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博　士　学　位　论　文

物理化学在芯片实验室中的几个应用

Several Applications of Physical Chemistry in Lab-on-Chip

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目 录

CONTENTS

摘 要

芯片实验室（Lab on a Chip）是一个多学科高度交叉的研究新领域，它采用微加工技术将传统的化学、生物实验室缩小并集成到芯片的尺度，为化学生物过程的研究提供一个微型化的平台。由于具有微型化、集成化、自动化、分析速度快、样品消耗少等诸多优点，芯片实验室在药物筛选、临床诊断、环境监测等领域具有巨大的应用前景。

芯片实验室的单元尺寸一般为微米至亚毫米量级，该尺度下许多物理化学规律将发生显著变化，表现在许多与表面相关的性质，如浸润性、电渗及表面张力等成为主要因素。研究芯片中这些特殊表面性质，对于进一步了解芯片中微观物理化学现象与规律及开发新的微单元操作技术具有重要意义。因此物理化学不仅为芯片实验室领域特殊的流体性质及表面现象提供理论解释，还为开发芯片的新技术和拓展应用范围提供技术支持。

微流控芯片（microfluidic chip）技术与微阵列芯片（microarray chip）技术是目前芯片实验室领域的研究热点和重点。本论文工作主要目的在于从物理化学角度出发，利用物理化学的原理和技术，针对微流控芯片的制作、样品预浓缩、分离检测等单元操作中遇到的问题提出新的解决方法；针对微阵列芯片中严重影响芯片质量的咖啡环效应进行研究和提出解决对策。具体研究内容如下：

1．微流控芯片快速制作方法研究

目前玻璃微流控芯片主要采用光刻工艺进行制备，存在成本较高、耗时较长等问题，因而限制了玻璃芯片在普通实验室的使用和研究。我们提出将电化学刻蚀方法应用于玻璃芯片的快速加工。采用体相存储有电解液、表面具有微结构的琼脂糖凝胶为模板，对玻璃基片表面的金属掩蔽层进行电化学刻蚀，可在 120 秒钟内获得宽度小于 50μm 的掩蔽层窗口，从而实现高精度的玻璃微通道的简便及快速加工。类似地，采用具有微缝图形的 PET 掩模板进行微细电解，可在 10 秒

钟内获得宽度小于 100μm 的掩蔽层窗口，结合湿法腐蚀与热键合，也可实现玻璃微流控芯片或微电极阵列的低成本与快速制作。

高分子聚合物芯片的模塑法批量制作需要大量模具，而目前缺乏芯片模具的快速制作技术。我们提出热压法快速制作 PMMA 阳模，并用于 PDMS 微流控芯片制作。该方法可在 30 分钟内获得一片高质量的芯片模具，同一模具的微凸起结构上不同位置的高度和宽度 RSD 分别为 1.46%和 0.42%，利用该模具批量制作的 PDMS 微流控芯片，微通道结构特征参数的 RSD 均小于 3%。

激光直写加工可降低微流控芯片的研究门槛，特别适合于设计思想快速多变的高聚物芯片研发阶段。我们建立CO_2激光直写快速加工PMMA芯片的工艺，30 分钟可制作一个芯片。考察了激光功率、扫描速度及加工次数等加工参数对芯片质量的影响，发现并解决了激光加工过程中重铸物所导致的通道堵塞问题。

2．微流控芯片的纳滤膜集成及应用

纳滤膜已在工业领域得到广泛应用，但主要采用压力驱动实现膜分离，在微流控芯片中，电驱动更容易集成与实施。我们研究纳滤膜在电场驱动下对不同离子与氨基酸的截留特性，结果表明所用商品化纳滤膜对不同价态的离子具有选择透过性，对FITC标记的氨基酸截留率接近100%。

提出采用CO_2激光烧蚀方法结合热键合技术将商品化纳滤膜与PMMA芯片集成制作一种预浓缩电泳芯片，并实现了氨基酸样品的在线浓缩与分离，浓缩倍数可达1000倍以上。

设计并制作用于界面聚合纳滤膜的玻璃微流控芯片，利用层流效应在微流控芯片中原位制备纳滤膜。

3．超亲水氧化铝的制备与机理研究

超亲水表面的制备与机理是目前固体表面物理化学领域的重要问题之一。我们提出并建立电化学阳极氧化法制作超亲水氧化铝表面的工艺。通过控制不同的氧化条件制备多种表面结构和粗糙度的氧化铝，系统研究表面结构与浸润性的构效关系，发现“ 鸟巢 ”结构的形成是氧化铝表面达到超亲水性的关键因素，从实验上，证明了Bico等人提出的“ 3D capillary effect ”理论模型。

4．超亲水/超疏水图案化表面在芯片实验室中的应用

“咖啡环效应”严重影响微阵列芯片的质量。我们提出并研究亲水/疏水图案化表面对“咖啡环效应”的抑制，发现图案化表面对“咖啡环效应”的抑制效果与组成图案两种表面的表面自由能差异成正比。从理论上和实验上证明了“咖啡环效应”的抑制作用是通过降低液滴边缘的蒸发速度，从而抑制从内向外的毛细管流。该结果除了可用于显著改善微阵列芯片上样品点均匀性外，还在涂料、色谱等方面具有重要的研究和应用意义。

开放式微流控芯片在操作上具有取样灵活等优点。我们提出将超亲水/超疏水图案化表面应用于制备开放式微流控芯片，并在芯片上组装琼脂糖凝胶，进行凝胶电泳分离的初步探索。

5．微流控芯片电泳系统的研制与应用

以倒置荧光显微镜为平台，搭建一套芯片电泳分离与检测系统。其中，自行设计和制作了用于芯片电泳的四路程控高压电源和与显微镜接口的小型PMT检测器，并将荧光显微镜的激发光源从汞灯改造成寿命更长，价格低廉的发光二极管。利用该系统考察了玻璃微流控芯片的电渗流特性，实现FITC等荧光样品的芯片夹流进样与电泳分离，FITC检测限为1×10^{-7} mol/L。

关键词：芯片实验室；微流控芯片；微阵列芯片；纳滤膜；超亲水/超疏水；咖啡环效应。

ABSTRACT

Lab-on-chip is a multi-disciplinary technology, which adopted microfabrication technology to miniaturize the traditional laboratory into chips and provided platforms for studying the processes of chemistry and biology. Due to the advantages such as miniaturization, integration, automotivation, fast-analysis and low-consumption, lab-on-chip has been found widespread application prospect in many fields including drugs screening, clinical diagnostics and environmental monitoring.

The structural unit size of chips is about micrometer to sub-millimeter, at this scale, many physical chemistry laws change significantly. One category of the prominent characteristics is surface phenomena, such as wettability, electroosmosis and surface tension, which will dominate many performances in micro-system. Studying on these surface characters would be helpful for understanding of the physical and chemical laws in micro-scale and developing new unit operations for micro-technology. Therefore, physical chemistry is an important subject closely related to lab-on-chip, which provides the theoretical and technical support for not only the special surface phenomena but also the development and application of micro chips.

Microfluidic chip and microarray chip were the researching hotspots and emphases of lab-on-chip. The main purpose of this thesis is to develop new unit manipulation methods and technologies in microfluidic chips such as fabrication, sample pretreatment, separation and detection based on principles and technologies of physicochemistry. Furthermore, coffee ring-stian effect, which could seriously influenced the quality of microarray chips was investigated and resolved by surfaces with special wettability. The main work and results are summarized as follow:

1. Rapid fabrication technology for microfluidic chips

A novel process technology based on electrochemical micromachining was

developed to fabricate rapidly glass-based microchips, in order to alleviate the cost- and time-consuming process of lithography during fabrication of glass microchips. Using the electrochemical etching process by means of patterned agarose mould, microchannel with width less than 50 micrometer could be fabricated in 120 seconds, and high-accuracy micromachining was achieved. Combined with wet-etching and thermal bonding technology, glass-based microchips could be rapidly fabricated with low-cost.

A hot embossing technology was develope to rapidly fabricate PMMA-based moulds for PDMS-based microchips, in order to solve problems that large numbers of moulds were necessary for mass production of polymer chips. Using this technology, a PMMA mould with high quality could be obtained in less than 30 minutes, and the relative standard deviation (RSD) value of the height and width of the convex structure in PMMA moulds was only 1.46% and 0.42%. Replicated from the PMMA mould, PDMS microfluidic chips could be fabricated, and the RSD value of the characteristic parameter of the micro-channel on the chips were all less than 3%.

CO_2 laser direct-write technology was established to fabricate PMMA-based microchips which could be done in 30 minutes, in order to keep up with the fast-changing designs in laboratory studies. The effect of laser power, frequency and writing speed on the quality of the chips was evaluated. Furthermore, we found and solved the problem that in thermal bonded chips, the recast layer would block the channel in the cross section area.

2. Integration of nanofiltration membrane with microfluidic chip

The rejection characteristics of inorganic ions and amino acids by nanofiltration membranes under an electric field were investigated. The results showed that the nanofiltration membrane had different permselectivity for the ions with different valences, and the rejection rate of FITC-labled amino acids reached almost 100%.

A PMMA-based microchip integrated with commercial nanofiltration membrane was developed through CO_2 laser ablation and thermal bonding. Electrophoretic preconcentration and separation of amino acids was achieved on the chip, FITC-labeled amino acids could be preconcentrated for more than 1000 times.

A glass-based microfluidic chip was designed and fabricated for preparation of nanofiltration membranes by means of interfacial polymerization. In-situ preparation of nanofiltration membrane was studied in the microchip based on the laminar flow effect.

3. Preparation and mechanism research of superhydrophilic aluminum oxides

An electrochemical method was developed to prepare superhydrophilic aluminum oxide surfaces. By controlling the electrochemical conditions, anodic aluminum oxide (AAO) films with different surface structures and roughenesses could be obtained, and the relationship between the morphology and wettability of the AAO films was investigated. We found that the formation of "bird's nest"-liked microstrucure was necessary for the superhydrophilicity. The results provided experimental evidence for "3D capillary effect" theory proposed by Bico et al.

4. Applications of superhydrophilic/superhydrophobic patterned surface in lab-on-chip

An etching technology based on oxygen plasma was developed to prepare hydrophilic/hydrophobic patterned surfaces. Suppressing the "ring-stain effect" with these patterned surfaces was proposed and investigated. We showed that "ring-stian effect" decreased with the increasment of surface free energy differences between hydrophilic and hydrophobic regions, so that the spot uniformity of microarrys could be improved significantly by superhydrophilic/superhydrophobic patterned surfaces. Furthermore, the ring-suppressing mechanism was explained theoretically and experimentally, and we finally revealed that coffee ring stain was suppressed through suppressing the outward capillary flow inside the drying droplet on patterned substrate,