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COMMUNICATION

The synthesis of warfarin using a reconfigurable-reactor platform integrated to a multiple variable optimization tool.

Jee Seong Kwak,[a,b] Nour Bizarri,[a] Sepideh Sharif,[a] Wenyao (Peter) Zhang,[a] Debasis Mallik,[a,b] and Michael G. Organ*[a,b]

Abstract: Optimization of the asymmetric synthesis of warfarin, an important anticoagulant, has been evaluated using a reconfigurable reaction platform capable of performing batch, continuous flow, and plug-flow synthesis. Further, this platform has been integrated with a novel, multidimensional, multiple variable analysis tool that can evaluate multiple critical quality attributes (CQA), percent conversion and enantiomeric excess in this case, from a single injection that is repeatedly recycled in a closed loop of chromatography columns, a detector and a heart-cut valve. Further, the new, integrated analysis system also facilitates validation of each QA, providing a high-level of confidence in analytical measurements, which are obtained without operator intervention.

The art of chemical synthesis has developed significantly over the last century. As a community, synthetic chemists have evolved from asking 'what can we make?' to 'what can't we make?' to 'how do we make selectively and efficiently only what we want?' Indeed, mighty synthetic challenges from palytoxin[1] to taxol[2] have all fallen as new transformations, reagents, and catalysts have been invented that made the synthetically unthinkable, possible. These two important natural products, and a large percentage of the rest of nature's molecular collection, have another foreboding element of complexity to them – chirality.

Nature's synthetic machine, i.e., the enzyme, is highly adept at making one enantiomer of a chiral molecule; this is not so simple for synthetic chemists. Indeed, what we do not see in the final manuscripts describing complex total syntheses are the countless attempts to make the key step that sets the chirality both high yielding and exquisitely selective for the right isomer. This is primarily because the current, state-of-the-art approach to solving the key enantioselective step is done in two separate operations. In one approach, the transformation itself is often developed and optimized around the substrate(s) before trying to make it enantioselective. The logic is reasonable; chiral and optically pure catalysts or chiral auxiliaries are often expensive, so one wants to make sure that the transformation itself works, and in high conversion.[3] Also, the development of a method to assess optical purity is itself an arduous task, so one would want to know in advance that the reaction being developed for optical assessment actually works. Unfortunately, quite often success in the racemic reaction does not carry over to the asymmetric version, for example when the process is adapted to employ a chiral metal catalyst, so it is back to the drawing board.

The above points conspire to halt the advancement of a chemical transformation with great conversion into an asymmetric version, but the opposite problem also exists. Conventional determination of ee (enantiomeric excess) typically involves purifying the product and then carrying it through optical assessment, for example using a chiral shift reagent by NMR spectroscopy, which means a reasonable amount of product (at least 10 mg) is required. If the approach to optimizing the critical asymmetric step involved first screening asymmetric transformations, then optimizing promising ones for yield, those which may well have outstanding ee, may be ignored. Reactions that only proceed a few percent, and may have complex mixtures at the end, will make isolating and assessing the product difficult. Consequently, a great chiral process that could well have been optimized for high yield will never be investigated further.

A major advance in asymmetric synthesis would be achieved if multiple CQAs (critical quality attributes), such as percent conversion and ee, for example, could be tracked in one combined synthetic and analytical operation. Current two-dimensional (2D) heart-cut chromatography affords the ability perform one round of chromatography, capture the analyte of interest, and run it through a second chromatographic operation thereby offering the capability of capturing two CQAs, however current methods have shortcomings.[4, 5]

In conventional 2D heart-cut techniques, a portion is cut from the first dimension run by a heart-cut valve based on a known retention time of a target analyte or based on a live feed from an inline detector.[6, 7] The portion is blindly sent to a second-dimension column for the analysis of a second CQA of the target analyte. The second CQA measurement, which plays an instrumental role in deciding whether synthetic conditions under investigation are worth pursuing further, could be erroneous if the target analyte undergoes chemical degradation during analysis.[8, 9] The evidence of such an analytical artefact is vital information that dictates the validity of the second CQA measurement. Current practice in high throughput screening of asymmetric reaction conditions turns a blind eye to such possibilities during conventional 2D analysis.

Furthermore, accuracy of the heart-cut process is critical in enantiomeric purity measurement as the ratio of the areas under the second dimensional peaks are taken blindly to calculate

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enantiomeric purity with an underlying assumption that the heart-cut portion bears the chiral target *exclusively*. In reality, careful adjustments in the heart-cut method are very often necessary to ensure that the chiral purity result truly comes from a pure target analyte. In an ideal world, the heart-cut portion would be re-injected into the first-dimension column to confirm if the heart-cut sample is pure (i.e., represents a single target analyte) or if the heart-cut method requires further optimization. Conventional 2D techniques require multiple discrete injections to carry out such an optimization process, which is a critical step especially when the matrix from which analytical samples originate contains traces of various reagents, catalysts, and solvents depending on the synthetic trial in question. Perfecting a heart-cut method through a single injection during an ongoing analysis is not supported by the design of the conventional 2D LC. Erosion of chirality during the ee measurement (i.e., on-analysis racemization) is another critical analytical artefact that standard high-throughput 2D techniques are forced to turn a blind eye towards.[10]

In this manuscript we describe the integration of a multi-dimensional chromatographic platform, which eliminates the above described persistent problems, with a multi-reaction platform. Further, we demonstrate the power of this combined synthetic/analysis tool as an asymmetric reaction optimization platform that combines reaction, analysis, and validation of the analytical method into a single, telescoped, automated operation.

Concept, Design and Details of Operation:

Details of the prototype reconfigurable reactor platform with a multidimensional chromatographic analysis tool is shown in Figure 1. V_5, which is a 6-port, 2-position (load and inject) injector valve, receives a reaction specimen (typically, a few microlitres in volume) in loop L_7 from one of three modes of reaction format: a batch reactor (R_1), a continuous flow reactor (R_2) or a plug-flow reactor (R_3). Pump P_7 then establishes fluid communication with loop L_7 at the inject configuration of valve V_5 and moves the specimen plug toward inlet port 1 of path-selector valve V_6, which then diverts the plug to column C_1 via ports 3 and 4 of heart-cut valve V_7. The fractions eluted off of C_1 are sent (via V_6) to the detector (D) (i.e., 1st-dimension analysis, which establishes the first CQA, e.g., % conversion) and then back to V_7 where the peak of interest is heart-cut in loop L_9 (after rotation). That material is then sent back through C_1 and the detector (i.e., 2nd-dimension analysis) to establish if any on-column degradation occurred during the 1st-dimension analysis. The peak of interest is again heart-cut, now in L_8 in V_7, and then sent off to C_2 for the second CQA evaluation, e.g., ee. The separated peaks (e.g., enantiomers) are sent through the same, single detector, *a major advantage of this technology*, thus establishing the 3rd-dimension. One of those peaks (e.g., one enantiomer) can then be recaptured in L_8 of V_7 and sent through C_2 and assessed for purity in the detector, again to assure that no on-column degradation occurred, which might be racemization in the case of chiral products (i.e., the 4th-dimension). All analytical modules (P_7, $V_{5\text{-}7}$, and D) are connected to a central controller, which transmits necessary instructions based on a live stream of data from the detector.

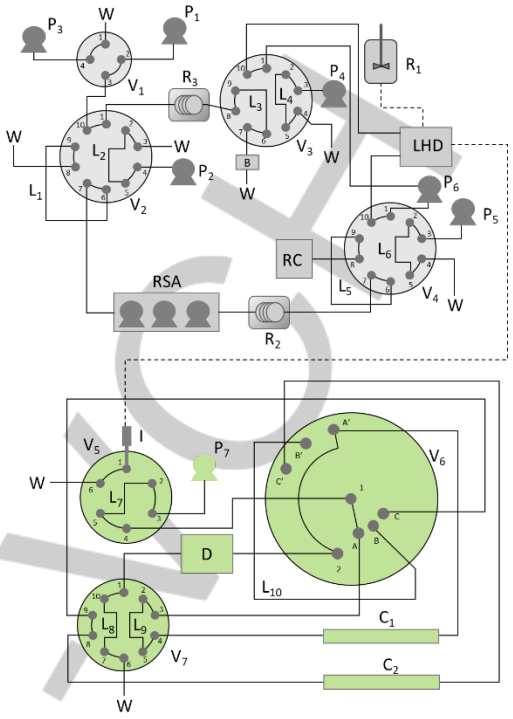

Figure 1. Reconfigurable-reaction platform integrated to multi-reaction-attribute analysis by multidimensional chromatography.
Legend. R_1=batch reactor; R_2=continuous-flow reactor; R_3=plug-flow reactor; RSA=Reagent Supply Assembly for flow experiments; RC= Reaction Sample Collection Device for flow-experiments; P_1, P_2=inert gas supply pumps for constructing reaction plugs for plug-flow experiments; P_3=pump for transporting samples from plug-flow experiments; P_4, P_5=pumps for analytical standards supply; P_6=sample transport pump; P_7=LC pump for analysis; V_1, V_2=valves for constructing sample plugs for plug-flow experiments; V_3, V_4=sampling valves; V_5=injector valve for analysis; V_6=LC path-selector valve for analysis; V_7=heart-cut valve for analysis; L_1, L_3, L_5=sampling loops for continuous-flow and plug-flow reactors; L_2=loop for forming gaseous boundaries for plug-flow experiments; L_4, L_6=loops for introducing analytical standards; L_7=injection loop; L_8, L_9=heart-cut loops; L_{10}=loop for equilibration; C_1, C_2=LC columns for multivariate analysis; I=injection port for multivariate analysis; D=detector; LHD=Liquid handling device; B=back-pressure regulator; W=waste. Solid lines indicate connectivity by physical flow-paths. Dashed lines indicate fluid communication is made by the robotic device of a liquid handling device (LHD) as necessary.

Simultaneous Optimization of Multiple Critical Quality Attributes in the Synthesis of Warfarin:

The transformation of interest for the simultaneous optimization of percent conversion and ee of warfarin, a commonly prescribed anticoagulant, is shown in Scheme 1.[11] A typical 4D chromatogram for this reaction is shown in Figure 2. The first dimension is typical of what any HPLC trace would look like for such a reaction after running through the C18 column (C_1, Figure 1). We see residual hydroxy coumarin (**1**) and benzylidene acetone (**2**), the desired warfarin product (**3**), the internal standard (IStd), and a couple of trace impurities (byproduct BP). When the third peak containing enantiomers **3a** and **3b** was heart-cut and recirculated through C_1, it gave rise to a single peak in the 2nd-dimension. This confirms that there is no on-column degradation, such as retro-Michael Addition that might return some of **1** and **2**. This gives confidence in the interpretation of percent conversion

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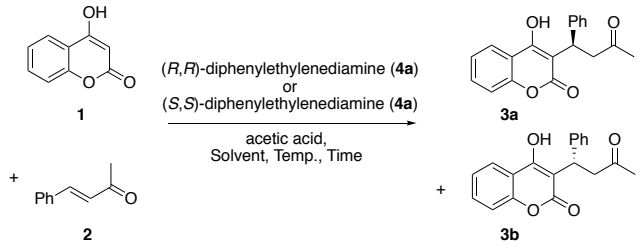

Scheme 1. Asymmetric synthesis of warfarin useed in Tables 1, 2 and 3.

obtained in the 1st-dimension. When this peak was heart-cut and sent through C_2 (the 3rd-dimension), the ee was found to be ~80%. The enantiomers were found to be stable for when **3a** was heart-cut and sent back through C_2 (the 4th-dimension), no racemization or other signs of degradation were observed.

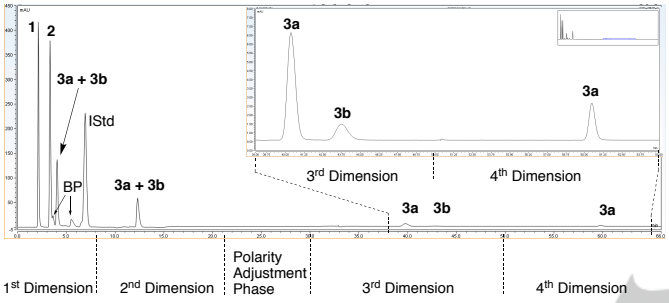

Figure 2. Sample 4-dimensional chromatograph for the optimization of the synthesis of warfarin. BP = unidentified byproduct, IStd = internal standard.

With the integrated recongifurational reactor / multi-dimensional, multiple-variable analysis tool in hand, the transformation was optimized first using the continuous flow reactor (R_2 in Figure 1, Table 1). While percent conversion was low and ee only moderate for all conditions attempted, these data demonstrate precisely the point made in the Introduction. Best conversion was attained in conditions that led, by a considerable distance, to the lowest ee (entry 9), while the highest ee was seen in one of the lowest conversion transformations (entry 6). Using traditional strategies to optimize first for yield and then ee would have favoured pursuing the conditions in entry 9 as the conditions to further optimize, despite leading to the worst enantioselectivity. Perhaps most impressive from these data is that a reaction that proceeded in as little as 2 percent conversion (entry 2) could be reliably analyzed *and validated* for both CQAs using this novel, integrated, mutli-dimensional chromatography technology.

Table 1. Continuous flow optimization of the asymmetric synthesis of warfarin.[1]

Entry	Sol-vent	BA (equiv)	Cat. Load (%)	Acid (equiv)	T (°C)	Res. Time (min)	% Conv.	ee (%)
1	A[2]	1.2	5	10	80	60	4	72
2	A[2]	1.2	5	10	80	20	2	66
3	B[3]	1	100	0	55	50	10	60
4	B[3]	1	30	0	55	50	13	77
5	B[3]	1	30	10	55	50	15	76
6	B[3]	1	30	0	55	50	8	78
7	B[3]	1	30	0	55	100	11	75
8	B[3]	1	30	10	55	50	12	75
9	B[3]	1	30	10	55	50	21	49
10	B[3]	1	30	10	55	50	13	71

[1] See SI for full details. Flow reactor ID = 0.03 inch. Solvent A = Dioxane. [2]Solvent B = MeOH:ACN (1:1).

Next, the transformation was optmized using plug-flow (Table 2), which offers the significant advantage of dramatically reducing the quantities of precious materials used during process optimization.[12] Increasing the amount of chiral catalyst (entries 7 vs 9) or acetic acid (entries 8 vs 9) improved the first CQA (i.e., % conversion), while leaving the second CQA (i.e., ee) unchanged. Interestingly, heating the plugs more did not lead to improved conversion as one might expect, but it did have a noticeable, generally negative impact on ee (entries 1-12 vs 13-25).

Table 2. Plug-flow optimization of the asymmetric synthesis of warfarin.[1,2]

Entry	Reactor ID (inch)	Cat. Load (%)	Acetic Acid (equiv.)	Temp (°C)	Res. Time (min)	% conv.	ee (%)
1	0.02	30	10	45	50	12	80
2	0.02	30	5	45	50	16	78
3	0.03	30	10	45	30	11	89
4	0.03	22.5	7.5	45	30	7	89
5	0.03	37.5	12.5	45	30	18	80
6	0.03	30	10	45	75	19	81
7	0.03	15	15	45	75	19	83
8	0.03	45	5	45	75	20	80
9	0.03	45	15	45	75	37	80
10	0.03	15	15	45	75	10	81
11	0.03	60	20	45	75	28	65
12	0.03	60	10	45	75	24	57
13	0.03	30	15	55	50	11	84
14	0.03	15	5	55	30	11	75
15	0.03	45	15	55	30	17	63
16	0.03	45	5	55	20	9	83
17	0.03	60	5	55	75	17	67
18	0.03	15	5	55	75	13	77
19	0.03	15	15	55	75	15	82
20	0.03	45	15	55	75	24	77
21	0.03	30	10	55	30	12	79
22	0.03	30	10	55	75	6	60
23	0.03	30	15	55	75	18	79
24	0.03	30	20	55	75	21	82
25	0.03	22.5	15	55	75	16	78

[1] See SI for full details. [2]Solvent = MeOH:ACN (1:1).

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With the flow results in hand, we set out to evaluate the batch component of this integrated synthesis/analaysis platform. One set of reaction conditions using both enantiomers of the chiral catalyst are shown in Figure 3. The time-course study illustrates very slow conversion of this process and that it follows near-linear kinetics. The ee of the process remains constant throughout the transformation, and was about the same level as seen the plug flow reactions that we performed at 45 ºC.

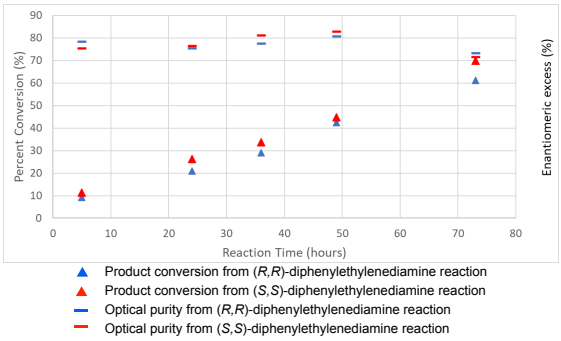

Figure 3. Batch Reactor optimization of the asymmetric synthesis of warfarin. Conditions: 1,4-dioxane, 22 ºC, **1** : **2**, 1 : 1.2, 0.05 equiv. **3a** or **3b**, 10 equiv. acetic acid. See SI for full details.

In summary, we have developed an integrated, reconfigurable-reactor, multi-dimensional chromatography platform for multiple reaction attribute optimization. Without ever leaving the sampling/analysis component, a single reaction sample can be evaluated for two or more attributes in unique chromatographic dimensions and each result validated by re-purposing the peak of interest back through the same (or another) column. This system operates in a hand-free way and uses live signals from the detector to ensure that the peaks of interest are heart-cut properly to give the most accurate and representative analytical outcome with which to optimize the synthetic chemistry. The versatility of this platform has been demonstrated with the simultaneous optimization of percent conversion and enantiomeric excess (ee) for the synthesis of warfarin, an important anti-coagulant. The optimization process could be readily navigated between batch and flow (both plug and continuous) reactors, illustrating its versatility.

Acknowledgements

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Keywords: flow chemistry • multi-dimensional chromatography • Warfarin • multiple critical quality attributes

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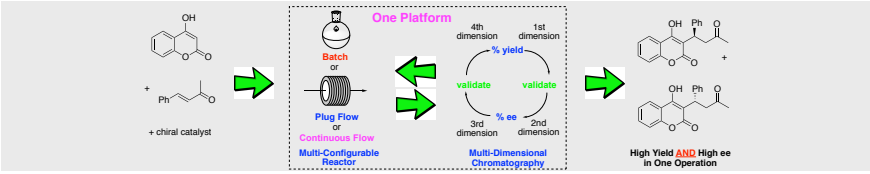

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Page No. – Page No.

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