CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 42, Issue 10, October 2014
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2014, 42(10), 1535–1538.

RESEARCH PAPER

Determination of Na^+, K^+ and Ca^{2+} of Apoplast of Poplar Stems by Atomic Absorption Spectrometry with Microdialysis Method

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Abstract: The dialysis for three species of populus stems apoplast was carried out with a microdialysis system, and the concentration of Na^+, K^+ and Ca^{2+} in the dialysate was directly measured by the graphite furnace atomic absorption spectrometry (GF-AAS). The flow rate of perfusate was 1 μL min^{-1} and the cut-off molecular weight of probe was about 30 kDa. The test technique was applied to the research of poplar biochemical physiology due to its perfect characteristic of real-time and non-destructive detection. The recovery of the method was 95.8%–103.1%. The content of Na^+ in *Populus wutunensis*, *Populus simonii* × *P.euphratica"* × *P.sp* and *Populus simonii* × *(Populus pyramidalis* + *Salix matsudana)* were 1034–1156 μg L^{-1}, 1493–1611 μg L^{-1} and 1586–1703 μg L^{-1}, respectively, K^+ was 1012–1237 μg L^{-1}, 941–964 μg L^{-1} and 1095–1201 μg L^{-1}, and Ca^{2+} was 4976–5237 μg L^{-1}, 4786–5042 μg L^{-1} and 5893–6142 μg L^{-1}. This method could provide reliable data for the investigation of the change of ion concentration of plant to all kinds of external environment stress.

Key Words: Popular; Apoplast; Microdialysis; Metal ion

1 Introduction

The plant apoplast is composed by the fiber of cell wall of outside of cell membrane, the microcrystal space and the cell space filled with water and air composition, also including the differentiation of xylem[1]. The apoplast of higher plant is a dynamic space, and many important physiological and biochemical processes and reactions such as solute transport, nutrient activation, resistance to adversity occurred in the apoplastic[2]. There is a close relationship between extracellular in vivo physiological mechanism of ion and the dynamic changes of the plant such as transpiration, cell signal transduction, and cell ion compartmentalization. However, because the apoplast of plant tissues (only a relatively small proportion of the total volume of plant tissue 8%–15%) cannot be separated easily from the plant tissue and there is a water and material balance with the cytoplasm in membrane, until now, there is no efficient method for the fast, simple, accurate determination of indexes of the plant apoplast. The present methods for the analysis of plant apoplast have some disadvantages such as easily polluted in extracting process, apoplastic components change, not on localization of the apoplast, and the obtained results could not reflect the quality of the outer body components of the dynamic changes[3]. To solve the above problems, microdialysis (MD) sampling technique was widely introduced for the sampling of various parts of animal and human samples[4], specially for in vivo analysis and continuous detection of extracellular fluid, due to its features such as living, in situ sampling, real-time and on-line detection[5]. The scheme of microdialysis principle is shown in Fig.1. Although the application of MD in plants sampling was rarely reported and only found it was used in the research of stems layer of Norway spruce, peas and plum fruit[6–9], some positive evaluation was given to the application

Received 12 June 2014; accepted 8 July 2014
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This work was supported by the National Natural Science Foundation of China (No. 30872004), and the Fundamental Research Funds for the Central Universities of China (No. DC12010305&12010204).

DOI: 10.1016/S1872-2040(14)60775-1

of MD in plant. A variety of physiological and biochemical reaction is directly influenced by the dynamic changes of ion in apoplast, by which the dynamic changes are close related to plant transpiration, cell signal transduction, cell ion compartmentation and other physiological mechanism. Calmodulin was considered as the multifunctional receptor protein of intracellular calcium signaling and the cell body of the second messenger. The free Ca^{2+} constitutes the calcium messenger system in plants and plays an important role in the life of plant, for instance, the response of high plants to the stimulation of outside environment. Under stress conditions, especially under salt stress, the concentrations of Na^+ and K^+ in plants determine the salt tolerance of plant.

Microdialysis (MD) was used to describe a similar extraction technology as the dialysis technology[10]. The basic principle of MD is the same as dialysis, in which the small molecules diffuse along the concentration gradient through a semipermeable membrane. As shown in Fig.1, a new concentric probe, with a semipermeable membrane material made of different molecular weight cut-off, was directly embedded in the tissue region to fill isotonic perfusion into the probe at a constant rate. When the fluid flows through the dialysis membrane at the probe tip, the bioactive substances with smaller molecular mass that can pass though the semipermeable membrane in the outer membrane of probe will diffuse across into the dialysis tubing according to the concentration gradient and then are continuously taken away by the perfusate, realizing the goal of sampling from the living tissue.

Since pure plant apoplast could be obtained conveniently and quickly with MD system[11], in this experiment, MD method was used to obtain three kinds of apoplast sap of poplar stems, and Na^+, K^+, Ca^{2+} concentrations in the samples were detected by atomic absorption spectroscopy to investigate the physiological and biochemical changes of Poplar in real time.

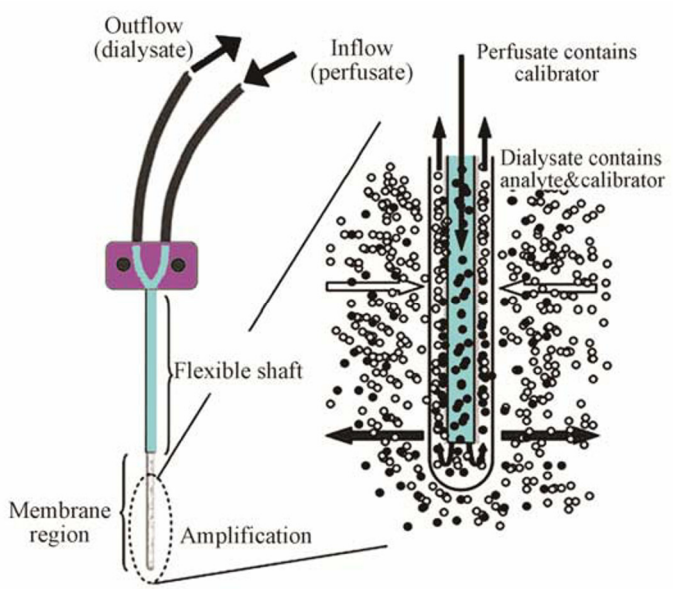

Fig.1 Schematic diagram of microdialysis probe of concentric design (The magnified membrane region illustrates net diffusion of a compound (analyte) of interest (open circles) into the probe, and the net diffusion of the calibrator (closed circles) from the probe to the extracellular space)[12]

2 Experimental

2.1 Instruments and reagents

Z-2000 atomic absorption spectrophotometer and hollow cathode lamps of calcium, potassium and sodium were purchased from Hitachi Company (Japan). CMA400 microdialysis devices (Sweden), micro injection pump (BASi, USA) and probe (polyacrylonitrile membrane, molecular weight cut-off of 30 kDa, BASi, USA) were used in the experiment.

2.2 Sample pretreatment

At the beginning of April, the cuttage strips of *Populus simonii×P.euphratica×P.sp, Populus simonii×Populus pyramidalis+Salix matsudana* and *Populus wutunenses* were collected from Shenyang and inserted in the flower pots (inner diameter 20 cm, high 16 cm, a strip per pot). Humus soil in forest, canopy for protection of rain and prevention of plant diseases and insect pests were used for the carefully cultivation of the three poplars.

The strips were watered fully every 4 days. After cultivated for 40 days, the cuttage strips were as high as 12–15 cm and the leaves of plant were 12–16. The microdialysis sampling was carried out for the 3 varieties. The test was repeated 3 times.

2.3 Microdialysis method

Microdialysis equipment adopted two sets, Swedish CMA400 and USA BASi microdialysis devices, including micro-injection pump, 3 injectors, 3 microdialysis probes, 3 liquid inlet pipes and 3 liquid outlet pipes. A liquid inlet pipe and a liquid outlet pipe were used to connect the inlet and outlet of the probe and Millipore-Q ultra-pure water as the perfusion was filled into the tube at 1 μL min^{-1}. When the liquid outlet pipe head began to water, the rigid guide tube at 30°–45° angle was inserted into the central plant stems to prevent damage of microdialysis probe. After implanted the probe, the liquid outlet pipe end was put into a centrifuge tube to collect the dialysate. Then the wound of the stem was sealed immediately with dental base acrylic resin liquid (take a small amount of self-curing powder, amount of denture base resin liquid mixing, and sticky) to prevent loss of the fluid, which could ensure the consistency of the perfusate flow rate