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Online sensing of palladium in flowing streams

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Rapid palladium (Pd) catalyzed deallylation of an uncoloured reagent within a flowing stream affords a dose dependent colour formation that can be used for convenient online analysis of trace levels of Pd contamination using a modified HPLC instrument. An application to the online sensing of Pd breakthrough from a flow through Pd adsorption cartridge is described. An alternative configuration of the instrumentation allows the rapid (<1 min) and accurate measurement of Pd levels within samples injected *via* a conventional HPLC autosampler.

Over the past 20 years, ongoing discovery of unique and valuable reactivity of palladium catalysts has led to their widespread adoption in organic synthesis.[1] Within the pharmaceutical industry, Pd catalysts are now routinely used from initial medicinal chemical synthesis, through process development, to commercial manufacturing.[2] In order to ensure that the final drug products are free of unwanted Pd residues, a number of new methods for the rapid and efficient remediation[3] and measurement of Pd-containing streams have been developed.[4] Such tools now allow comparatively rapid selection of appropriate treatment conditions for Pd sequestration and removal, with convenient analytical methods in some cases allowing for rapid measurement of Pd concentration at the point of use, without the need for traditional ICP-MS measurement that requires expensive and specialized equipment, laboratories, and personnel.

Koide and co-workers have developed fluorometric and colorimetric reagents that exploit the Pd catalysed Tsuji–Trost deallylation of an uncoloured substrate to produce a fluorescent and/or coloured product, with the intensity of the signal being proportional to Pd concentration.[5b,c] Recent improvements allowed colour formation in rapid 'burst' reactions of limited duration, affording a signal corresponding to Pd concentration that does not continue to grow over time,[5a] a valuable attribute for 'on the spot' laboratory or pilot plant investigations. Reaction duration, sensitivity, and signal intensity can be adjusted by varying the proportions of the substrate, reducing agent, and other reaction components, making these colour-forming Pd chemodosimeters well suited for monitoring residual Pd levels in the batch production of pharmaceuticals, where testing at several defined points during each production run is required.[5d,e] However, the approach is less well suited for continuous production *via* flow chemistry, where an online analysis method would be preferred.

Continuous processing is becoming increasingly important in pharmaceutical manufacturing, sometimes offering advantages in yield, purity, safety, production costs and space requirements.[6] Continuous removal of metals from solutions by flow through an adsorption column has long been used in pharmaceutical production; however, convenient online analysis methods to continuously monitor trace metal contamination have heretofore not been available. Realizing that the rapid and linearly responsive nature of our Pd chemodosimeters could form the basis for such an online analysis system, we investigated the development of flow chemistry-based approaches for online analysis of Pd in flowing streams containing pharmaceutical intermediates or active pharmaceutical ingredients (APIs).

We have recently identified reaction conditions where the colour formation is completed in less than a minute (Fig. 1a), affording a rapid measurement of Pd that could potentially be useful for online analysis. A flow chemistry version of this assay system was investigated using a conventional quaternary pump HPLC instrument, as illustrated in Fig. 1b. Pump A delivers a cocktail solution containing the substrate, ammonium acetate, phosphine ligand and antioxidant (BHT) in ethanol, while pump B delivers a borohydride solution in aqueous NaOH (both solutions are stable up to 7 days at room temperature, longer if refrigerated). The flow rate and/or reaction coil length can be adjusted so that there is sufficient residence time for the colour forming reaction to be completed before the stream enters the UV-vis diode array detector, which reads the absorbance at λ_{abs} = 580 nm.

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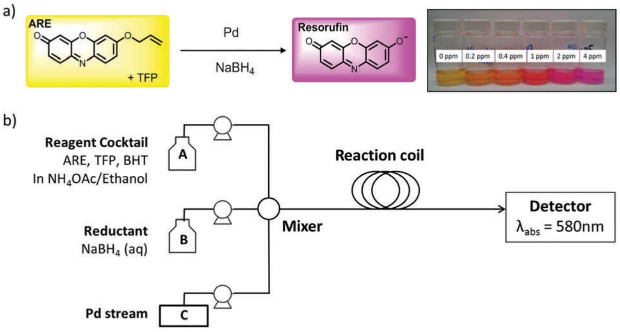

Fig. 1 (a) Quantitative analysis of Pd using catalysis-based colorimetric assay, (b) schematic diagram of the online Pd analyzer using a quaternary HPLC system; reagent cocktail: 32 μM allyl resorufin ether (ARE), 250 μM tri(2-furyl)phosphine (TFP), 40 ppm butylated hydroxytoluene (BHT), 800 mM NH_4OAc in absolute ethanol.

To establish proof of principle, the HPLC system was set to supply a constant flow of the cocktail solution (channel A, 0.8 mL min^{-1}) and the borohydride solution (channel B, 0.1 mL min^{-1}). A 10 μg mL^{-1} (94 μM) solution of Pd(OAc)$_2$ in ethanol was added into the flowing stream *via* pump C at a flow rate between 0 and 0.1 mL min^{-1}. To avoid a change in the overall flow rate upon addition of the Pd stream, a compensating flow of ethanol was added *via* pump D such that the combined flow of channels C + D was always 0.1 mL min^{-1}, and the combined flow from channels A + B + C + D was always 1.0 mL min^{-1}. As the flow of the incoming Pd stream was varied, the absorbance at λ_{abs} = 580 nm was monitored. As illustrated in Fig. 2, a programmed flow gradient from low (0 ppm) to high (10 ppm) Pd levels was directly translated into a change in absorbance at λ_{abs} = 580 nm, with a delay of ∼50 seconds due to the residence time of the system. This system shows excellent dose and time responsiveness, suggesting potential utility for monitoring the changing levels of Pd within a flowing stream.

A close examination of the dose response of the highest step gradient in Fig. 2 suggests some possible nonlinearity that could arise from an incomplete reaction. Using the standard reaction coil consisting of 100 cm of 0.17 mm internal diameter stainless steel tubing, the total flow rate arising from pumps A, B, C and D was proportionally reduced in a stepwise manner from 5 mL min^{-1} to 0.6 mL min^{-1}, corresponding to a change in the available reaction time from about 15 seconds to about 2 minutes. (Note: the ∼1.2 mL dwell volume of the HPLC system arising from internal mixers and other fluidics makes a substantial contribution to the overall reaction volume.) As shown in Fig. 3a, very little conversion to the coloured product is observed at λ_{abs} = 580 nm for the highest 5 mL min^{-1} flow rate, owing to lack of sufficient residence time for completing the reaction. Slowing the flow leads to an increased extent of the reaction, which reaches a maximum at 0.6 mL min^{-1}, although it should be noted that flow rates of 1.5 mL min^{-1} or below show over 90% completion of the reaction.

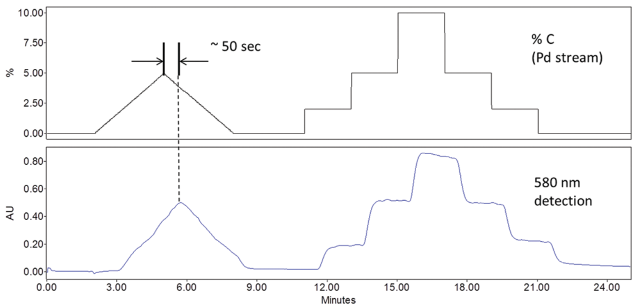

Fig. 2 Demonstration of online Pd detection by direct introduction of a Pd stream through an HPLC pump, using the experimental setup illustrated in Fig. 1b. Signal intensity at λ_{abs} = 580 nm (bottom trace) reflects real time Pd concentration changes (top trace); mobile phase A: cocktail solution, B: 10 mM sodium borohydride in 1 N NaOH, C: 10 ppm palladium solution in ethanol, and D: ethanol; gradient: 80% A, 10% B, C + D = 10%; total flow rate: 1.0 mL min^{-1}.

Similarly, the effect of coil length on the extent of the reaction is illustrated in Fig. 3b. A reaction coil with 150 cm length (ID: 0.51 mm) was added between the column compartment and the UV-vis detector to ensure that chemical conversion is completed before reaching the UV-vis detector. The coil was progressively cut and the absorbance was noted. Removal of 10 or 20 cm of coil had no effect on the extent of colour formation, although further reductions in coil length led to a decreased extent of the reaction. Thus, under these fixed flow rate conditions (combined flow = 1.5 mL min^{-1}), the majority of reaction occurs within the HPLC dwell volume and a 130 cm reaction coil provides sufficient time for completing the reaction. This length was selected for the subsequent breakthrough curve experiments for Pd removal by flow through adsorption.

It is worth noting that the color-generation reaction is completed fast within the HPLC system volume, therefore this online detection approach should readily be applicable to other HPLC setups with different types of tubing.

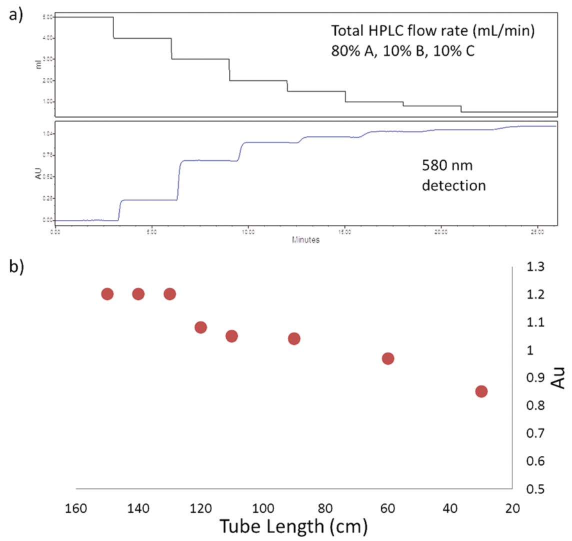

Fig. 3 Effect of (a) flow rate and (b) the length of the reaction coil (flow rate: 1.5 mL min^{-1}) on the extent of the color-forming reaction and signal response. Mobile phase A: cocktail solution, B: 10 mM sodium borohydride in 1 N NaOH, C: 10 ppm palladium solution in ethanol, and D: ethanol. Gradient: 80% A, 10% B, 10% C.

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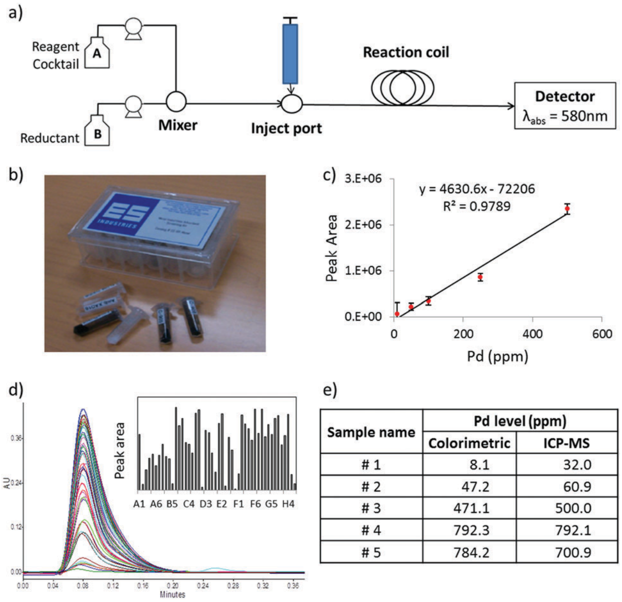
Inspired by the sharply delineated responsiveness of this system for detecting rapid changes in Pd concentration, we investigated the potential application to the flow injection analysis (FIA) of Pd. In this setup, illustrated in Fig. 4a, samples containing different levels of palladium are injected by the HPLC autosampler, entering a stream of cocktail (channel A) and borohydride (channel B) solutions, at which point the catalytic deallylation reaction begins, continuing as the injected sample traverses the length of the reaction coil before finally entering the UV-vis detector for quantitation. The injection of a set of Pd standards showed excellent sensitivity and linearity of detection (Fig. 4c) encouraging us to apply this FIA Pd analysis method to the high throughput selection of a suitable process adsorbent for the removal of Pd from a liquid stream, a frequently encountered problem in pharmaceutical process research.

An aqueous Pd containing solution was added to a set of 48 micro-tubes, each containing a different adsorbent.[7] After equilibration, an aliquot of the supernatant solution from each vial was placed in an HPLC vial without dilution and analysed using the FIA setup pictured in Fig. 4a. An injection cycle time of only 0.5 min allows all 48 samples to be analysed within half an hour.

The intensity at λ_{abs} = 580 nm indicates the level of palladium remaining within each sample following resin treatment (Fig. 4d), with wells E6, D2, E3, A2 and B5 exhibiting the most effective Pd removal, consistent with results obtained using off-line analysis and spot checking with conventional ICP-MS (Fig. 4e).



Sample name	Pd level (ppm)	
	Colorimetric	ICP-MS
# 1	8.1	32.0
# 2	47.2	60.9
# 3	471.1	500.0
# 4	792.3	792.1
# 5	784.2	700.9

Fig. 4 (a) Schematic diagram of the online Pd analyzer in injection mode (note that the length of the reaction coil is reduced to 50 cm to optimize the peak shape), (b) commercially available resin screening kit containing 48 adsorbents, (c) linearity and sensitivity graphs (detection limit: 10 ppm of Pd standard), (d) high-throughput online Pd analysis to identify the hits of resin screening (inset: visual selection of hits), and (e) comparison table of Pd concentration measured by colorimetry and ICP-MS.

In order to further explore the application of this technique to online analysis of flowing streams, the best performing adsorbent from Fig. 4 (well A2; Pall AKS 9 adsorbent) was packed into a 10 cm column and used in the remediation of a Pd-containing stream. Flow-through adsorbent cartridges are often used for the removal of Pd contamination in pharmaceutical manufacturing, with offline methods typically used for assaying the effectiveness of Pd removal.[8] Upon addition of a new cartridge, Pd concentration at the outlet stream remains very low, showing a breakthrough of Pd as the adsorbent capacity of the cartridge becomes overwhelmed. Given the difficulty of online monitoring of low level Pd, current manufacturing approaches often rely on time or volume based estimates of this breakthrough point, adding a safety margin to avoid unexpected breakthrough of contaminating Pd, which also leads to inefficient use of adsorbent cartridges. Online analysis can potentially permit the switch to a fresh cartridge only when actual Pd breakthrough is detected. The experimental setup shown in Fig. 5a illustrates this approach, with an external pump supplying flow of a 100 ppm $Pd(OAc)_2$ solution through the adsorbent cartridge at 4 mL min^{-1}, and 10% of the exiting flow being diverted to the analysis stream of the HPLC setup in a manner similar to the approach used in Fig. 1b. Initially, the solution exiting the cartridge leads to no signal generation at λ_{abs} = 580 nm, indicating efficient Pd removal. As flow of the Pd solution through the cartridge continues, a distinctive increase in the signal at about 37 minutes heralds the onset of Pd breakthrough (Fig. 5b), at which point switching to a fresh column would be warranted.

In a pharmaceutical manufacture setting, feedback control based on the signal at λ_{abs} = 580 nm can be used to trigger automatic switching of flow from the saturated column to a fresh column. Given the ~1 min time delay between column exit and the detection of the corresponding absorption signal reflecting Pd concentration, a delay loop would be required to

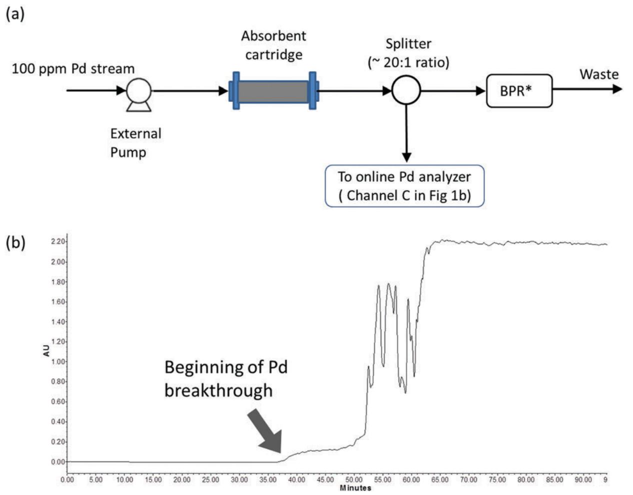


Fig. 5 (a) Schematic diagram of the online Pd analyzer for a resin treatment cartridge (* BPR: back pressure regulator) and (b) demonstration of a real-time Pd breakthrough curve.

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achieve the precisely timed valve actuation that would enable real time responsiveness. This approach could allow for continuous processing using a two column system, with more efficient use of adsorbent cartridges by actual detection of breakthrough rather than conventional time or volume based estimates. In addition, this approach also avoids unanticipated failures and catastrophic breakthrough of Pd into the stream containing the desired API or intermediate.

In summary, we herein report the use of a fast catalysis based colorimetric assay for flow-chemistry-based Pd monitoring using modifications of basic HPLC equipment. A system capable of continuous monitoring of Pd within a flowing stream has been demonstrated, addressing a recognized gap in pharmaceutical development and manufacturing. A modified experimental design allows for rapid (30 seconds) analysis of Pd samples injected from conventional HPLC vials or microplates, thereby enabling rapid and convenient Pd analysis by non-experts.

Instrumentation. The online Pd detection experiments were carried out on an Agilent 1100 HPLC instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, a sample manager, a column manager, and a photodiode array UV-vis detector. Waters Empower software was used for instrument control and data processing.

For the metal analysis by ICP-MS, depending on the concentration range of the element, either a Perkin-Elmer Elan 6000 quadrupole ICP-MS spectrometer (Perkin-Elmer, Norwalk, CT) or a Thermo Finnigan Element 2 high-resolution ICP-MS spectrometer (Finnigan, Bremen, Germany) was used.

Resin screening procedure.[7b] *Chemicals and reagents.* All commercial chemicals used were of analytical grade. HPLC grade 200 proof ethanol, DMSO, 1000 ppm Stock Pd standard were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Tri-(2-furyl)phosphine (TFP), butylated hydroxytoluene (BHT), resorufin and NH_4OAc were all purchased from Sigma–Aldrich (St. Louis, MO). Ultrapure water was obtained from a Milli-Q Gradient A10 from Millipore (Bedford, MA, USA).

General protocol for preparing the cocktail solution for testing. A reaction cocktail was prepared by mixing 100 mM NH_4OAc in ethanol (500 mL) with 800 μM solution of allyl resorufin ether (ARE) in 200 proof ethanol (20 mL) and 3 mM TFP in DMSO, with 250 ppm BHT in DMSO (40 mL).

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