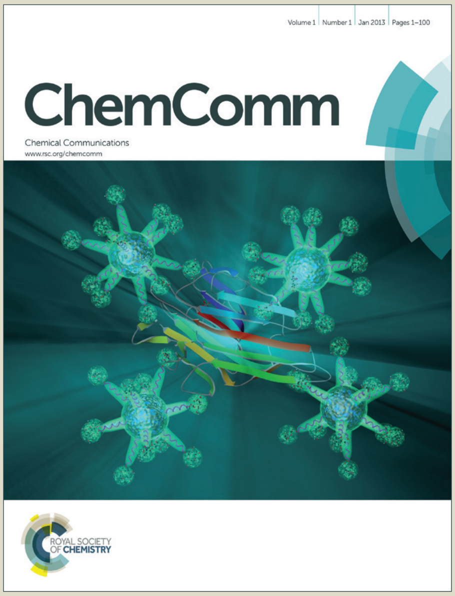
ChemComm

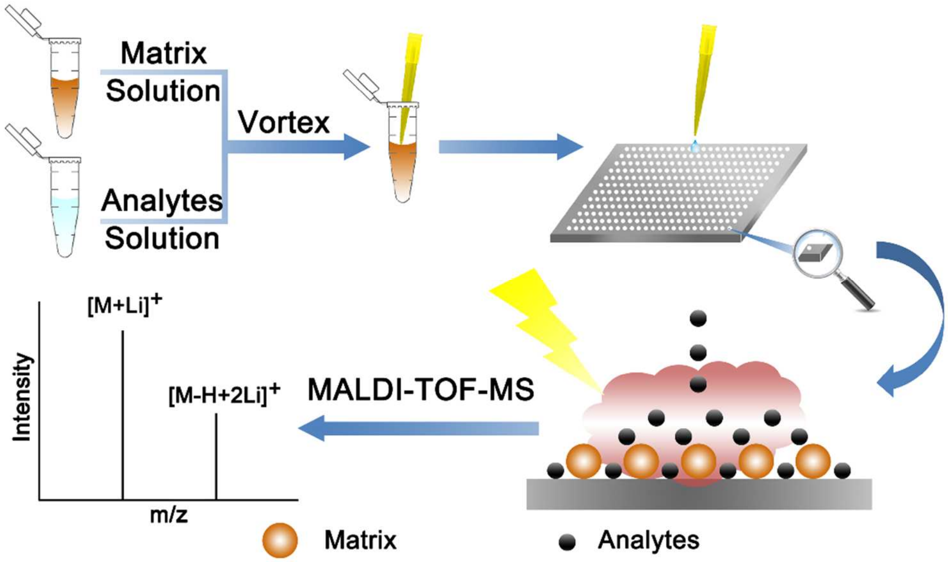
Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Graphical Abstract



Lithium-rich composite metal oxide material as SALDI matrix for high throughput analysis of small molecules

Journal Name RSCPublishing

COMMUNICATION

Lithium-rich composite metal oxide used as SALDI-MS matrix for the determination of small biomolecules

Cite this: DOI: 10.1039/x0xx00000x

Ze Li, Yi-Wei Zhang, Yue-Long Xin, Yu Bai, Heng-Hui Zhou, Hu-Wei Liu*

Received 14th September 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

Lithium-rich composite metal oxide was evaluated as novel matrix for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-MS). The low background interference and lithium-rich feature made it a high efficient matrix for the analysis of small molecules with high reproducibility, sensitivity and strong salt tolerance.

Matrix-assisted laser desorption/ionization (MALDI) has been widely used in the analysis of large biomolecules such as peptides, proteins, nucleic acids, and synthetic polymers due to the incorporation of highly efficient organic matrices 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid (CHCA) since it was introduced in the late 1980s.[1] These matrix facilitated soft and efficient desorption/ionization of the analytes by directly absorbing lased UV light and transferring that energy to the analysed molecules. Nevertheless, the range of applications, notably in the low molecule weight (LMW) region (< 700 Da), was limited as the LMW organic matrices generally populated the low mass spectral region with matrix-derived interferences. Moreover, inhomogeneous crystallization among organic matrices and analytes often contributed to poor ionization reproducibility.

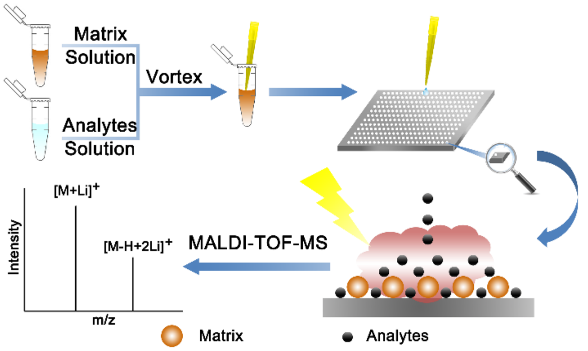
In light of the inherent challenges associated with organic matrices, new efforts have been made in the area of inorganic matrices. Tanaka et al. first described the use of cobalt particles in glycerol as a possible matrix for laser desorption.[2] Sunner later reported 2 μm activated carbon particles in glycol as matrix, and coined the technique, Surface-assisted laser desorption/ionization - mass spectrometry (SALDI-MS).[3] Unlike organic matrices, these new inorganic matrices exhibited low background interference and facilitated homogeneous sample deposition, which contributed to improved shot-to-shot reproducibility. Furthermore, recent advances in the areas of nano-materials have enabled SALDI-based approaches using a variety of inorganic materials, including carbon,[4-7] silicon,[8,9] metal,[10-12] metal oxide,[13-19] even metal-organic frameworks (MOFs).[20] A wide variety of functionalized nano-materials, especially gold-based materials have been also reported as useful SALDI matrices.[21] Actually, more and more attention has been paid to the development of various SALDI matrices.[22, 23]

Metal nanoparticles, such as gold,[10, 24, 25] silver, [11] platinum[12], palladium[26] have been evaluated as potential SALDI matrices. In addition, sensitivity and mass range extension have been further improved by surface etching.[12] However, surface etching has also introduced new issues facing matrix stability and consequently reproducibility. Compared with metal particles, metal oxide powders provide better stability as SALDI matrices. Commonly used metal oxide including Fe_xO_y,[17] Co_xO_y, CuO, NiO,[16] ZnO,[15] TiO_2,[18] MgO,[19] SnO_2, WO_3,[13] have all been explored as possible SALDI matrices. Wu have demonstrated that laser desorption/ionization mass spectra of oligosaccharides with MgO can be obtained with a sensitivity in the nanomole range.[19] Arakawa have reported the analysis of β-cyclodextrin with detection of limit (LOD) at picomole level with ZnO.[15] However, to the best of our knowledge, efforts to evaluate composite metal oxides as novel SALDI matrices with improved sensitivities have not been reported yet.

Lithium-rich composite metal oxides have been widely used in the manufacture of lithium-ion batteries.[27] Indeed, various lithium-rich materials with different compositions and morphologies have been synthesized, but the applications of these materials in MS technology have not been explored. More pertinently, lithium salts (LiCl, $LiNO_3$) have been already widely used in tissue fixation for MALDI imaging.[28, 29] Moreover, lithium salt-based matrices yield valuable lipid structural information based on the facile formation of lithium-lipid adducts, although its use toward identifying other biomolecules has remained limited.

In this work, we proposed lithium-rich composite metal oxide as SALDI matrix for the first time, and $Li_{1.2}Mn_{0.54}Ni_{0.18}Co_{0.13}O_2$ was chosen for the analysis of small biomolecules by SALDI-MS. Furthermore, a robust array of small biomolecules including drugs, oligosaccharides, lipids, peptides were chosen to evaluate the matrix performance. The method is very fast and convenient, which is conducted by mixing the material with the analyte solutions, vortex, then piped on to MALDI target for detection. The basic workflow is revealed in Scheme 1.

The lithium-rich material was synthesized by a facile sol-gel method using ethylenediaminetetraacetic acid (EDTA) and citric acid as chelating agents, which have been reported previously.[30] Details of the material synthesis and characterization are described in the ESI†. A transmission



Scheme 1 SALDI-MS using lithium-riched composite metal oxide matrix.

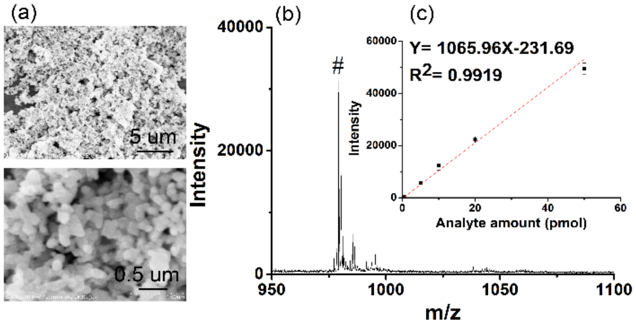


Fig. 1 (a) SEM image of lithium-riched composite metal oxide matrix, (b) a typical SALDI mass spectra of α-cyclodextrin at 5 pmol, (c) the calibration curve of α-cyclodextrin.

electron microscope (TEM) image of the material (Fig. 1(a)) shows that the materials are homogeneously distributed as spherical particles with average size of 200-300 nm. The performance of this lithium-rich composite metal oxide as a potential SALDI matrix was first evaluated with a standard analyte, α-cyclodextrin, and further compared with three other common metal oxides (MnO_2, NiO, Co_3O_4). Fig. S2 (ESI†) shows that the analytes formed alkali metal adducts instead of protonated ions with all the metal oxide as matrices. That is because carbohydrates have a high affinity to interact with alkali metals following the order Cs>K>Na>Li>H, which has been noted elsewhere.[24] Different from three common metal oxides, when the lithium-rich material was used as matrix, lithium-analyte adducts were observed besides common sodium, potassium-analyte adducts. The phenomenon showed great prospect using lithium-rich composite metal oxide as matrix for the reason that the lithium ion intensity in this matrix is much wealthier than the natural sodium and potassium ions in other materials.

Matrix concentration was optimized (Fig. S3, ESI†) for maximizing the desorption and ionization efficiency for SALDI-MS, and 1 mg/mL were finally chosen as working concentration. In order to further improve the ionization efficiency, TFA was added in the mixed solution of analyte and matrix, because it facilitates the release of lithium ions from the lithium-rich matrix, improving the formation and predominance of lithium adducts over sodium and potassium adducts. Thus, the matrix could act both as ionization assistant agent and energy mediator at the same time. Under optimized conditions, matrix performance parameters were quantitatively evaluated with α-cyclodextrin as a standard benchmarking analyte (Fig. 1(b)). In this case, observed peaks were predominantly [M+Li]$^+$ and [M-H+2Li]$^+$. The method showed good reproducibility, linearity (Fig. 1(c)), limit of detection and salt tolerance. Intra- and inter-spot reproducibility determined with 5 picomole α-cyclodextrin (n=6) were 5.5% and 6.5% RSD, respectively. The LOD was found to be 5 femtomole, a substantial improvement over ZnO matrices which are reported to have detection limits near 1 picomole.[15] The analytes (5 pmol) can be detected (S/N >3) even in 0.5 M NaCl solution, which is much better than organic matrix. Furthermore, the material demonstrated excellent stability and equivalent performance after three month's storage in deionized mater. As a result, the material would be a promising new SALDI matrix.

SALDI-MS has become a popular tool for the analysis of LMW molecules due to its reduced background noise in the low-mass region.[26, 31] To fully demonstrate the feasibility of the novel lithium-based matrices under robust applications, a variety of standard analytes such as drugs, oligosaccharides, lipids, and peptides were further analyzed. Fig. 2(a) shows that the lithium-rich composite metal oxide resulted in less matrix interferences in the low mass regions (< 500 Da). Two major peaks, m/z 330.62 and m/z 458.80 were observed, responding to [gliclazide+Li]$^+$ and [glipizide+Li]$^+$ respectively. Similarly, three cyclodextrins (α, β, γ) were all detected in their lithium adducts without fragmented ions and protonated molecular ions in the mass spectra (Fig. 2(b)). We also explored the feasibility of this matrix for the analysis of other oligosaccharides including maltopentaose, maltohexaose, maltoheptaose (Fig. 2(c)). Three major peaks represented three [M+Li]$^+$ ions and minor peaks were [M-H+2Li]$^+$. Moreover, mass-dependent desorption efficiency can be observed as the signal intensity reduced with mass is increased. Compared with the references on the analysis of oligosaccharides with SALDI reported by several groups.[19, 24, 32] LOD in the femtomole range were observed for the three maltoses in present study, which is much lower than that with MgO matrices.[19] These results demonstrated the material to be a soft and high efficient matrix for the detection of oligosaccharides, and this method can be utilized to detect glycans without labelling, making the analysis much easier and faster than the existing ESI-MS method.[33]

Besides oligosaccharides, three standard lipids (Fig. 2(d)) and four peptides (Fig. S4, ESI†) were also detected. It has been reported that lipids can be detected with SALDI recently.[15] Fig. 2 (d) shows that SM (d18:1/17:0) and laccer (8:0) are observed as their lithium adducts as major peaks. Moreover, TG (18:1/14:0/17:1) standard was also observed as sodium adducts. Results for the detection of peptides showed some differences (Fig. S4, ESI†), and some peptides were observed as fragment ions. In detail, both lys-bradykinin and bradykin were detected as fragments [M-Me+H]$^+$with a loss of 15 Da, which is due to the loss of $-CH_3$, whereas the latter was also observed as a minor peak [M-H+2Li]$^+$. Meanwhile, substance P was observed as common [M+Li]$^+$ ions and neurotensin was observed as [M-H+2Li]$^+$. However, when the matrix was used for the analysis of larger peptides with molecular weight more than 2000 Da, no signals were obtained, which may due to low desorption efficiency. Therefore, the matrix was useful for the analysis of small biomolecules. The material was further used for the analysis of complex real samples (Fig. S5, ESI†). Olive oils were directly dissolved in acetic ether, and then diluted with methanol without any other preparation. Satisfactory signals can be observed at the concentration as low as 50 μg/mL. Detected ions were mainly in the 800-1000 Da mass range, which mostly belong to TAGs.[34] It could be concluded that this method could find more applications for complex real sample analysis.

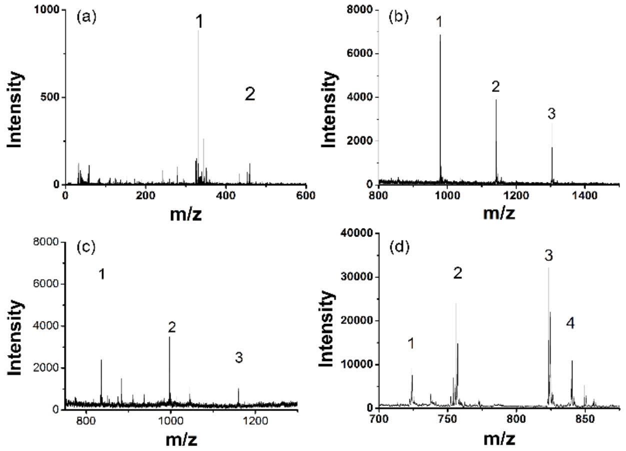


Fig. 2 SALDI mass spectra of (a) two drugs (10 pmol): 1. gliclazide $[M+Li]^+$ (m/z 330.62), 2. glipizide $[M+Li]^+$ (m/z 458.80); (b) three oligosaccharides (5 pmol): 1. α-cyclodextrin $[M+Li]^+$ (m/z 980.46), 2.β-cyclodextrin $[M+Li]^+$ (m/z 1142.70), 3. γ-cyclodextrin $[M+Li]^+$ (m/z 1304.95); (c) three maltose (10 pmol): 1. maltopentaose $[M+Li]^+$ (m/z 834.85), 2. ,maltohexaose $[M+Li]^+$ (m/z 996.99), 3. maltoheptaose$[M+Li]^+$ (m/z 1159.09); (d) three lipids (5 pmol): 1. SM (d18:1/17:0) $[M+Li]^+$ (m/z 723.69), 2. laccer (8: 0), $[M+Li]^+$ (m/z 756.67), 3. TG (18:1/14:0/17:1), $[M+Li]^+$ (m/z 823.97), and 4. TG (18:1/14:0/17:1), $[M+Na]^+$ (m/z 839.95).

In summary, we proposed a new SALDI matrix, lithium-riched composite metal oxide, and investigated its abilities for the detection of small biomolecules (< 2000 Da). The use of TFA as addictive facilitated the releasing of lithium ions from matrix. Due to the formation of lithium-analyte adduct, ionization efficiency were greatly improved. Therefore, the matrix could act both as ionization assistant agent and energy mediator at the same time. Satisfactory signals were observed for the analysis of various biomolecules including drugs, oligosaccharides, lipids and peptides. Low noise interference, high salt tolerance, good reproducibility made the lithium-riched composite a very promising SALDI matrix. Further work on the influence of material composition and morphology is still under investigation. .

This study was supported by the National Natural Science Foundation of China (No. 21175005 and 21175008).

Notes and references

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. E-mail: hwliu@pku.edu.cn; Fax: +86-10-62751708; Tel: +86-10-62754976

† Electronic Supplementary Information (ESI) available: Experimental details, figures and tables. See DOI: 10.1039/c000000x/

1. K. Dreisewerd, *Anal. Bioanal. Chem.*, 2014, **406**, 2261-2278.
2. K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida and T.Yoshida, *Rapid Commun. Mass Spectrom.*, 1988, **2**, 151-153.
3. Y. C. Chen, J. Shiea and J. Sunner, *J. Chromatogr. A*, 1998, **826**, 77-86.
4. X. Z. Zhou, Y. Y. Wei, Q. Y. He, F. Boey, Q. C. Zhang and H. Zhang, *Chem. Commun.*, 2010, **46**, 6974-6976.
5. J. Zhang, X. L. Dong, J. S. Cheng, J. H. Li and Y.S. Wang, *J. Am. Soc. Mass Spectrom.*, 2011, **22**, 1294-1298.
6. T. Lu and S. V. Olesik, *Anal. Chem.*, 2013, **85**, 4384-4391.
7. S. M. Chen, H. Z. Zheng, J. N. Wang, J. Hou, Q. He, H. H.Liu, C. Q. Xiong, X. L. Kong and Z. X. Nie, *Anal. Chem.*, 2013, **85**, 6646-6652.
8. N. H. Finkel, B. G. Prevo, O. D. Velev and L. He, *Anal. Chem.*, 2005, **77**, 1088-1095.
9. M. Dupré, Y. Coffinier, R. Boukherroub, S. Cantel, J. Martinez and C. Enjalbal, *J. Proteomics*, 2012, **75**, 1973-1990.
10. Y. F. Huang and H. T. Chang, *Anal. Chem.*, 2006, **78**, 1485-1493.
11. T. C.Chiu, L. C. Chang, C. K. Chiang and H. T. Chang, *J. Am. Soc. Mass Spectrom.*, 2008, **19**, 1343-1346.
12. H. Kawasaki, T. Yonezawa, T. Watanabe and R. Arakawa, *The J. Phys. Chem. C*, 2007, **111**, 16278-16283.
13. T. Kinumi, T. Saisu, M. Takayama and H. Niwa, *J. Mass Spectrom.*, 2000, **35**, 417-422.
14. K. H. Lee, C. K. Chiang, Z. H. Lin and H. T. Chang, *Rapid Commun. Mass Spectrom.*, 2007, **21**, 2023-2030.
15. T. Watanabe, H. Kawasaki, T. Yonezawa and R. Arakawa, *J. Mass Spectrom.*, 2008, **43**, 1063-1071.
16. C. R. McAlpin, K. J. Voorhees, A. R. Corpuz and R. M. Richards, *Anal. Chem.*, 2012, **84**, 7677-7683.
17. S. Taira, I. Osaka, S. Shimma, D. Kaneko, T. Hiroki, Y. Kawamura-Konishi and Y. Ichiyanagi, *Analyst*, 2012, **137**, 2006-2010.
18. G. Piret, D. Kim, H. Drobecq, Y. Coffinier, O. Melnyk, P. Schmuki and R. Boukherroub, *Analyst*, 2012, **137**, 3058-3063.
19. S. K. Kailasa and H. F. Wu, *Microchim. Acta.*, 2013, **180**, 405-413.
20. Y. H. Shih, C. H. Chien, B. Singco, C. L. Hsu, C. H. Lin and H. Y. Huang, *Chem. Commun.*, 2013, **49**, 4929-4931.
21. R. Liu, J. F. Liu, X. X. Zhou and G. B. Jiang, *Anal. Chem.*, 2011, **83**, 3668-3674.
22. K. P. Law and J. R. Larkin, *Anal. Bioanal. Chem.*, 2011, **399**, 2597-2622.
23. C. K. Chiang, W. T. Chen and H. T. Chang, *Chem. Soc. Rev.*, 2011, **40**, 1269-1281.
24. C.L. Su and W.L. Tseng, *Anal. Chem.*, 2007, **79**, 1626-1633.
25. Y. F. Huang and H. T. Chang, *Anal. Chem.*, 2007, **79**, 4852-4859.
26. M. C. Cheng, K. M. Chi and S. Y. Chang, *Talanta*, 2013, **115**, 123-128.
27. S. Dou, *J. Solid State Electrochem.*, 2013, **17**, 911-926.
28. C. D. Cerruti, D. Touboul, V. Guérineau, V. W. Petit, O. Laprévote and A. Brunelle, *Anal. Bioanal. Chem.*, 2011, **401**, 75-87.
29. R. L. Griffiths, J. Sarsby, E. J. Guggenheim, A. M. Race, R. T. Steven, J. Fear, P. F. Lalor and J. Bunch, *Anal. Chem.*, 2013, **85**, 7146-7153.
30. Z. Q. Deng and A. Manthiram, *J. Phys. Chem. C*, 2011, **115**, 7097-7103.
31. K. Shrivas and H. F. Wu, *Rapid Commun. Mass Spectrom.*, 2008, **22**, 2863-2872.
32. T. Fujita and K. Shibamoto, *Chem. Lett.*, 2013, **42**, 852-853.
33. S. H. Walker, L. M. Lilley, M. F. Enamorado, D. L. Comins and D. C. Muddiman, *J. Am. Soc. Mass Spectrom.*, 2011, **22**, 1309-1317.
34. Q. Shen, W. Dong, M. Yang, J. T. Baibado, Y. Wang, I. Alqouqa and H. Y. Cheung, *Food Res. Int.*, 2013, **54**, 2054-2061.