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Article

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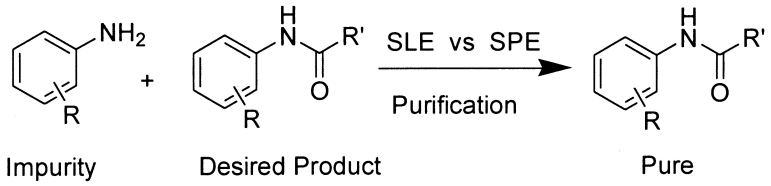

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Articles

Scope and Limitations of Solid-Supported Liquid−Liquid Extraction for the High-Throughput Purification of Compound Libraries

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This paper reports some of the advantages and limitations of solid-supported liquid−liquid extraction (SLE) for the rapid purification of organic compound libraries. Issues of solvent compatibility, compound compatibility, and technical methods are addressed. In addition the prospective use of calculated log *P* values is investigated to determine which impurities will be effectively removed by this technique. In addition SLE is shown to be complementary and in some cases superior to solid-phase extraction (SPE) methods for library purification. This is especially true when the desired products have functionality equivalent to that of the impurity to be removed.

Introduction

As parallel synthesis becomes more commonplace among synthetic chemists, rapid purification of library compounds has become a bottleneck in the synthetic process. Traditional methods of compound purification such as aqueous extraction and chromatography become cumbersome as the number of simultaneously processed compounds increases. As a result, many innovative purification methods have recently been disclosed that make automated workup of crude solution-phase reactions possible. These include, but are not limited to, solid-phase extraction (SPE), resin scavenging, fluorous phase extraction, and chemical tagging of reagents.[1] However, the most common method traditionally used for cleanup of organic reactions is aqueous extraction.[2] Recently, there have been a number of methods developed for the aqueous extraction of large numbers of organic reactions in parallel. Unfortunately, most of these techniques suffer from drawbacks such as expensive robotics, inability to cope with emulsions, and restrictions to solvents that are denser than water.[3]

In late 1997 Johnson and co-workers first reported the use of a versatile extraction method, referred to as SLE (solid-supported liquid−liquid extraction), for the purification of combinatorial libraries.[4] The technique involves supporting an aqueous buffer on a bed of coarse mesh, calcinated diatomateous earth sold under the product name Hydromatrix. Since this first report, a number of groups have reported the use of Hydromatrix for the parallel purification of compound libraries.[5] In this paper we outline some of the advantages and limitations of SLE for the rapid purification of organic compound libraries and compare SLE to SPE in a library application.

Methods

Hydromatrix was originally invented by NASA/JPL in the mid 1980s as a tool for the isolation of organic materials from aqueous samples that were collected for environmental monitoring.[6] The product is currently marketed by Varian Sample Preparations Products and has primarily been used to extract organic soluble drugs from aqueous plasma or urine samples for PK determinations and controlled substance monitoring.[7] The introduction of this inexpensive ($63.00/kg) and versatile material offers many advantages to previously reported methods of parallel aqueous extraction.

Varian offers prepacked Hydromatrix cartridges, called Chemelut, in volumes from 0.3 to 300 mL. Varian also manufactures 96-well plates prepacked with Hydromatrix. Additionally, Hydromatrix can easily be packed into any desired format, making it compatible with a wide range of robotic platforms.[4,8] The general method for SLE in the Chemelut format is shown in Figure 1.

The Chemelut cartridge is placed over a collection tube and primed with aqueous buffer. Our laboratories have found SLE to be compatible with buffers (2.0 N H_2SO_4 to 1.0 N NaOH) in a wide range of pH values. The amount of aqueous buffer added depends on the amount of Hydromatrix in the tube. Generally 1 g (0.25 g/mL) of Hydromatrix will support 2 mL of aqueous buffer. Alternatively, each 1.0 mL (0.25 g/mL) of unpacked Hydromatrix will support 0.5 mL of aqueous material. The aqueous material is allowed to adsorb onto the column for about 2 min. The crude organic reaction mixture is then added to the column and allowed to settle into the Hydromatrix. Additional organic solvent (1.5 mL/mL of Hydromatrix) is then added to elute the organics into the collection tube.

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Table 1. Solvent Mixtures Compatible with SLE

solvent mixture	% of water-miscible solvent usable for SLE[a]	solvent mixture	% of water-miscible solvent usable for SLE
CH_2Cl_2/MeOH	20% MeOH	toluene/THF	70% THF
CH_2Cl_2/acetone	20% acetone	toluene/DMF	30% DMF
CH_2Cl_2/DMF	10% DMF	EtOAc/DMF	10% DMF
CH_2Cl_2/DMA	20% DMA	EtOAc/THF	70% THF
CH_2Cl_2/NMP	20% NMP	EtOAc/*i*-prOH	60% *i*-prOH
CH_2Cl_2/THF	70% THF	EtOAc/MeOH	10% MeOH
CH_2Cl_2/CH_3CN	10% CH_3CN	Et_2O/THF	50% THF

[a] These values reflect the highest percentage of the more water-miscible solvent that can be applied to the SLE column with out causing aqueous breakthrough.

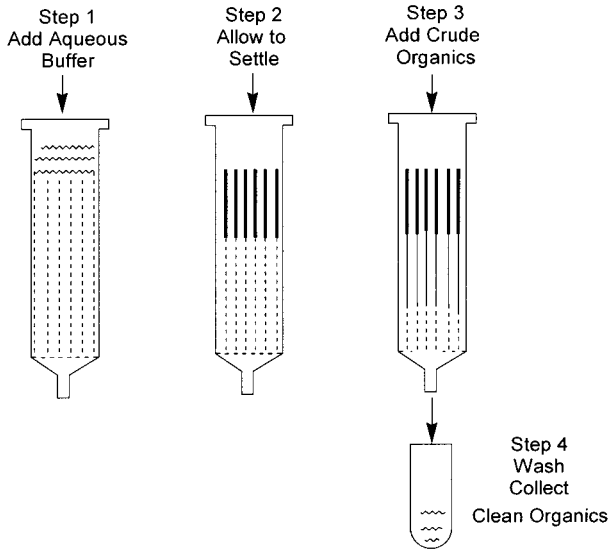


Figure 1.

Instructions that accompany the Chemelut cartridges recommend no pressure be applied to the column. However, we have found that if the columns are not overloaded with aqueous buffer, a −5 psi vacuum applied to the column speeds extraction and does not result in aqueous material passing through the column.

It has been reported that a potential advantage of SLE over traditional aqueous extraction is that more than one theoretical plate of separation could be achieved.[4] However, when SLE is compared to traditional aqueous extraction experimentally, the degree of purification appears to be equivalent to that of a single extraction using a separatory funnel.

Plate Formatting

Hydromatrix can be easily packed into filter plates for the simultaneous processing of up to 96 compounds. Typically, Polyfiltronics 96-deep-well GF/C filter plates (1.5 mL of Hydromatrix) or Robbins' Flexchem 48-well filter plates (3.5 mL of Hydromatrix) are used. The Hydromatrix is simply poured onto the filter plate, leaving about 3 mm of headspace at the top of the wells for solvent addition. Aqueous buffer is then added (0.6 mL for 96-well plates and 1.2 mL for 48-well plates). Crude organic reaction mixtures (0.75 mL for 96-well plates, 2.0 mL for 48-well plates) are then transferred from reaction plates to the filter plate using a multichannel pipet, Robbins' Hydra, or other robotic liquid-transfer tools.

In early work some of the wells would clog with salts that were insoluble in the organic reaction medium. This problem was overcome by adding 200−400 uL of aqueous buffer directly to the crude reaction mixture prior to its addition to the SLE plate. The SLE plate is then washed with organic solvent (2.0 mL for 96-well plates, 4.0 mL for 48-well plates), eluting the compounds into the collection plate.

Solvent Compatibility

One of the advantages of SLE over extraction using hydrophobic membranes[5e] is that it is fully compatible with a wide range of solvents, regardless of density. Extractions are readily performed in any solvent that is not miscible with water. In addition, because emulsions are not an issue with SLE, solvent mixtures can be used regardless of density.

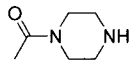
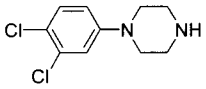
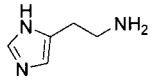
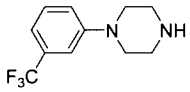
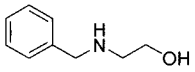
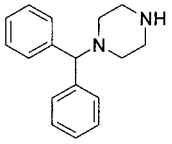
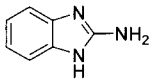
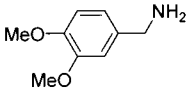
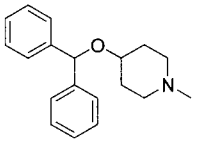
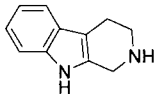
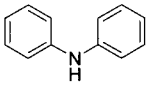
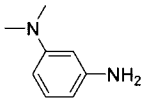
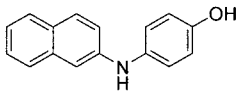
Because many reactions are run in water-soluble solvents such as THF, DMF, and the like, it would be advantageous to know what proportion of water-soluble solvent is compatible with SLE prior to a library purification. Instructions published by Varian suggest that no more than 10% aqueous soluble material can be added to the Hydromatrix for an effective SLE. When tested experimentally, the effective composition was highly variable depending on the solvent (Table 1).

Chemelut cartridges (5 mL) were loaded with 2.5 mL of 0.5 N $CuSO_4$ for ease of visualization. Organic solvent mixtures (10 mL) were then passed through the column using a vacuum of −10 kPa. The effluent was collected and visually inspected for aqueous $CuSO_4$. The highest percentage of aqueous soluble solvent, which did not show $CuSO_4$ in the effluent, was recorded in Table 1. In the case of DMF a 10% maximum concentration is appropriate. However, for a solvent such as THF, compositions as high as 70% can be tolerated. These observations should enhance the utility of SLE for library purification by eliminating the necessity of removing the reaction solvent and redissolving prior to extraction. Instead, the reaction can simply be diluted with the appropriate organic solvent and extracted by SLE directly.

What Can SLE Remove?

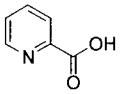
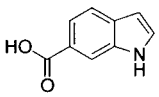
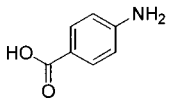
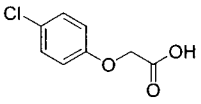
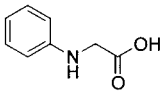
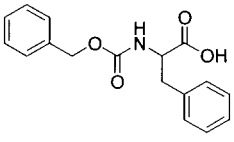
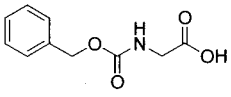
Previous reports show acidic SLE is effective in removing a wide variety of amines from crude organic reactions.[4,5,8] However, there are some reported cases where the technique failed to remove the desired byproduct.[4] This is not surprising given the varying water solubilities of the impurities in question. Because it would be beneficial to determine which

Table 2. Amine Sequestration by Acidic SLE[a]

Amine	ClogP	% Amine Removed by SLE 1N HCl (1N H_2SO_4)	Amine	ClogP	% Amine Removed by SLE 1N HCl (1N H_2SO_4)
	-0.94	>99%		2.25	99%
	-0.82	>99%		2.62	95% (99%)
	0.51	>99%		3.07	99%
	1.09	>99%			
	1.28	88% (97%)		3.26	<2% (26%)
	1.71	>99%		3.46	6% (6%)
	1.89	>99%		4.19	<2% (<2%)

[a] All extractions were run using CH_2Cl_2/DMF (10:1) as the organic solvent.

Table 3. Acid Sequestration by Basic SLE[a]

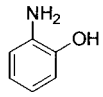
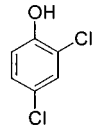
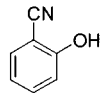
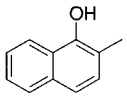
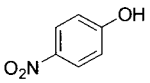
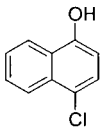
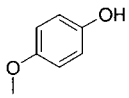
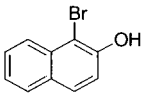
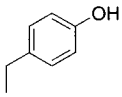
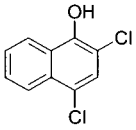
Acid	ClogP	% Acid Removed by SLE 1N NaOH	Acid	ClogP	% Acid Removed by SLE 1N NaOH
	0.35	>99%		1.17	>99%
	0.41	>99%		1.63	>99%
	0.8	>99%		3.03	>99%
	0.86	>99%			

[a] All extractions were run using CH_2Cl_2/DMF (10:1) as the organic solvent.

reagents SLE could effectively remove prior to library synthesis, a correlation between calculated log *P* values for a given compound with SLE efficiency was investigated (Table 2).

A number of amines (0.25 mmol) with varying ClogP values[10] were dissolved in 3 mL of 10% DMF in DCM and added to 6 mL Chemelut cartridges that were preincubated with 1.0 N H_2SO_4 (2.5 mL). The compounds were then eluted

Table 4. Phenol Sequestration by Basic SLE[a]

Phenol	ClogP	% Phenol Removed by SLE 1N NaOH	Phenol	ClogP	% Phenol Removed by SLE 1N NaOH
	0.66	>99%		2.72	>99%
	0.98	>99%		3.01	86%
	1.44	>99%		3.22	>99%
	1.61	>99%		3.49	>99%
	2.35	>99%		3.84	>99%

[a] All extractions were run using CH_2Cl_2/DMF (10:1) as the organic solvent.

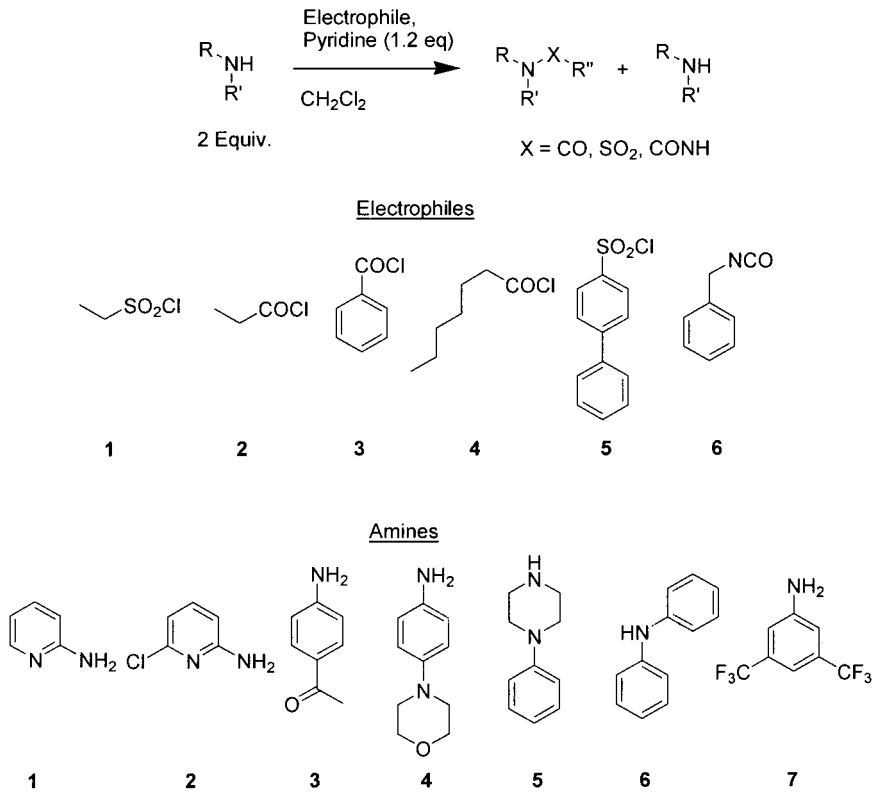


Figure 2.

with 10 mL of 10% DMF in DCM. The quantities of amine eluted were then determined by HPLC analysis, using toluene as an internal reference standard.

Complete removal of amine was accomplished in cases where the amine had a ClogP of <3.1. In cases where the amine had a ClogP value of >3.1, SLE was inefficient in removing the amine. With this information in hand, it is easy to prospectively determine if SLE will be an effective way of removing library inputs by simply calculating the ClogP values for the compounds before synthesis.

The efficiency with which basic SLE removed acids and phenols from organics was also tested. Surprisingly, even

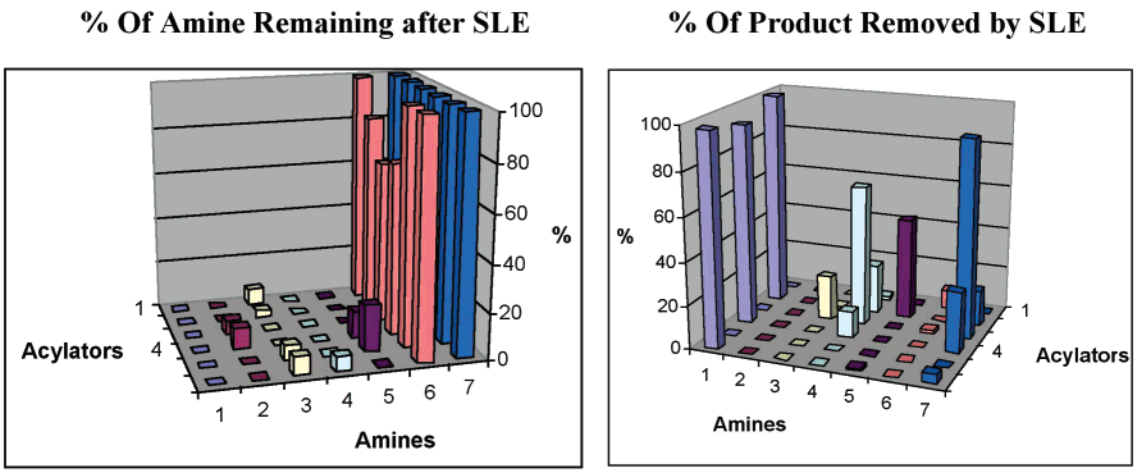


Figure 3. All amounts were determined by HPLC at 214 nm using toluene as an internal reference standard.

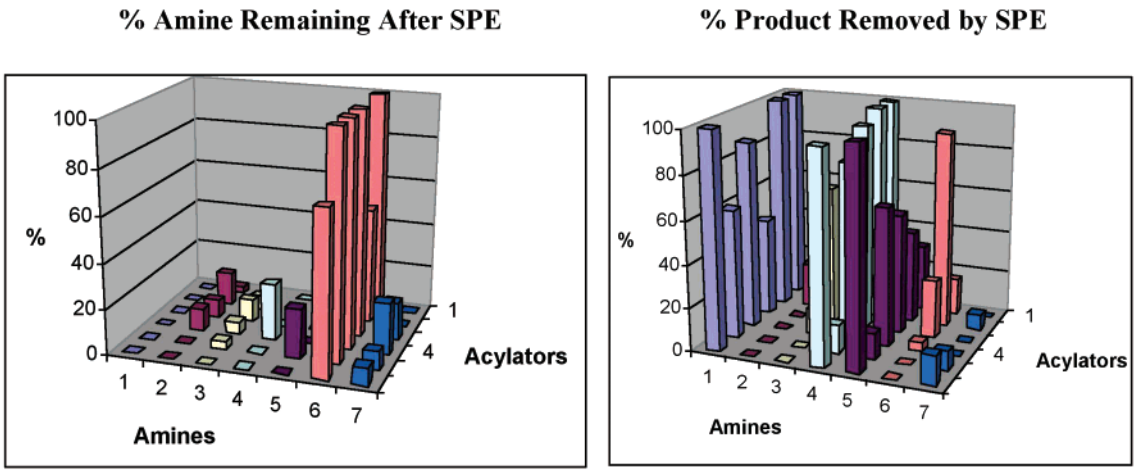


Figure 4. All amounts were determined by HPLC at 214 nm using toluene as an internal reference standard.

very nonpolar acids and phenols could be effectively removed using a basic SLE extraction (Tables 3 and 4).

The acid or phenol (0.25 mmol) was dissolved in 3 mL of 10% DMF in DCM, and added to 6 mL Chemelut cartridges that were preincubated with 1.0 N NaOH (2.5 mL). The organics were then eluted with 10 mL of 10% DMF in DCM. The quantities of acid or phenol eluted were then determined by HPLC analysis, using toluene as an internal reference standard. In this experiment all of the phenols and carboxylates tried were effectively sequestered by the SLE conditions used; therefore, no correlation between ClogP and SLE efficiency could be determined. However, it is noteworthy that even very nonpolar acids and phenols were effectively sequestered by SLE, making SLE a versatile method for the removal of these impurities.

Comparing SLE to SPE

To demonstrate the utility of SLE for library purification, the technique was tested on a small library of compounds.[9] In this library excess amines were coupled to a variety of acid chlorides, sulfonyl chlorides, or isocyanates. The amines used were selected to demonstrate the scope and limitations of SLE purification compared to ion exchange purification (Figure 2).

A solution of an acid chloride, sulfonyl chloride, or isocyanate (0.25 mmol) in CH_2Cl_2 (1.0 mL) and pyridine (0.5 mmol) was added to the columns of a 48-well Robbins' filter-bottom microtiter plate. Excess amines (0.5 mmol in CH_2Cl_2) were then added across the rows of the plate and shaken for 14 h at ambient temperature. A second 48-well filter plate was then filled with Hydromatrix (3.5 mL/well), pretreated with 1.0 N H_2SO_4 (1.0 mL), and stacked onto a 48-well collection plate. Toluene (0.75 mmol) was added to the crude reactions as an internal standard, and the wells were analyzed by HPLC. The contents of the reaction plate were then transferred with a multichannel pipet onto the SLE plate, and the organics were eluted into the collection plate with 5% MeOH in CH_2Cl_2 (3.0 mL). The reactions were analyzed by HPLC, and the amounts of amide product and residual amine were determined.[11] The results are shown in Figure 3 below.

In this library the excess amines were effectively removed from the products except in the case of amines **6** and **7**. However, the result is anticipated by the amines' ClogP values of 3.46 and 3.07, respectively. All amines with ClogP values of <3.0 were effectively removed from the reaction. As far as product recovery, all but 6 of the 42 products were recovered in $>80\%$ yield. (Two of these products were found

to be very soluble in 1.0 N H_2SO_4. The other four compounds were not soluble in 5% MeOH in CH_2Cl_2 and precipitated on top of the extraction columns.)

A second library was synthesized by the same procedure. However, in this case the compounds were purified by addition of acidic ion-exchange resin (Dowex 50WX8-400; 300 mg), followed by shaking for 3 h and filtration. In this case all of the excess amines were effectively removed from the reactions except amine **6**. Presumably the *N*,*N*-diphenylamine is not basic enough to be extracted by the resin. However, in the case of this library, the 18 products containing a basic nitrogen were sequestered on the resin as well, resulting in poor product recovery. This demonstrates clearly one of the advantages of SLE over ion exchange methods for library purification. Because SLE is a solubility-driven purification method, libraries can be built in which the products contain the same functionallity as the byproducts to be removed, as long as there is a significant difference in solubility between the two. In this way, ion exchange methods and SLE are somewhat complementary techniques.

Conclusions

In summary we have demonstrated the effectiveness of SLE for the efficient parallel removal of organic acids and bases from organic reactions. In addition the prospective use of calculated log *P* values allows one to predetermine which impurities will be effectively removed by this technique. It has also been shown that the aqueous soluble solvent concentrations in the organic phase of these extractions can greatly exceed that previously reported, increasing the useful scope of the SLE technique. SLE has also been shown to be complementary, and in some cases superior, to ion exchage methods in cases where the desired products have functionality equivalent to that of the impurity to be removed.

References and Notes

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