Urinary Assay Using Micro Flow Injection Analysis

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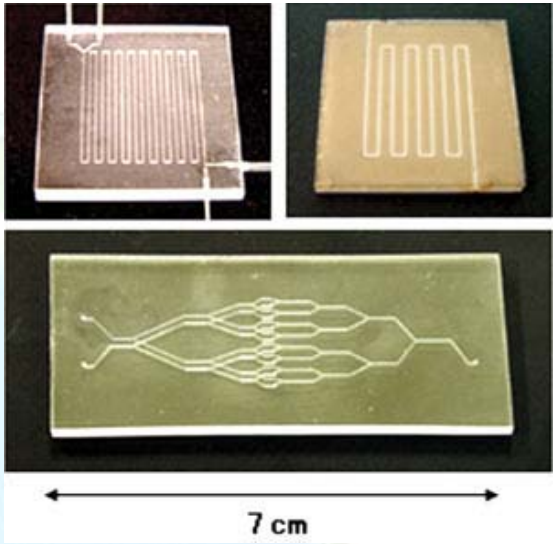
Micro Total Analysis System

➢Progress in μTAS

- Improvement of precise machining techniques
- Miniaturization of detectors



Micro Total Analysis System (μTAS)
Analytical system is developed on the chip

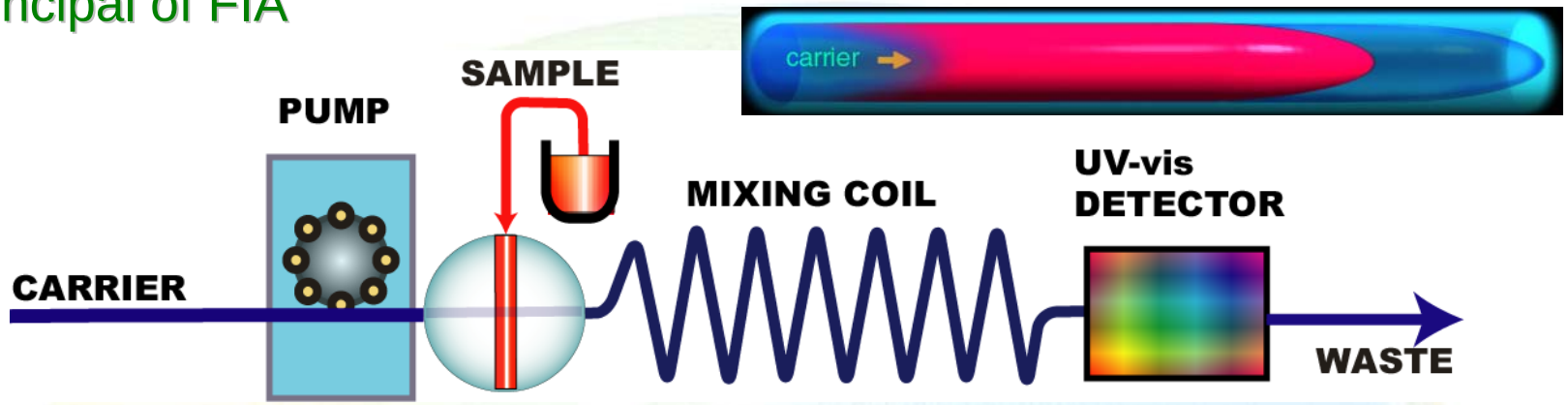
Lab-on-Chip
Sample and reagent injection, and reaction
Chemical laboratory is realized on the micro chip

μTAS → Portable analytical instruments
Decrease in sample, reagent, and waste volume
Shortening of assay time

Most of present μTAS is based on the flow injection analysis (FIA)

Flow Injection Analysis (FIA)

➢Principal of FIA



Liquid sample and reagent are added to the system, allowing the sample/reagent to be mixed and reacted.

The resulting product forms a concentration gradient corresponding to the concentration of the analyte in the sample.

When the reaction is color reaction, the absorbance of the product is to be measured with UV-VIS detector.

Purpose of the Present Work

➢Subject of the Present μTAS

Small versatile detector, such as UV-VIS, has disadvantages of selectivity and sensitivity. ⟷ High performance detector has disadvantages of size and cost.

The novel μTAS by small detectors with selectivity and sensitivity is required.

➢Purpose of the Present Work

Urinary assay system for the point of care testing (POCT) is investigated by μFIA technique.

- Urinary glucose and urinary protein are target analyte.
- UV-VIS is used as the detector.

Urine is multi-components system.
↓
Selectivity is required to the system.

→ Separation of glucose and protein is combined to the μFIA system as the pre-treatment part.

Determination of Urinary Glucose and Protein

➢Relationship between Urinary Glucose and Diabetes

	Healthy	Border	Diabetes
Before meal	～100 mg/dL	～100 mg/dL	100 mg/dL～
After meal	～100 mg/dL	～500 mg/dL	500 mg/dL～

➢Decision of Urinary Protein Test with Conventional Test Paper

Un-Detected	Border	Detected
～300 μg/mL	300～1000 μg/mL	1000 μg/mL～

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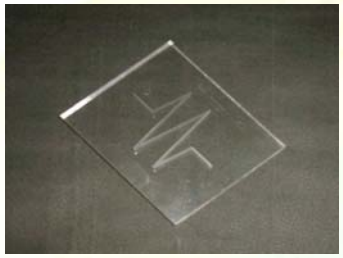
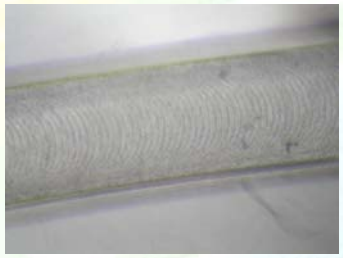
Experimental

➢Fabrication of the μFIA Chip

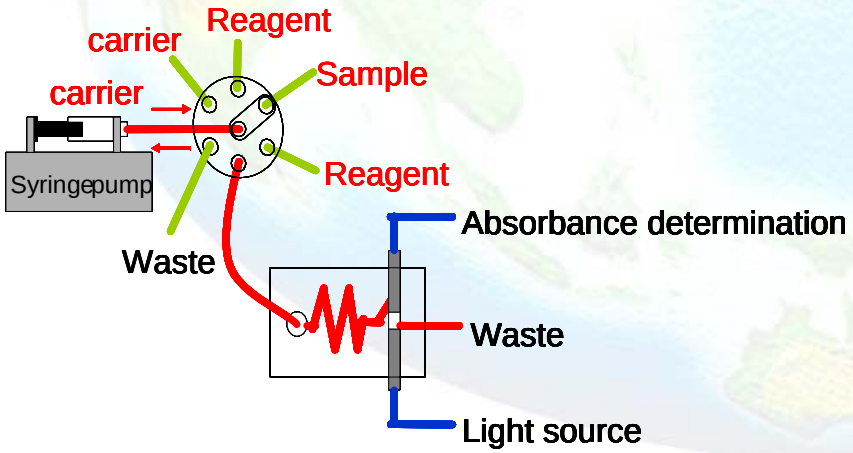
μFIA chip was fabricated on PMMA plate with precise machining center.

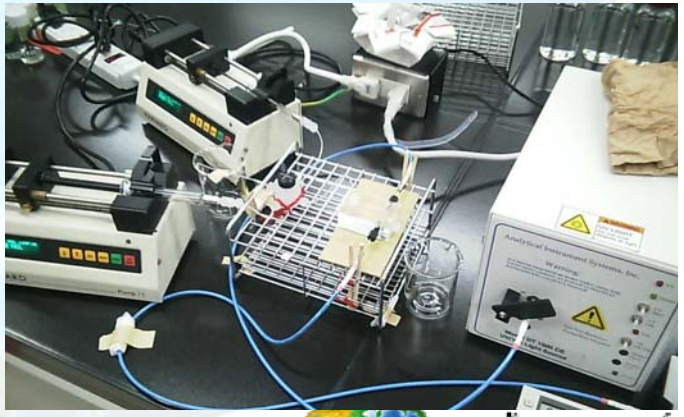




Width is 500 μm.

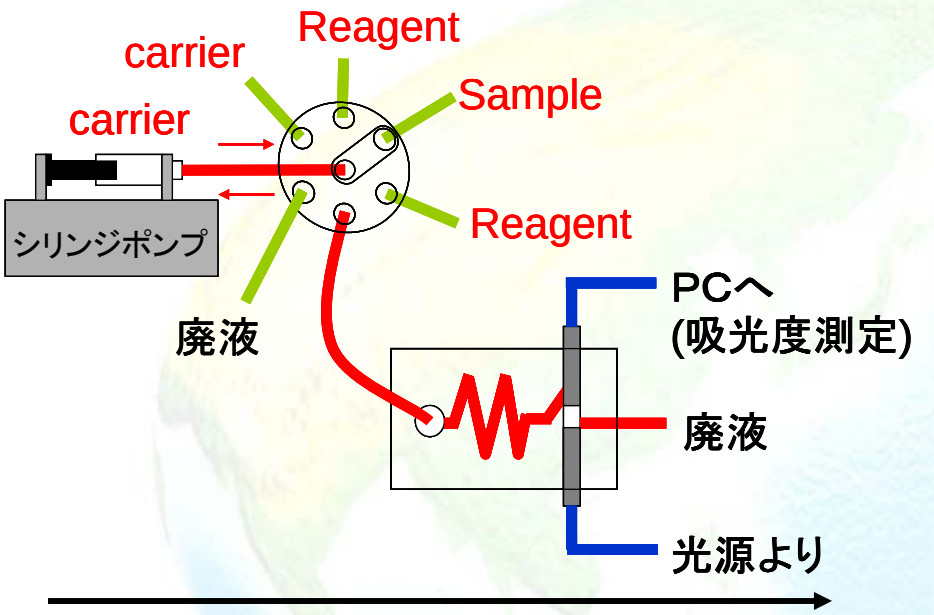
➢μFIA System



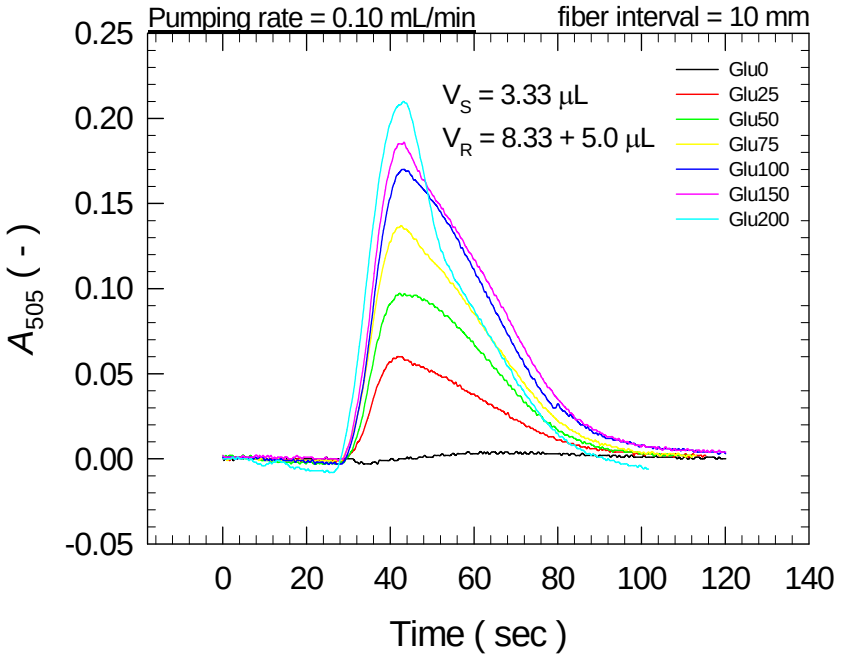


Single Component Assay with μFIA without Separation Part

➢Single Glucose Assay



Carrier	Reagent 8.33 μL	Sample 3.33 μL	Reagent 5.00 μL	Carrier



Typical FI peak was obtained for ca. 50 sec

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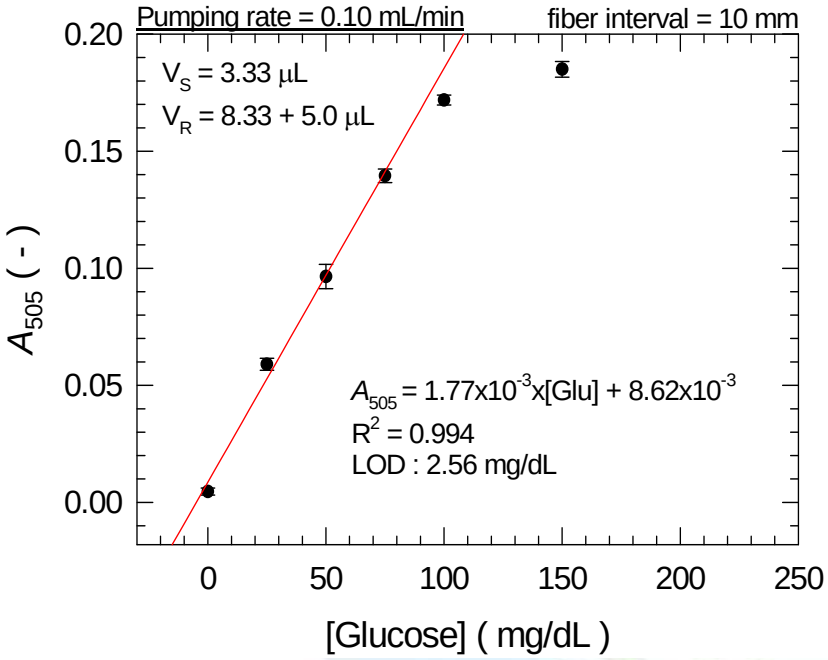
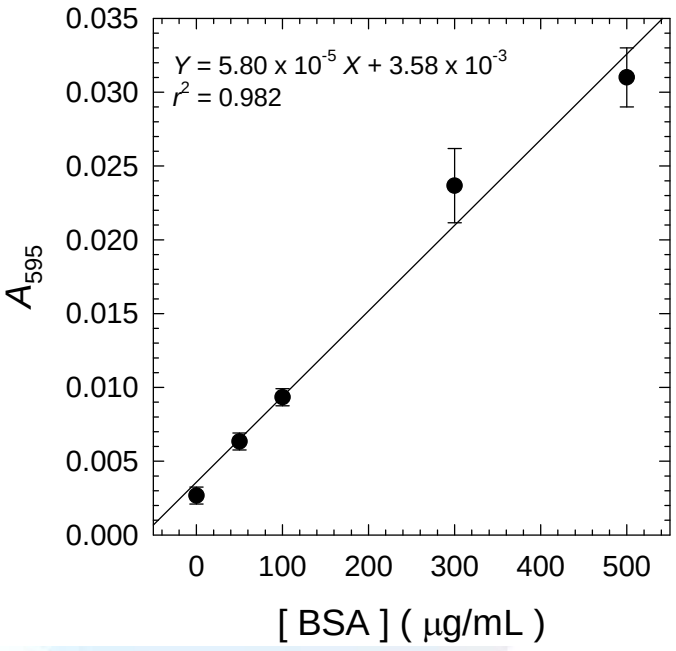
Single Component Assay with μFIA without Separation Part

➢Calibration Curve for Single Glucose



➢Calibration Curve for Single Protein



Present μFIA system works well for single component assay system

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Multi-Component Assay with μFIA without Separation Part

➢Determination in Artificial Urine

Component	Conc.
NaCl	1.0 g/dL
Urea	1.0 g/dL
Creatinine	0.05 g/dL
Albumin	300 μg/mL
Glucose	80 mg/dL
Acetone	160 mg/dL
Sodium Nitrite	0.1 mg/dL
Human Hemoglobin	30 μg/dL

When the concentrations of glucose and protein are determined using calibration curve obtained by single component system,

166 μg/mL of protein is determined.

34.9 mg/dL of glucose is determined.

The interference with other components progresses.

The separation of the analyte from other urinary components is necessary prior to the detection.

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Adsorptive Separation System

Adsorption or Ion exchange are suitable to be combined with FIA system.

Activated alumina is used
for glucose separation.

Adsorption is achieved in pH = 3~11.
Elution is achieved with water.

Hydroxyapatite is used
for protein separation.

Buffer solution should be used, because both pH and ionic strength affect to the adsorption.

Adsorption is achieved with low buffer concentration (pH < 6.5).

Elution is achieved with high buffer concentration (pH > 6.3).

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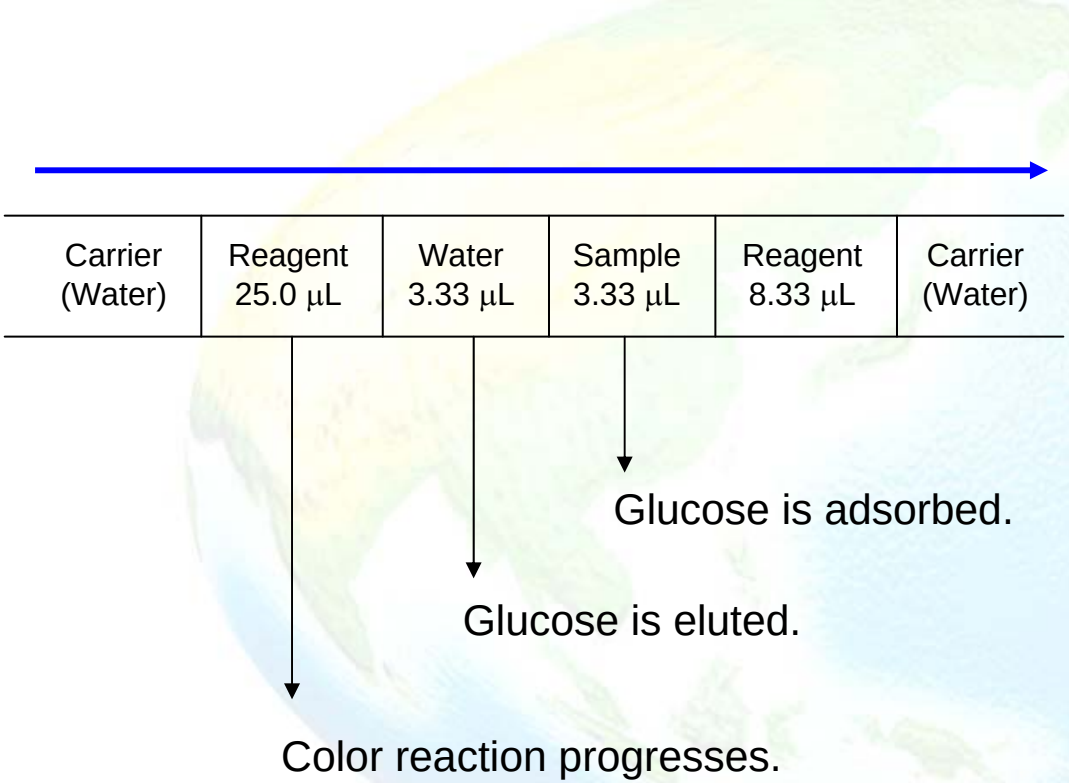
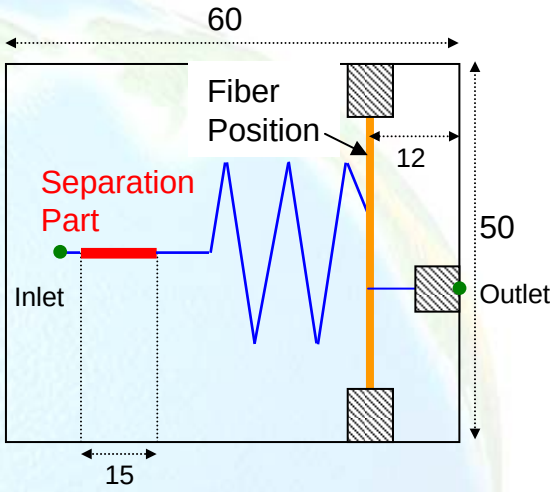
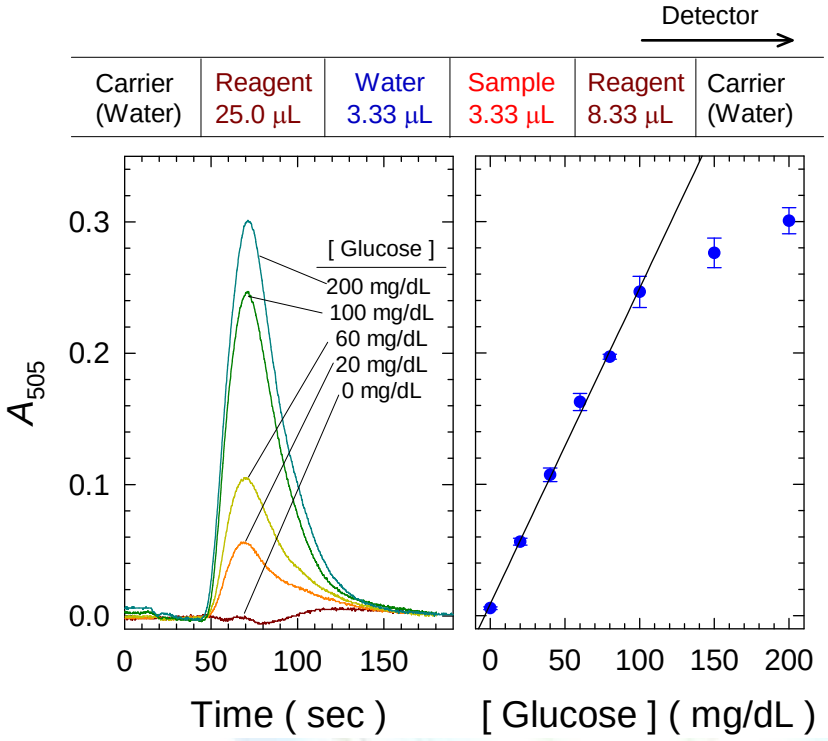
Urinary Glucose Assay with μFIA with Separation Part

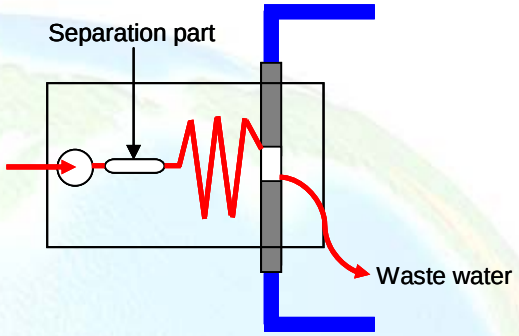
➢Scheme of μFIA chip





Urinary Glucose Assay with μFIA with Separation Part



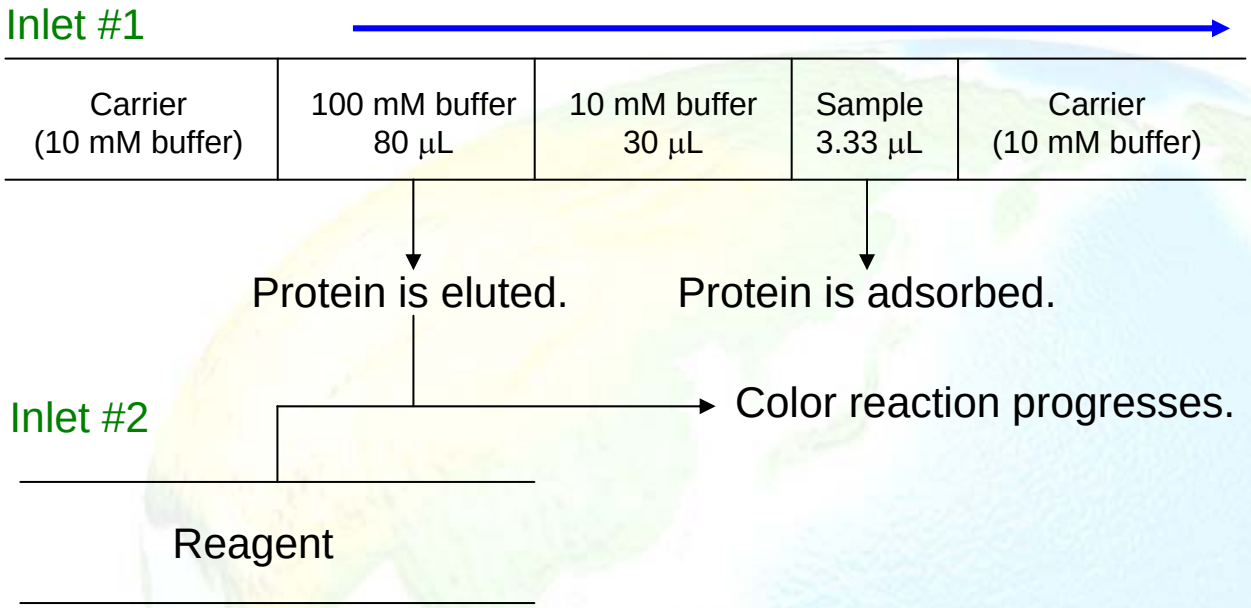


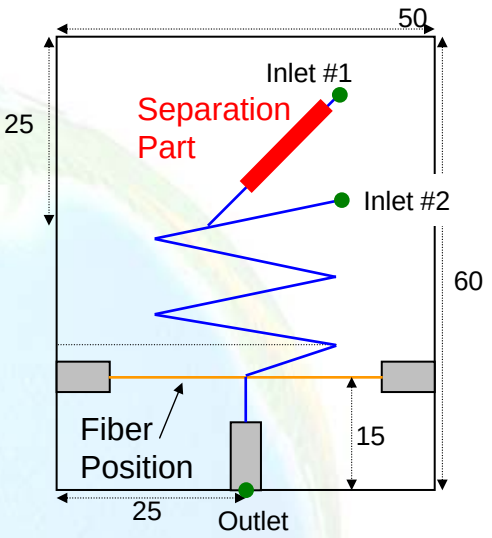
The glucose can be determined after the adsorption-elution process with activated alumina adsorbent.

When the artificial urine ([Glu] = 80 mg/dL) was applied, 85.3 mg/dL of glucose was determined.

The interference with other components can be suppressed with the present simple analytical system.

Urinary Protein Assay with μFIA with Separation Part

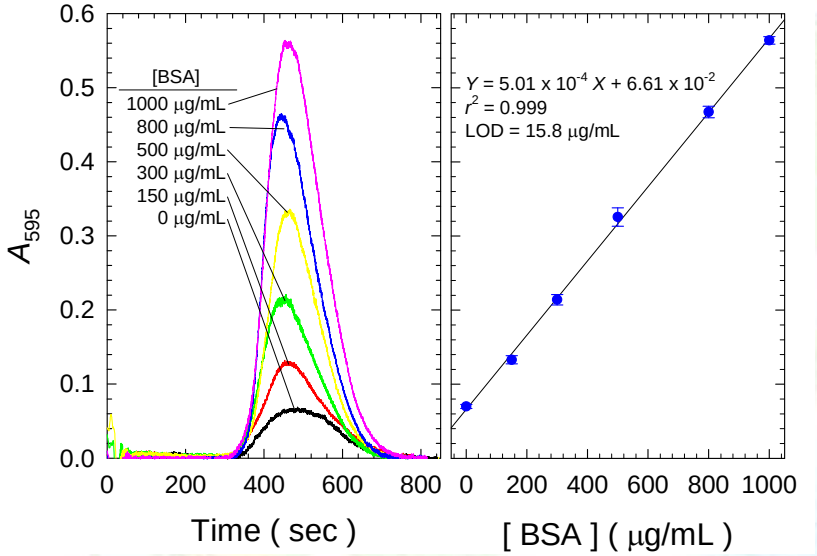




Two inlets, for sample and reagent stream, were used since hydroxyapatite was dissolved by acidic reagent solution.

The protein eluted from the separation part was reacted with the reagent after the confluence.

Urinary Protein Assay with μFIA with Separation Part



The protein can be determined after the adsorption-elution process with hydroxyapatite.

The pH adjustment is important for the effective adsorption-elution.
The dilution with 10 mM buffer is required to adjust pH value for the artificial urine.

When the artificial urine ([BSA] = 300 μg/mL) was applied after 21 times dilution, 252 μg/mL of protein was determined.

The interference with other components can be suppressed with the present simple analytical system.

Conclusion

Urinary assay system with μFIA system was investigated, with following results.

(1) μFIA system can be developed on the PMMA plate, based on the spectrophotometry.

(2) Interference with the co-existing components in the urine progresses with the conventional protocol.

(3) The actual concentration of the analyte can be determined, when the separation part is combined to the μFIA system.

(4) The present μFIA system with separation part can be the template for the simple analytical system with the sensitivity and selectivity.

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