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Continuous Multiple Liquid–Liquid Separation: Diazotization of Amino Acids in Flow

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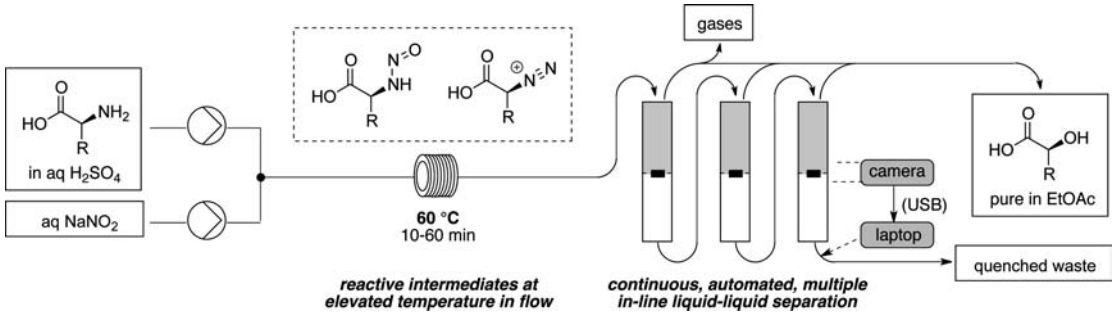
ABSTRACT



A second-generation laboratory-scale, modular liquid–liquid separation device based on computer-controlled high-pressure pumps and a high-resolution digital camera has been invented. The diazotization of amino acids to produce valuable chiral hydroxyacids is demonstrated in flow for the first time. The use of a triple-separator system in conjuction with the developed diazotization process allows the safe and efficient production and automated isolation of multigram quantities of valuable chiral hydroxyacids.

In the past decade, continuous-flow chemistry has emerged as a powerful complement to batch chemistry on the laboratory scale due to relative advantages some of which include: increased safety, more accurate temperature control, ease of reaction scale-up, and amenability to automation.[1] The rapid emergence of new enabling technologies such as tube-in-tube gas reactors,[2] photochemical reactors,[3] copper tube reactors,[4] flow IR cells,[5] agitating cell and ultrasound reactors,[6] and cryo-flow reactors,[7] have rapidly and dramatically expanded the potential scope of flow chemistry's applications.

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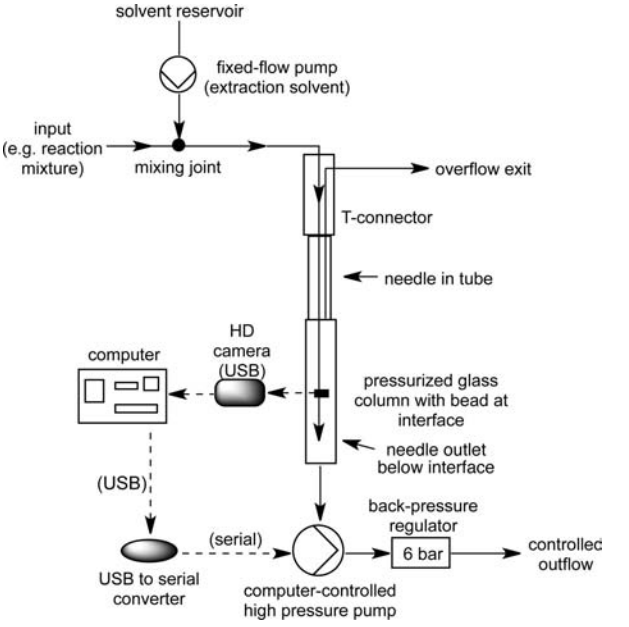
One of the chief focuses of our research group has been the development of enabling technologies for flow chemistry which reduce manual downstream processing tasks such as isolation and purification. For example, we and others have advocated the use of polymer-supported reagents and scavengers to capture impurities in-line,[8] allowing either the direct collection of pure material from flow reactors or direct use of the reaction stream in multistep sequences.[9] In certain "simple" workup processes such as extraction and acid or base washes, however, liquid-phase workup procedures would be preferable to solid-phase procedures if they could be readily conducted in flow.

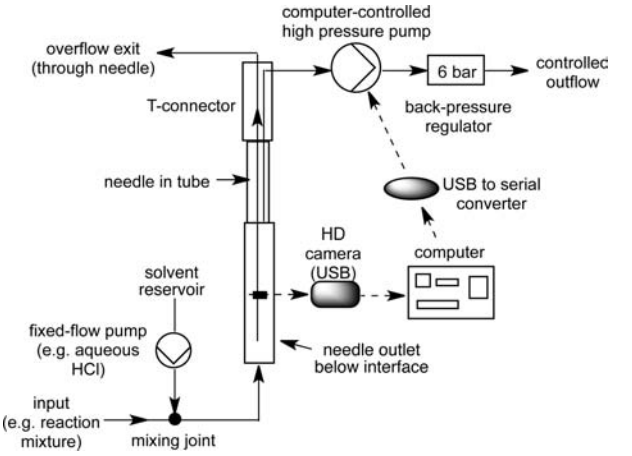
We and other groups have therefore sought to develop tools for automated in-line aqueous workups and phase separations for flow synthesis as a complementary downstream processing technique to solid-phase techniques.[10] Recently, we reported the invention of a syringe pump and camera-based liquid–liquid phase separation tool and its application to reactions requiring a single phase separation.[11] Here, we disclose the development of a modular second-generation high-pressure pump system which allows *solvent-independent*, *multiple consecutive* phase separations and its application to the diazotization of amino acids in flow.

The basic schematic for a single extractor is shown above (Scheme 1). The reaction stream from a flow reactor enters the first extractor which mixes the reaction stream with an extraction solvent. The biphasic stream then enters the top of a small (3 mm diameter) glass separating column

Scheme 1. Detailed Schematic of a Single Extractor (Configuration A: Heavy Phase Further Processed)



Scheme 2. Detailed Schematic of a Single Extractor (Configuration B: Lighter Phase Further Processed)



through a stainless steel tube within a T-piece connector. Inside the phase-separating column is a small, colored polymer float of an intermediate density between the organic and aqueous phases such that it remains at the interface. The bottom of the phase-separating column is connected to a computer-controlled high-pressure pump with a back pressure regulator (75–100 psi). When the flow rate of the high-pressure pump is lower than that of the mixed incoming streams, the upper layer overflows out of the top of the separating column. The computer uses a digital high-resolution camera to monitor the colored bead in the column and provides damped feedback to the high-pressure pump according to the level of the interface

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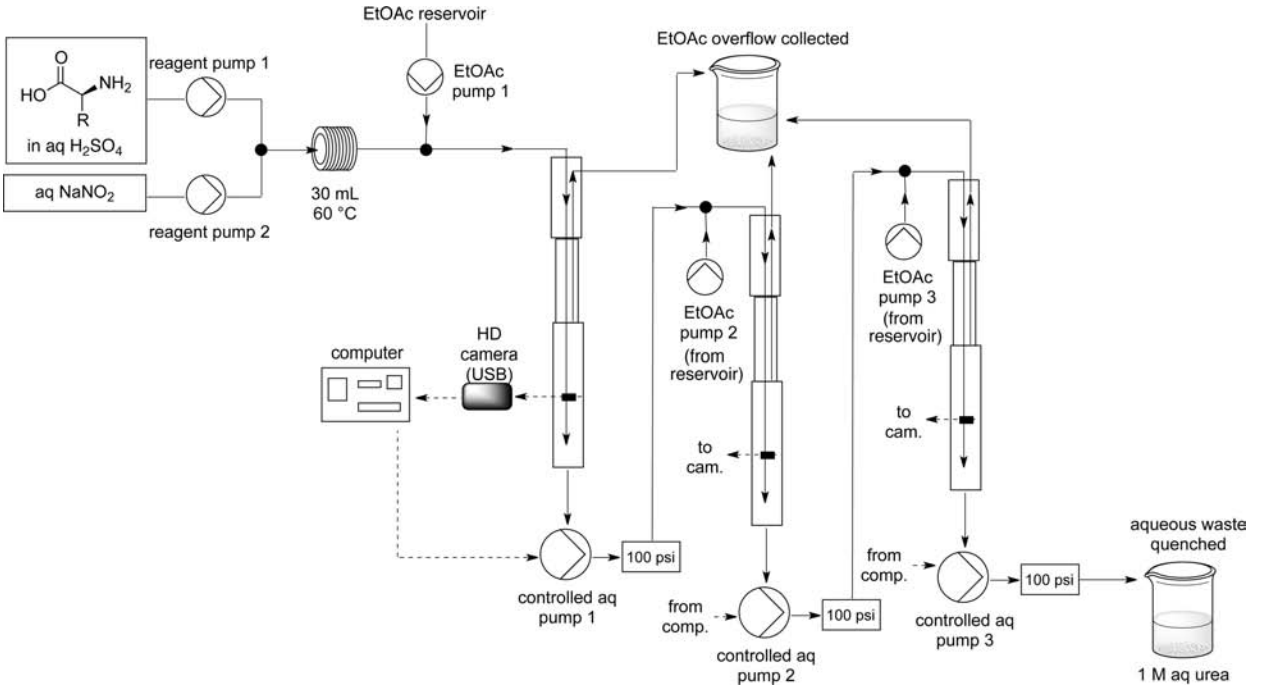
Scheme 3. Flow Diazotization with Multiple Extraction Setup[a]



[a] A solution of amino acid in aq sulfuric acid is mixed with an aqueous solution of sodium nitrite in a Uniqsis flow reactor and reacted in a heated coil. The exit stream is treated by three ethyl acetate extractors in series, and the aqueous exit stream is allowed to collect into a flask containing urea to quench excess sodium nitrite.

relative to the height of the column selected by the user. The computer increases the exit flow rate when the interface is too high and reduces the flow rate when the interface is too low (see Supporting Information for source code, photos, and video). Due to the damped feedback and a rapid computer communication rate, the level of the interface is essentially indefinitely stable.

While the configuration described and shown in Scheme 1 specifically takes the more dense phase on for further processing (e.g., DCM in a DCM/aqueous mixture or H_2O in a H_2O/Et_2O mixture), it is also possible to take the lighter phase on for further processing using the configuration shown in Scheme 2.

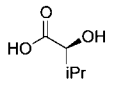
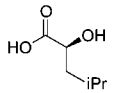
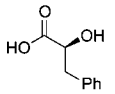
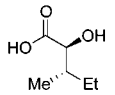
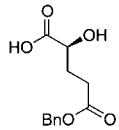
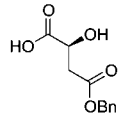
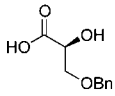
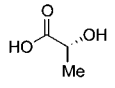
Since each separator has an input and two controlled outputs, several can be placed in series for multiple separations by connecting the output of one to the input of another. The control program has been written to allow a single computer and camera to control three or more separators in series simultaneously based on the user's one-time definition of the columns of interest and column configuration. The final output can be connected to another flow reactor for further reactions, as the final flow rate remains controlled and accurate due to the final high-pressure pump.

We decided to test the multiple separator system in conjuction with the flow synthesis of chiral α-hydroxyacids (Scheme 3), which are highly useful chiral synthons and valuable precursors to numerous natural products and pharmaceutical agents. Importantly, the hydrophobic hydroxyacid, hydroxyvaline (**1**, Table 1), has an experimentally determined partition coefficient of ~0.74 between ethyl acetate and water, necessitating multiple extractions. In batch, the classic diazotization of amino acids is typically conducted at 0 °C due to the release of gas and allowed to stir for several hours to allow the reaction to go to completion.[12] The use of flow apparatus allowed us to safely conduct the diazotization reactions at 60 °C, allowing the reactions to complete between 10 and 60 min in the reactor coil before direct extraction. Impressively, the separators' control program was unaffected by the interfacial turbulence caused by the vigorous evolution of nitrogen gas through the first extractor column, surviving several 24+ h runs of the diazotization reaction without manual assistance.

The results of flow diazotization, displacement, and multiple extraction of select amino acids on scales >1 g are summarized in Table 1. The flow preparation and extraction was highly efficient and remained stereoselective at the elevated temperature used in most instances. The diazotizations were typically conducted at a concentration of 0.25 M postreagent-mixing (Method A, see Supporting Information); higher dilution and higher flow rates were necessary to keep more hydrophobic substrates and their products solubilized (Method B). In some cases, acetone was introduced to prevent crystallization of the less polar hydroxyacid products within the coil (Method C).

(12) For representative examples, see: (a) Deechongkit, S.; You, S.; Kelly, J. W. *Org. Lett.* **2004**, *6*, 497–500. (b) Koppenhoefer, B.; Schurig, V. *Org. Synth.* **1988**, *66*, 151. (c) Fu, S. J.; Birnbaum, S. A. I.; Greenstein, J. P.; Fu, J.; Birnbaum, M. *J. Am. Chem. Soc.* **1954**, *76*, 6054. (d) Fischer, E. *Berichte* **1906**, 489.

Table 1. Summary of the Results of Various Diazotization Reactions in Flow

product	scale (s/m)	yield	er/dr	method
 (**1**) *from L-Val*	20 g	91%	>96:4[a]	A
 (**2**) *from L-Leu*	1 g	89%	~97:3[a]	A
 (**3**) *from L-Phe*	1 g	91%	>99:1[a]	B
 (**4**) *from L-Ile*	1 g	92%	>95:5[b]	A
 (**5**) *from Bn-L-Glu*	2 g	81%	>95:5[c]	C
 (**6**) *from Bn-L-Asp*	2 g	91%	>95:5[d]	C
 (**7**) *from Bn-L-Ser*	1 g	64%	80:20[c,e]	B
 (**8**) *from D-Ala*	8 g	34%	>90:10[a]	A

[a] Determined by chiral HPLC of the corresponding benzyl ester by comparison with a racemic standard. [b] Determined by NMR. [c] Determined by NMR after derivitization of the corresponding benzyl ester to both Mosher ester diastereomers. [d] Determined by NMR after derivitization of the corresponding methyl ester to both Mosher ester diastereomers. [e] From starting material of >95:5 *er* checked by Mosher amide analysis after derivization to the corresponding methyl ester (see Supporting Information).

Notably, due to the short retention time of the reactions and the rapid extraction of product from the acidic and oxidizing aqueous layer, benzyl ether and ester protecting groups largely survived the reaction conditions, with deprotected byproducts washing out in the aqueous stream. The reaction could be conducted continuously on 20+ g scales over 24 h+ periods without manual intervention or detriment to yield. The enantiomer of hydroxyvaline derived from D-valine generated by this process was used as the starting material for our total synthesis of (−)-enniatin B.[13]

The poor yield for alanine (**8**, Table 1) is explained by the extreme product water solubility The degradation in the enantiomeric ratio of the hydroxyacid derived from serine (**7**) appears to be due to the inductive effect of the β-oxygen atom, which likely impedes S_N2 substitution of the intermediate α-lactone, increasing the relative rate of ring-opening by acyl substitution. In all cases, the combination of the flow reactor and the multiple extraction system made each reaction sequence exceedingly simple to execute: a solution of amino acid in aqueous sulfuric acid and a stock solution of aqueous sodium nitrite were connected to a flow reactor, the pumps were started, and a final solution of pure α-hydroxyacid in ethyl acetate was obtained, dried, and concentrated. The aqueous phase, which was automatically extracted and quenched in line, was simply discarded. It should be noted that conducting the same 20 g scale reaction in batch would require much more careful thermal control and the manual manipulation of over 3 L of solvent in a very large separating funnel.

In summary, a device capable of multiple liquid−liquid extractions/phase separations has been developed and applied to the first examples of amino acid diazotization in flow on multigram scales. The device is easy to assemble manually, and instructions along with the source code for the machine are provided. Applications of the device in other chemical reaction sequences are currently being investigated in our laboratory and will be disclosed in due course. We believe the use of this device will greatly accelerate synthetic preparations in research laboratories and enable more complex multistep sequences in the future.

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Supporting Information Available. Detailed machine design with photos, an explanatory video showing the full setup in operation, Python code with explanatory comments, experimental details, compound characterization, and supporting spectra are provided as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.