Integrated Micro Bio Systems and High Performance Liquid Chromatographic System on Chip

KITAMORI Takehiko

Department of Applied Chemistry, School of Engineering, The University of Tokyo, Tokyo 113-8656, Japan

Key words: micro bio system, high performance liquid chromatographic system, integration, chip

CLC number: O658

Document code: A

Article IC: 1000-8711 2004/04-0335-03

1 Micro chemical system on chip

Micro integrated chemical systems are expected as promising ultra high throughput chemical and bio processors with extremely small volume. Our research groups have proposed and developed the original methodologies for micro integration of general chemical systems as well the electrophoresis analysis in which many groups of this micro technology are interested. They are as follows: 1 micro unit operation, MUO; 2 continuous flow chemical processing, CFCP; 3 2D and 3D multi-phase laminar flow network; 4 thermal lens microscope, TLM.

The first two items MUO and CFCP are the general methods for the design of micro chemical system. Unit operations such as mixing, reaction, extraction and others in micro space are developed by using micro channels and they are able to combine each others for constructing total chemical process like analytical and synthesis systems. The third one is controlling method of micro fluid chemical process and chemical reaction. And the last one TLM is ultra sensitive detection method for ymol 10^-24 mol level determination of non-fluorescent molecules.

These methods have enabled the integration of any kinds of chemical and bio systems on microchips almost freely although the conventional electrophoresis chips are limited in application range. We have integrated about seventy kinds of systems on chips and they have been applied to practical applications and have been utilized as novel tools for chemical sciences.

Concerning practical applications analytical chips for environmental and clinical diagnosis, bioassay chips using living cells and E. coli chemi-cal synthesis chips were developed. These devices proved that the integrated micro system was a few orders of faster and superior performances comparing to the corresponding conventional macro systems. These superior characteristics are elicited by fast and efficient molecular and energy transfers due to the physical characteristics of microspace such as large specific interface area, short diffusion time, small heat capacity, and so on. Some diagnosis systems and chemical plants have already been served in pilot operations. Especially a large scale gel particle plants 20 m x 20 m x 4 m were successfully miniaturized to shoebox size 2 m x 1 m x 1.2 m though the production of 30 t/year was the same. Some national research projects have been launched for industrialization of the micro chemical and bio chip technologies in Japan.

2 Life and function support μ-system

These technologies were applied to micro bio systems from the beginning of the research project. There are many reports concerning cell culture on microchips but cell culture on microchips is not so easy because the micro space road stress on the cells and the cultivated cells sometimes loose their normal and healthy functions. Therefore the development of the cell function maintenance system is very important as well as the life supporting system on microchips.

Temperature control oxygen and medium supply are the main requirement of life support. Micro Peltier device for local temperature control was developed. Plural elements such as cell culture extraction enzymatic reaction and chemical reaction systems are need to integrate on small space for a micro bioassay system on a micro-
chip. These elemental systems require different
temperatures. The developed micro Peltier tem-
perature control devices enable the very local
temperature control and adequate temperature
for cell culture could be kept even the different
temperature systems were close-in. And our mi-
cro fluidic control system was applied to provide
oxygen and medium supply to the confined living
cells.

These systems were integrated on a micro-
chip and a life supporting system could be de-
veloped. However the cultivated living cells some-
times lose their normal functions. For example the
primary culture hepatic cells were successfully
cultivated however they lost the normal func-
tions of hepatic cells. They felt the shear stress of
the laminar flow and changed their figures. We
found optimal flow rate for culture the hepatic
cells keeping their normal functions and the con-
tions were checked by the production of albu-
min. The cells which changed the healthy figures
did not show the albumin production.

Furthermore coating technique of inner sur-
face of micro culture vessel is also very nervous i-
to the living cells. We applied our surface modi-
fication technologies to coat the inner sur-
face by optimal materials for the individual kind
of cells.

3 Micro bioassay systems

The developed life support and function ma-
intenance systems of cells were applied to various
kinds of micro bioassays such as toxicity check
and side reaction check of medicines. And the
micro bio systems were also applied to basic cell
biology experiments.

For example macrophages from mouses
were cultivated on a microchip and their release
dynamics of NO after stimulation by lipopolysac-
charide LPS. Usually direct monitoring of cel-
lar release is very difficult. Because the quantity
of the released substances is in quite trace levels
and the trace substances easily dispersed in large
volume of culture vessels. However in our micro
bioassay system not only the cell culture but the
extraction and subsequent processes of the cellular
release such as enzymatic and chemical reactions
and separation proceeded in micro volume contin-
uously and the trace substances did not disperse
but even concentrated in the smaller volume. The
total number of living cells was very small about
four hundreds hence the cultivation time was
very short. Actually two to three hours were suf-
ficient. The release dynamics of the NO at fmol
levels per cell from the macrophage was success-
fully monitored.

The primary cultivated hepatic cells were ap-
plied to check the individual sensitivity to the side
reaction of anti cancer medicine. The healthy he-
patic cells under the adequate culture conditions
were exposed to the anti cancer medicine. The
sensitivity was checked by death rate of the cells.
This assay was completed by using several hun-
dreds of hepatic cells and the method was proved
to be very useful. The converse case that is the
assay of medicinal effect of the anticancer
medicine was also successful by using hepatoma
cells.

4 Micro high performance liquid chroma-
tograph and other instruments

Even detection and determination are trou-
blesome subjects for micro chemical and bio tech-
nologies identification of chemical species on a
microchip is still a more difficult problem. We
have developed TLM for ultra sensitive detection
and determination for non-fluorescent species but
TLM’s selectivity is the same as absorption
spectrometry and thus very poor. Therefore the
combination of TLM and a fine separation method
which can identify specimen is one of the solu-
tions of this difficulty. Actually we proved that
the TLM determination and solvent extraction
method resulted sub-nmol analysis. And then de-
sirably the combination of TLM with other sepa-
ration analyses for general use will widen the ap-
plication range of the micro chemical and bio
technologies.

High performance liquid chromatography
HPLC is one of the most desirable separation
methods. But integration of HPLC on a microchip
has been unreal because of some technical rea-
sons. The main reasons are the high pressure and
dead volume of connector between a high pressure
pump and a microchip. They are the reasons why
the monolithic column and capillary electrochromatographic CEC methods have been studied. But if a column packed HPLC system became available various kinds of techniques which are developed for separation analysis can be introduced into the micro chemical and bio technologies.

Therefore we have been trying to integrate a "packed micro channel" HPLC on a chip. And recently a high pressure durable and dead volume free connector and a rotary sampling valve at nano-liter levels were successfully developed and we realized the on-chip micro HPLC system. The plate number was above ten thousands and good separation ability was proved. As the system is pressure driven not only reversed phase separations like CEC but also normal phase separations are possible. The combination on-chip micro HPLC and UV-TLM detection system will be a powerful analytical system for micro chemical and bio technologies.

Biographical Sketch

Name    KITAMORI Takehiko    Birth date    June 1 1955    Nationality    Japanese
Affiliation    Department of Applied Chemistry School of Engineering The University of Tokyo 7-3-1 Hongo Bunkyo-ku Tokyo 113-8656 Japan
Tel    +81-3-5841-7231    Fax    +81-3-5841-6039    E-mail    kitamori@icl.t.u-tokyo.ac.jp
Title    Professor
Academic degrees
1980    B. S. The University of Tokyo Department of Pure and Applied Sciences Course of Physics and Mathematics
1989    D. Eng. The University of Tokyo
Business experiences and academic positions
1980    Research Staff Energy Research Lab Hitachi Ltd.
1989    Assistant Professor The University of Tokyo
1990    Lecturer The University of Tokyo
1991    Associate Professor The University of Tokyo
1998    Professor The University of Tokyo
        Project Leader of "Integrated Chemistry Project" Kanagawa Academy of Science and Technology Additional Post
Research fields and themes
Integration of chemical system on microchips applied laser spectroscopy for ultrasensitive detection analytical chemistry meso space chemistry
Memberships
Chemical Society of Japan American Chemical Society Japan Society of Spectroscopy Physical Society of Japan SPIE