**Development of an evaluation system for L-tyrosinase activity based on a flow injection analysis**

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An evaluation system based on a flow injection analysis for tyrosinase activity was developed for screening of tyrosinase inhibitors from natural products, and the system was applied to screening of the inhibitors from herbal medicines.

Tyrosinase (monophenol monooxygenase, EC 1.14.18.1, from mushroom) was purchased from sigma (Japan), and was covalently immobilized onto alkylaminated CPG (Controlled-Pore glass) as previously described [1]. The immobilized preparations were packed into a small polymer column and then mounted in a water-jacketed holder. The enzyme column (0.1 ml packed volume) was used as a recognition element for L-tyrosine. A schematic diagram of the flow system was shown in Fig. 1. The system was assembled with a double-plunger pump (flow rate 1.0 ml/min), a Teflon rotary injection valve equipped with a 100 μl sample loop, the tyrosinase column surrounded by the water jacket maintained at 303 K, the flow-through type of an oxygen electrode. Phosphate buffer solution (50 mM, including 1.0 M NaCl, pH 6.5) as the carrier was continuously pumped through the system.

A 100 μl of 1.0 mM L-tyrosine solution was injected into the FIA system, and decrease in dissolved oxygen caused in the tyrosinase-catalyzed reaction was monitored by the polarographic oxygen electrode and then recorded. Subsequently, a 100 μl of 1.0 mM L-tyrosine solution was introduced into the system followed by injection of sample solutions (screening source). Inhibition ratio of the sample was evaluated from a comparison between the activity before the sample injection and after. At this step, if the effect of the inhibitor holds out, no activity of tyrosinase will be obtained when L-tyrosine solution was injected after adding of L-tyrosine. In contrast, if the inhibitors react temporarily, reactivation of the tyrosinase will be observed by injecting L-tyrosine after introduction of one.

Then, we investigated the inhibition effect of kojic acid being well known as inhibitors of L-tyrosinase with use of the proposed FIA system. A 100 μl of L-tyrosine solution was injected into the system followed by a 100 μl of 5 mg/ml kojic acid solution. No noticeable activity of L-tyrosinase was observed. However, L-tyrosinase was reactivated by injection of L-tyrosine again. These results indicated that kojic acid should inhibit the L-tyrosinase activity completely, but the effect should be temporarily. Therefore, L-tyrosine inhibitors having long-term inhibition for L-tyrosinase activity were screened from herbal medicines with use of the FIA system and the results were shown in Table 1. These results indicate that many promising inhibitors of L-tyrosinase of which activity was stronger than that of kojic acid could be monitored with use of this FIA system based on an evaluation of reactivation of the enzyme.

[1] Y. Iida, T. Kikuchi, and I. Satoh, *Sens. Actuators B*, **91**, 175-179 (2003).

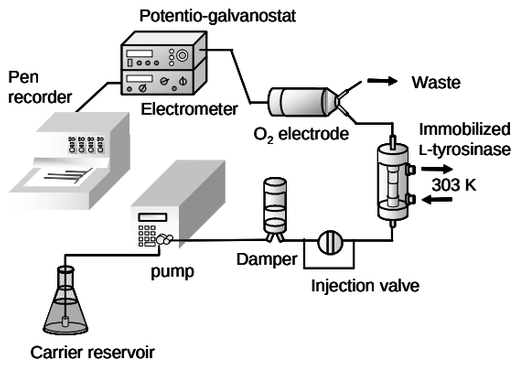


Fig. 1 Schematic diagram of the FIA system.

Table 1 Evaluation of inhibition and reactivation ratios of various herbal medicines against an L-tyrosinase activity.

| Medicinal Plants | Inhibitory potency: Inhibition ratio | Inhibitory potency: reactivation ratio |
|---|---|---|
| ***Hydrangea macrophylla*** **(folium)** | **+++** | **+++** |
| ***Glycyrrhiza uralensis*** **(radix)** | **+++** | **+++** |
| ***Sophora flavescens*** **(radix)** | **++** | **++** |
| ***Curcuma longa*** **(rhizoma)** | **++** | **+++** |
| ***Morus alba*** **(cortex)** | **++** | **+++** |
| ***Rheum palmatum*** **(rhizoma)** | **++** | **+++** |
| ***Eriobotrya japonica*** **(folium)** | **++** | **+++** |
| ***Cimicifuga dahurica*** **(rhizoma)** | **++** | **++** |
| ***Sweria japonica*** **(herba)** | **+** | **++** |
| ***Cassia angustifolia*** | **+** | **+++** |
| ***Polygala tenuifolia*** **(radix)** | **+** | **+++** |
| ***Carthamus tinctorius*** **(flos)** | **+** | **+++** |
| ***Aconitum carmichaeli*** **(tuber)** | **+** | – |
| ***Ostrea gigas*** **(testa)** | **+** | **–** |
| ***Magnolia obovata*** **(cortex)** | **+** | **+++** |
| ***Anemarrhena asphodeloides*** **(rhizoma)** | **–** | **–** |
| ***Scutellaria baicalensis*** **(radix)** | – | – |
| ***Platycodon grandiflorum*** | **–** | **–** |
| ***Paeonia lactiflora*** | **–** | **–** |
| ***Arctostaphylos uva-ursi*** **(folium)** | – | – |
| ***Coptis chinensis*** **(rhizoma)** | **–** | **–** |
| **Kojic acid** | **+++** | – |

Inhibition ratio: +++ 71-100 %, ++ 41-70 %, + 11-40 %, - 0-10 %

reactivation ratio: - 71-100 %, + 41-70 %, ++ 11-40 %, +++ 0-10 %