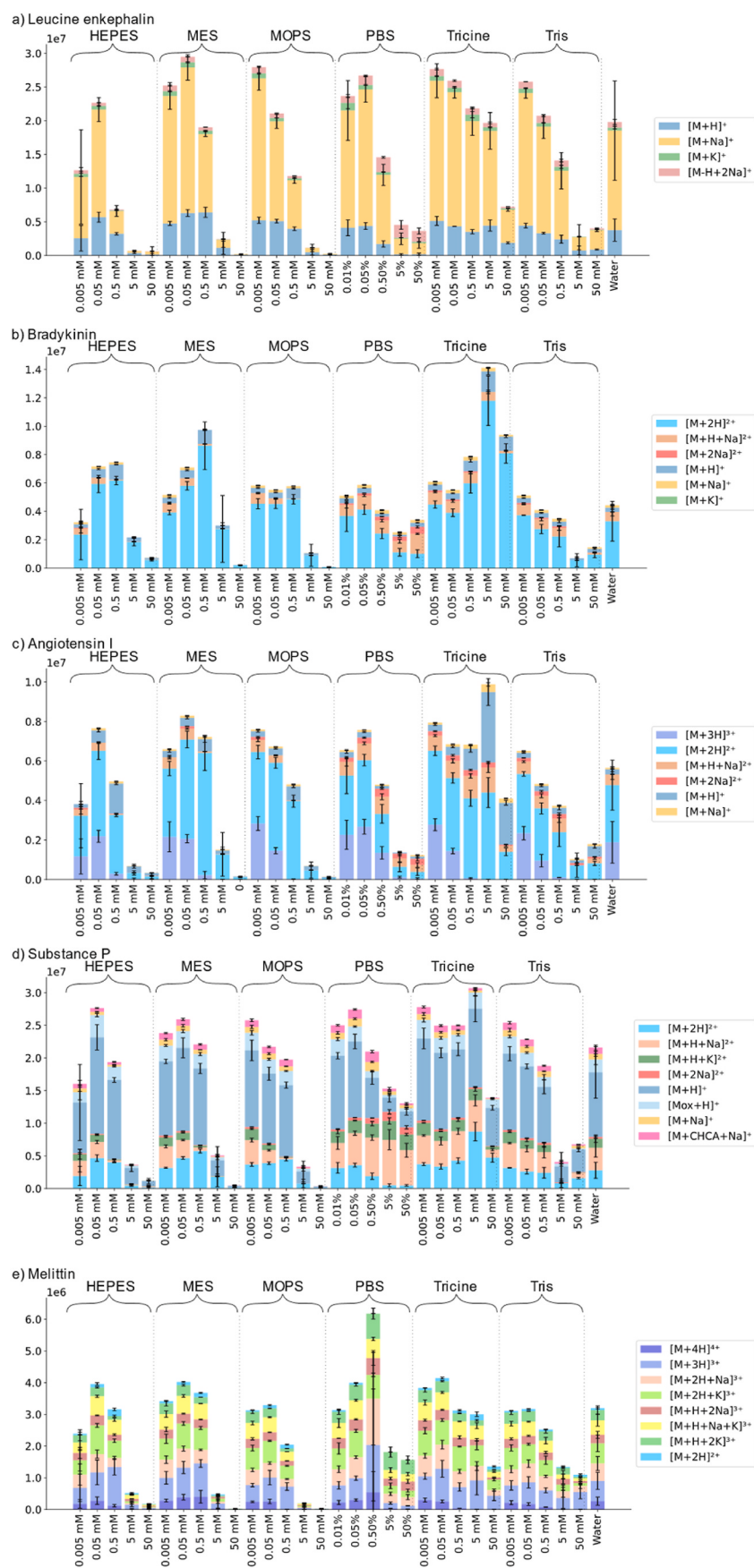


Fig. 1. Influence of buffer compounds on peptide ion signal for a) leucine enkephalin, b) bradykinin, c) angiotensin I, d) substance P and e) melittin. The error bars denote the standard deviation between replicates ($n=3$ for additives, $n=15$ for water) or the data point range for $n=2$ where the signal is below the signal-to-noise threshold of 3 (see [SI Table 3](#)). Ion signals with minor contributions to the total ion signal ($<5\%$ of total ion signal in all samples), e.g. from rare adduct ion formation, were omitted for clarity.

compared to an axial-ToF instrument that was used for the above study. For peptide analysis, 100 mM tris was successfully used in solid MALDI MS analysis in combination with various detergents [41]. This potentially higher resilience of solid MALDI against buffer compounds compared to LAP-MALDI can be partly attributed to the crystallisation process which can purify the sample. In contrast, the presence of buffers can potentially impede crystallisation, which also depends on the matrix employed [39].

ESI and nanoESI have been reported to yield protein spectra with acceptable signal-to-noise in 100 mM tris buffer [42], although with a signal loss of more than an order of magnitude. For the acoustic droplet ejection (ADE) [43] as implemented on the ECHO® MS system, a residual concentration of 10 μ M tris was acceptable [44].

Apart from suppressing analyte ion signal intensity, buffers can also have an impact on operational conditions, particularly in high-throughput screening (HTS) workflows. For example, the ECHO® MS system requires adjustment of the ADE conditions depending on the buffer composition [44]. The data presented here for LAP-MALDI MS, which has been previously shown to be capable to acquire data at a speed of up to 50–60 samples per second [29], were acquired using the same conditions for all additives and no adjustments were required.

3.2. Metal salts

The presence of various salts in biological matrices was mimicked by adding several concentrations of sodium, potassium, magnesium and calcium salts to study the effect on signal intensity. As expected, the mode of ionisation shifted from mainly protonated molecules for low salt concentrations to sodiated and potassiated molecules for higher sodium/potassium salt concentrations (see Fig. 2). This shift resulted in lower signal intensity for the protonated species, and at higher salt concentrations even the sum over all observed analyte ions is significantly decreased. It should be noted that the presence of potassium appears to be more detrimental than the presence of sodium. In contrast, magnesium chloride and calcium chloride did suppress overall analyte ion signal intensity (above a concentration of 0.05–0.5 mM) without forming magnesium or calcium adducts. Ammonium chloride gave the best results of all salts analysed independent of the type of peptide. Here, it should be noted that 0.5 and 0.05 mM ammonium chloride generally provided higher protonated and overall analyte signals than the water control. Apart from these, some other lower (<5 mM) salt concentrations showed slightly higher overall peptide ion signal intensities than the water control.

To exclude an effect of the chloride anion, acetate salts of sodium, magnesium and ammonium were analysed. For salts containing the same cation, e.g. acetate and chloride salts of magnesium, the same trend was observed. Thus, the influence of the anion on analyte signal intensity was minor compared to the influence of the cation. For analyses in negative ion mode this might be different. However, magnesium acetate always gave slightly more intense overall peptide ion signal intensities compared to the chloride whereas ammonium chloride gave higher intensities compared to the acetate. It is recommended not to use sodium acetate with solid-state MALDI as its hygroscopic nature results in wet sample surfaces [38], which is naturally not an issue for LAP-MALDI.

The recorded analyte ion signal intensities did not vary linearly with the salt concentration in all cases. For instance, the ion signal intensity of leucine enkephalin decreases with increasing ammonium chloride concentration (lowest at 50 mM) while the highest overall ion signal of angiotensin I for this chloride was obtained at a concentration of 50 mM.

In LAP-MALDI MS mostly multiply charged peptide ions are observed, which were reported to form less adducts than singly protonated molecules [24]. This behaviour was also reported for nESI where slightly less sodium adduction was observed for higher charge states [9]. For the peptide mixture used in this study (see SI Fig. 3), the protonated-to-sodiated molecular signal ratio was greater for higher

charge states of angiotensin I and bradykinin than for their singly charged ion species while this effect is absent for large parts of the data for SubP.

Typical salt concentrations in biological samples are summarised in SI Table 5. In biofluids, sodium is typically present in concentrations around 5–150 mM. Potassium is present between 5 and 50 mM while magnesium and calcium are less abundant with calcium below 5 mM and magnesium below 1 mM. For the MS analysis of biological samples by LAP-MALDI (as well as by other ionisation techniques), the natural occurrence of sodium can therefore be a limiting factor for mass spectral quality compared with the other salts investigated. Hence, strategies to reduce the sodium concentration of biological samples are recommended for LAP-MALDI MS analysis.

3.3. Salt remediation

Additives are often used in MALDI and ESI to alleviate the effects of salts without resorting to extensive sample clean-up. The addition of ammonium salts can decrease metal cation adducts for peptide analysis in conventional MALDI MS [45] and liquid MALDI MS [46], and ammonium acetate is commonly used in ESI [47–49]. The previously used peptide mixture was mixed with a 10-mM NaCl solution, and various ammonium salt and L-serine solutions were added to study the effect on the protonated, sodiated and overall peptide signal intensity (see SI Fig. 5). For some additives, like ammonium acetate (see Fig. 3), the same trend was observed for all peptides. In contrast, for ammonium tartrate, Ang and Brdk display a similar pattern while the other three analytes follow a different trend. Neither analyte basicity nor molecular size seem to be a predictor for these patterns.

Even for aqueous peptide mixture solutions without any NaCl, several additives enhanced the protonated molecule signal intensities (see Fig. 4). L-serine significantly increased the protonated and total ion signal intensity for Ang and Brdk when used at high concentrations. Most ammonium salts worked best at low concentrations but suppressed ion signals at higher concentrations.

The use of the 10-mM NaCl solution clearly suppressed not only protonated but also overall ion signal intensity for all analytes (see Fig. 4 and SI Fig. 5). For LeuEnk, SubP and Mel none of the additives were able to restore the ion signal to levels obtained in pure water. For Brdk and Ang, the peptide ion signal intensity recovered upon the addition of some additives and even exceeded the pure-water intensities at some additive concentrations.

Ammonium dihydrogen phosphate added to pure aqueous solutions reduced peptide ion signals with increasing concentrations but provided for most additive concentrations an increased Brdk and Ang ion signal intensity compared to pure water solutions. Added to the NaCl solution, the Brdk and Ang ion signals increased with the additive concentration and were greater than in pure water at the three higher concentrations. For conventional solid MALDI MS analysis of tryptic digests, the addition of around 10 mM ammonium dihydrogen phosphate increased peptide ion signals and reduced matrix clusters [15]. Higher and lower concentrations were found to be less effective [15]. Other studies reported increased peptide signals between 5 and 20 mM [50] and 0.5 and 50 mM [16]. Similar effects were observed for tryptic digest analysis by liquid vacuum MALDI, using concentrations between 10 and 100 mM [46]. Thus, LAP-MALDI MS data presented here follow a similar trend of signal enhancement. Although phosphate is not compatible with liquid chromatography [51], it can be a valuable additive for MALDI MS analysis.

There were no substantial ion signal-enhancing effects for ammonium acetate, which was even detrimental at higher concentrations. In nanoESI under native conditions, 7 M ammonium acetate was required to obtain a visible reduction of sodium adducts in protein analysis and no effect was typically obtained at 100 mM [9]. The NaCl concentration investigated here might have been not high enough to observe a similar effect. However, in standard-flow ESI a reduction in protein ion signal is

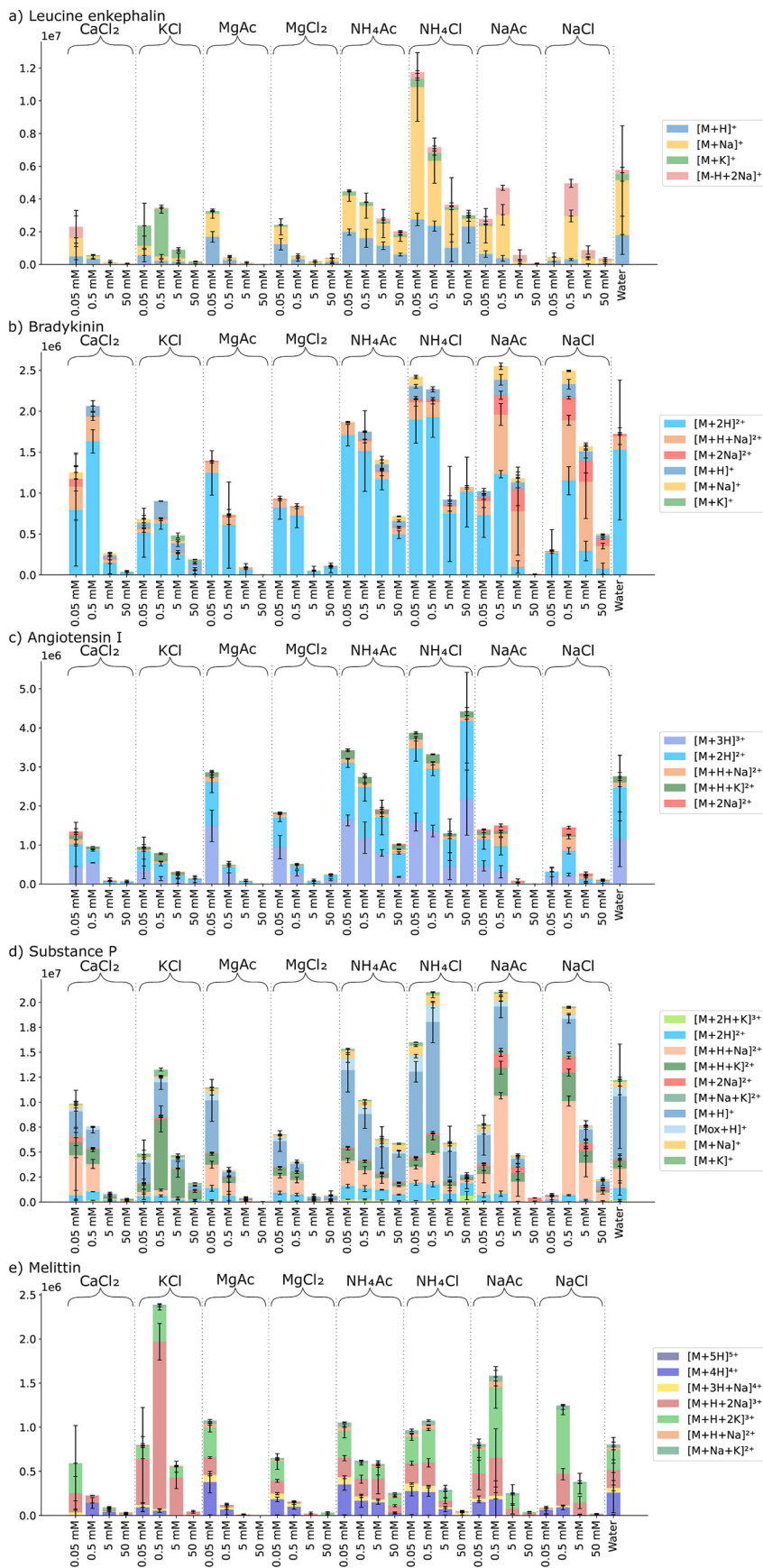


Fig. 2. Influence of salts on peptide ion signal for a) leucine enkephalin, b) bradykinin, c) angiotensin I, d) substance P and e) melittin. The error bars denote the standard deviation between replicates ($n=3$ for additives, $n=15$ for water) or the data point range for $n=2$ where the signal is below the signal-to-noise threshold of 3 (see SI Table 4). Ion signals with minor contributions to the total ion signal ($<5\%$ of total ion signal in all samples), e.g. from rare adduct ion formation, were omitted for clarity.

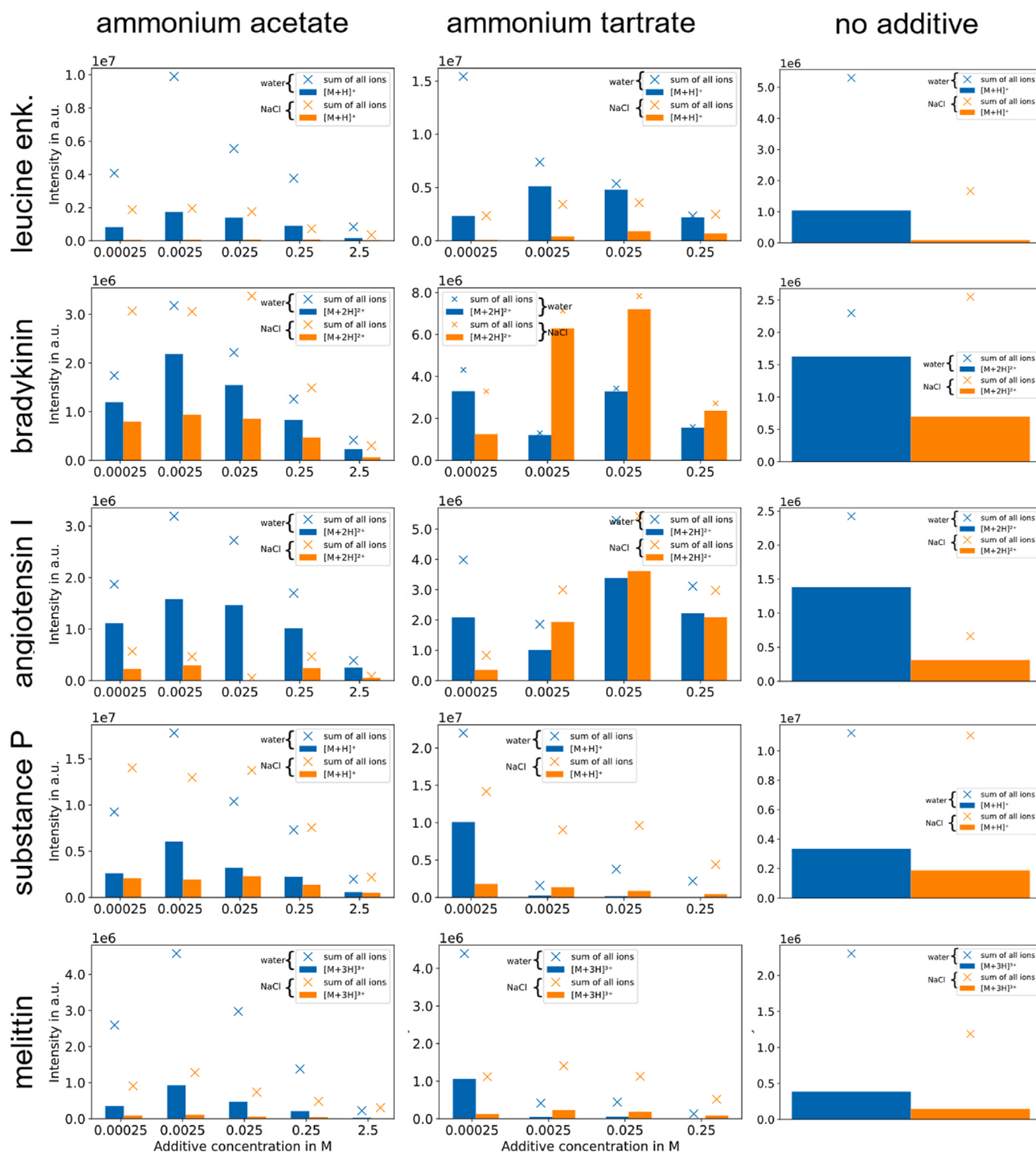


Fig. 3. Influence of ammonium acetate and ammonium tartrate on ion signal intensity in pure water and a 10-mM NaCl solution.

observed with increasing ammonium acetate concentration [52] which is in line with the data presented here. Hence, an influence of the initial droplet size created during the ablation might play a role in adduct formation. In solid MALDI, up to 10 mM did not have significant effects on the analyte ion signal in the analysis of small molecules [28].

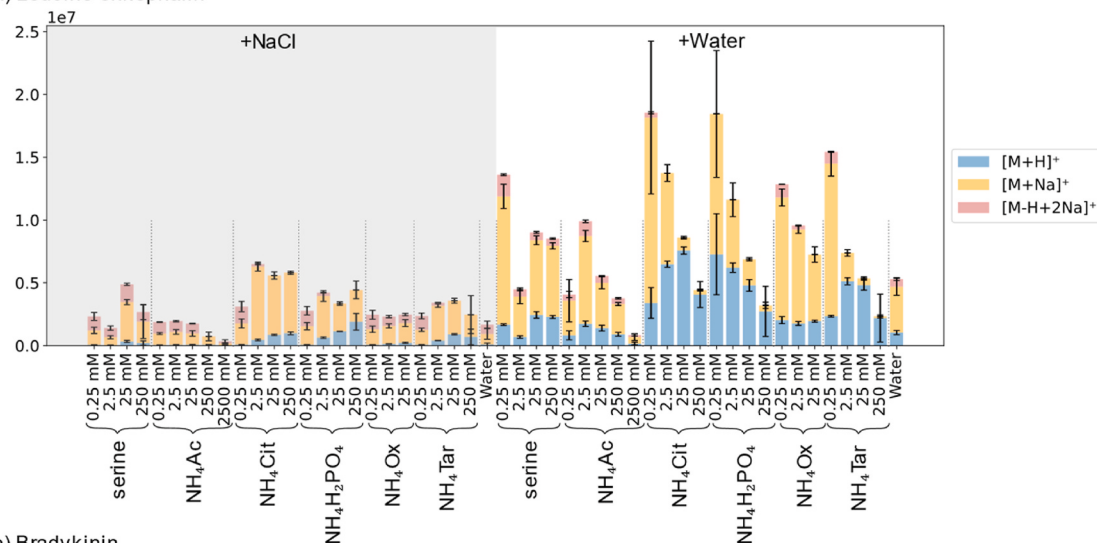
The addition of ammonium citrate significantly increased protonated signal intensity for Ang and Brdk in the presence of NaCl. Ammonium citrate is known to reduce sodium adducts [53] and enhance signal intensity [51] in solid MALDI MS analysis. Citrate was found to be more

effective than other ammonium salts [45] although a reduction in signal was reported above 5 mM [16].

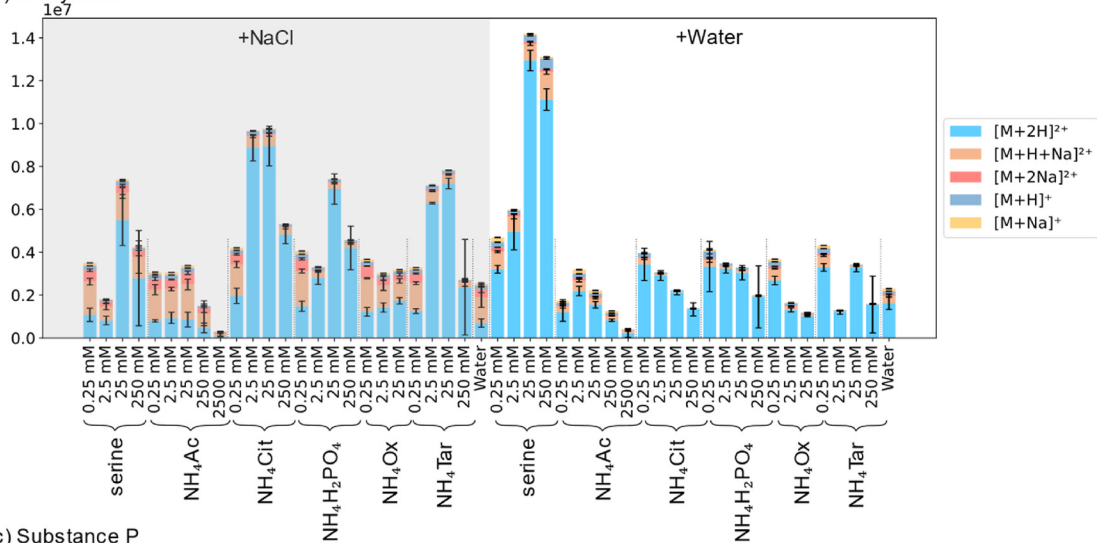
Similar to citrate, oxalate and tartrate salts were investigated as sterically hindered ions are thought to result in less adducts than smaller ammonium salts [17] and provide several ammonium ions per molecule (see SI Fig. 4). Ammonium tartrate follows similar trends as citrate whereas oxalate is slightly inferior with regard to analyte ion signal intensities.

Finally, the addition of L-serine was analysed and a significant

a) Leucine enkephalin



b) Bradykinin



c) Substance P

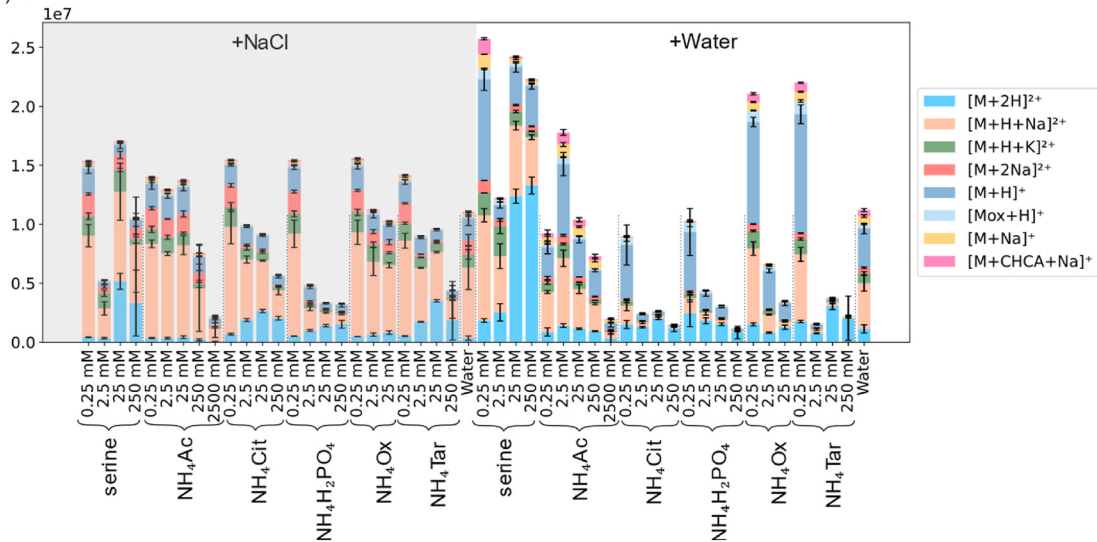


Fig. 4. Influence of the addition of ammonium salts and L-serine on ion signal intensity of peptides in a 10-mM NaCl solution and pure water, respectively, for a) leucine enkephalin, b) bradykinin and c) substance P. The error bars denote the standard deviation between replicates ($n=3$ for additives, $n=15$ for water with NaCl, $n=9$ for water) or the data point range for $n=2$ where the signal is below the signal-to-noise threshold of 3 (see SI Table 6). Ion signals with minor contributions to the total ion signal ($<5\%$ of total ion signal in all samples), e.g. from rare adduct ion formation, were omitted for clarity.

increase in protonated Brdk and Ang signals was observed at around 100 mM. This is consistent with conventional solid MALDI, for which the addition of serine improved the signal-to-noise ratio for protonated Ang species by a factor of 4 [14]. A similar effect was reported for DESI MS with L-serine-enhanced protein signal [54] and reduced sodium adducts even if used at less than stoichiometric amounts [55]. However, at higher concentrations (mM) a decrease in signal was observed [55], which is not in agreement with the data presented here. In ESI MS, the addition of ten times more serine than NaCl led to a reduction of Na adducts and an improvement in the signal-to-noise ratio for native proteins [56]. As no effect was observed above 2 mM sodium, it was hypothesised that the direct binding of Na to the amino acid prevents remediation by serine [56]. In the LAP-MALDI MS data presented here, an increase in peptide ion signal was observed for Brdk and Ang when serine was added in excess compared to sodium. No clear effect was visible for the other peptides analysed.

3.4. Surfactants

Surfactants are amphiphilic substances often used in biological sample preparations to help protein solubilisation, prevent adsorption [38] and aggregation [57] and generally assist with the analysis of hydrophobic peptides [58] and proteins [59]. Surfactants have a wide range of properties [60], e.g. being denaturing or not, and hence, need to be tailored to a specific application. Different types can be distinguished according to their molecular structure: anionic, cationic, zwitterionic or non-ionic. For this study, non-ionic (OGP), zwitterionic (CHAPS) and anionic (SDC) surfactants were analysed (see SI Table 7). Commonly used polymeric surfactants, e.g. Tween-20, triton (X-100 and 114) and NP40 alternative, were not further investigated as they caused a wide polymeric distribution over a large range of the mass spectrum which interfered with analyte detection.

With increasing surfactant concentration, the surface tension of the sample decreases and the droplet becomes unstable (see SI Fig. 6). Samples can spread out (and completely wet the sample plate surface), and the resulting film does not yield any analyte signal. With lower surfactant concentrations stable droplets can be obtained. The surface tensions for the investigated surfactants are summarised in SI Table 7. At the critical micelle concentration, these surface tensions are similar, although it must be noted that surface tension also depends on various other parameters such as electrolyte concentration [61], which can affect droplet stability in 'real-life' samples. Although droplet stability is somewhat correlated with surface tension and therefore inversely with surfactant concentration (see SI Table 8), SDS yields stable droplets even at the highest concentration investigated. Hence, other factors might play a role such as the affinity of the surfactant molecules to the stainless-steel sample plate. To circumvent unstable droplets, other sample plate surfaces were investigated, and a Bruker AnchorChip™ plate was used for analysis. Its hydrophobic surface area, surrounding the small hydrophilic sample spot areas, effectively prevented sample spreading and all samples (up to 10% w/v) yielded stable liquid MALDI sample droplets (see SI Fig. 6). Results obtained from stable samples on both plates are in good agreement apart from SDC, for which a discrepancy was observed at 0.1% (see Fig. 5 and SI Fig. 7).

For the surfactants, the variability between replicates was higher than for other experiments (see Fig. 5) as surfactant samples are more difficult to handle and the plate holder was not optimised for accommodating large plates with a microtiter plate format. In general, the lowest surfactant concentrations gave similar results to the water control. Samples containing 5% SDS solidified upon laser radiation, probably caused by solvent evaporation leading to a concentration of SDS beyond its solubility. For all surfactants, intense additional peaks (see SI Fig. 8) were observed which are not present in the water control and are dependent on the surfactant concentration. Putative assignments of surfactant clusters (mainly $[n\cdot M+H]^+$ and $[n\cdot M+Na]^+$, where M is the surfactant molecule) can be found in SI Table 9. For SDS, similar peaks

have been reported for ESI, although at higher SDS concentrations [62, 63].

Regarding analyte ion signal intensities, the same trend can be observed for all analytes for the addition of ASB, CHAPS and SDS. In most cases, ion suppression was observed around the 0.01% level (see Fig. 5). At this and higher concentrations ASB gave the worst results of all surfactants investigated while CHAPS led to slightly more intense analyte ion peaks compared to SDS. For the other surfactants, more diverse results were obtained. With increasing SDC concentration the signal intensity for LeuEnk, Ang and Mel decreased. For SubP, the opposite was observed apart from the highest concentration, at which no analyte ion signal could be obtained. As the peptides were analysed together as a mixture, no variation in sample preparation or analysis can account for these differences. The nature of the analytes and their relative differences in the ionisation process are the most probable origins of this behaviour. Comparing the isoelectric points (see SI Table 1), Brdk, SubP and Mel are significantly more basic than Ang and LeuEnk. However, this difference was not reflected in the SDC data. OGP was the only surfactant that provided analyte ion signals at 5%. Ang, Brdk and LeuEnk showed the strongest ion signal at 0.01% OGP; for Melittin 1% OGP was best.

Although detergents are widely used for biological sample preparations, most MS ionisation techniques require careful detergent removal. In ESI MS, surface-active compounds are generally detected at higher ion signal intensities due to their location at the droplet surface during droplet fission [64], which can suppress non-surface-active analytes. In conventional MALDI, the addition of surfactants can impede crystallisation [65].

For solid MALDI MS, contradictory results for the influence of surfactants on analyte signal are reported in the literature. In one study, no peptide or protein ion signals were obtained at 0.1% SDS or CHAPS but at 1% SDS or CHAPS protein ion signal was detected [66]. In other studies it was found that SDS concentrations of up to 0.1% [67] or 0.6% [42] were tolerable but interferences were seen at 1% [67]. CHAPS was found to be incompatible with solid MALDI MS analysis [67,68]. For solid MALDI MS analysis of acetylcholine, up to 0.6% CHAPS showed no significant effect on ion signal intensity [28]. Data obtained by LAP-MALDI MS show peptide ion signals at 0.1% SDS or CHAPS, but no signals at 1% of either for any of the peptides analysed. In an early study using solid MALDI, it was hypothesised that at low surfactant concentrations protein ion pairs were formed with the surfactant while at surfactant concentrations above the critical micelle concentration, the protein studied was well solubilised in micelles [66]. It was also suggested that higher surfactant concentrations lead to crystallisation of the surfactant around the matrix and therefore decrease the energy transfer to the sample [66]. In LAP-MALDI, crystallisation does not occur but increased surfactant concentrations can still change laser absorption due to a lower surface concentration of matrix molecules. For solid MALDI MS analysis of OGP-containing samples, it was reported that 0.1 and 1% OGP concentrations were suitable for peptide analysis [67] while the use of up to 5% OGP was reported for other studies [68]. This is in good agreement with the data reported here. Additionally, peak broadening and a mass shift were observed for some proteins when using OGP in solid MALDI MS [59]. This might indicate the formation of adducts. However, for LAP-MALDI MS analysis no OGP adducts were observed.

In a comparative MS study, ESI was found to be one order of magnitude less tolerant against surfactants than solid MALDI [68] with the exception of CHAPS, which ESI tolerated up to 1% [68]. Other studies showed that no protein ion signal was observed at 1% CHAPS [63] or only weak signal at 0.6% CHAPS [42]. In another study using proteins, SDS gave only 10% analyte ion signal compared to the water control at a concentration of 0.01% [63] but in a different study weak analyte ion signals were observed at 1.4% and good signal was obtained at 0.3% [42]. LAP-MALDI MS data presented here show comparatively good peptide ion signals at 0.001% SDS but weaker signals at 0.01–0.1% and therefore are in the same range as the literature values for ESI.

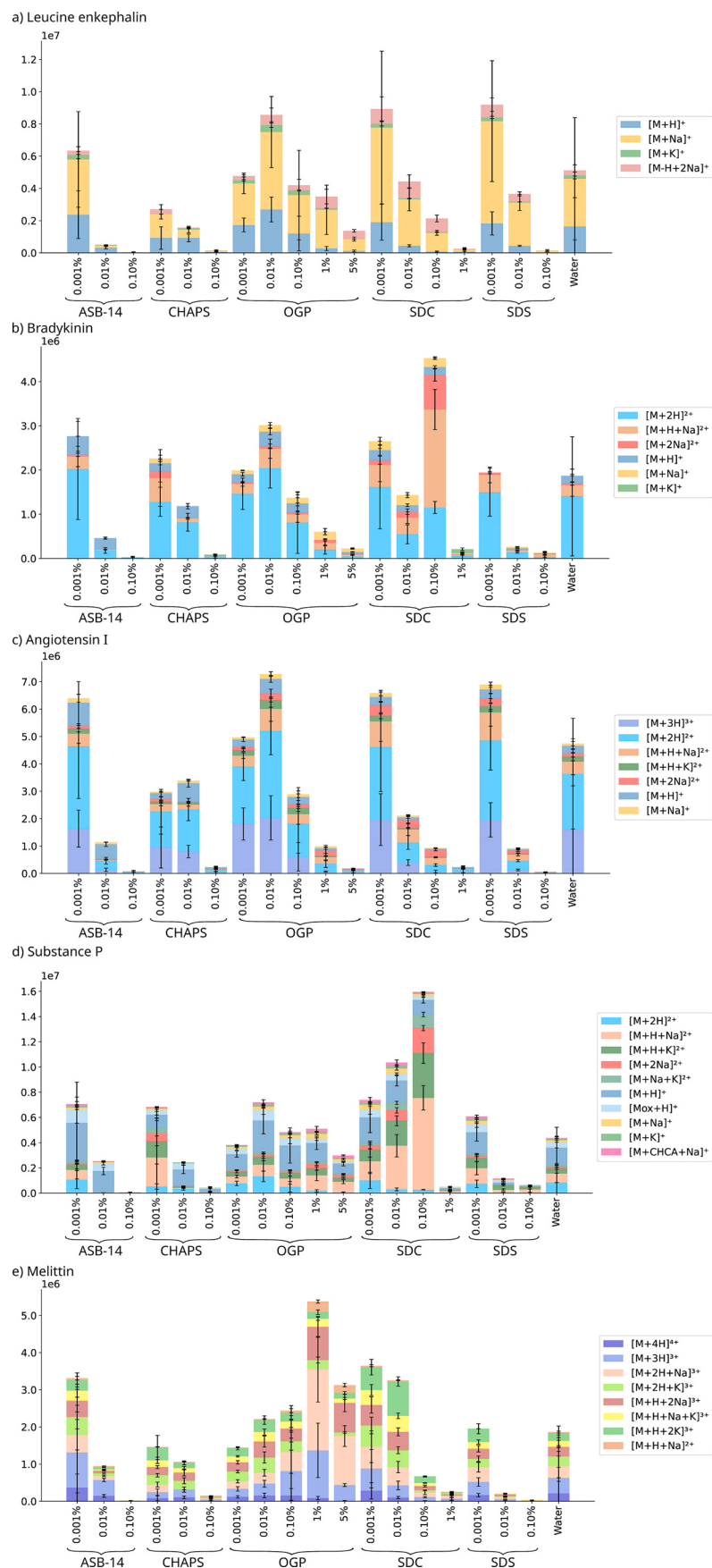


Fig. 5. Influence of surfactants on peptide ion signal for a) leucine enkephalin, b) bradykinin, c) angiotensin I, d) substance P and e) melittin. The error bars denote the standard deviation between replicates ($n=3$ for additives, $n=9$ for water) or the data point range for $n=2$ where the signal is below the signal-to-noise threshold of 3 (see SI Table 10). Ion signals with minor contributions to the total ion signal ($<5\%$ of total ion signal in all samples), e.g. from rare adduct ion formation, were omitted for clarity.

Non-ionic saccharides gave best analyte ion signal in ESI compared with other surfactants as less background ions were generated, less Na adducts were observed and analyte ion signal suffered less suppression [63]. This is in very good agreement with the data presented here. In ESI, OGP still yielded analyte ion signal at 1% surfactant [63] while for LAP-MALDI MS peptide ion signal could still be observed at 5% OGP.

In HTS assays non-ionic surfactants are generally used at 0.01–0.1% to prevent aggregation [57]. As shown for OGP, LAP-MALDI MS does not suffer from severe ion suppression at these concentrations and it is assumed that this is also true for small molecules often used as screening targets. However, a shift from traditional assay detergents like triton and tween to surfactants more compatible with mass spectrometry is necessary.

3.5. Other compounds

BSA is used in cell-based assays to reduce nonspecific binding but is not advised for biochemical screens as it binds many compounds that are viable drug leads [69]. In small concentrations (2.5 pmol/ μ L, app. 0.02%) only a minor suppression of analyte ion signal is observed (see Fig. 6). This is comparable to other ionisation techniques like MALDI [28] and IR-MALDESI [70] for which no significant impact up to 0.01% was noticed. At higher concentrations, unresolved protein peaks dominate the mass spectra. However, resolved peaks can be probably obtained using different instrument settings [23].

DMSO is often used to store compound libraries, so HTS assays contain small amounts. At 1% DMSO, no ion signal suppression is observed for any analyte and for 5% only minor effects are noticeable. After dilution of the target compounds in assay buffer, no adverse effects on LAP-MALDI MS analysis are expected. In solid MALDI, DMSO is known to help with crystallisation [71,72], although heterogeneous crystallisation is observed at higher concentrations [73], and up to 1% is used to enhance tissue images of drugs [74].

The addition of 0.1 mM EDTA did not adversely affect LAP-MALDI MS analysis. At 1 mM suppression of analyte ion signal is observed, although the extent of signal reduction depends on the analyte and at 10 mM signal can still be obtained. Solid MALDI MS analyses suggest a reduction of analyte signal by approximately 50% at 50 mM [28], so is more tolerant against this additive. However, a small molecule was used as a test compound compared to peptides used in this study. In ESI MS, the addition of EDTA led to a significant improvement of phosphopeptide detection but caused issues with chromatographic separation and spray stability due to precipitation [51].

The use of acids in positive ionisation mode is thought to have a positive impact on analyte detection owing to the increased abundance of protons for ionisation and is therefore routinely used as an additive in ESI [75,76]. However, in LAP-MALDI MS the signal intensity for all analysed peptides decreased at 1% formic acid compared to water albeit at different factors and a significant decrease for all analytes is observed at 10%. As standard MALDI MS workflows for identification of micro-organism recommend 35% formic acid [77], LAP-MALDI appears more susceptible to formic acid than conventional MALDI. When using TFA, droplets were not as stable and resulted in spread samples which increased signal variability (see Fig. 6). Nevertheless, analyte ion signal could be obtained for all dilutions (0.1–10% TFA on target). TFA is widely used as a mobile phase additive in liquid chromatography of peptides and proteins but is known to suppress protein ion signals in ESI by forming ion pairs with basic analytes [78]. Reported suppression factors range from 10 [76] to 250 [78] and can only be partially mediated [78]. Hence, LAP-MALDI MS is less affected by TFA.

If 1 M urea is added to the sample, significant ion signal suppression is observed and nearly no signal can be detected at 4 M. In solid MALDI no protein signal can be obtained at 8 M urea [19] and for nanoESI 4 M urea clogged the emitter [79]. However, 0.5 M was found to be compatible with nanoESI MS analysis and severe suppression occurred at around 2 M [79]. If urea is used in the sample preparation, sufficient

dilution is necessary to allow LAP-MALDI MS analysis.

For ammonium sulphate, ion signal intensity was influenced differently for the different analytes. For Ang, no suppression was observed at 10 and 100 mM, whereas Brdk and LeuEnk showed reduced ion signals for sodiated peaks which resulted in an overall decreased ion signal (see Fig. 6). For SubP and Mel, severe ion signal suppression was observed even at 10 mM. The poor performance of the 1 M sample for all analytes was attributed to crystallisation. After the MS analysis, some samples were found to be solidified which is thought to be caused by solvent evaporation due to the laser irradiation and heating of the inlet tube which increased the additive concentration beyond solubility. Ammonium sulphate is commonly used for protein precipitation at concentrations between 800 and 3200 mM [80].

4. Conclusion

A variety of sample additive compounds were tested for compatibility with LAP-MALDI MS analysis. Although some differences between the analysed peptides were observed, general trends could be deduced.

Amongst the investigated buffer compounds tricine showed the best ion signal intensities for all analysed peptides over a concentration range from 0.005 to 5 mM on target. Owing to the dilution with the LAP-MALDI matrix, a higher initial concentration during sample preparation can be chosen.

In the presence of salts, the mode of ionisation is changing from mainly protonated analyte molecules to the formation of salt adducts. Depending on the analyte and the concentration of salts (generally <0.5 mM) the overall analyte ion signal intensity might be enhanced compared to the signal obtained in pure water. As naturally occurring concentrations of salts, especially NaCl, are comparably high, strategies for salt removal are necessary to avoid lower signal-to-noise levels in LAP-MALDI MS analysis.

One relatively inexpensive and fast method to reduce salt effects is the addition of salt sequestering agents to the sample. Ammonium salts and L-serine were added to the peptide mix in pure water and in 10 mM NaCl. Signal intensity in salt-containing samples could not be completely restored (to the level of pure water) but some improvement was visible for most peptides. Best ion signal recovery was obtained by ammonium citrate when used stoichiometrically or in 10-times excess of NaCl. Addition of 0.25 mM of various additives also led to improvements of ion signal intensities in general, i.e. even in the samples without added NaCl. This might be due to the presence of salts in the 'pure' samples. The use of higher concentrations led to a decrease in analyte ion signal intensity. Interestingly, the addition of serine at higher concentrations (25–250 mM) led to signal increases in both sample sets, with and without added NaCl, in particular for the multiply charged analyte ion species.

The direct analysis of samples containing polymeric surfactants is unsuitable for LAP-MALDI MS. Even other types of surfactants create a range of surfactant-related ions which might interfere with analyte detection. In general, concentrations greater than 0.01% suppressed analyte detection. An exception is OGP, which yielded analyte ion signals over a comparably wide concentration range and is therefore recommended for analysis by LAP-MALDI MS. For some peptides, 0.1% SDC also resulted in good analyte ion signal intensity, although the mode of ionisation changed from protonation to sodiation.

Last, several compounds often used in sample preparation were tested for their suitability with LAP-MALDI MS analysis. For acids, FA rather than TFA should be chosen as higher signal intensities were obtained with the former for all peptides. DMSO often used for compound storage is not impeding analysis at concentrations normally present after reconstitution ($\leq$ 5%). BSA used as a model for protein addition lowers analyte signal intensity but again in most laboratories only small concentrations are expected. For the use of urea in the sample preparation, its concentration should be lowered before LAP-MALDI MS analysis as 1 M decreases analyte ion signal intensity by more than 50%.

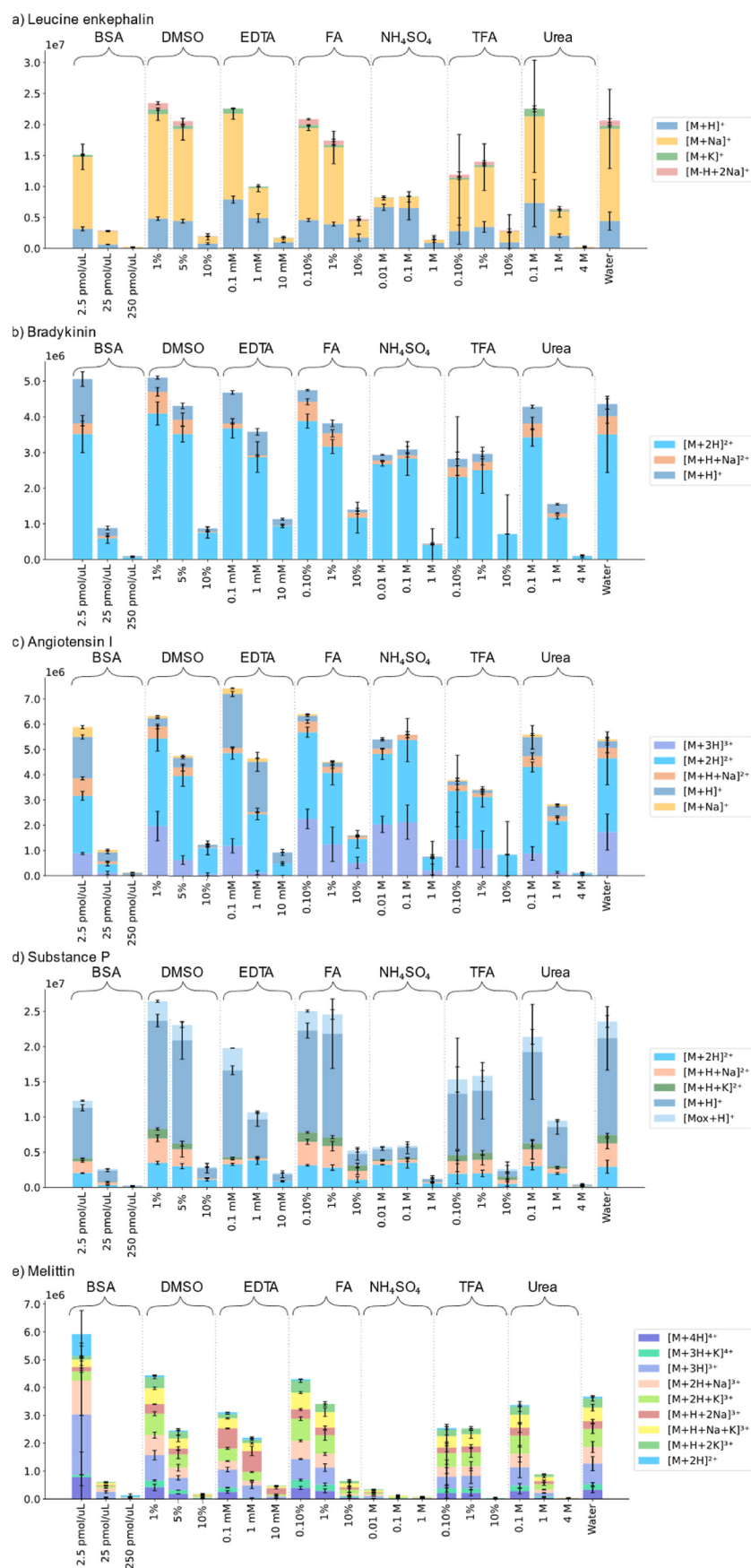


Fig. 6. Influence of compounds often used in biological sample preparation on peptide ion signal for a) leucine enkephalin, b) bradykinin, c) angiotensin I, d) substance P and e) melittin. The error bars denote the standard deviation between replicates (n=3 for additives, n=9 for water) or the data point range for n=2 where the signal is below the signal-to-noise threshold of 3 (see [SI Table 11](#)). Ion signals with minor contributions to the total ion signal (<5% of total ion signal in all samples), e.g. from rare adduct ion formation, were omitted for clarity.

Author statement

Henriette Krenkel: methodology, software, formal analysis, investigation, writing, visualization.

Jeffery Brown: resources, writing – review & editing.

Michael Morris: resources, writing – review & editing, funding acquisition.

Rainer Cramer: conceptualization, methodology, resources, writing, visualization, supervision, funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rainer Cramer reports financial support and equipment, drugs, or supplies were provided by Waters Corporation. Rainer Cramer reports a relationship with BSPR that includes: board membership.

Data availability

Data supporting the results reported in this paper are openly available from the University of Reading Research Data Archive at <https://researchdata.reading.ac.uk/id/eprint/464>.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijms.2023.117134>.

References

- [1] H. Metwally, R.G. McAllister, L. Konermann, Exploring the mechanism of salt-induced signal suppression in protein electrospray mass spectrometry using experiments and molecular dynamics simulations, *Anal. Chem.* 87 (2015) 2434–2442.
- [2] H. Maity, A.N. Muttathukattil, G. Reddy, Salt effects on protein folding thermodynamics, *J. Phys. Chem. Lett.* 9 (2018) 5063–5070.
- [3] C. Küttemeyer, M. Froeck, H.D. Werlein, B.M. Watkinson, The influence of salts and temperature on enzymatic activity of microbial transglutaminase, *Food Control* 16 (2005) 735–737.
- [4] C. Park, R.T. Raines, Quantitative analysis of the effect of salt concentration on enzymatic catalysis, *J. Am. Chem. Soc.* 123 (2001) 11472–11479.
- [5] M.J.V. Clausen, H. Poulsen, Sodium/potassium homeostasis in the cell, in: L. Banci (Ed.), *Metallomics and the Cell*, Springer Netherlands, Dordrecht, 2013, pp. 41–67.
- [6] J.P. Morth, B.P. Pedersen, M.J. Buch-Pedersen, J.P. Andersen, B. Vilsen, M. G. Palmgren, P. Nissen, A structural overview of the plasma membrane Na⁺, K⁺-ATPase and H⁺-ATPase ion pumps, *Nat. Rev. Mol. Cell Biol.* 12 (2011) 60–70.
- [7] L.M. Biga, S. Dawson, A. Harwell, R. Hopkins, J. Kaufmann, M. LeMaster, P. Matern, K. Morrison-Graham, D. Quick, J. Runyeon, 3.1 the cell membrane, in: *Anatomy & Physiology*, OpenStax/Oregon State University, 2019.
- [8] F. Beaudry, P. Vachon, Electrospray ionization suppression, a physical or a chemical phenomenon? *Biomed. Chromatogr.* 20 (2006) 200–205.
- [9] A.T. Iavarone, O.A. Udekwu, E.R. Williams, Buffer loading for counteracting metal salt-induced signal suppression in electrospray ionization, *Anal. Chem.* 76 (2004) 3944–3950.
- [10] J.C. DeMuth, S.A. McLuckey, Electrospray droplet exposure to organic vapors: metal ion removal from proteins and protein complexes, *Anal. Chem.* 87 (2015) 1210–1218.
- [11] J.T. Hopper, K. Sokratous, N.J. Oldham, Charge state and adduct reduction in electrospray ionization-mass spectrometry using solvent vapor exposure, *Anal. Biochem.* 421 (2012) 788–790.
- [12] J. Hu, Q.Y. Guan, J. Wang, X.X. Jiang, Z.Q. Wu, X.H. Xia, J.J. Xu, H.Y. Chen, Effect of nanoemitters on suppressing the formation of metal adduct ions in electrospray ionization mass spectrometry, *Anal. Chem.* 89 (2017) 1838–1845.
- [13] R.L. Griffiths, J. Bunch, A survey of useful salt additives in matrix-assisted laser desorption/ionization mass spectrometry and tandem mass spectrometry of lipids: introducing nitrates for improved analysis, *Rapid Commun. Mass Spectrom.* 26 (2012) 1557–1566.
- [14] T. Nishikaze, M. Takayama, Disappearance of interfering alkali-metal adducted peaks from matrix-assisted laser desorption/ionization mass spectra of peptides with serine addition to alpha-cyano-4-hydroxycinnamic acid matrix, *Rapid Commun. Mass Spectrom.* 21 (2007) 3345–3351.
- [15] Y. Ucal, A. Ozpinar, Improved spectra for MALDI MSI of peptides using ammonium phosphate monobasic in MALDI matrix, *J. Mass Spectrom.* 53 (2018) 635–648.
- [16] X. Zhu, I.A. Papayannopoulos, Improvement in the detection of low concentration protein digests on a MALDI TOF/TOF workstation by reducing alpha-cyano-4-hydroxycinnamic acid adduct ions, *J. Biomol. Tech.* 14 (2003) 298–307.
- [17] Y.F. Zhu, N.I. Taranenkov, S.L. Allman, S.A. Martin, L. Haff, C.H. Chen, The effect of ammonium salt and matrix in the detection of DNA by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.* 10 (1996) 1591–1596.
- [18] R. Chen, W. Xu, C. Xiong, X. Zhou, S. Xiong, Z. Nie, L. Mao, Y. Chen, H.C. Chang, High-salt-tolerance matrix for facile detection of glucose in rat brain microdialysates by MALDI mass spectrometry, *Anal. Chem.* 84 (2012) 465–469.
- [19] S. Wang, Z. Xiao, C. Xiao, H. Wang, B. Wang, Y. Li, X. Chen, X. Guo, (E)-Propyl alpha-cyano-4-hydroxyl cinnamate: a high sensitive and salt tolerant matrix for intact protein profiling by MALDI mass spectrometry, *J. Am. Soc. Mass Spectrom.* 27 (2016) 709–718.
- [20] E.B. Monroe, B.A. Koszciuk, J.L. Losh, J.C. Jurchen, J.V. Sweedler, Measuring salty samples without adducts with MALDI MS, *Int. J. Mass Spectrom.* 260 (2007) 237–242.
- [21] R.W. Garden, L.L. Moroz, T.P. Moroz, S.A. Shippy, J.V. Sweedler, Excess salt removal with matrix rinsing: direct peptide profiling of neurons from marine invertebrates using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *J. Mass Spectrom.* 31 (1996) 1126–1130.
- [22] O.J. Hale, R. Cramer, Collision-induced dissociation of doubly-charged barium-cationized lipids generated from liquid samples by atmospheric pressure matrix-assisted laser desorption/ionization provides structurally diagnostic product ions, *Anal. Bioanal. Chem.* 410 (2018) 1435–1444.
- [23] P. Ryumin, R. Cramer, The composition of liquid atmospheric pressure matrix-assisted laser desorption/ionization matrices and its effect on ionization in mass spectrometry, *Anal. Chim. Acta* 1013 (2018) 43–53.
- [24] R. Cramer, A. Pirkel, F. Hillenkamp, K. Dreisewerd, Liquid AP-UV-MALDI enables stable ion yields of multiply charged peptide and protein ions for sensitive analysis by mass spectrometry, *Angew. Chem. Int. Ed. Engl.* 52 (2013) 2364–2367.
- [25] S.E. Lellman, R. Cramer, Bacterial identification by lipid profiling using liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry, *Clin. Chem. Lab. Med.* 58 (2020) 930–938.
- [26] O.J. Hale, M. Morris, B. Jones, C.K. Reynolds, R. Cramer, Liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry adds enhanced functionalities to MALDI MS profiling for disease diagnostics, *ACS Omega* 4 (2019) 12759–12765.
- [27] H. Krenkel, E. Hartmane, C. Piras, J. Brown, M. Morris, R. Cramer, Advancing liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry toward ultrahigh-throughput analysis, *Anal. Chem.* 92 (2020) 2931–2936.
- [28] J. Chandler, C. Haslam, N. Hardy, M. Leveridge, P. Marshall, A systematic investigation of the best buffers for use in screening by MALDI-mass spectrometry, *SLAS Discov* 22 (2017) 1262–1269.
- [29] H. Krenkel, J. Brown, K. Richardson, E. Hoyes, M. Morris, R. Cramer, Ultrahigh-throughput sample analysis using liquid atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry, *Anal. Chem.* 94 (2022) 4141–4145.
- [30] I. Sinclair, M. Bachman, D. Addison, M. Rohman, D.C. Murray, G. Davies, E. Mouchet, M.E. Tonge, R.G. Stearns, L. Ghislain, S.S. Datwani, L. Majlof, E. Hall, G.R. Jones, E. Hoyes, J. Olechno, R.N. Ellson, P.E. Barran, S.D. Pringle, M. R. Morris, J. Wingfield, Acoustic mist ionization platform for direct and contactless ultrahigh-throughput mass spectrometry analysis of liquid samples, *Anal. Chem.* 91 (2019) 3790–3794.
- [31] C.M.H. Ferreira, I.S.S. Pinto, E.V. Soares, H.M.V.M. Soares, (Un)suitability of the use of pH buffers in biological, biochemical and environmental studies and their interaction with metal ions – a review, *RSC Adv.* 5 (2015) 30989–31003.
- [32] N.E. Good, G.D. Winget, W. Winter, T.N. Connolly, S. Izawa, R.M.M. Singh, Hydrogen ion buffers for biological research, *Biochemistry* 5 (1966) 467–477.
- [33] N.E. Good, S. Izawa, [3] Hydrogen ion buffers, in: *Methods Enzymol.*, Academic Press, 1972, pp. 53–68.
- [34] W.J. Ferguson, K.I. Braunschweiger, W.R. Braunschweiger, J.R. Smith, J. J. McCormick, C.C. Wasmann, N.P. Jarvis, D.H. Bell, N.E. Good, Hydrogen ion buffers for biological research, *Anal. Biochem.* 104 (1980) 300–310.
- [35] E.A. Peroza, E. Freisinger, Tris is a non-innocent buffer during intein-mediated protein cleavage, *Protein Expr. Purif.* 57 (2008) 217–225.
- [36] W.A. Bubba, H.A. Berthon, P.W. Kuchel, Tris buffer reactivity with low-molecular-weight aldehydes: NMR characterization of the reactions of glyceraldehyde-3-phosphate, *Bioorg. Chem.* 23 (1995) 119–130.
- [37] K.K. Mock, C.W. Sutton, J.S. Cottrell, Sample immobilization protocols for matrix-assisted laser-desorption mass spectrometry, *Rapid Commun. Mass Spectrom.* 6 (1992) 233–238.

- [38] K.O. Börnsen, Influence of salts, buffers, detergents, solvents, and matrices on MALDI-MS protein analysis in complex mixtures, in: J.R. Chapman (Ed.), *Mass Spectrometry of Proteins and Peptides: Mass Spectrometry of Proteins and Peptides*, Humana Press, Totowa, NJ, 2000, pp. 387–404.
- [39] U. Kallweit, K.O. Börnsen, G.M. Kresbach, H.M. Widmer, Matrix compatible buffers for analysis of proteins with matrix-assisted laser desorption/ionization mass spectrometry, *Rapid Commun. Mass Spectrom.* 10 (1996) 845–849.
- [40] Y. Guo, M. Forbush, T.R. Covey, L. Ghislain, C. Liu, High-throughput analysis from complex matrices: acoustic ejection mass spectrometry from phase-separated fluid samples, *Metabolites* 11 (2021).
- [41] F. Gharahdaghi, M. Kirchner, J. Fernandez, S.M. Mische, Peptide-mass profiles of polyvinylidene difluoride-bound proteins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the presence of nonionic detergents, *Anal. Biochem.* 233 (1996) 94–99.
- [42] M.K. Mandal, L.C. Chen, Z. Yu, H. Nonami, R. Erra-Balsells, K. Hiraoka, Detection of protein from detergent solutions by probe electrospray ionization mass spectrometry (PESI-MS), *J. Mass Spectrom.* 46 (2011) 967–975.
- [43] I. Sinclair, R. Stearns, S. Pringle, J. Wingfield, S. Datwani, E. Hall, L. Ghislain, L. Majlof, M. Bachman, Novel acoustic loading of a mass spectrometer: toward next-generation high-throughput MS screening, *J. Lab. Autom.* 21 (2016) 19–26.
- [44] T. Hasegawa, R.M. Imamura, T. Suzuki, T. Hashiguchi, T. Nomura, S. Otsuguro, K. Maenaka, M. Sasaki, Y. Orba, H. Sawa, A. Sato, T. Okabe, T. Nagano, H. Kojima, Application of acoustic ejection MS system to high-throughput screening for SARS-CoV-2 3CL protease inhibitors, *Chem. Pharm. Bull. (Tokyo)* 70 (2022) 199–201.
- [45] J.M. Asara, J. Allison, Enhanced detection of phosphopeptides in matrix-assisted laser desorption/ionization mass spectrometry using ammonium salts, *J. Am. Soc. Mass Spectrom.* 10 (1999) 35–44.
- [46] R. Cramer, S. Corless, Liquid ultraviolet matrix-assisted laser desorption/ionization – mass spectrometry for automated proteomic analysis, *Proteomics* 5 (2005) 360–370.
- [47] H. Hernandez, C.V. Robinson, Determining the stoichiometry and interactions of macromolecular assemblies from mass spectrometry, *Nat. Protoc.* 2 (2007) 715–726.
- [48] U.H. Verkerk, P. Kebarle, Ion-Ion and ion-molecule reactions at the surface of proteins produced by nanospray. Information on the number of acidic residues and control of the number of ionized acidic and basic residues, *J. Am. Soc. Mass Spectrom.* 16 (2005) 1325–1341.
- [49] L. Konermann, Addressing a common misconception: ammonium acetate as neutral pH “buffer” for native electrospray mass spectrometry, *J. Am. Soc. Mass Spectrom.* 28 (2017) 1827–1835.
- [50] I.P. Smirnov, X. Zhu, T. Taylor, Y. Huang, P. Ross, I.A. Papayanopoulos, S. A. Martin, D.J. Pappin, Suppression of alpha-cyano-4-hydroxycinnamic acid matrix clusters and reduction of chemical noise in MALDI-TOF mass spectrometry, *Anal. Chem.* 76 (2004) 2958–2965.
- [51] D. Winter, J. Seidler, Y. Ziv, Y. Shiloh, W.D. Lehmann, Citrate boosts the performance of phosphopeptide analysis by UPLC-ESI-MS/MS, *J. Proteome Res.* 8 (2009) 418–424.
- [52] G. Wang, R.B. Cole, Effect of solution ionic strength on analyte charge state distributions in positive and negative ion electrospray mass spectrometry, *Anal. Chem.* 66 (1994) 3702–3708.
- [53] U. Pielek, W. Zurcher, M. Schar, H.E. Moser, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a powerful tool for the mass and sequence analysis of natural and modified oligonucleotides, *Nucleic Acids Res.* 21 (1993) 3191–3196.
- [54] R. Javanshad, A.R. Venter, Effects of amino acid additives on protein solubility – insights from desorption and direct electrospray ionization mass spectrometry, *Analyst* 146 (2021) 6592–6604.
- [55] R. Javanshad, E. Honarvar, A.R. Venter, Addition of serine enhances protein analysis by DESI-MS, *J. Am. Soc. Mass Spectrom.* 30 (2019) 694–703.
- [56] D.J. Clarke, D.J. Campopiano, Desalting large protein complexes during native electrospray mass spectrometry by addition of amino acids to the working solution, *Analyst* 140 (2015) 2679–2686.
- [57] N. Thorne, D.S. Auld, J. Inglese, Apparent activity in high-throughput screening: origins of compound-dependent assay interference, *Curr. Opin. Chem. Biol.* 14 (2010) 315–324.
- [58] G.A. Breaux, K.B. Green-Church, A. France, P.A. Limbach, Surfactant-aided, matrix-assisted laser desorption/ionization mass spectrometry of hydrophobic and hydrophilic peptides, *Anal. Chem.* 72 (2000) 1169–1174.
- [59] K.O. Börnsen, M.A. Gass, G.J. Bruin, J.H. von Adrichem, M.C. Biro, G.M. Kresbach, M. Ehrat, Influence of solvents and detergents on matrix-assisted laser desorption/ionization mass spectrometry measurements of proteins and oligonucleotides, *Rapid Commun. Mass Spectrom.* 11 (1997) 603–609.
- [60] J.E. Coligan, Commonly used detergents, *Curr. Protoc. Protein Sci.*, Appendix 1 (2001). Appendix 1B.
- [61] E. Fuguet, C. Rafols, M. Roses, E. Bosch, Critical micelle concentration of surfactants in aqueous buffered and unbuffered systems, *Anal. Chim. Acta* 548 (2005) 95–100.
- [62] I.F. Shieh, C.Y. Lee, J. Shiea, Eliminating the interferences from TRIS buffer and SDS in protein analysis by fused-droplet electrospray ionization mass spectrometry, *J. Proteome Res.* 4 (2005) 606–612.
- [63] O.R.R. Loo, N. Dales, P.C. Andrews, The effect of detergents on proteins analyzed by electrospray ionization, in: J.R. Chapman (Ed.), *Protein and Peptide Analysis by Mass Spectrometry*, Humana Press, Totowa, NJ, 1996, pp. 141–160.
- [64] N.B. Cech, C.G. Enke, Practical implications of some recent studies in electrospray ionization fundamentals, *Mass Spectrom. Rev.* 20 (2001) 362–387.
- [65] R.R. Loo, J.A. Loo, Matrix-assisted laser desorption/ionization mass spectrometry of hydrophobic proteins in mixtures using formic acid, perfluorooctanoic acid, and sorbitol, *Anal. Chem.* 79 (2007) 1115–1125.
- [66] F.M.L. Amado, M.G. Santana-Marques, A.J. Ferrer-Correia, K.B. Tomer, Analysis of peptide and protein samples containing surfactants by MALDI-MS, *Anal. Chem.* 69 (1997) 1102–1106.
- [67] N. Zhang, L. Li, Effects of common surfactants on protein digestion and matrix-assisted laser desorption/ionization mass spectrometric analysis of the digested peptides using two-layer sample preparation, *Rapid Commun. Mass Spectrom.* 18 (2004) 889–896.
- [68] J. Funk, X. Li, T. Franz, Threshold values for detergents in protein and peptide samples for mass spectrometry, *Rapid Commun. Mass Spectrom.* 19 (2005) 2986–2988.
- [69] M.R. Arkin, M.A. Glicksman, H. Fu, J.J. Havel, Y. Du, Inhibition of protein-protein interactions: non-cellular assay formats, in: S. Markossian, A. Grossman, K. Brimacombe, M. Arkin, D. Auld, C. Austin, J. Baell, T.D.Y. Chung, N.P. Coussens, J.L. Dahlin, V. Devanarayan, T.L. Foley, M. Glicksman, J.V. Haas, M.D. Hall, S. Hoare, J. Inglese, P.W. Iversen, S.C. Kales, M. Lal-Nag, Z. Li, J. McGee, O. McManus, T. Riss, P. Saradjian, G.S. Sittampalam, M. Tarselli, O.J. Trask Jr, Y. Wang, J.R. Weidner, M.J. Wildey, K. Wilson, M. Xia, X. Xu (Eds.), *Assay Guidance Manual*, Eli Lilly & Company and the National Center for Advancing Translational Sciences, 2004. Bethesda (MD).
- [70] A.J. Radosevich, F. Pu, D. Chang-Yen, J.W. Sawicki, N.N. Talaty, N.L. Elsen, J. D. Williams, J.Y. Pan, Ultra-high-throughput ambient MS: direct analysis at 22 samples per second by infrared matrix-assisted laser desorption electrospray ionization mass spectrometry, *Anal. Chem.* 94 (2022) 4913–4918.
- [71] J.J.A. van Kampen, P.C. Burgers, R. de Groot, A.D.M.E. Osterhaus, M.L. Reedijk, E. J. Verschuren, R.A. Gruters, T.M. Luiders, Quantitative analysis of HIV-1 protease inhibitors in cell lysates using MALDI-FTICR mass spectrometry, *Anal. Chem.* 80 (2008) 3751–3756.
- [72] J.H. Lee, H.S. Choi, K.A. Nasr, M. Ha, Y. Kim, J.V. Frangioni, High-throughput small molecule identification using MALDI-TOF and a nanolayered substrate, *Anal. Chem.* 83 (2011) 5283–5289.
- [73] M.S. Unger, M. Blank, T. Enzlein, C. Hopf, Label-free cell assays to determine compound uptake or drug action using MALDI-TOF mass spectrometry, *Nat. Protoc.* 16 (2021) 5533–5558.
- [74] S. Schulz, D. Gerhardt, B. Meyer, M. Seegel, B. Schubach, C. Hopf, K. Matheis, DMSO-enhanced MALDI MS imaging with normalization against a deuterated standard for relative quantification of dasatinib in serial mouse pharmacology studies, *Anal. Bioanal. Chem.* 405 (2013) 9467–9476.
- [75] J.H. Eriksson, R. Mol, G.W. Somsen, W.L. Hinrichs, H.W. Frijlink, G.J. de Jong, Feasibility of nonvolatile buffers in capillary electrophoresis-electrospray ionization-mass spectrometry of proteins, *Electrophoresis* 25 (2004) 43–49.
- [76] M.C. Garcia, A.C. Hogenboom, H. Zappey, H. Irth, Effect of the mobile phase composition on the separation and detection of intact proteins by reversed-phase liquid chromatography–electrospray mass spectrometry, *J. Chromatogr. A* 957 (2002) 187–199.
- [77] High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories, in: S.Q. van Veen, E.C.J. Claas, J. Kuijper (Eds.), *J. Clin. Microbiol.* 48 (2010) 900–907.
- [78] F.E. Kuhlmann, A. Appfel, S.M. Fischer, G. Goldberg, P.C. Goodley, Signal enhancement for gradient reverse-phase high-performance liquid chromatography–electrospray ionization mass spectrometry analysis with trifluoroacetic and other strong acid modifiers by postcolumn addition of propionic acid and isopropanol, *J. Am. Soc. Mass Spectrom.* 6 (1995) 1221–1225.
- [79] M.K. Mandal, L.C. Chen, Y. Hashimoto, Z. Yu, K. Hiraoka, Detection of biomolecules from solutions with high concentration of salts using probe electrospray and nano-electrospray ionization mass spectrometry, *Anal. Methods* 2 (2010) 1905–1912.
- [80] P. Wingfield, Protein precipitation using ammonium sulfate, *Curr. Protoc. Protein Sci.*, Appendix 3 (2001). Appendix 3F.