

基于可逆加成-断裂链转移聚合的孕酮分子印迹膜制备与检测

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2017-08-17 收稿, 2017-09-20 修回, 2017-09-22 接受, 2017-11-09 网络版发表

国家自然科学基金(20771015)和高等学校学科创新引智计划(B07012)资助

摘要 基于可逆加成-断裂链转移(reversible addition fragmentation chain transfer, RAFT)聚合原理, 通过紫外光引发方式, 以2-(十二烷基硫代碳酸酯基)-2-甲基丙烯酸(DDMAT)作为唯一的控制试剂, 在DDMAT修饰的金膜表面合成了针对孕酮分子的分子印迹物聚合物(molecularly imprinted polymers, MIPs)薄膜, 并作为表面等离子体共振(SPR)传感器的识别单元. 通过现场原位监控MIPs薄膜生长动力学, 使其厚度得到有效控制. 聚合动力学研究表明, DDMAT在紫外光引发聚合过程中起到引发剂和链转移试剂双重作用. 对MIPs薄膜表面进行接触角、红外光谱、扫描电子显微镜表征, 结果表明通过紫外光引发的方式能够有效地将MIPs薄膜接枝到DDMAT修饰的金膜表面, 且MIPs薄膜厚度均一, 其表面布满的纳米尺寸孔径的孔穴, 为识别孕酮分子提供了通道. 在pH 7.4的PBS缓冲液中对浓度范围 10^{-12} ~ 10^{-7} mol/L的孕酮样品进行检测, 结果表明该MIPs薄膜修饰的传感器对孕酮分子具有较高的灵敏度, 检测限为 3.24×10^{-13} mol/L(信噪比, $S/N=3$), 且具有良好的选择识别性能和重复使用性能; 稳定性实验结果显示, MIPs薄膜修饰的SPR传感器芯片在 N_2 气保护下, 室温存储30 d对低浓度的孕酮分子样品仍具有良好的信号响应. 实际水体及人工尿液中测得孕酮回收率为92.9%~96.5%, 说明此传感器可用于实际样品测定.

关键词 可逆加成-断裂链转移聚合, 分子印迹物, 孕酮, 水相兼容, 表面等离子体共振传感器

分子印迹聚合物(molecularly imprinted polymers, MIPs)是模拟自然界抗原-抗体作用原理, 人工合成的针对模板分子具有选择性识别能力的高分子材料, 其内在形成的识别孔穴在识别位点、空间尺寸上都与模板分子相吻合^[1]. 目前, 大多数MIPs制备仍采用传统的自由基聚合(traditional free radical polymerization, TFRP), 主要因为该方法可选取的单体广泛且反应条件温和. 但是TFRP由于不可逆的链转移终止反应的发生, 使所得的MIPs分子量分布较宽、均一性差、部分识别位点被包埋^[2], 这将进一步影响MIPs的

识别能力和吸附能力. 此外, 为了保持MIPs在水环境中的稳定性和识别位点, 采用TFRP制备MIPs中使用的交联剂比例较高, MIPs难以对目标分子进行快速识别. 为了克服上述缺点, 研究人员将可逆加成-断裂链转移聚合(reversible addition-fragmentation chain transfer, RAFT)引入MIPs的制备过程. RAFT是活性/可控自由基聚合方式的一种, 能够通过增长链自由基与高分子聚合物链之间的动态平衡过程, 使增长链自由基发生可逆链转移形成“休眠”状态的高分子链, 当新单体加入时原高分子链能够继续增

引用格式: 王洋, 焦少勤, 陈小丽, 等. 基于可逆加成-断裂链转移聚合的孕酮分子印迹膜制备与检测. 科学通报, 2018, 63: 68–77

Wang Y, Jiao S Q, Chen X L, et al. Preparation and detection of progesterone by molecularly imprinted film based on reversible addition fragmentation chain transfer polymerization (in Chinese). Chin Sci Bull, 2018, 63: 68–77, doi: 10.1360/N972017-00875

长^[3]。相比TFRP方法,采用RAFT方法制备的MIPs结构均一,选择性和吸附脱附更快^[4],且不需要高比例的交联剂同样可以保持MIPs在水环境中的稳定性,实现在水相中对目标物检测^[5,6]。

MIPs作为传感器的敏感材料是分子印迹技术应用的一个重要方向。MIPs与生物材料相比,具有较高的选择性,良好的稳定性,耐酸、碱和有机溶剂等特点,非常适合作为化学传感器的识别元件^[7]。将MIPs与传感器结合的方法主要有:旋涂法^[8]、液滴涂布法^[9]和原位聚合法^[10]。原位聚合法是通过在传感器表面进行适当的化学修饰后,采用适当的引发方式在传感器表面进行MIPs制备。原位聚合的优点在于MIPs与传感器表面结合牢固,MIPs同质性(孔隙、光学等)更好^[11]。光聚合是原位聚合MIPs中常用的引发方式,适用于不同的传感器表面^[12~14]。

孕酮是人体内重要的激素,影响人生殖系统、神经系统和大脑发育^[15,16],目前作为更年期激素治疗的一种药品。但是过量的消费孕酮药品会随着人体排泄物进入到环境中,导致水体的污染。已报道的关于孕酮MIPs多与高效液相色谱或气相色谱结合^[17~19],样品需要前期处理且无法直接对水中样品直接检测。Lee等人^[20]采用苯胺和间氨基苯磺酸共聚制备了孕酮的MIPs薄膜取得了良好的检测效果,该MIPs修饰的电极响应信号随MIPs薄膜厚度增加而增大,但是该方法难以实现对薄膜厚度的精确控制。本文以甲基丙烯酸(MAA)为功能单体、孕酮作为模板分子、二甲基丙烯酸乙二醇酯(EGDMA)为交联剂、2-(十二烷基硫代碳酸酯基)-2-甲基丙烯酸(DDMAT)作为唯一的控制试剂,在无额外的光引发剂或催化剂条件下,采用紫外光引发的方式在表面等离子体共振(SPR)传感器芯片表面制备MIPs薄膜;结合SPR原理实现了对MIPs薄膜生长的精确控制,并对其在水环境中对模板分子识别能力进行检测分析。

1 实验

(i) 试剂及仪器。孕酮(98%,阿拉丁试剂公司)、睾酮(98%,阿拉丁试剂公司)、雌酮(99%,百灵威);甲基丙烯酸(MAA, 99%,百灵威)和二甲基丙烯酸乙二醇酯(EGDMA, 98%,百灵威)蒸馏后使用;巯基乙胺(95%,阿拉丁试剂公司)、*N,N'*-二环己基碳二亚胺(DCC, 99%,麦克林试剂公司)、2-(十二烷基硫代碳酸酯基)-2-甲基丙酸(DDMAT, 97%,麦克林试剂

公司)、二苯甲酮(BP, 99%, Acros Organics)、正十二烷基硫醇(98%,百灵威)、无水乙醇(分析纯,北京市通广精细化工公司)、乙腈(分析纯,北京市通广精细化工公司)、乙酸(分析纯,北京市通广精细化工公司)、浓盐酸(优级纯,北京化工厂)、磷酸氢二钠(分析纯,北京化工厂)、磷酸二氢钠(分析纯,北京化工厂)、氯化钠(分析纯,北京化工厂);水为二次蒸馏水。红外光谱仪(Nicolet 6700,赛默飞世尔,美国);接触角测量仪(JC2000C,上海中晨数字技术设备有限公司,中国);扫描电子显微镜(SEM, Zeiss SUPRATM55 SAPPHIRE,德国);表面等离子体共振装置为本实验室自行搭建的Kretschmann结构角度调制型表面等离子体仪,光源为632.8 nm;UV-LED点光源照射机(UVEC-4 II, 3 W/cm², LAMPLIC,深圳市蓝谱里克科技有限公司,中国)。

(ii) SPR芯片的表面修饰及MIPs薄膜制备。采用真空镀膜方式在LaSFN9玻璃基片上镀金膜50 nm,将刚蒸镀好的LaSFN9基片置于巯基乙胺的无水乙醇溶液中,巯基乙胺浓度为5 mmol/L,静置24 h后取出,用无水乙醇冲洗3次,氮气吹干备用;将14.6 mg的DDMAT和16.5 mg的DCC溶于40 mL无水乙腈中,将在巯基乙胺浸泡24 h的LaSFN9基片取出,用无水乙腈冲洗3次,氮气吹干后备用。孕酮(31.4 mg), MAA (34 μ L)加入4 mL无水乙腈中,超声混合,室温下静置3 h,使其形成模板-单体复合物;之后向该溶液中加入交联剂EGDMA(338.5 μ L)和链转移试剂DDMAT(15 mg),通氮气10 min除去溶液中的氧气;将芯片安装到SPR设备上,通入反应溶液,对聚合过程进行实时监控,SPR原位监控原理见表S1和图S1。紫外光照射30 min后停止反应,聚合动力学见图S2。反应结束后通入磷酸盐缓冲液(PBS, pH 7.4)冲洗芯片表面约10 min,之后通入洗脱溶液(PBS(pH 7.4): 乙酸=9:1, 体积比),洗脱约33 min,之后再通入PBS (pH 7.4)冲洗约75 min。非印迹物(NIPs)薄膜制备方法相同,只是制备过程中不加入孕酮分子。

(iii) DDMAT在光聚合中作用。为了验证DDMAT在光聚合过程中是否同时起到光引发剂和链转移试剂的作用。本文设计一组对照实验,根据本课题组^[21]前期工作结果,对照实验1选择BP作为光引发剂,在正十二烷基硫醇修饰的金膜表面制备MIPs薄膜。具体方法如下:将新蒸镀50 nm金膜的LaSFN9玻璃基片浸泡在正十二烷基硫醇的无水乙醇

溶液中,正十二烷基硫醇浓度为1 mmol/L,浸泡24 h后取出,用无水乙醇冲洗3次,氮气吹干后备用.孕酮(31.4 mg),MAA(34 μ L)加入4 mL无水乙醇中,超声混合,室温下静置3 h,之后加入交联剂EGDMA(338.5 μ L)和光引发剂BP(7.3 mg),通氮气10 min除去溶液中的氧气.将芯片安装到SPR设备上,通入反应溶液,紫外光源照射20 min后停止光照,通过SPR现场监测记录反应过程中反射率值的变化.对照实验2选择DDMAT作为光引发剂,在DDMAT修饰的金膜上制备MIPs薄膜.具体方法如下:孕酮(31.4 mg),MAA(34 μ L)加入4 mL无水乙醇中,超声混合,室温下静置3 h,之后加入交联剂EGDMA(338.5 μ L)和光引发剂DDMAT(15 mg),通氮气10 min除去溶液中的氧气.将芯片安装到SPR设备上,通入反应溶液,紫外光源照射20 min后停止光照,通过SPR现场监测记录反应过程中反射率值的变化.

(iv) 接触角检测. 分别对裸金、巯基乙胺-Au、DDMAT-巯基乙胺和MIPs薄膜修饰后的芯片表面进行接触角检测,焦距为0.7倍,液体样品为二次蒸馏水,进样量1.0 μ L,采用JC2000C自带软件中的拟合分析法分别对所成图像进行角度分析.

(v) SEM检测. 制备好的MIPs和NIPs薄膜,室温下干燥后,经过切割制备电子显微镜样品;由于薄膜样品均为导电性能不佳的材料,进行样品检测前需在样品表面进行喷金处理,以减小因表面电荷富集导致无法获得样品表面形貌信息.

(vi) 红外光谱检测. 采用Nicolet 6700双通道偏振模式对MIPs和NIPs薄膜进行检测,以裸金作为背景通过差谱方式获得MIPs和NIPs薄膜的红外信息.

(vii) SPR检测. SPR对MIPs薄膜和NIPs薄膜的检测均在室温下进行,检测样品均采用20 mmol/L磷酸盐缓冲液稀释得到,缓冲液中NaCl浓度为150 mmol/L,缓冲液pH分别由浓盐酸和氢氧化钠调节得到;所测得反射率变化数值 $\Delta R\%$ ($\Delta R\% = \Delta R \times 100\%$)为3次平行检测结果的平均值.

2 结果与讨论

2.1 DDMAT在光聚合中的作用

对照实验1中以BP为光引发剂,在正十二烷基硫醇修饰的金膜表面制备MIPs薄膜,紫外光源开启后反射率先上升,这是由于紫外光照射引起反射光强

值增加,随后反射率下降,表明金膜表面开始有薄膜生长,如图1(a1)所示.紫外照射20 min后停止光照,反射率快速下降(图1(a2)),紫外光源关闭引起的反射率快速下降;反射率继续下降(图1(a3)),表明停止光照后MIPs薄膜继续生长,这是由于反应溶液中仍有一定的活性自由基存在.60 min后反射率值趋于稳定,表明反应溶液中的活性自由基基本消耗完毕,MIPs薄膜停止生长,如图1(a4)所示.对照实验2中,以DDMAT为光引发剂,在DDMAT修饰的金膜表面制备MIPs薄膜.如图1(b)所示,通入反应溶液后,将反应池中乙醇溶液逐渐排除,反射率略有下降(图1(b1)).之后反射率逐渐趋于平稳(图1(b2)),表明反应池溶液成分趋于一致,同时表明反应液在632.8 nm光源照射下不会发生反应.随后开启紫外光源照射20 min,反射率开始下降(图1(b3)),表明在紫外光激发下溶液发生反应,有MIPs薄膜开始生长.这一现象也进一步表明在紫外光照射下溶液中产生活性自由基,一般来讲,这一活性自由基是由DDMAT中C-S键在紫外光照射下断裂产生的^[22],因此DDMAT在紫外光照射下可以起到光引发剂的作用.紫外光照射20 min后停止光照,反射率迅速下降,一方面由于紫外光关闭后光强降低导致反射率数值下降,另一方面,反应溶液中存在大量的活性自由基保证反应继续进行,如图1(b4)所示.随后,反射率继续下降,表明在停止光照后溶液中仍不断地有活性自由基产生保证MIPs薄膜继续生长(图1(b5)).这一实验现象与RAFT反应机理保持一致^[23,24],如图1(c)所示.对照实验结果表明,与传统的紫外光引发剂BP不同,DDMAT在光聚合反应中同时起到光引发剂和链转移试剂作用.

2.2 接触角表征

分别对金(Au)膜表面、巯基乙胺-Au表面、DDMAT-巯基乙胺-Au表面、MIPs薄膜-DDMAT-巯基乙胺-Au的表面进行接触角检测,得到接触角角度分别为93.01°, 23.22°, 89.75°和75.94°,如图2所示.巯基乙胺分子一端为-SH基团,另一端为亲水性的-NH₂基团;当巯基乙胺与金膜接触时,-SH基团通过Au-S键与金膜结合,末端亲水性的-NH₂基团暴露在表面,液滴接触其表面迅速铺展开,其接触角值23.22°.DDMAT分子一端含有-COOH基团,另一端为疏水性的-CH₃基团;通过DCC可以将DDMAT中的-COOH

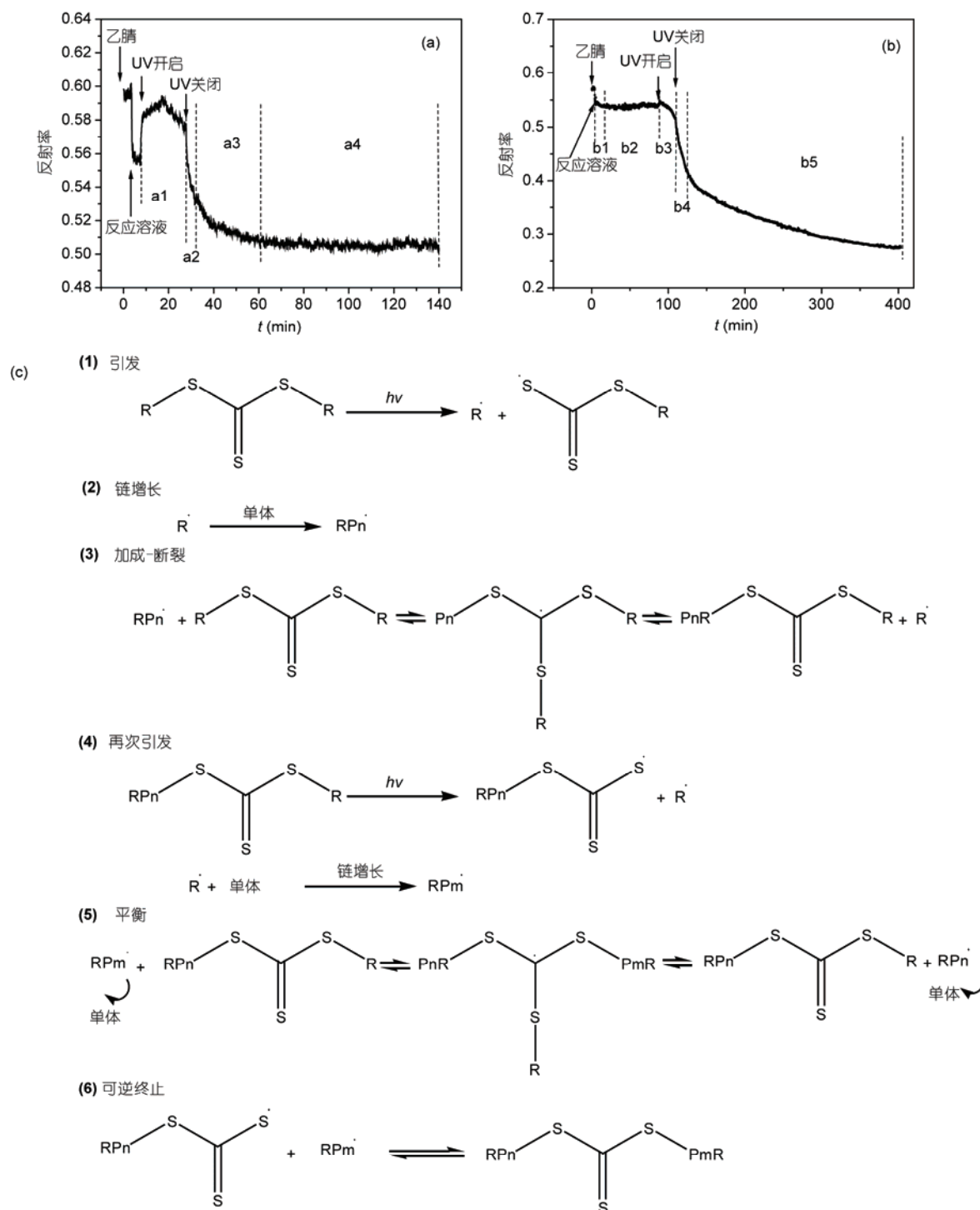


图1 SPR动力学监测紫外光引发聚合过程. (a) BP为光引发剂的聚合动力学; (b) DDMAT为光引发剂的聚合动力学; (c) DDMAT在紫外光引发下发生反应的机理示意图^[23]

Figure 1 The SPR kinetics of photo-polymerization by UV initiated. (a) The polymerization kinetics with BP as an initiator; (b) the polymerization kinetics with DDMAT as an initiator; (c) the reaction mechanism of in the presence of DDMAT under UV irradiation^[23]

与巯基乙胺末端的 NH_2 通过脱水分子方式结合, 此时疏水性的 $-\text{CH}_3$ 基团暴露在外侧, 接触角值为

89.75° . 当MIPs薄膜修饰到DDMAT-巯基乙胺-Au表面后, 其接触角 $75.94^\circ (<90^\circ)$, 表明MIPs薄膜具有较

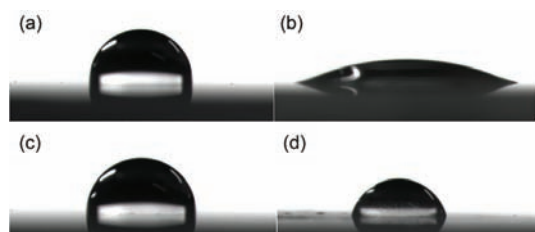


图2 接触角表征. (a) Au表面; (b) 巯基乙胺-Au表面; (c) DDMAT-巯基乙胺-Au表面; (d) MIPs薄膜- DDMAT-巯基乙胺-Au表面

Figure 2 Characterization of contact angle. (a) Au surface; (b) cysteamine-Au surface; (c) DDMAT-cysteamine-Au surface; (d) MIPs film- DDMAT-cysteamine-Au surface

好亲水性.

2.3 SEM表征

利用SEM对洗脱后的MIPs薄膜和NIPs薄膜表面进行检测,结果如图3所示. MIPs薄膜表面布满纳米尺寸的通道,这些通道有利于模板分子进出MIPs薄膜,而NIPs薄膜则没有观察到类似的通道存在.通过对MIPs薄膜的断截面进行SEM检测,可知其厚度约为200 nm(图3(c)). SEM结果表明, MIPs和NIPs薄膜成功地接枝到芯片表面.

2.4 红外光谱表征

分别对MIPs薄膜和NIPs薄膜进行了红外光谱分析.其中, $1731\sim 1735\text{ cm}^{-1}$ 的吸收峰为单体MAA和交联剂EGDMA中C=O的伸缩振动峰; 1265 cm^{-1} 处特征峰为C-O伸缩振动峰.红外光谱结果进一步表明MIPs和NIPs薄膜成功地接枝到芯片表面,且两者具有相似的化学结构.

2.5 pH对MIPs薄膜吸附性能的影响

MIPs薄膜修饰的SPR芯片分别对pH 5.0, 6.0, 7.4, 8.0和9.0缓冲溶液配制的 10^{-10} mol/L 孕酮样品溶液进

行吸附检测,结果表明MIPs薄膜在pH 7.4和8.0时对孕酮分子有较好的响应信号,在pH 6.0时响应信号最低. MAA及其均聚物等电点在pH 6.0~7.0^[25],这可能导致MIPs薄膜在pH 6.0环境下接近等电点,使MIPs薄膜处于收缩状态,进而影响孕酮分子进入MIPs内部与识别位点相作用,如图5所示.

2.6 SPR对MIPs薄膜吸附特性

聚合后采用洗脱液对MIPs薄膜进行洗脱,洗脱后共振角角度为 72.07° 与洗脱前共振角角度 72.44° 相比,共振角角度减小了 0.37° ,如图6(a)所示.这主要是因为模板分子洗脱后在薄膜上留下孔穴,引起折射率发生变化导致的.采用pH 7.4缓冲液配制 $10^{-12}\sim 10^{-7}\text{ mol/L}$ 的孕酮样品溶液,在固定角度 68.8° 位置进行吸附动力学监测,样品吸附顺序由低浓度到高浓度,每个样品监测时间为25 min.如图6(b)所示,反射光强随着样品通入MIPs薄膜表面逐渐增强,这是由于模板分子进入MIPs薄膜导致其折射率发生变化,进而导致SPR共振角角度随之增加.反射率变化值 $\Delta R\%$ 与检测孕酮样品浓度在 $10^{-12}\sim 10^{-7}\text{ mol/L}$ 成线性关系,通过线性拟合计算检测限为 $3.24\times 10^{-13}\text{ mol/L}$ ($n=3$ 次测量结果, $RSD=2.7\%\sim 7.1\%$),如图6(c)所示.共振角角度扫描显示,当MIPs薄膜吸附 10^{-7} mol/L 浓度的孕酮分子样品后,共振角角度增加到 72.14° ,与洗脱前角度 72.44° 接近,表明MIPs薄膜中的印迹孔穴基本被模板分子占据, MIPs薄膜的吸附能力接近饱和(图6(d)).

选择了两种与孕酮结构相似的睾酮和雌酮作为干扰物对MIPs薄膜的选择性进行了检测.如图7(a)所示,通入睾酮和雌酮样品后,反射率逐渐增加,但是通入PBS(pH 7.4)溶液后反射率迅速下降,其反射率变化值 $\Delta R\%$ 分别为0.12%和0.09%,与吸附相同浓度的孕酮所产生的反射率变化值 $\Delta R\%=0.34\%$ 相比较低.

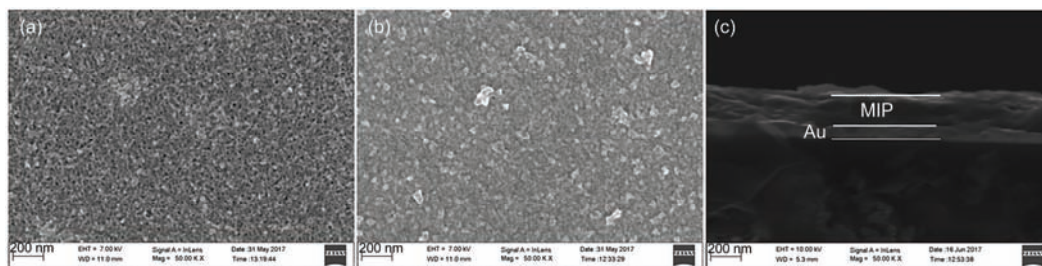


图3 SEM图像. (a) MIPs薄膜表面; (b) NIPs薄膜表面; (c) MIPs薄膜截面图

Figure 3 SEM images. (a) MIPs film surface; (b) NIPs film surface; (c) cross section of MIPs film

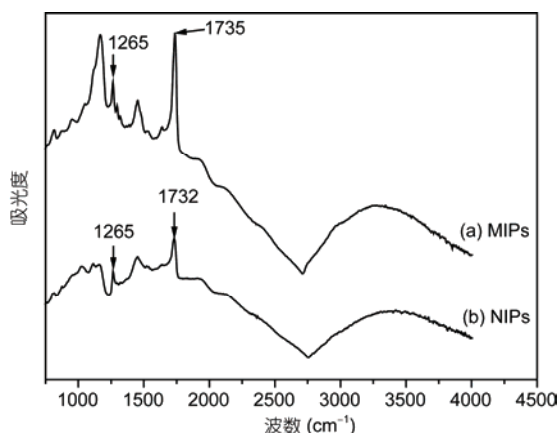


图4 红外光谱图. (a) MIPs薄膜; (b) NIPs薄膜

Figure 4 Infrared spectroscopy. (a) MIPs film; (b) NIPs film

这说明干扰物在MIPs薄膜中的保留时间短, 干扰物分子与MIPs薄膜内部形成的印迹孔穴大小, 作用位点不够吻合, 结合能力差. 吸附相同浓度的孕酮分子所产生的反射率变化值约为睾酮的2.83倍, 雌酮的3.78倍. 实验结果表明该MIPs薄膜对模板分子孕酮具有良好的选择识别能力.

对照实验选择NIPs薄膜修饰的SPR芯片对 10^{-10} mol/L孕酮分子样品溶液进行吸附检测, 结果如图7(b)所示. 当样品流经NIPs薄膜表面, 反射率变化缓慢, 与MIPs薄膜相比吸附相同浓度的孕酮分子样品溶液引起的反射率变化值 ΔR 只有0.17%, 这是由于NIPs薄膜中缺少有效的识别孔穴和识别位点, 且NIPs薄膜表面紧实, 孕酮分子很难进入薄膜内部. 吸附相同浓度的孕酮分子样品, MIPs薄膜产生的响应信号 $\Delta R\%$ 约为NIPs薄膜产生的响应信号的1.9倍, 如

图7(c)所示. 对照实验结果进一步表明, MIPs薄膜的特异性吸附与内部形成的印迹孔穴和识别位点相关.

2.7 MIPs薄膜重复性和稳定性检测

为了进一步评估MIPs薄膜的性能, 对MIPs薄膜的稳定性从重复使用和存储时间两方面进行考察. 选择 10^{-11} mol/L的孕酮溶液样品进行重复吸附检测, 每次吸附孕酮样品16 min, 之后通入PBS(pH 7.4)缓冲液进行脱附直至反射光强恢复到起始光强数值, 再进行下一次吸附. 通过在固定角度检测吸附动力, 比较吸附过程中反射率差值 $\Delta R\%$, 如图8(a)所示. 重复吸附5次反射率差值 $\Delta R\%$ 平均值为0.68%, 相对标准偏差RSD=2.75%. 结果表明, 该MIPs薄膜具有良好的重复使用性能, 重复吸附同浓度样品5次, 响应信号无明显变化. 将制备好的芯片室温 N_2 下保存30 d, 对 10^{-11} mol/L孕酮样品进行吸附检测, 结果表明MIPs薄膜在该条件下存放30天仍保持良好的响应信号, 其响应信号 $\Delta R_{30}\%$ 约为新鲜制备的MIPs薄膜响应信号 $\Delta R_0\%$ 的81%, 如图8(b), (c)所示.

2.8 实际样品中检测

为了进一步考察MIPs薄膜的性能, 分别取民用自来水、地下水和人工尿液作为实际考察样品. 采用乙腈溶液配制浓度为 10^{-4} mol/L的孕酮溶液, 用民用自来水对其进行逐级稀释, 得到含有 10^{-11} mol/L孕酮的样品溶液. 以民用自来水为背景溶液, 对SPR对稀释后的孕酮样品溶液进行检测. 采用同样的稀释和检测方法, 对MIPs薄膜修饰的SPR芯片在地下水和

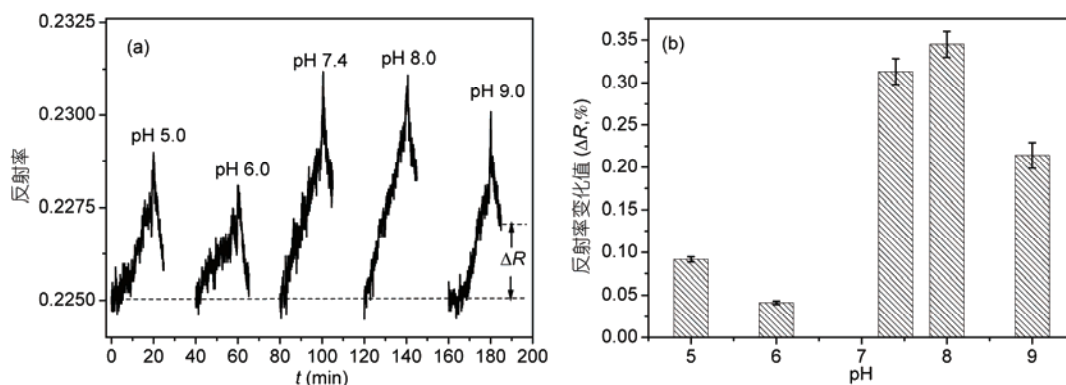


图5 pH 对MIPs吸附孕酮的影响. (a) MIPs薄膜在pH 5.0~9.0下吸附 10^{-10} mol/L孕酮分子动力学; (b) MIPs薄膜在pH 5.0~9.0下吸附 10^{-10} mol/L孕酮分子反射率变化值, RSD=3.3%~7.1%, $n=3$ 次测量结果

Figure 5 Effects of pH on MIPs binding progesterone. (a) The kinetics of binding 10^{-10} mol/L progesterone at pH 5.0~9.0 by MIPs film; (b) the reflectivity changes after binding 10^{-10} mol/L progesterone at pH 5.0~9.0 by MIPs film, RSD=3.3%~7.1%, $n=3$

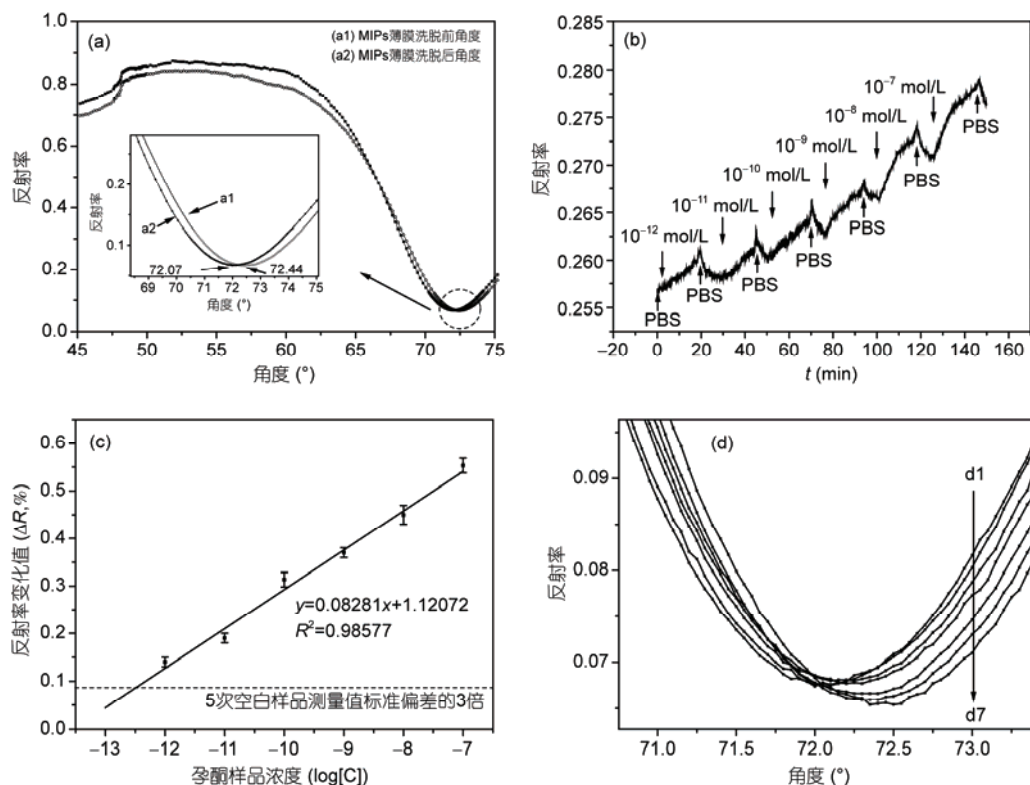


图6 MIPs薄膜的SPR表征. (a) MIPs薄膜洗脱前后共振角角度变化: (a1) 洗脱前共振角角度; (a2) 洗脱后共振角角度, 插图共振角部分放大. (b) MIPs薄膜吸附 10^{-12} ~ 10^{-7} mol/L孕酮样品动力学. (c) 反射光强变化值 ΔR %与孕酮样品浓度线性拟合, $RSD=2.7\%\sim 7.1\%$, $n=3$ 测量结果. (d) 共振角曲线: (d1) MIPs薄膜洗脱后共振角角度; (d2)~(d7) 吸附 10^{-12} ~ 10^{-7} mol/L孕酮样品溶液共振角曲线

Figure 6 Characterized MIPs film by SPR. (a) The resonance angle changes of MIPs film. (a1) The resonance angle of MIPs film before washing; (a2) the resonance angle of MIPs film after washing, inset graph is the enlarged part of resonance angles. (b) The kinetics of binding 10^{-12} ~ 10^{-7} mol/L progesterone on MIPs film. (c) Linearity between reflectivity changes (ΔR %) and progesterone concentrations, $RSD=2.7\%\sim 7.1\%$, $n=3$ measurements. (d) Resonance angle curves: (d1) the resonance angle of MIPs film after washing, (d2)~(d7) resonance angle curves after binding 10^{-12} ~ 10^{-7} mol/L progesterone on MIPs film

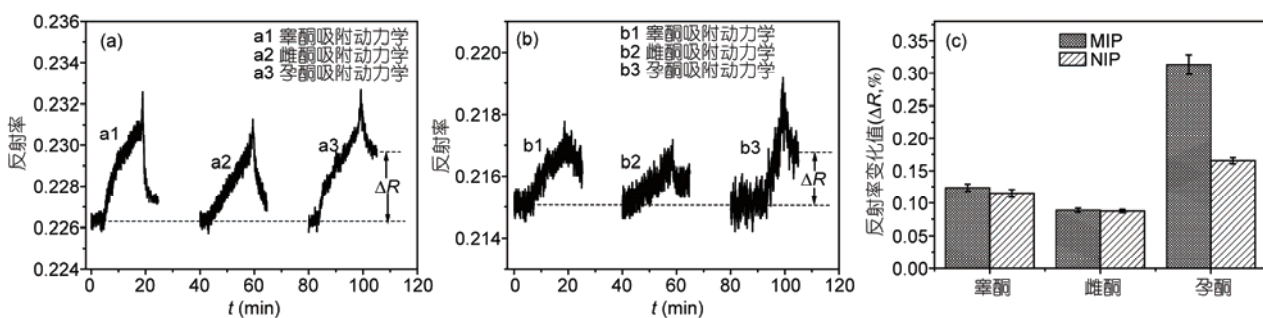


图7 MIPs和NIPs薄膜选择性. (a) MIPs薄膜吸附 10^{-10} mol/L睾酮(a1), 10^{-10} mol/L雌酮(a2), 10^{-10} mol/L孕酮(a3)动力学; (b) NIPs薄膜吸附 10^{-10} mol/L睾酮(b1), 10^{-10} mol/L雌酮(b2), 10^{-10} mol/L孕酮(b3)动力学; (c) MIPs薄膜和NIPs薄膜分别吸附 10^{-10} mol/L睾酮, 10^{-10} mol/L雌酮, 10^{-10} mol/L孕酮样品后反射率变化值 ΔR %, $RSD=2.9\%\sim 5.0\%$, $n=3$ 检测结果

Figure 7 Selectivity of MIPs and NIPs films. (a) The kinetics of binding 10^{-10} mol/L testosterone (a1), 10^{-10} mol/L estrone (a2), 10^{-10} mol/L progesterone (a3) on the MIPs film. (b) The kinetics of binding 10^{-10} mol/L testosterone (b1), 10^{-10} mol/L estrone (b2), 10^{-10} mol/L progesterone (b3) on the NIPs film. (c) The reflectivity changes ΔR % after binding 10^{-10} mol/L testosterone, 10^{-10} mol/L estrone, 10^{-10} mol/L progesterone on the MIPs and NIPs films respectively, $RSD=2.9\%\sim 5.0\%$, $n=3$

人工尿液环境下对孕酮样品响应进行评估, 结果如表S2所示, 所测样品回收率为92.9%~96.5%, 表明该

MIPs薄膜修饰的传感器能够应用于实际样品中孕酮的检测。

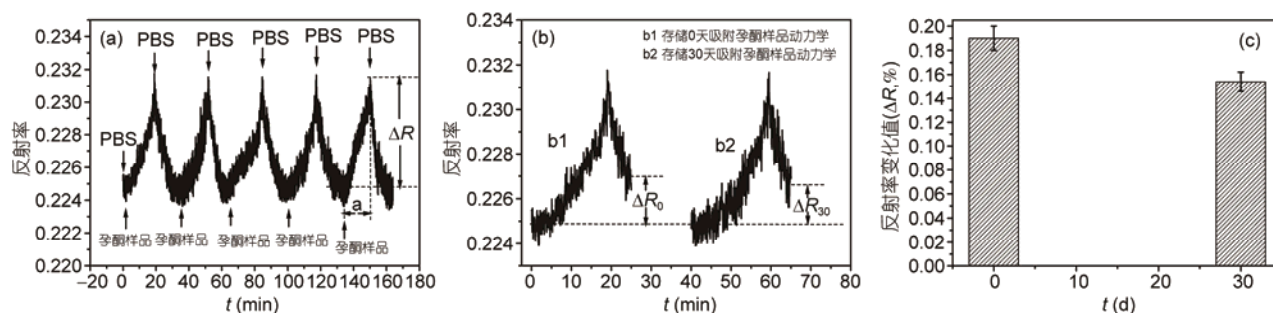


图 8 MIPs薄膜重复吸附-脱附和稳定性检测. (a) MIPs薄膜重复吸附 10^{-11} mol/L孕酮样品动力学. (b) MIPs薄膜稳定性: (b1) 存储0 d吸附 10^{-11} mol/L孕酮样品动力学; (b2) 存储30 d吸附 10^{-11} mol/L孕酮样品动力学. (c) MIPs薄膜存储0和30 d吸附 10^{-11} mol/L孕酮样品反射率变化值 $\Delta R\%$, RSD=5.2%~5.3%, $n=3$ 检测结果

Figure 8 Adsorption-desorption and stability detections of MIPs film. (a) The kinetics of adsorption-desorption of 10^{-11} mol/L progesterone on the MIPs film. (b) The stability detection of MIPs film: (b1) binding 10^{-11} mol/L progesterone on the MIPs film after storing 0 d; (b2) binding 10^{-11} mol/L progesterone on the MIPs film after storing 30 d. (c) The reflectivity changes $\Delta R\%$ after binding 10^{-11} mol/L progesterone on the MIPs films after storing 0 and 30 d, RSD=5.2%~5.3%, $n=3$

3 结论

本文基于RAFT反应机理采用紫外光引发的方式在SPR传感器芯片表面原位制备了针对孕酮分子的MIPs薄膜. 采用SPR实时监测方法, 实现了对MIPs薄膜在传感器表面生长的精确控制. 采用该MIPs薄膜作为识别单元的SPR芯片能够在水相环境中对孕酮分子进行有效识别, 且具有灵敏度高、选择性好、稳定性好的特点. 该方法为拓展水相兼容的MIPs薄

膜制备提供了一种新的思路和方法, 而且该方法同样也适用于电化学电极、石英晶体微天平芯片, 纳米金探针等表面修饰, 具有广泛的科研和应用前景. 同时, 本文考察了DDMAT在紫外光引发聚合过程中的作用, 实验结果表明DDMAT在这一过程中起到光引发剂和链转移试剂的双重作用. 这对深入了解DDMAT试剂在聚合过程的作用提供了新的线索和依据, 也为重新认识和利用链转移试剂开启了一扇新的大门.

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补充材料

表 S1 乙腈溶液中对 SPR 芯片的拟合参数

图 S1 乙腈溶液中共振角随薄膜厚度变化(0~200 nm)以及 70.11°位置原位监测反射率变化拟合曲线

图 S2 SPR 原位监测 MIPs 薄膜生长动力学

表 S2 实际水体中孕酮回收率测定

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Summary for “基于可逆加成-断裂链转移聚合的孕酮分子印迹膜制备与检测”

Preparation and detection of progesterone by molecularly imprinted film based on reversible addition fragmentation chain transfer polymerization

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Molecularly imprinted technique is a versatile and straightforward way to produce artificial receptors for a given target molecule. The molecularly imprinted polymers (MIPs) have received widespread attention and application, owing to their high physical stability, remarkable robustness and low cost. Despite the tremendous progress made in this field, many changes still remain to be solved. In general, MIPs are prepared by traditional free radical polymerization. However, this approach causes problems such as low-affinity binding, high diffusion barrier, low-rate mass transfer. In particular, it has been demonstrated that most of the MIPs fail to exhibit specific recognition towards the target molecules in aqueous environments although they can be successfully applied to recognize the target molecules in organic solvent media, which severely limits their potential application in some fields. In this work, a new method to prepare MIPs film for the detection of progesterone based on reversible addition-fragmentation chain transfer (RAFT) polymerization is presented. 2-Methyl-2-[(dodecylsulfanylthiocarbonyl)sulfanyl]propanoic acid (DDMAT) was employed as the sole control agent in the UV polymerization. The SPR sensor chip with 50 nm Au was firstly immersed in an ethanol solution of cysteamine (5 mmol/L) for 24 h. The cysteamine modified chip was washed by pure ethanol solvent repeatedly and dried under a stream of nitrogen. Then the chip was placed in 40 mL acetonitrile solution with 14.6 mg DDMAT and 16.5 mg *N,N'*-dicyclohexylcarbodiimide (DCC) for 24 h. After that, the chip was washed with acetonitrile several times and dried under a stream of nitrogen. The MIPs film was grafted by UV irradiated without adding additional photo initiator or catalyst in the presence of progesterone (31.4 mg), methacrylic acid (33.4 μ L), ethylene glycol dimethacrylate (338.5 μ L) and DDMAT (15 mg) in 4 mL acetonitrile. The growth process of the MIPs film was monitored by surface plasmon resonance (SPR) *in situ*. A mixture of PBS (pH 7.4) buffer and acetic acid (v:v=9:1) was used as a washing solution to remove the template molecules from the MIPs film. Results indicated that DDMAT acted as both photo initiator and chain transfer agent during the polymerization. The MIPs film was characterized by contact angle measurements, infrared spectroscopy and scanning electron microscope (SEM). Analysis of SPR spectroscopy showed that the MIPs film coated sensor displayed good sensitivity and selectivity to progesterone compared to other analytes with a similar structure. The response of the progesterone ranged from 10^{-12} to 10^{-7} mol/L with a limit of detection (LOD) of 3.24×10^{-13} mol/L ($S/N=3$) in PBS buffer (pH 7.4). The MIPs film coated sensor also showed good reusability and stability, and kept about 81% of its original response after 30 d of storage under N_2 protection at room temperature. In addition, the SPR sensor was successfully used to determine progesterone in tap water, underground water and artificial urine samples with recoveries from 92.9% to 96.5%, which indicated the SPR sensor can be applied for the real sample detection. This method will expand the surface modification of the MIPs film on the surface of different sensors and is a great candidate for rapid, simple and label-free detection of molecules in aqueous solution. Finally, this study opens a door to re-know the roles of chain transfer agents (DDMAT, in this study) in synthesizing MIPs and we believe that the properties of various chain transfer agents will be excavated and utilized in future study.

reversible addition-fragmentation chain transfer, molecularly imprinted polymers, progesterone, water-compatible, surface plasmon resonance

doi: 10.1360/N972017-00875