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Application of polymeric high-internal-phase-emulsion-coated stationary-phase columns in open-tubular capillary electrochromatography

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ABSTRACT: The application of polymeric high-internal-phase emulsion (polyHIPE) capillary coatings for open-tubular analytical separation columns was demonstrated in this study for the first time. Multiple polystyrene-*co*-divinylbenzene polyHIPE layers with an average total depth of 1.73 μm were coated onto internal capillary surfaces to create open-tubular columns (20 cm coating and 32.5 cm effective length). With these columns for open-tubular capillary electrochromatography, ethylbenzene and pentylbenzene were separated. Although the overall separation capacity of the produced columns was low, the polyHIPE coatings improved the analyte peak shape, decreased the total run time, and improved the peak symmetries relative to comparable unmodified open-tubular columns. In addition, the use of these novel polyHIPE columns led to the use of 30% less organic modifier. These columns have the potential to improve the shelf life of open-tubular columns typically used in capillary electrochromatography. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 44237.

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INTRODUCTION

Polymeric high-internal-phase emulsion (polyHIPE) materials have been successfully demonstrated as sensors,[1] water-purification supports,[2–6] and tissue culture supports.[7,8] Emulsions are classed as high internal phase when the internal droplet phase is greater than 74% of the total emulsion volume.[9] Monolithic structures result when the aqueous phase is the internal phase and the organic phase forms the continuous phase. Typically, these monolithic polyHIPEs are macroporous with pore sizes greater than 10 μm.[9–13] In separation science, polyHIPEs have been successfully applied as heavy-metal adsorption supports for water purification[2–6] and with modifications using iron oxide to demonstrate their use in batch format.[4,5] PolyHIPEs within convective interaction media discs[14] and stainless steel columns[15–17] have demonstrated their applicability as stationary phases in flow-through mode due to their large pore sizes. These separations have been predominantly demonstrated with large biomolecules; nanoparticle (NP) separation has also been reported, albeit with very poor resolution.[18]

Although monolithic polyHIPE columns have proven advantageous as stationary phases, providing lower backpressures and decreased wall effects,[19] they have been limited by their low surface areas (5–20 m^2/g), which causes poor chromatographic performance.[14] Because of their poor performance and limitations in reproducibility, the application of these macroporous columns for small-molecule analysis has focused on their application for the preconcentration of analytes before analysis with a traditional packed column; for example, for the preconcentration of polyaromatic hydrocarbons, repeatability with the same column was measured at under 10%, and column-to-column reproducibility was determined to be under 13%.[20] Additionally, knowledge of the roles of porogenic solvent in determining the final polyHIPE morphology have enhanced their repeatability.[21] Building on this knowledge, we recently demonstrated a polystyrene-*co*-divinylbenzene (PS-*co*-DVB) polyHIPE housed within 1.0 mm *i.d.* silcosteel tubing, applied to the successful isocratic separation of small molecules under a pressure-driven flow.[22] The chromatographic performance of the polyHIPE column was evaluated by the separation of selected alkylbenzenes with observed efficiencies of 1565 to 3087 N/m. Column-to-column

reproducibility was shown with retention factor variation between 2.6 and 6.1% for two separately prepared columns; this illustrated that the technical limitations of polyHIPE columns are being reduced but are not yet fully resolved. Unfortunately, the peak widths did not approach those obtainable with commercial columns, with baseline widths of approximately 2 min for ethylbenzene and over 8 min for pentylbenzene.

With polyHIPE materials in small-molecule separation in capillary electrochromatography (CEC), their chromatographic performance capabilities have been demonstrably enhanced.[23,24] Unfortunately, repeatability concerns after *in situ* fabrication and pore morphology variation have persisted.[25–27] Variations in the pore size diameter are particularly significant in CEC applications because pore size reduction can cause electrical double-layer overlap. This results in electroosmotic flow collapse and negatively affects electrolyte flow with a corresponding impact on separation.[27] A potential strategy to overcome this severe limitation is the use of capillaries coated but not filled with a stationary phase. The advantages of coated columns include flexibility in the stationary-phase type, control and stabilization of electroosmotic flow, no pressure limitations, and reduced column bleeding (observed mainly with packed columns). These advantages have resulted in the increasing investigation of coated columns for CEC application. Recently, it has also been demonstrated that the fabrication of coated columns by multiple-layer polymerization can lead to increased crosslinking and a higher proportion of mesopores; this provides an additional strategy to enhance their chromatographic performance.[28]

Nanomaterials have dominated open-tubular capillary electrochromatography (OTCEC) separations, with many variants of NPs explored, including vinyl benzyl chloride,[29,30] aminated latex,[31–33] and inorganic NPs, such as gold[34,35] and metal oxide.[36] Nanomaterial-coated columns in CEC have shown impressive separation capabilities and have highlighted the potential for novel OTCEC columns. Polymer-coated columns present a potential alternative method to NP-coated columns for further reducing wall effects and decreasing blockages. Within the OTCEC column, a polymer layer can be polymerized on the internal capillary surface; this increases analyte interactions with the stationary phase. Hollow polyHIPEs have been demonstrated for microfluidic devices,[37] although such a technique would prove challenging to repeat in a capillary format, whereas 11-mm diameter open-tubular (OT) polyHIPEs have also been fabricated. This demonstrated the scalability of hollow polyHIPEs and illustrated that OT polyHIPE fabrication in chromatographic dimensions should be possible. Indeed, polymer-coated columns have been developed in OTCEC and been shown to improve column selectivity,[38,39] although emulsion template (polyHIPE) polymers have not been explored in OTCEC-coated columns. PolyHIPE application in electrochromatographic separation should result in enhancements in the chromatographic performance criteria relative to pressure-driven chromatographic separation. In this study, novel OTCEC polyHIPE-coated columns were fabricated by the polymerization of multiple thin layers, which were successfully applied to electrochromatographic separation, with an illustrative separation of the alkyl benzenes ethylbenzene and pentylbenzene. Significant improvements in the peak widths for both compounds were observed; this highlighted the potential of multilayer polyHIPE separation phases in OTCEC format for the separation of small molecules.

EXPERIMENTAL

Millipore ultrapure water purified to a resistance of greater than 18 MΩ cm was used in all instances. Calcium dihydrochloride (>99%) and Span 80 were purchased from Fluka (Sigma-Aldrich, Tallaght, Ireland), 3-glycidoxypropyl trimethoxysilane (≥98%), potassium persulfate, styrene (≥99%), divinylbenzene (80%) isomeric mix, ethylbenzene, pentylbenzene, acetonitrile (ACN), sodium phosphate monobasic, and sodium tetraborate decahydrate were purchased from Sigma-Aldrich (Tallaght, Ireland) and used as received. Fused silica tubing (*i.d.* = 100 μm and *o.d* = 360 μm) was supplied by CM Scientific (West Yorkshire, United Kingdom).

Instrumentation

Morphological characterization was carried out with a Hitachi S-3400N scanning electron microscope, and all samples were gold-sputtered with a 750T sputter coater (Quorum Technologies, United Kingdom). A KD Scientific syringe pump was used for flow-through. Electrophoretic experiments were demonstrated with an Agilent 7100 capillary electrophoresis system, whereas the columns were rinsed with a Dionex Ultimate 3000 capillary liquid chromatography instrument.

Silanization of Fused Silica Tubing

A fused silica capillary with an inside diameter of 100 μm was prepared by washing with acetone for 5 min and then dried under nitrogen for 10 min. The dried capillary was treated with 0.2 *M* NaOH for 30 min and then rinsed with water for 5 min before treatment with 0.2 *M* HCl for 5 min. Finally, the capillary was washed with water and acetone for 5 min each before it was dried with nitrogen for 10 min. The previous procedures were carried out at 1 μL/min with a syringe pump. The capillary was filled with a 50% v/v solution of 3-(trimethoxysilyl) propyl methacrylate in acetone. The capillary was sealed with silicone septa. Silanization was carried out in a water bath at 60 °C for 20 h. The silanized fused silica capillary was rinsed with acetone for 5 min at 1 μL/min before it was dried with nitrogen.

Preparation of the PS-*co*-DVB Emulsion

The emulsion preparation of the PS-*co*-DVB polyHIPE was adapted as previously outlined.[21] Briefly, the aqueous phase (15 mL of ultrapure deionized water, 0.03 g of potassium persulfate, and 0.10 g of calcium chloride dihydrate) and organic phase (1.333 mL of styrene, 0.333 mL of divinylbenzene, and 0.329 g of Span 80) were prepared separately and homogenized by a vortex. The organic phase was placed into a 250 mL round-bottom flask connected to the overhead stirrer (set to 350 rpm) and under N_2 supply. The aqueous phase was added dropwise with a hypodermic syringe. The white emulsion that formed upon the addition of the aqueous phase was stirred for 20 min. The emulsion was transferred into an airtight syringe

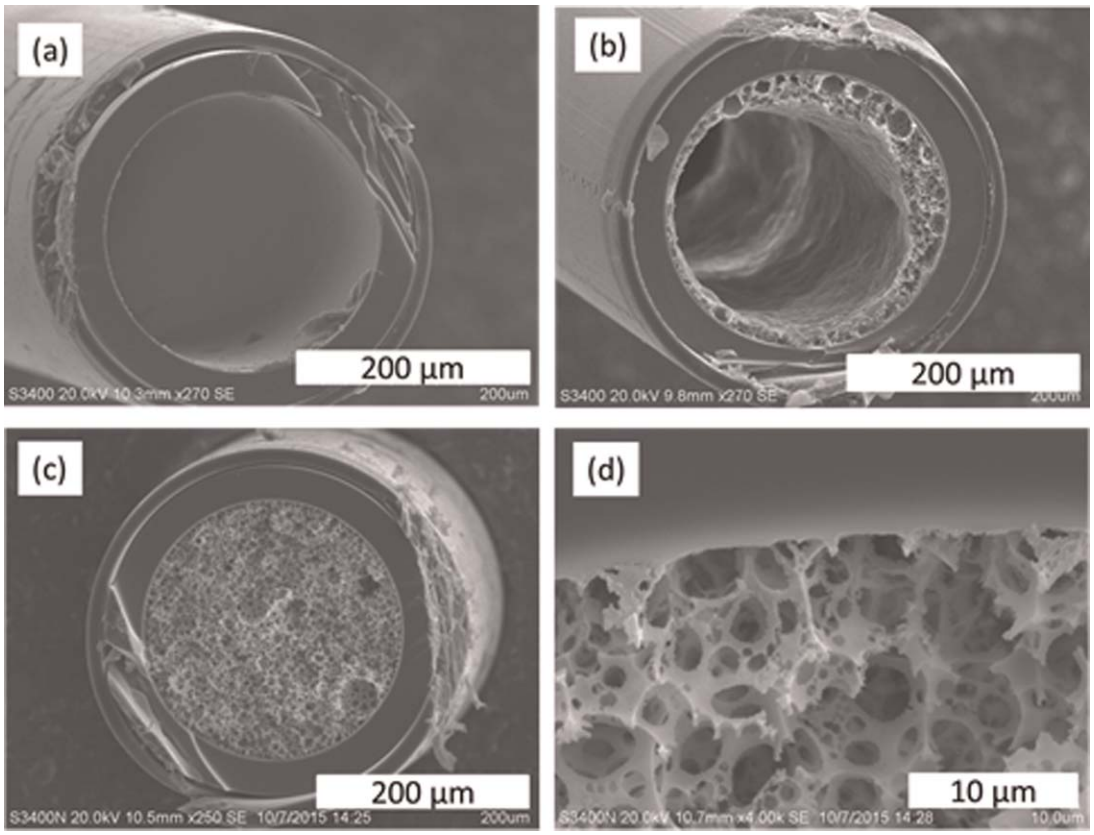

Figure 1. Polymerization of an emulsion-coated capillary in which the columns were polymerized for (a) 1, (b) 2, and (c,d) 4 h. The original magnifications were (a–c) 270 and (d) 4000×.

to form coated columns, and the remainder was transferred into 1.5 mL centrifuge tubes for characterization.

Fabrication of PS-*co*-DVB polyHIPE Emulsion-Coated Capillaries for CEC

To establish the polymerization time required to form coated columns, shorter columns (10 cm) were initially produced. These columns underwent one emulsion filling step (10 cm columns filled entirely) and were polymerized for 1, 2, and 4 h. To establish separation parameters, columns of 41 cm were produced and followed multiple coating and polymerization steps. For multiple coating and polymerization steps, the 41 cm capillary was filled up to only 20 cm and polymerized for 1 h at 60 °C in a water bath. The column was then attached to a capillary liquid chromatography instrument and washed with methanol until excess emulsion was removed. For multiple coatings, the entire process was carried out twice. A detection window 8.5 cm from the uncoated side of the capillary was formed by the removal of 1 cm of the polyimide coating. This resulted in an effective capillary length of 32.5 cm with a 20 cm polyHIPE coating.

Electrochromatographic Conditions

Buffers were pH-adjusted as required with dilute NaOH and prepared daily. The working buffer consisted of 5 m*M* sodium tetraborate decahydrate and 2.5 m*M* sodium phosphate monobasic in 40% ACN at pH 9. Standards were made to 1% v/v in ACN. Both buffer and samples were degassed and filtered into sample vials before analysis with Acrodisc 0.45 µm nylon membrane syringe filters. Analysis was carried out with an Agilent 7100 CE at 214 nm for alkyl benzene detection. Electrokinetic injections of 5 kV for 3 s at 25 °C were applied to OTCEC columns with a total length of 41 cm, a coated length of 20 cm, and an effective length of 32.5 cm.

RESULTS AND DISCUSSION

Visualization of the PolyHIPE-Coated OTCEC Columns

The optimal polymerization time to fabricate a single layer of polyHIPE on the internal capillary surface was determined by the polymerization of emulsion coatings on short columns (10 cm) at different times. The scanning electron microscopy results in Figure 1(a–c) show that as expected, when the polymerization time was increased, a thicker layer of polyHIPE material was present on the capillary surface. A uniform polyHIPE layer was difficult to fabricate; nonetheless, the 2 h coating was found to be optimum; this resulted in the most uniform coating. A typical polyHIPE morphology was clear in Figure 1(d). However, when this fabrication time was used for longer columns, high-backpressure measurements, up to 60 bar, resulted, possibly due to blockages within the column.

To reduce blockages formed by single-polymerization strategies, multiple sequential thin films of emulsion were deposited and polymerized on the inner capillary walls. The optimum coating was established when the capillary was coated sequentially three times with a 1 h polymerization time for each layer, as

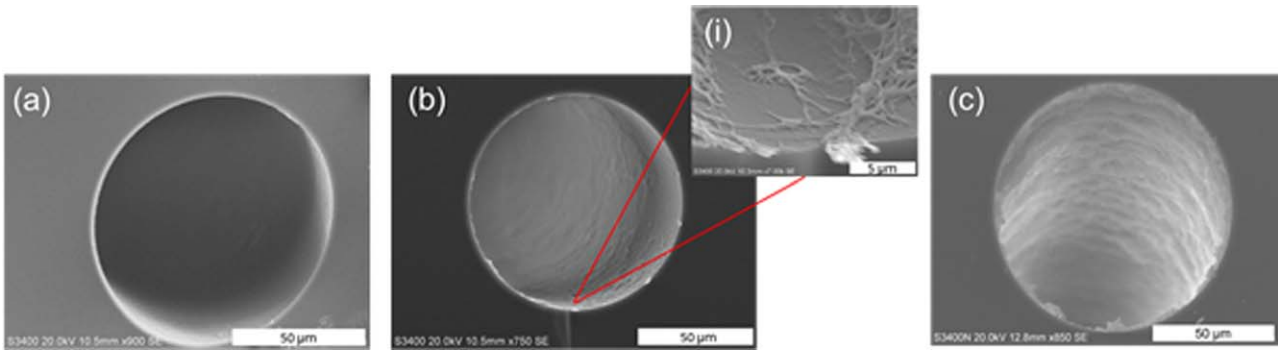

Figure 2. PolyHIPE-coated columns (100 μm in diameter): (a) one coat of the polyHIPE emulsion, (b) two coats of the polyHIPE emulsion with (i) an enlarged image illustrating the scattered polyHIPE film and (c) three coats of the polyHIPE film. The scale bars are (a–c) 50 μm and (i) 5 μm. [Color figure can be viewed at wileyonlinelibrary.com.]

illustrated in Figure 2(c). No visually distinct polymer layer was observed in the first coating of the polyHIPE emulsion [Figure 2(a)]. Upon the second coating [Figure 2(b,i)], it was observed that a scattered film of polyHIPE was attached onto the surface of the fused silica capillary. The average film diameter after three coatings was found to be 1.73 ± 0.44 μm across three different columns (measured with ImageJ). The polyHIPE layers did not result in the traditional void and windows observed in the polyHIPE materials. Instead the materials resembled a lace pore structure typically observed when additional porogens are used.[21]

Chromatographic Separation of Alkyl Benzenes on Layer-by-Layer Fabricated Static PS-*co*-DVB PolyHIPE OTCEC Columns

Single-layer-coated PS-*co*-DVB columns were used for the separation of ethylbenzene and pentylbenzene. Unfortunately, separation was not achieved, and the analytes were observed to co-elute, as shown in Figure 3(a). It was hypothesized that this probably resulted because the polyHIPE layer was not thick enough to provide a selective separation.

We fabricated the second PS-*co*-DVB layer by coating a singly coated capillary (previously polymerized for 1 h) for a second hour before the removal of excess emulsion. As previously observed for multiple-layer polymer coatings,[28] the increased polyHIPE coating resulted in improved separation, as shown in Figure 3(b). However, as illustrated in Figure 2(b,i), the coating layer was not even, with patches evident where no coating was evident. It was hypothesized that the layering method would result in an evenly distributed capillary coating once sufficient layers were deposited. On application of a third coat of the polyHIPE emulsion, the separation was indeed enhanced, with successful baseline separation of ethylbenzene and pentylbenzene achieved with resolution (Rs) of 2.80, as shown in Figure 3(c). The increasing selectivity observed was attributed to increased analyte interaction with the polyHIPE layer present. It was therefore hypothesized that the macroporous structure of the polyHIPE coating would enable the analyte molecules to permeate throughout the coating depth. Although baseline separation was achieved, a narrow migration time between analytes was observed. This was expected because of the amount of stationary phase present. The aim was not to achieve an equivalent separation to chromatographic columns filled completed with the polyHIPE stationary phase but to demonstrate layer-by-layer fabrication of the polyHIPEs and their potential as OTCEC columns. The OTCEC columns in this study demonstrated significant advantages relative to comparable polyHIPE CEC monolithic columns;[24] this resulted in shorter run times and better peak shapes with lower organic modifier concentrations, albeit with lower overall efficiencies. The PS-*co*-DVB CEC column studied by Tunç *et al.*[19] resulted in a peak asymmetry (As) of 2.1 for butylbenzene; in contrast, in this study, pentylbenzene achieved a peak A_s of 1.2. Although Tunç *et al.* improved the peak shape and run time with acrylic-based polyHIPEs for CEC, the resolution between analytes was reduced as a consequence.[19] Similarly, compared with pressure-driven chromatographic separation utilising a column filled with PS-*co*-DVB polyHIPE stationary phase, the electrochromatographic separation with the OTCEC polyHIPE column presented here also demonstrated a significant enhancement in the chromatographic performance.[23] The baseline peak width for ethylbenzene decreased from 2 min to just under 30 s, whereas the pentylbenzene peak width fell from approximately 8 min to just 1 min; this decreased the run time from 25 to 6 min. Meanwhile, the separation efficiency was comparable for ethylbenzene and pentylbenzene for both separations: 2332 and 1810 N/m for both compounds, respectively, with pressure-driven chromatography and 1966 and 3460 N/m for both compounds, respectively, with electrochromatographic separation with the OTCEC column presented here.

In addition, a high concentration of organic modifier was used; although a run time decrease was observed, this severely shortened the capillary column lifetime. The negative effect of organic modifier in CEC significantly curtailed the technique's applicability, as CEC column inefficiencies frequently arise because of physical blockages in the columns, which reduce current flow.[40] In the OTCEC polyHIPE separation presented here, the run time was shortened when both the peak symmetry ($A_s = 1.2$ for pentylbenzene) and baseline resolution (>2) between analytes was maintained, and this did not require typical high concentrations of organic modifier. This highlighted the potential of these novel polyHIPE materials for use in OTCEC.

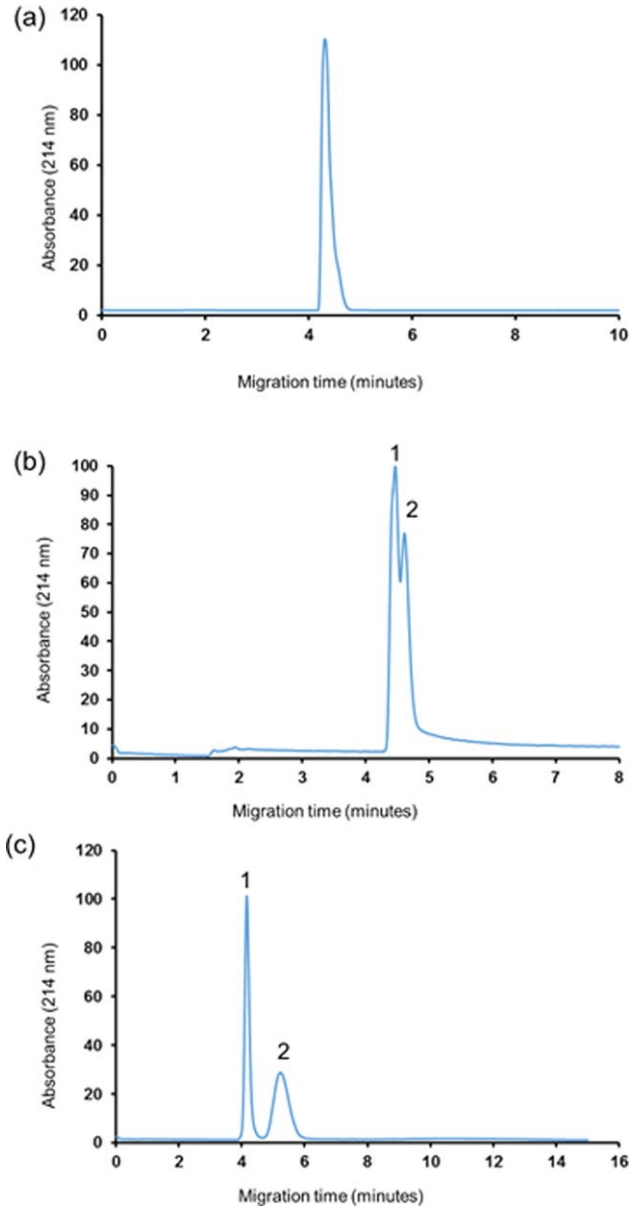

Figure 3. Separation of 1% v/v ethylbenzene and pentylbenzene. The buffer was 5 m*M* sodium tetraborate decahydrate and 2.5 m*M* monobasic sodium phosphate at pH 9 (40% ACN). Electrokinetic injection was performed at 5 kV for 3 s at 25 °C with 100 µm *i.d.* columns with a 20 cm polyHIPE coating. The effective length was 32.5 cm, and the total length was 41 cm. The presented separations show (a) singly coated, (b) doubly coated, and (c) triply coated columns. [Color figure can be viewed at wileyonlinelibrary.com.]

CONCLUSIONS

PS-*co*-DVB polyHIPE emulsion coatings were initially investigated at various polymerization times. However, it was established that shorter polymerization times and multiple coats were optimal for even coverage of the polyHIPE layers on the capillary walls. Upon an increase in the polyHIPE emulsion layers, baseline separation of ethylbenzene and pentylbenzene was achieved. This separation represented the first successful application of polyHIPE OTCEC columns. Despite the low separation capacity, polyHIPEs in OTCEC format resulted in multiple interaction sites throughout the macroporous structure in the internal capillary walls. This resulted in a higher resolution and a more symmetrical peak shape when a reduced organic modifier was used; this extended the applicability of OTCEC. The separation observed in this study highlighted the potential use of polyHIPE-coated columns in future OTCEC separations. Potential alternative applications for these OT polyHIPE columns are as substrates upon which to immobilize metallic NPs as a catalytic flow-through reactor[41] or for advanced bioscreening,[42] as they provide a facile fabrication method to generate OT polymer-coated capillaries. This alleviates the high-backpressure problems typically associated with this type of column.[43] In continuing work, our group will focus on increasing the surface area of the polyHIPE layers and tailoring the surface chemistry of the coated layer with selective NPs to increase the selectivity and broaden the applicability.

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