Bioanalytical applications of low-volume enzyme assays

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Microreactors in chemistry

DOWNSIZING CHEMISTRY

Chemical analysis and syntheses on microchips promise a variety of potential benefits

Michael Freemantle

T he notion of putting a conventional, general-purpose chemistry laboratory onto a single microchip is possibly fanciful, but the miniaturization of chemical and physical processes and their integration onto such a chip for a specific application are a definite reality.

The development of microscale devices that can process and analyze minuscule amounts of samples and reagents is exciting the interest of increasing numbers of chemists. According to some, it could revolutionize chemical analysis and synthesis in the same way that microchips have revolutionized computers and electronics.

"The field is one that is growing very rapidly," says George M. Whitesides, a chemistry professor at Harvard University. "The first real applications will very probably be in analytical systems."

Many companies are racing to develop and commercialize tiny devices that will be used in chemistry. That fact has significant implications for instrument companies, which will have to go from a business of a few expensive sales to bulk sales of inexpensive systems.

According to Richard D. Klaus, vice president of Hewlett-Packard and general manager of its Chemical Analysis Group, the emerging lab-on-a-chip technology will revolutionize the drug discovery process in the same way that the processes of miniaturization and integration have recast the microelectronics industry.

The pharmaceutical industry is the main driver for developing this technology right now, observes J. Michael Ramsey, a corporate fellow and group leader at C. R. Bard, Inc.

Tiny reactors and ancillary devices may work where large equipment would be too risky or costly

MICROREACTORS FIND NEW NICHEs

Normally, chemical engineers take bench chemists' concepts and devices handling liters of reagents, and scale them up to industrial plants. But a tiny system into commercially available products should take about five to 10 years, says Wolfgang Ehrfeld, managing director of the Institute of Miniaturization Mass Goods (IMM) in Germany.

This static mixer, right, shows a fly's eye, is of truly microscopic dimensions. Such devices are being developed for use with downsized reactors, and high-exothermic or exothermic reactions, where conventional sized equipment would prove too dangerous.

Microvolume enzyme assays

- Enzymatic microreactors
- Micro and nanotiter plates
- Biosensors
- Electrophoretic assays
Objectives

To develop assays for enzyme-catalyzed reactions
- fast and low-cost
- microvolume
- capillary electrophoresis driven mixing and separation
- DAD and CMOS-based imaging detection
Electrophoretically mediated microanalysis

EMMA – electrophoretically mediated microanalysis, useful for fast reactions; first described by J Bao and FE Regnier (1992) J Chromatogr 608, 217. Several applications for enzymatic reactions have been presented.

Apparatus

- Capillary
- Cover slip
- Glue
- Phosphor
- Cylindrical lens
- Condenser lens
- Lens
- Glass slide
- Running buffer
- CMOS imager
- Deuterium lamp
- Filter 200 nm
EMMA visualization of GSH oxidation

\[ \beta > \alpha \]

collaboration with Maria Kulp
EMMA process visualization

Real time image acquisition for UV absorption detection in capillary electrophoresis.

injection of GSH followed by injection of H$_2$O$_2$
25 mM phosphate buffer, 25 mM SDS, pH 7.5
fused silica capillary, length 70 mm, i.d. 75 μm, potential -7 kV
window length 14 mm, UV detection at 200 nm

collaboration with Maria Kulp
Tyramine oxidase (TAO)

\[ \text{tyramine} + \text{O}_2 + \text{H}_2\text{O} \rightarrow p\text{-hydroxyphenylacetaldehyde} + \text{NH}_3 + \text{H}_2\text{O}_2 \]

\[ \text{KM (tyramine)} = 0.38 \text{ mM} \]
TAO: substrate specificity screening

- Substrates + enzyme
- Substrate-enzyme overlap
- Enzyme + substrates and products being separated

![Graph showing absorbance over time](image-url)
Penicillinase assay

$KM$ (penicillin G) = 3.7 mM
GOx in a plug-plug process

\[ \beta\text{-D-glucose} + \text{O}_2 \rightarrow \text{D-glucono-1,5-lactone} + \text{H}_2\text{O}_2 \]

\[ \beta\text{-D-glucose} + \textbf{1,4-benzoquinone} \rightarrow \text{D-glucono-1,5-lactone} + \textbf{hydroquinone} \]
1,4-Benzquinone reduction

GOx

0.01

0.1

0.5 mg ml\(^{-1}\)

245 nm (substrate)

290 nm (product)
Parallel capillary CE set-up
Parallel capillary electrophoresis

Reaction catalyzed by penicillinase (β-lactamase)
DAD vs CMOS detection

- Diode Array Detector (DAD)
  - commercialized for CE
  - complex architecture
  - enables capture of entire UV-Vis spectra in real time

- Complementary Metal Oxide Semiconductor (CMOS)
  - new class of miniaturized capillary detectors
  - integrates photoelements and electronics on single chip
  - enables multiplexing of capillaries
  - improved sensitivity
Advantages of electrophoretic assays

- Low volume of enzyme sample
- Low expenditure of chemicals
- Less waste produced
- Allow automation
- Higher throughput
- High resolution
- Implementation of existing CE instrumentation
Conclusions

- Electrophoretically mediated microanalysis allows monitoring of enzyme-catalyzed reactions in nanolitre volumes.

- CMOS active pixel sensors enable parallel enzymatic assays.
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