Talanta 105 (2013) 417–421

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Fast nucleotide identification through fingerprinting using gold nanoparticle-based surface-assisted laser desorption/ionisation

Miguel Larguinho [a,b], José L. Capelo [b,c], Pedro V. Baptista [a,*]

[a] CIGMH, Departamento Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal
[b] BioScope Group, Faculty of Sciences of Ourense, University of Vigo, 32004 Ourense, Spain
[c] REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

ARTICLE INFO

Article history:
Received 20 April 2012
Received in revised form
17 October 2012
Accepted 22 October 2012
Available online 12 November 2012

Keywords:
Gold nanoparticles
SALDI-TOF
Nucleotides
Glycidamide
DNA adducts

ABSTRACT

We report a method centred on gold nanoparticle-based surface-assisted laser desorption/ionisation for analysis of deoxynucleotides and alkylated nucleobases. Gold nanoparticles allow for enhanced analysis capability by eliminating undesired signature peaks; thus more elegant mass spectra can be attained that allow identification by nucleotide mass fingerprint. The resulting fingerprinting patterns on the spectra are compared and associated with the presence of different nucleotides in the sample. This method can be easily extended to modified nucleotides implicated in genome lesions due to exposure to environment chemicals, such as DNA adducts (e.g. guanine adducts). The use of gold nanoparticles for surface-assisted laser desorption/ionisation can be an useful tool to resolve common issues of background noise when analysing nucleic acids samples.

1. Introduction

Matrix-assisted laser desorption/ionisation (MALDI) is a soft ionisation technique used in mass spectrometry for detection of the characteristic *m/z* values of different molecules on a single spectrum. A pulsed laser is used to ionise the analyte via irradiation of a matrix/analyte mixture, where the addition of an organic matrix permits a more controlled energy transfer from the laser to the sample [1–3]. The main disadvantage of using an organic matrix is the elevated number of *m/z* interferences added to the spectrum, mainly in the low mass region (<500 Da), which may often mask the analyte's signature [4]. In order to attain spectra with a minimum number of interfering *m/z* signals, organic matrix-free methods have been proposed, including the use of noble metal nanoparticles [4–8]. This approach has been broadly named as surface-assisted laser desorption/ionisation (SALDI). Several nanomaterials with different compositions, with emphasis on gold nanoparticles (AuNPs), have been proposed as substituents of organic matrices for SALDI based analysis of peptides and proteins [5,8–11]. Recently, surface-assisted laser desorption/ionisation time-of-flight (SALDI-TOF) analysis of nucleic acid molecules and nucleotides has also been reported [12], overcoming issues related to the difficulty of detection of DNA molecules using time-of-flight (TOF) analysers due to the high molecular weight.

Nucleotides act mainly as building blocks for the synthesis of DNA and RNA within organisms. Increased levels of free nucleotides/modified nucleotides in body fluids (e.g. blood, urine) may be used as biomarkers of different pathologies, health conditions or exposure to chemical agents [13,14]. Despite the advantages of laser desorption/ionisation based techniques for nucleotide biomarker analys is, these molecules often exhibit low molecular weight (<600 Da) and organic matrices commonly generate spectra with high background noise. We present a method for identification of nucleotides using AuNP-based SALDI-TOF fingerprinting. By comparing unique signature peaks in different mass spectra, we were able to associate a given fingerprint to a specific nucleobase present in a mixture. We further demonstrate the use of this approach for the detection of DNA adducts, which can be used as biomarkers of exposure to chemicals.

2. Material and methods

2.1. Materials

Tetrachloroauric acid, trisodium citrate and glycidamide were purchased from Sigma-Aldrich (USA). Deoxynucleotide triphosphate (dNTPs) were purchased from Fermentas (Canada). All solutions and dilutions were made using ultrapure water from a millipore MilliQ system.

* Corresponding author. Tel./fax: +351 212 948 530.
E-mail address: pmvb@fct.unl.pt (P.V. Baptista).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.10.072

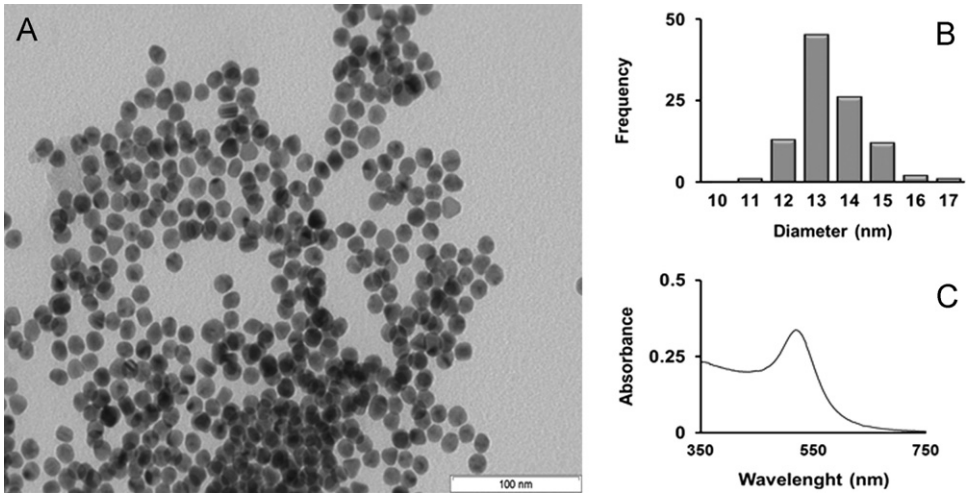

Fig. 1. Characterisation of synthesised gold nanoparticles. (A) TEM image of the synthesised AuNPs; (B) size distribution obtained using the TEM analysis; and (C) UV/vis spectrum of the AuNP solution.

2.2. Gold nanoparticles synthesis and characterisation

Gold nanoparticles were synthesised following the citrate reduction method described by Turkevich [15] and extensively used in our research group [16–18]. Briefly, 250 mL of an aqueous solution of 1 mM $HAuCl_4$ is brought up to a boil, with constant and vigorous agitation. Upon reflux, 25 mL of an aqueous solution of 38.8 mM trisodium citrate is added and, after a change in colour is observed, reflux is continued further for 10 min. Afterwards, the solution is cooled down to room temperature, with continuous agitation, for 2 h. Characterisation was carried out by transmission electron microscopy (TEM) imaging and UV–vis spectrophotometry. TEM imaging of the colloidal solution shows spherical NPs (Fig. 1A) with a mean diameter of 13 nm, as shown in the size distribution (Fig. 1B). The synthesised batch presents a plasmon band centred at around 520 nm (Fig. 1C) and the determined NPs concentration was 14 nM.

2.3. In vitro alkylation reactions

Deoxyguanosine triphosphate (dGTP) was incubated with glycidamide (GA) to produce glycidamide–guanine (GA–Gua) adducts. *In vitro* alkylation reactions were performed in a final volume of 20 μL at 37 °C for 48 h using a final concentration of 1 mM dGTP and 5 mM GA. Additionally, control mixtures containing only GA, dGTP, and dGTP+GA were also prepared. Following incubation, the reaction mixtures were heated up to 100 °C for 15 min to release the alkylated guanines [19].

2.4. Sample application and mass spectrometry measurements

Before spotting on the MALDI plate, AuNPs were centrifuged and concentrated up to 4.5x the initial concentration. Sterile water was used to dilute samples to the desired concentrations on the MALDI plate.

The dried-droplet method was used for sample application; equal volumes of analyte and AuNPs were thoroughly mixed and then spotted onto the MALDI plate. For the nucleotide fingerprint identification, the final concentrations on the plate were 500 μM of analyte and 15 nM of AuNPs. For sensitivity assessment, the concentration of AuNPs was decreased and maintained at 0.1 nM, while the concentration of analyte was varied between 0 and 250 μM. Measurements were made on an Ultraflex TOF/TOF System (Bruker Daltonics, Germany) equipped with a reflectron.

3. Results and discussion

3.1. Nucleotide identification using AuNPs for SALDI-TOF

Nucleotide analysis with MALDI-TOF is usually carried out in negative-ion mode due to the analyte affinity towards the formation of negative molecular ions in the gas-phase [12]. Some commercially available matrices (3-hydroxypicolinic acid, sinapinic acid, super-dihydroxybenzoic acid, 2-(4'-hydroxybenzeneazo) benzoic acid, α-cyano-4-hydroxycinnamic acid (CHCA) and 2',4', 6'-trihydroxyacetophenone) were tested for nucleotide ionisation. Results show that only CHCA is allowed for the assignment of *m/z* signals to a corresponding molecular structure, i.e. identification of molecular ion peaks, when acquiring in negative-ion mode, whereas the remaining organic matrices failed to yield conclusive identification signals (see Supplementary data).

Using AuNPs to assist in ionisation do not allow to unequivocally attribute an existing *m/z* signal to a given nucleotide when acquiring in negative-ion mode. The resulting spectra for any of the different dNTPs are very similar, presenting a high intensity peak at 74.21 Da, which cannot be associated to the mass/charge (*m/z*) ratio of any known structures that might have been present in the sample. Conversely, when acquiring in positive-ion mode, specific patterns are observed for each of the analysed nucleotides. Fig. 2 shows the mass spectra for different deoxynucleotides, when using AuNPs to assist in the ionisation process. Fig. 2A presents the mass spectrum for the AuNP solution, where the characteristic peaks at 196.96 Da, 236.44 Da, 393.93 Da and 590.89 Da attributed to gold clusters may be observed [5]. When AuNPs are mixed with the deoxynucleotide sample, the intensity of these characteristic peaks often plummets, resulting in clearer spectra, allowing identification of specific nucleobase signals with low background noise. This way, identification of deoxynucleotides in positive-ion mode is accomplished in a rather straightforward and rapid manner, as signature peaks that may mask the nucleotide fingerprint are removed from the spectrum. If we further analyse the spectra in Fig. 2B–E, we notice common m/z signals in all spectra (337.54 Da, 365.55 Da, 377.55 Da, 421.40 Da, 603.36 Da, 631.30 Da—highlighted with a black asterisk) with variable relative intensities, which may be related to structural similarities between all analytes, i.e. the pentose (deoxyribose) and the phosphate groups. In addition to these signals, some specific signature peaks for each nucleobase can also be easily attributed as the related peak differs according to the analysed

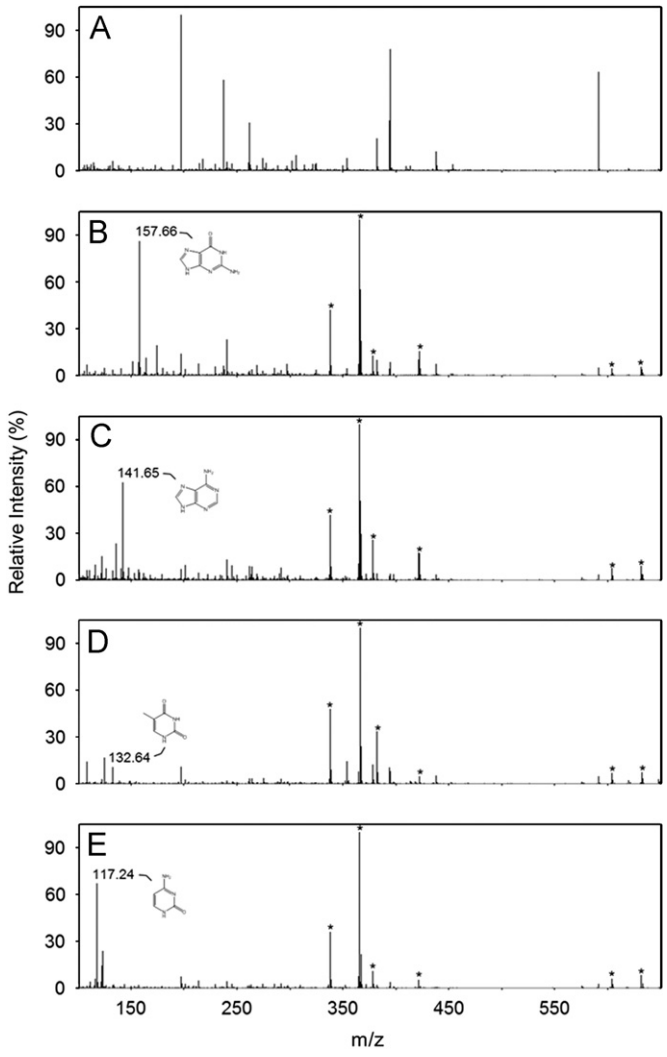

Fig. 2. Gold nanoparticle based ionisation of dNTPs. The SALDI-TOF spectra of dNTPs obtained using AuNPs for ionisation. (A) AuNPs with a final concentration of 15 nM; (B) mass spectrum for dGTP; (C) mass spectrum for dATP; (D) mass spectrum for TTP; and (E) mass spectrum for dCTP. Black asterisks highlight signature peaks common to all nucleotides. Specific peaks for each nucleotide indicated with the mass value (Da) and structural information displayed next to the label. Final concentration of dNTPs is 500 μM and AuNPs is 15 nM.

nucleotide (157.66 Da for guanine—Fig. 2B; 141.65 Da for adenine—Fig. 2C; 132.64 Da for thymine—Fig. 2D; 117.24 Da for cytosine—Fig. 2E).

To evaluate this specific nucleotide identification via fingerprinting using AuNP assisted desorption/ionisation, different nucleotides were mixed in equimolar proportions and ionised using AuNPs—see Fig. 3. For each case, the specific patterns can be visualised (highlighted in Fig. 3 with black asterisks), depending on the presence of distinct nucleobases in the mixture (Fig. 3A–D). Regarding ion formation, guanine and cytosine bases are clearly favoured (Fig. 3C), as they present a considerably more intense signal. Thymine shows the lowest relative intensity of the analysed nucleotides (see Fig. 3B and D). These results support the association between *m*/*z* signals in the mass spectra and the presence of different nucleobases in the sample. Despite this association, these signals present a deviation, when in comparison to the molecular weights of different nucleobases. These differences are too great to be explained by calibration errors of the apparatus, but are too high to be considered as cation adducts. At present there is no data in the literature that could help clarifying the underlying reason for this deviation, but this might be somehow related to the AuNPs used for assisting ionisation, whose energy transfer to the sample may induce different ion combinations that have, thus far, not been characterised.

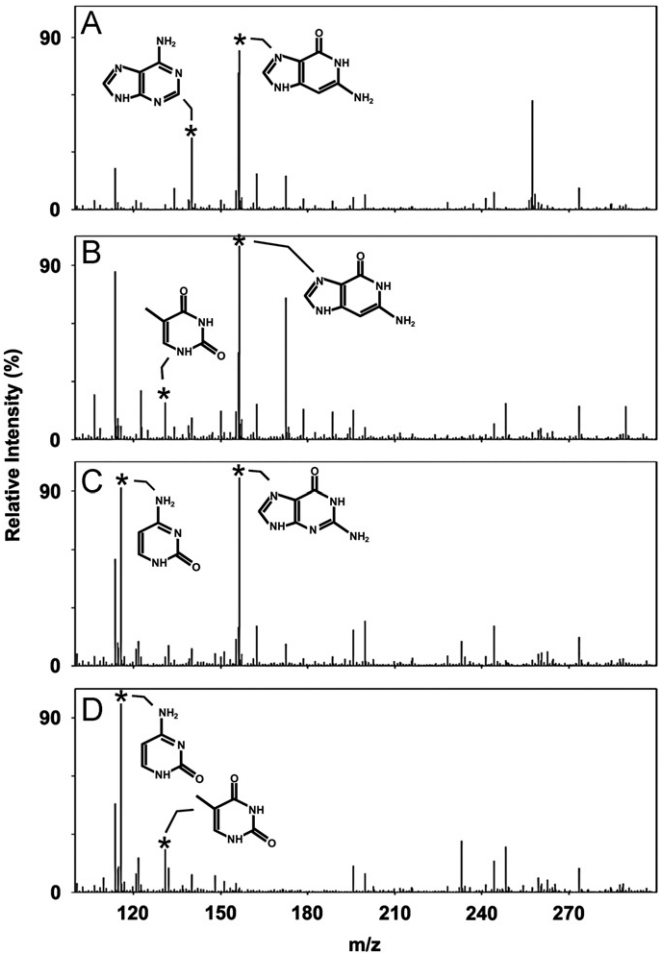

Fig. 3. AuNP-based SALDI-TOF spectra of equimolar mixtures of dNTPs. (A) dATP and dGTP; (B) TTP and dGTP; (C) dCTP and dGTP; and (D) dCTP and TTP. Specific peaks for each nucleotide are highlighted in the mass spectra with black asterisks and chemical information of the various nucleotides displayed next to it. Final concentration of dNTPs is 500 μM and AuNPs = 15 nM.

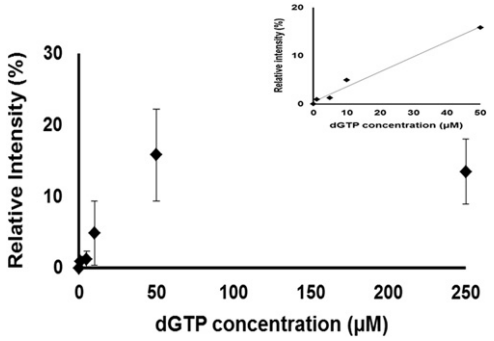

Fig. 4. Semi-quantification of dGTP via AuNP-SALDI. The relative intensity of the peaks is plotted against the dGTP concentration from data attained for the observed mass peak at 157.66 Da as an indicator to the presence of guanine. Final concentration of AuNPs is 0.1 nM.

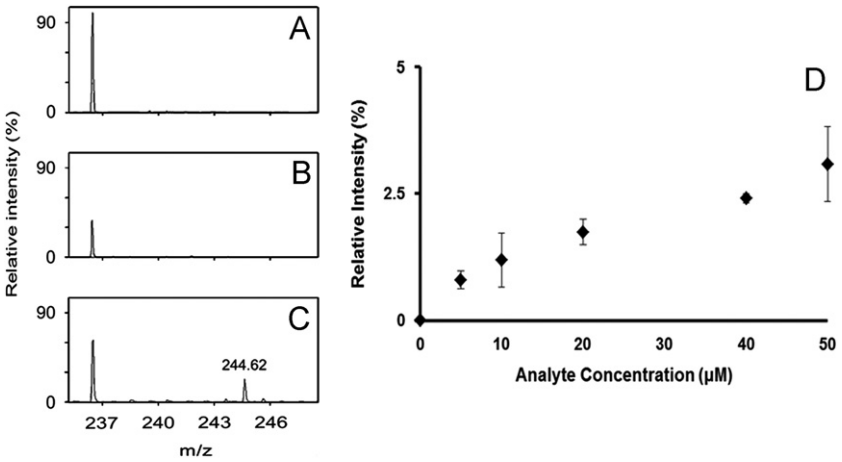

Fig. 5. AuNP-SALDI for identification of nucleotide adducts. AuNPs based SALDI approach was used to identify a glycidamide–guanine DNA adduct. Mass spectra attained within the considered mass range for (A) AuNPs and glycidamide; (B) AuNPs and dGTP; (C) AuNPs and dGTP, previously reacted with glycidamide. A specific signature peak observed only in spectrum C, at 244.62 Da associated to the presence of GA–Gua DNA adduct; (D) Plot of relative intensity vs. analyte concentration for the observed mass peak at 244.62 Da. Final concentration of AuNPs is 0.1 nM and glycidamide is 2.5 mM.

3.2. *Influence of analyte concentration*

Because laser desorption/ionisation is a destructive ionisation technique with some instrumental variability, we assessed the influence of analyte concentration on the signal intensity together with spot-to-spot reproducibility. For this, the final AuNP concentration was kept at a constant concentration of 0.1 nM, which presented the lowest spot-to-spot variability (see Supplementary data-II), and we varied the concentration of analyte (dGTP) from 0 to 250 μM. We then analysed the relative intensities of the identified peak (157.66 Da), considering that the most intense peak in each spectrum represents 100%. Fig. 4 shows an increase in the intensity of *m/z* signals for an increasing analyte concentration up to 50 μM, where the signal intensity starts to stabilise up to the maximum concentration of analyte used (250 μM). The inset shows a closer look into the first concentrations, until 50 μM, where there appears to be a linear relation between the peak relative intensity and analyte concentration. These results show that, upon optimisation of the procedure and active calibration of the detection set-up, an actual quantification assay might be possible. We chose the 0–50 μM concentration range to analyse glycidamide–guanine adducts.

3.3. *Detection of glycidamide–guanine adducts*

Upon a successful identification of deoxynucleotides in positive-ion mode using AuNPs for ionisation, we applied the described AuNP-SALDI approach to enhance detection of a most relevant analyte that can be used as exposure biomarker. Glycidamide (GA) is a well-known alkylating agent capable of generating guanine-derived DNA adducts. GA is an epoxide derived from acrylamide (AA) biotransformation via cytochrome P450 monoxygenase pathway. Genotoxicity induced by AA has been reported that has been frequently associated to the N7–GA–Gua adduct resulting from a nucleophilic attack of GA to the guanine nitrogen centres on DNA molecule [19–22].

In vitro alkylation reaction and sample spotting on the MALDI plate were performed as described in the experimental section. Based on the GA molecular weight (87 Da) and previously described structures for GA–DNA adducts [19,23], the GA–Gua adduct ought to present a specific peak in the 244–245 Da mass range (considering the guanine's characteristic signature of 157.66 Da attained beforehand plus 87 Da). Fig. 5 shows the obtained AuNP-based SALDI spectra for GA—Fig. 5A; dGTP—Fig. 5B; and dGTP+GA—Fig. 5C. All spectra present a considerably intense mass peak at 236.44 Da, associated to AuNPs. Fig. 5C shows a mass peak at 244.62 Da, which is not present in any other sample and can be attributed to the presence of guanine adducts. Fig. 5D reveals a linear trend within the 0–50 μM concentration range for dGTP+GA. The proposed AuNP-based SALDI approach allows identification of GA–Gua adducts through fingerprinting down to 5 μM, which corresponds to 5 pmol on the MALDI plate. However, this concentration may not correspond to the actual GA–Gua adduct final concentration as this is directly related to the alkylation reaction yield.

4. Conclusions

We report a rapid and easy methodology for identification of nucleotides through specific nucleotide fingerprint by using gold nanoparticles for SALDI-TOF-MS. We further demonstrate the application of this technique to the detection of a biologically relevant DNA adduct resulting from guanine alkylation of glycidamide. To the best of our knowledge, this is the first time the use of gold nanoparticles for the specific fingerprinting of DNA adducts via SALDI-TOF-MS is reported. Based on the data here presented, this methodology seems to be extremely promising for nucleotide analysis, namely as a rapid and straightforward approach to biomarkers of exposure to mutagenic agents without the need to use radioisotope labelling as in currently available techniques. The use of gold nanoparticles further contributes for lower background noise generally associated with organic matrices used in MALDI-TOF analysis of nucleic acids. Future studies are required towards evaluation of quantification capability together with characterisation of the limit of detection when using real biological samples, where sample preparation and pre-concentration would be required.

Acknowledgements

We acknowledge Fundação para a Ciência e Tecnologia (MCES) for financial support: CIGMH; PTDC/QUI-QUI/112597/2009; PTDC/CTM-NAN/109877/2009 SFRH/BD/64206/2009 for M. Larguinho. J. L. Capelo is grateful to Xunta de Galicia (Spain) for the Isidro Parga Pondal Programme and financial support: Projects 09CSA043383PR and IN825H.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.10.072.

References

[1] M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, Int. J. Mass Spectrom. Ion Processes 78 (1987) 53–68.
[2] M. Karas, F. Hillenkamp, Anal. Chem. 1988 (1988) 2301–2303.
[3] K. Tanaka, H. Waki, W. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 2 (1988) 151–153.
[4] D.S. Peterson, Mass Spectrom. Rev. 26 (2007) 19–34.
[5] J.A. McLean, J.A. Stumpo, D.H. Russel, J. Am. Chem. Soc. 127 (2005) 5304–5305.
[6] T. Yonezawa, H. Kawasaki, A. Tarui, T. Watanabe, R. Arakawa, T. Shimada, F. Mafuné, Anal. Sci. 25 (2009) 339–346.
[7] H. Kawasaki, T. Sugitani, T. Watanabe, T. Yonezawa, H. Moriwaki, R. Arakawa, Anal. Chem. 80 (2008) 7524–7533.
[8] Y. Huang, H. Chang, Anal. Chem. 78 (2006) 1485–1493.
[9] R. Arakawa, H. Kawasaki, Anal. Sci. 26 (2010) 1229–1240.
[10] C. Chiang, W. Chen, H. Chang, Chem. Soc. Rev. 40 (2011) 1269–1281.
[11] C. Chiang, N. Chiang, Z. Lin, G. Lan, Y. Lin, H. Chang, J. Am. Soc. Mass Spectrom. 21 (2010) 1204–1207.
[12] Y. Huang, H. Chang, Anal. Chem. 79 (2007) 4852–4859.
[13] J.H. Banoub, P.A. Limbach, Mass Spectrometry of Nucleosides and Nucleic Acids, CRC Press, Boca Raton, 2010.
[14] D. Bullinger, H. Fröhlich, F. Klaus, H. Neubauer, A. Frickenschmidt, C. Henneged, A. Zell, S. Laufer, C.H. Gleiter, H. Liebich, B. Kammerer, Anal. Chim. Acta 618 (2008) 29–34.
[15] J. Turkevich, Gold Bull. 18 (1985) 86–91.
[16] P. Baptista, G. Doria, D. Henriques, E. Pereira, R. Franco, J. Biotechnol. 119 (2005) 111–117.
[17] G. Doria, R. Franco, P. Baptista, IET Nanobiotechnol. 1 (2007) 53–57.
[18] J. Conde, J. de la Fuente, P. Baptista, J. Nanobiotechnol. 8 (2010) 5.
[19] G. Gamboa da Costa, M. Churchwell, L.P. Hamilton, L.S. Von Tungeln, F.A. Beland, M.M. Marques, D.R. Doerge, Chem. Res. Toxicol. 16 (2003) 1328–1337.
[20] C. Martins, N.G. Oliveira, M. Pingarilho, G. Gamboa da Costa, V. Martins, M.M. Marques, F. Beland, M.I. Churchwell, D.R. Doerge, J. Rueff, J.F. Gaspar, Toxicol. Sci. 95 (2007) 383–390.
[21] A. Besaratinia, G.P. Pfeifer, J. Natl. Cancer Inst. 96 (2004) 1023–1029.
[22] D. Segerbäck, C.J. Calleman, J.L. Schroeder, L.G. Costa, E.M. Faustman, Carcinogenesis 16 (1995) 1161–1165.
[23] J. Backman, R. Sjöholm, L. Kronberg, Chem. Res. Toxicol. 17 (2004) 1652–1658.