行政院國家科學委員會補助專題研究計畫成果報告

81

※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※
※ 以質譜方法研究烹調油煙所產生的 ※
※ 揮發性有機物與女性肺癌之相關性 ※
※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：☑個別型計畫　□整合型計畫
計畫編號：　NSC 90-2320-B-040-047
執行期間：　90 年 9 月 1 日 至 91 年 7 月 31 日

計畫主持人：張耀仁
共同主持人：李輝
計畫參與人員：王建良，吳蓉莉

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

執行單位：中山醫學大學毒理學研究所

中華民國 **91** 年 **10** 月 **25** 日

行政院國家科學委員會專題研究計畫成果報告

以質譜方法研究烹調油煙所產生的揮發性有機物與女性肺癌之相關性

The role of volatile organic compounds form cooking oil fumes under domestic conditions on the lung cancer in Taiwanese women -using mass spectrometry

計畫編號：NSC90-2320-B-040-047
執行期限：90 年 9 月 1 日至 91 年 7 月 31 日
主持人：張耀仁　中山醫學大學毒理所
共同主持人：李輝　中山醫學大學毒理所
計畫參與人員：王建良，吳蓉莉 中山醫學大學毒理所

1. 摘要

本研究以自製的微電灑塑膠質譜晶片分析 DNA 鍵結物，追查烹調油煙所產生的揮發性有機物可能產生的氧化性傷害與對 DNA 鍵結物之影響，以探討其與女性肺癌之相關性。研究中，自製的質譜晶片操作在超低流速下(<0.1 μL/ min)；與正常電灑法之質譜相比較：自行研發的微電灑質譜晶片不但使用較少的樣品，較快的分析速度與結果，更有 3.5 倍高的濃度靈敏度及約 100 倍高的質量靈敏度。將此質譜晶片應用於 MDA- DNA adducts 快速分析，我們發現：在含有乙醛(酒精代謝)的環境下，原本主要的 DNA adducts 產物 MDA-dG 戲劇化轉變成 MDA-dA，並有極高的生成率。MDA-dA 的結構推測可能為 9-(2'-deoxyribosyl)- 6-(3,5-diformayl-4-methy-1, dihydro-1-pyridyl) purine (M_2AA-dA).

關鍵詞：揮發性有機化合物，DNA 鍵結物

Abstract

In this project, a disposible plastic nanoelectrospray chip for mass spectrometric analysis has been developed for detection DNA adducts with aldehydes derived from lipid peroxidation. The prototype nanoflow electrospray chips used for these studies were fabricated on the PMMA polymer that contained two main elements, sample chamber plate and graphite-coated nanoelectrospray emitter. The nanoflow electrospray chip was operated under low-flow rates (<0.1μL/ min). In the comparison experiment of conventional ESI/MS vs. nanoelectrospray chip, the spectra are similar, but the total amount of sample consumed in nanelectrospray chip was over an order of magnitude less than the standard ESI setup, while the intensity of the base peak was about 3.5 times greater. The device was applied to rapid identification MDA- DNA adducts. In our studies, the major product of DNA adducts almost changed to deoxyadenosine (MDA-dA) and the yield increased drastically under higher acetaldehyde concentration. It was identified by nanoelectrospray chip - MS, indicate that acetaldehyde and MDA can react together in a synergistic manner and generate hybrid adducts 9-(2'-deoxyribosyl)-6-(3,5-diformayl 4 - methy-1, 4-dihydro-1-pyridyl) purine (M_2AA-dA).

Keywords: volatile organic compounds, DNA adduct,

2. Introduction:

Humans often exposed to a wide array of chemical compounds unknowingly, then those compounds or their metabolites may be interacted with biological macromolecules such as proteins, RNA, and DNA. Interactions with DNA can lead to a new covalent bond between the carcinogen and DNA, leading to damaged DNA and so-called DNA adducts. If these damages are not enzymatically repaired, they can be the cause of mutations and eventually lead to chemically induced carcinogenesis[1,2]. Modification of the nucleobases in DNA is

Microchip technology has recently been applied to capillary electrophoresis (chip-CE), generating an extremely powerful separation and sample pretreatment tool for rapid analyses (typically less than 1 min) [22,23]. Separations have been combined on-chip with sample dilution, derivatization, enzyme digestion, and a set of independent manifolds all integrated onto a single chip leading to multiplexed analyses[24-34]. Transport of bio logical cells using direct current electro kinetic effects in a chip-based capillary format was also demonstrated. A recent report has shown the separation and isolation of cultured cervical carcinoma (HeLa) cells from normal blood cells using electro phoresis on a bioelectronic chip[34-35]. Thus, sample pretreatment can be automated within an integrated device, a feature that could offer significant advantages in sample pre paration for mass spectrometry, particularly if the chip can be incorporated as part of the electrospray source.

This paper is the primary development of a compact and versatile nanoelectrospray (nanoESI) chip using a low dead volume connection that enables direct coupling to mass spectrometry. The advantages of this low dead volume connection have been examined. The sensitivity and analytical characteristics of the respective interfaces were also evaluated for the analysis of standard biological samples. The practical application of these devices for rapid identification MDA-DNA adduct is investigated.

3. Experimental Section:

Reagents and Materials All reagents and solvents were purchased and used without further purification. Calf thymus DNA, horse heart myoglobin and 1,1,3,3-tetra-methoxypropane was purchased from Sigma Chemical Co. (St Louis, MO). Acetalal dehyde (purity >99%) and methanol, 2-propanol of HPLC grade were purchased from J. T. Baker (Phillipsburg, NJ, USA) used without further purification. Deion ized water (Milli-Q water system, Millipore Inc., Bedford, MA. USA) was used in the preparation of the samples and buffer solution. All fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Modification of calf thymus DNA with MDA Calf thymus DNA was modified by reacting it with MDA essentially as described by Vaca et al[4]. The MDA solution was prepared by acid hydrolyzing freshly distilled 1,1,3,3-tetramethoxypropane (0.02 g) in aqueous hydrogen chloride (1 ml, 0.1 mol /L) at 40°C for 45 min. Following the hydrolysis, the pH of the mixture was adjusted to 4.6 by the addition of aqueous ammonium acetate. Accordingly, MDA (30μg) was reacted with 250μg calf thymus DNA and(or)200μL acetaldehyde. The DNA samples were then incubated for 5 days at 37°C. Aqueous ammonium acetate was added, and the DNA was precipitated with cold ethanol (1 ml) and kept in the cold for 1 h. The precipitate was collected by centrifugation (1200 rpm), washed thorou ghly with cold ethanol. Finally,the product-DNA adduct was then treated by Nuclease P1(20μg) and alkaline phosphatase (1.5 unit). The modified DNA was stored at 4°C before analyzed by electrospray mass spectrometer.

Instrumentation The samples were subjected to ESI–MS or ESI–MS/MS analysis on a mass spectrometer: a Finnigan MAT LCQ quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA). In normal direct infusion experiments, the solution with 5.0ul /min flow rate was supplied to ESI emitter using a Cole-Parmer 100 syringe pump with a gas-tight Hamilton syringe connected to the capillary through TFL tube. The solution was electrosprayed from ESI emitter at approximately 4.5 kV, in the positive-ion mode, and is sampled by the capillary, which is typically headed to a temperature of 200℃. The heated capillary (400 μm i.d., 11.5 cm long) helps complete the desolvation process and serves as the nozzle for the supersonic expansion of the gas into the next chamber. The autotune mode and nebulization gas was used for

considered to be a major step in the genesis of cancer. While the role of covalent DNA modification in cancers caused by exposure to environmental carcinogens has become clear, an important issue is the products of endogenous metabolism play a role in the formation of covalently modified DNA. Aerobic metabolism can lead to peroxidation of membrane lipids through free radical mediated processes that are initiated and propagated by ubiquitous biological oxidants including transition metal ions and molecular oxygen[3].

In general, DNA of living organisms is susceptible to continuous structural modification by both xenobiotic compounds and endogenously produced metabolises. Cyclic adducts of nucleic acid bases produced by reactions with a,b-unsaturated aldehydes and dicarbonyl compounds constitute a well known example of such modifications. Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and prostaglandin biosynthesis that is mutagenic and carcinogenic. MDA reacts with DNA to form variety adducts [4]. The order of reactivity MDA with DNA bases is guanine>adenine>cytosine>thymine. It has been detected in liver, white blood cells, pancreas, and breast from healthy human beings at levels ranging from 1–120 per 10^8 nucleotides.

The analysis of DNA adducts, the molecular biomarkers indicative of potential cellular damage, is very important. The analytical method used for the analysis of carcinogen DNA adducts must provide two features: the methodology has to be sensitive, i.e., it should provide the sensitivity necessary to detect adducts in the picogram to femtogram range, and it must enable us to elucidate the structure of the formed adducts. Several analytical techniques, including mass spectrometry[5,6], ^{32}P-postlabeling[4,7],and immunochemical[8] techniques have been employed to DNA adducts. Each technique offers advantages and disadvantages based on a combination of sensitivity and specificity. The most sensitive method up to now to establish the presence of adducts is ^{32} P-postlabelling. These procedures are highly sensitive, being capable of detecting adduction levels on the order of 1 in 10^{10} normal nucleotides. However, it can not provide the structural information needed for unambiguous identification[9,10]. In addition, DNA that has been heavily adducted or otherwise cross-linked can be resistant to the enzymatic hydrolysis procedures that release adducted nucleotides. Furthermore, ^{32}p-postlabelling method is dangerous for it is radioactive. Accordingly, development of highly safe, sensitive and selective method to identify DNA adducts is important.

Mass spectrometry, especially using electrospray ionization (ESI) [11], is a powerful method to elucidate the structures of unknown DNA adducts. ESI has been used for analysis in a wide range of disciplines, including environmental, biological, and clinical samples. ESI/MS facilitate the formation of multiply charged ions from high molecular weight biopolymers. It has proven to be a gentle method for the characterization of macrobiomolecules such as proteins or oligonucleotides. The combination of liquid chromatography interfaced with mass spectrometry (LC/MS) has been used to identify and quantify DNA adducts in animal and human tissues produced in response to exogenous carcinogens and endogenous metabolic processes[12-16]. The development of electrospray interfaces that can operate under low-flow rates makes the coupling of nanoscale separation with ES-MS possible. Several groups have reported coupling capillary liquid chromatography or capillary electophoresis (CE) to MS for the analysis of pharmaceutical compounds, deoxyribo nucleoside adducts, and peptides[17-19]. By interface nanoscale separation and nano electrospray, the mass sensitivity could be further improved. Because of their high sensitivity, longer sampling time, and femtomole amounts of sample consumption, nanoelectrospray-MS are being more and more routinely used for the analysis of biological samples[20,21].

maximizing analyte ion signals at normal ESI experiments. Mass spectra were obtained with averaging over 20 scans, and each scan was built-up from three microscans, taking a maximum injection time of 200 ms.

The prototype nanoelectrospray chips (Fig 1) was configured in-house. The chips were fabricated on the two piece of PMMA polymer plates that was thermal bonding carried out at 104℃ for 10min, then cooled to 80℃ for 10min. It was contained the sample vial and a 40μm i.d. 100μm o.d. graphite coated fused silica tip as the nanoelectrospray emitter. The graphite coated capillary tip was smeared with marker pen following with graphite coating using a soft pencil (more details can be found in Results and Discussion).

The nanoelectrospray chip-MS setup was showed in Fig 2. Briefly, it consisted of a high-voltage power supply connected to sample vial on the chip and operated at constant-voltage mode. This nanoelectrospray chip assembly could be manipulated in x, y, and z directions via the micrometer screws. A stainless steel wire was inserted into sample vial or contracted with the graphite coated nanoelectrospray tip for electrical contact. For the nanoelectrospray chip emitter, ESI voltage ranged from 1500 to 2500V. The fused silica capillary was positioned at a distance less than 2 mm from the entrance hole of the heated transfer capillary. The actual position was found to be not a critical factor. Nebulization gas was not necessary and the heated transfer capillary was kept at a temperature of 200℃. ESI-MS spectra were acquired at full scan mode.

4. Results and Discussion:

Chip/MS . The prototype nanoelectrospray chip used for these studies was disposable because it was fabricated on the PMMA polymer. The nanoelectrospray chip operates under very low-flow rates (<0.1μL/min). It was evaluated by infusing horse heart myoglobin in 0.1% (v/v) formic acid, 50% methanol solution. Spectra were obtained with an average of 20 scans, and the heated transfer capillary was kept at a temperature of 200℃. Fig. 3 shows the results obtained from the horse heart myoglobin at 10 pmol/ul using standard electrospray source (Fig. 3a) and a home made nanoelectrospray chip (Fig. 4b). The spectra are similar, with multiply charged molecular ions ranging from the 9+ to 29+. The total amount of sample consumed in nanoflow electrospray chip was over an order of magnitude less than the standard ESI setup, while the intensity of the base peak was about 3.5 times greater.

The use of graphite-coated emitter is important for nanoelectrospray chip-MS [37,38]. For the graphite-coated nanoelectrospray emitter, ESI voltage ranged from 1500 to 2500V; however, ESI voltage was needed more than 4500V for the native fused silica nanoelectrospray emitter. Because of the discharge was occurred between the capillary tip and the inlet of the mass spectrometer at high electric field strengths. If the blue glow discharge was visible at the tip, and would be produced chemical noises into MS, then the sensitivity was reduced.[14,17,21].

The procedure for making graphite coated capillary column is very simple. The tip of the fused silica capillary was first smeared with a marker pen. This step is critical because it was quite difficult to coat the tip without the layer of marker pen ink. To make it easier to monitor the following graphite coating process, marker pen of black color should be avoided. After drying, the capillary tip was then smeared with graphite using a soft pencil. It is noteworthy that it takes less than 1 minute to finish a graphite-coated fused silica capillary tip.

The obvious advantage of this method is that it allows, in contrast to the other MS methods employed, analysis of DNA adducts without further derivatization. Similar LC/ESI/MS methods based on the use of an isotopically modified adduct as an internal standard have been introduced. Further,

nanoelectrospray chip that will incorporate nanoscale liquid-phase separations such as electrophoresis and electrochromatography are being increasingly recognized as a convenient means of manipulating and analyzing small quantities of material.

MDA-DNA modification. Although modifcation of the nucleobases in DNA by both xenobiotic compounds and endogenously produced metabolises is considered to be a major step in the genesis of cancer. It is still unknown which type of modifcation is most efficient for the induction of the series of events eventually leading to cancer, since most carcinogens react with DNA at several different sites and with the formation of more than one product. It result uncertainty about the role of specifc adducts, even though some reports have been published about the effect of major and minor DNA modification.

So far, the possibility of MDA being an endogenous human carcinogen is clear. It is known to form adducts in reaction with deoxyguanosine (dG), deoxyadenosine (dA), and deoxycytidine (dC). Reportedly, MDA-DNA adducts was common detected in the human livers cells and most of them are MDA-dG.[39] In our experiments, MDA hydrolyzed from 1,1,3,3-tetra-ethoxypropane was reacted with calf thymus DNA for 5 days at 37℃. However, the DNA-adduct yield was still very low. It could be a mild reaction because MDA is a byproduct of endogenous biosynthesis. Previously, Gomez-Sanchez et al. reported that MDA prepared by hydrolysis of 1,1,3,3-tetra-ethoxypropane may be cleaved to acetaldehyde[40]. in acetaldehyde condition. After the addition of acetaldehyde, the yield is drastically increased the major product of DNA adducts is drastically changed to deoxyadenosine (MDA-dA) that was identified by nanoelectrospray chip-MS.

Acetaldehyde is a compound, which is producted by ethanol metabolism or during lipid peroxidation [41,42]. Most acetaldehyde metabolism takes place in liver. From our studies, it was suggested the role of acetaldehyde may be very important in the formation of MDA-DNA adduct. Acetaldehyde can enhance the reactivity adenine nucleosides and increase the amounts of DNA adduct drastically. It seems that acetaldehyde could not bind to DNA along. The product was identified as 9-(2'-deoxyribosyl)-6-(3,5-diformayl-4-meth y-1,4-dihydro-1-pyridyl) purine (M_2AA - dA) by ESI/MS in Fig5(b). In the positive ion electrospray mass spectrum of M_2AA-dA, the protonated molecular ion peak was observed at m/z 388.9 and the most abundance ion. The fragment recorded at m/z 252 corresponds to the cleavage of ribosyl moiety from the protonated molecular ion.

The DNA adduct was most likely formed by an initial condensation of two molecules of MDA with one molecule of acetaldehyde followed by reaction of the condensation product with the exocyclic amino group of 2'deoxyadenosine according to electrospray mass spectrum (Fig 4). In conclusion, these results indicate that acetaldehyde and MDA can react together in a synergistic manner and generate hybrid adducts (M_2AA-adducts) and further suggest that M_2AA adducts maybe represent a major species of adducts formed in the liver during the ethanol metabolism.

5. Conclusion :

In this paper, we show the results of feasibility studies using nanoelectrospray chip- MS to provide the specificity needed for direct analysis of putative endogenous DNA adducts. By the addition of acetaldehyde, the major product of MDA-DNA adducts almost changed to deoxyadenosine (MDA-dA) and the yield increased drastically. These results indicate that acetaldehyde and MDA can react together in a synergistic manner and generate hybrid adducts. It suggests that M_2AA adducts maybe represent a major species of adducts formed in the liver during the ethanol metabolism in vivo. The use of nanoelectrospray design, with small flow

rates of 20–100 nL/min provides better than 10 times the sensitivity of conventional ESI-MS. Besides, the nanoelectrospray chip is disposable. In addition, we show the way in which nanoelectrospray chip-MS can be used to validate ^{32} P- postlabeling procedures for adduct identification. Future, nanoelectrospray chip that will incorporate nanoscale liquid-phase separations such as electrophoresis or electrochromatography are being increasingly recognized as a convenient means of manipulating and analyzing small quantities of material. As a high throughput method for its detection and quantitation by increasing specificity through structural characterization of assay substrates and reaction products, it will be extremely useful for screening large populations.

6. References

1. L.J. Marnett, in: K. Hemminki, A. Dipple, D.E.G. Shuker, F.F. Kadlubar, D. Segerba¨ck, H. Bartsch (Eds.). (1994) DNA Adducts: Identification and Biological Significance, IARC, Lyon pp. 151–163.

2. Cantoni, O., Sestili, P. and Cattabeni, F. (1989) Calcium chelator Quin 2 prevents hydrogen-peroxide-induced DNA breakage and cytotoxicity. Eur. J. Biochem., 2 , 209-212.

3. Jaruga, P., Zastawny, T. H., Skokowski, J., Dizdaroglu, M. and Olinski, R. (1994) Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. FEBS Letter, 341, 59-64.

4. Vaca C.E., Vodicka P. and Hemminki K. (1992) Determination of malonaldehyde-modified 2-deoxyguanosine-3-monphosphate and DNA by ^{32}P-postlablelling 13,4, 593-599.

5. Chaudhary AK, Nokubo M, Marnett LJ, Blair IA. (1994) Analysis of the malondialdehyde-2'-deoxyguanosine adduct in rat liver DNA by gas chromatography/electron capture negative chemical ionization mass spectrometry. Biol Mass Spectrom. 23, 457-64.

6. Giese R.W. (1997) Detection of DNA adducts by electron capture mass spectrometry, Chem. Res. Toxicol.,10, 3.

7. Bann R. A, Steenwinkel M.S.T, Asten S., Roggband R, Delft J.H.M. (1997) The use of benzo [a] pyrene diolepoxide-modified DNA standards for adduct quantification in ^{32}P-postlabelling to assess exposure to polycyclic aromatic hydrocarbons: application in a biomonitoring study. Mutation Res.378, 41-50.

8. Leuratti C., Singh R., Lagneau C., Farmer P.B., Marnett L.J., Shuker D.E.G. (1998) Quantitation of malondialdehyde–DNA dam-age in human tissues using a sensitive immunoslot blot assay, Proc. Am. Assoc. Cancer Res. 39 286.

9. Esmans E.L., Broes D. Hoes I, Lemiere F., Vanhoutte K. (1998) Liquid chromatography - mass spectrometry in nucleside, nucleotide and modified nucleotide characterization. J Chroma. A 794 109-127.

10. Laurent Leclercq, Christian Laurent, and Edwin De Pauw* (1997) High-Performance Liquid Chromatography/Electrospray Mass Spectrometry for the Analysis of Modified Bases in DNA: 7-(2-Hydroxyethyl)Guanine, the Major Ethylene Oxide-DNA Adduct, Anal. Chem. 69(10); 1952-1955.

11. Fenn J.B., Mann M., Meng C.K., Wong S.F., Whitehouse C.M. (1989) Electrospray ionization for mass spectrometry of large biomolecules. Science, 246, 64-71.

12. Chaudhary AK. Reddy GR. Blair IA. Marnett LJ. (1996) Characterization of an N6-oxopropenyl-2'-deoxyadenosine adduct in malondialdehyde-modified DNA using liquid chromatography/electrospray ionization tandem mass spectrometry. Carcinogenesis. 17(5):1167-70.

13. Guengerich F. P. (1997) Analysis of 1,N2-Ethenoguanine and 5,6,7,9-Tetrahydro -7-hydroxy-9-oxoimidazo[1,2-a]purine in DNA Treated with 2-Chlorooxirane by High Performance Liquid Chromatography / Electrospray Mass Spectrometry and Comparison of Amounts to Other DNA Adducts, Chemical Research in Toxicology 10(2); 242-247.

14. Doerge D. R., Yi P. , Churchwel M. I. , Preece S. W., Langridge J. Fu P.P. (1998) Mass spectrometric analysis of 2'-deoxyribonucleoside and 2'-deoxyribonucleotide adducts with aldehydes derived from lipid peroxidation. Rapid Commun. Mass Spectrom. 12, 1665–1672.

15. Doerge D. R., Churchwel M. I., Fang J.-L., and Beland F. A. (2000) Quantification of Etheno-DNA Adducts Using Liquid Chromatography, On-Line Sample Processing,

and Electrospray Tandem Mass Spectrometry, Chem.Res. Toxico. 13(12); 1259-1264.

16. Hakala K. Auriola S. Koivisto A. Lonnberg H. (1999) Mass spectrometric (HPLC/ESI--MS/MS) quantification of pyrimidoand 2 over black square]; [1 and 2 over black square],3-a]purin-10(3H)-one, a guanine adduct formed by reaction of malondialdehyde with DNA. Journal of Pharmaceutical & Biomedical Analysis. 21(5):1053-61.

17. Ding J.and Vouros P. (1997) Capillary Electrochromatography and Capillary Electrochromatography - Mass Spectrometry for the Analysis of DNA Adduct Mixtures, Anal.Chem. 69(3); 379-384.

18. Vanhoutte, K., Van Dongen, W., Hoes I., Lemière, F., Esmans, E. L., Van Onckelen, H., Van den Eeckhout E., Van Soest R. E., and Hudson A. J. (1997) Development of a Nanoscale Liquid Chromatography/Electrospray Mass Spectrometry Methodology for the Detection and Identification of DNA Adducts, Anal. Chem. 69(16); 3161-3168.

19. Filip Lemière and Eddy L. Esmans (1998) Analysis of DNA Adducts in DNA Hydrolysates by Capillary Zone Electrophoresis and Capillary Zone Electrophoresis-Electrospray Mass Spectrometry, Analytical Chemistry; 1996; 68(20); 3575-3584.

20. Apruzzese W.A., Vouros P. (1998) Analysis of DNA adducts by capillary methods coupled to mass spectrometry: a perspective. J Chroma.A 794 97-108.

21. Gangl E.T., Turesky R.T.,Vouros P. (1999) Determination of in Vitro- and in Vivo-Formed DNA Adducts of 2-Amino-3-methylimidazo[4,5-f]quinoline by Capillary Liquid Chromatography/ Microelectrospray Mass Spectrometry, Chem.Res. Toxico. 12(10); 1019-1027.

22. Harrison, D. J.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C. S.; Manz, A. (1993) Science 1993, 261, 895-897.

23. Jacobson SC.and Ramsey J.M.* (1996) Integrated Microdevice for DNA Restriction Fragment Analysis, Anal. Chem. 68, 720-723.

24. Chiem N. Harrison DJ. (1997) Microchip-based capillary electrophoresis for immunoassays: analysis of monoclonal antibodies and theophylline. Anal. Chem. 69, 373-8.

25. Koutny LB. Schmalzing D. Taylor TA. and Fuchs M.* (1996) Microchip Electrophoretic Immunoassay for Serum Cortisol, Anal. Chem. 68,18-22.

26. Hadd AG. Raymond DE. Halliwell JW. Jacobson SC. Ramsey JM. Microchip device for performing enzyme assays. (1997) Anal. Chem. 69(17):3407-12.

27. Munro NJ. Huang Z. Finegold DN. and Landers JP.* (2000) Indirect Fluorescence Detection of Amino Acids on Electrophoretic Microchips, Anal Chem. 72, 2765-2773.

28. Waters LC. Jacobson SC. Kroutchinina N. Khandurina J. Foote R.S. and Ramsey J.M.* (1998) Microchip Device for Cell Lysis, Multiplex PCR Amplification, and Electrophoretic Sizing, Anal. Chem. 70, 158-162.

29. McCormick RM. Nelson RJ. Alonso-Amigo MG. Benvegnu DJ. Hooper HH. Microchannel electrophoretic separations of DNA in injection-molded plastic substrates. (1996) Anal. Chem. 69,2626-30.

30. Fister JC. Jacobson SC. DavisLM. and Ramsey JM.* (1998) Counting Single Chromophore Molecules for Ultrasensitive Analysis and Separations on Microchip Devices, Anal. Chem.; 70(3); 431-437.

31. Woolley AT. Sensabaugh GF. Mathies RA. (1997) High-speed DNA genotyping using microfabricated capillary array electrophoresis chips. Anal. Chem. 69, 2181-6.

32. Schrum DP. Culbertson CT. Jacobson SC. and Ramsey JM.* (1999) Microchip Flow Cytometry Using Electrokinetic Focusing, Anal. Chem. 71, 4173-4177.

33. Cheng J. Sheldon EL. Wu L. Heller MJ. O'Connell JP. (1998) Isolation of cultured cervical carcinoma cells mixed with peripheral blood cells on a bioelectronic chip. Anal. Chem. 70(11):2321-6.

34. Khandurina J. McKnight TE., Jacobson SC. Waters LC. Foote RS. and Ramsey JM.* (2000) Integrated System for Rapid PCR-Based DNA Analysis in Microfluidic Devices, Anal. Chem. 72, 2995-3000.

35. Gary A. Schultz,* Thomas N. Corso, Simon J. Prosser, and Sheng Zhang .(2000) A Fully Integrated Monolithic Microchip Electrospray Device for Mass Spectrometry, Analytical Chemistry; 72(17); 4058-4063.

36. McClain MA. Culbertson CT. Jacobson SC. and Ramsey JM.* (2001) Flow Cytometry of Escherichia coli on Microfluidic Devices, Anal. Chem.73, 5334-5338.

37. Chang Y.Z. and Her G.R. (2000) Sheathless Capillary Electrophoresis / Electrospray Mass Spectrometry Using a Graphite-Coated Fused-Silica Capillary, Anal. Chem.72, 626-630.

38. Chang Y.Z. Chen Y.R. and Her G.R.*(2001) Sheathless Capillary Electrophoresis / Electrospray Mass Spectrometry Using a Graphite-Coated Tapered Fused-Silica Capillary with a Beveled Edge, Anal. Chem. 73, 5083-5087.

39. Chaudhary AK. Nokubo M. Reddy GR. Yeola SN. Morrow JD. Blair IA. Marnett L.J. (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science. 265(5178)1580-2.

40. Gomez-Sanchez, A., Hermosin, I., Lassaletta, J.M., and Maya, I. (1993) Cleavage and oligomerization of malondialdehyde. Tetrahedron 49, 1237-1250.

41. Ohya T. (1994) Reaction of malondialdehyde with deoxyribonucleosides in the presence of acetaldehyde. Biological & Pharmaceutical Bulletin. 17(10):1411-3.

42. Le Curieux F. Pluskota D. Munter T. Sjoholm R. Kronberg L. (1998) Formation of a fluorescent adduct in the reaction of 2'-deoxyadenosine with a malonaldehyde-acetaldehyde condensation product. Chemical Research in Toxicology. 11(9) 989-94.

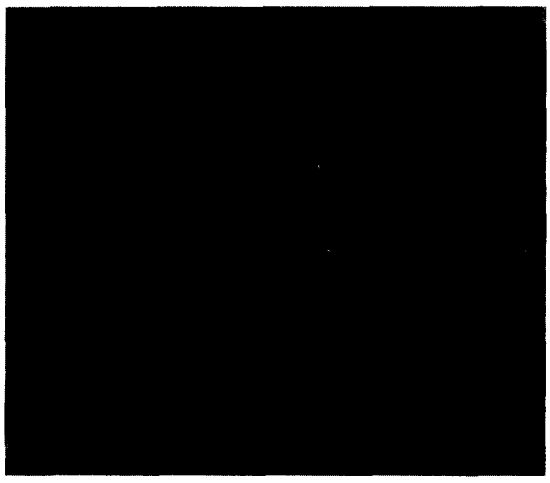

Figure1. Photograph of nanoelectrospray chip with graphite-coated tip.

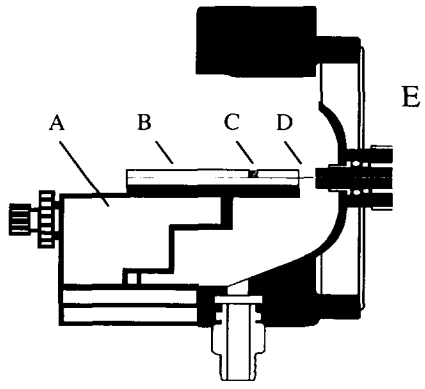

Figure 2. Schematic diagram of the setup of nanoelectrospray chip with LCQ Mass Spectrometry (not to scale). (A) x, y, z positor (B) chip (C) Sample vial (D) carbon-coated tip (E) API interface of LCQ Mass Spectrometry.

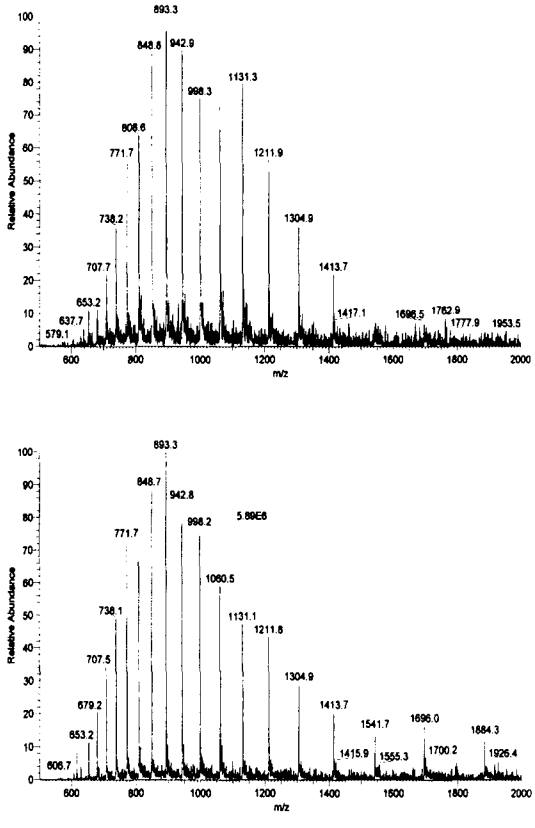

Figure 3. Mass spectrum of 10 pmol/ul horse heart myoglobin obtained with: (a) standard electrospray; (b) home made nanoflow electrospray chip.

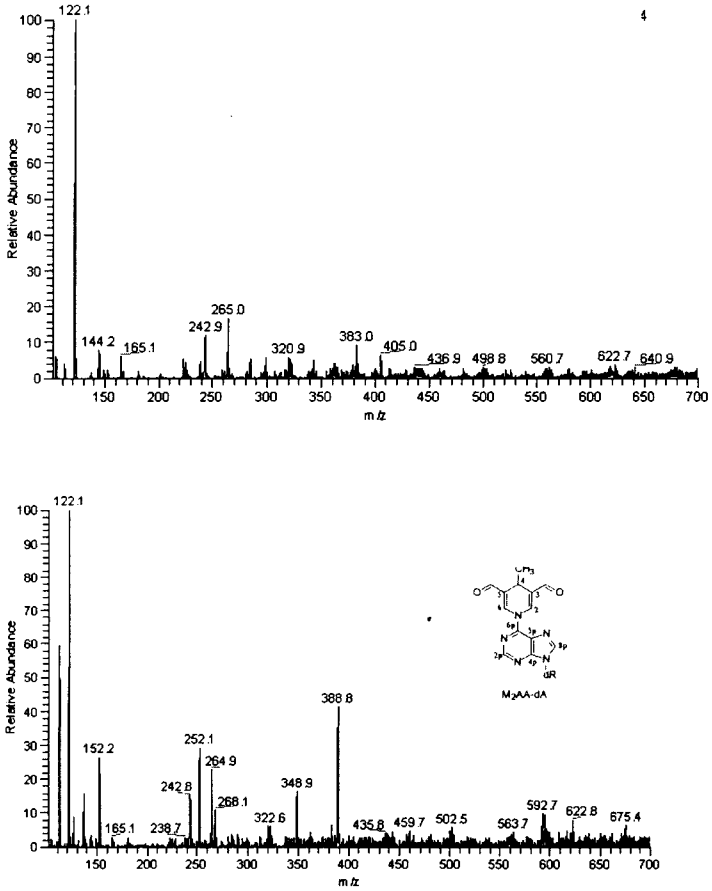

Figure 4. Mass spectrums of calf thymus DNA in the reaction with: (A) MDA; (B) MDA and acetaldehyde

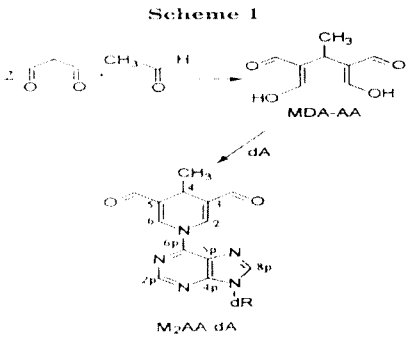

Figure 5. Mechanism of acetaldehyde interaction with calf thymus DNA through conjugation with MDA.