Simple and Economical High-Throughput Equilibrium Dialysis System

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High-throughput technology (HTT) has transformed a broad and diverse range of research areas from DNA sequencing to compound synthesis to reaction protocol optimization. The small molecule pharmaceutical industry is perhaps one of the most salient examples of this transformation. With the introduction of automated dispensing systems, thousands of pharmaceutical candidates can be synthesized simultaneously in multiwell microtiter plates, replacing the arduous task of synthesizing individual compounds in separate reaction flasks. Coupling this technology with high-throughput analytical equipment, the pace at which viable drug candidates can be identified and optimized has greatly accelerated.[1]

Given the potential benefits of HTT for small molecule drug development, the extension of these strategies to a polymeric biomaterials application may seem natural considering both areas of research share similar organic synthesis processes. As such, we and others have adopted high-throughput strategies to facilitate the creation of polymer libraries for use in various biomedical research.[2−4] In particular, we have adopted HTT to facilitate the synthesis, purification, characterization, and screening of a combinatorial library of polymeric biomaterials. Each step is amenable to high-throughput processing within 96-well microtiter plates, with the exception of polymer purification from within the individual reaction wells. In smaller scale experiments, where the number of polymers to purify is modest and each polymer solution is on the milliliter scale or greater, traditional equilibrium dialysis is a reliable and commonly used method for purifying a high molecular weight (MW) product. However, in high-throughput experiments on the microliter scale, transferring individual reaction batches into separate dialysis tubes can quickly become unmanageable and inefficient.

Several high-throughput equilibrium dialysis systems were recently reported in the literature or are currently available commercially.[5,6] Some were specifically designed to purify a desired high MW product from reaction batches, while others were designed for use in protein binding assays but could likely be adapted for a purification application. All of the systems, however, are either cumbersome to assemble, require access to specialized equipment (e.g., centrifuge), involve costly investment in start-up equipment, or require dialysis from a custom-made multiwell plate to and from which samples would need to be transferred.

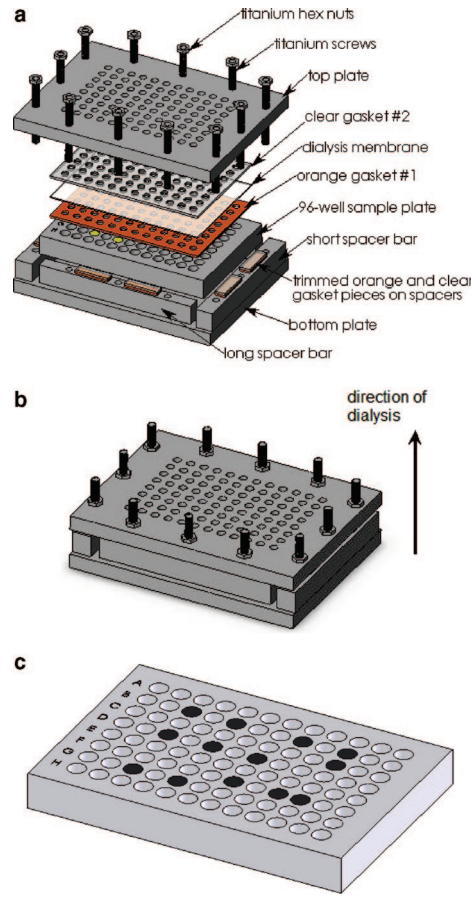

Figure 1. High throughput equilibrium dialysis system (a) Exploded view of dialysis apparatus including 96-well microtiter sample plate. After dialysis, the high molecular weight retentate remains in each well of the microtiter plate. (b) Assembled view of dialysis apparatus. Upon assembling the apparatus, the system is flipped over in the dialysis bath prior to commencing dialysis (see Supporting Information for the dialysis protocol) making the direction of dialysis as shown. (c) Well assignment of tartrazine and dextran-rhodamine for dialysis experiments (black wells). All other wells contained an equivalent volume (200 μL) of 0.1N HCl.

In this report we describe a robust high-throughput equilibrium dialysis apparatus aimed at addressing the aforementioned limitations associated with the reported and commercially available systems. The dialysis system reported herein was designed to purify a high molecular weight polymer from a reaction mixture comprising a 2-fold excess of a low MW molecule that represents reaction byproducts, reagents, and other contaminants. Moreover, the dialysis apparatus can be economically constructed from commercially available materials and easily fabricated in an average machine shop. Attributes of the reported system

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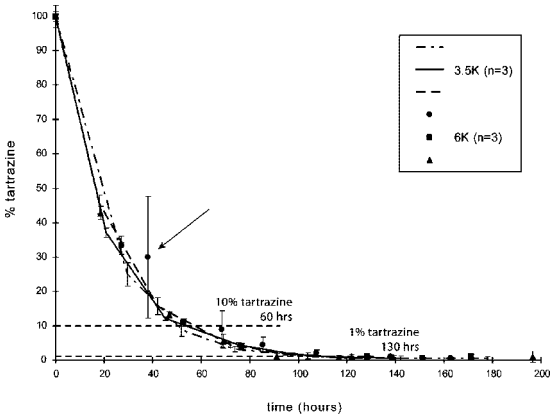

Figure 2. Dialysis profiles of tartrazine from wells initially containing 50 μM tartrazine in 0.1 N HCl. Tartrazine dialyzed to less than 10% after approximately 60 h and less than 1% after approximately 130 h. Arrow marks the time point at which dialysis was delayed because of an air bubble trapped in one well of the top plate. Upon removal of the air bubble, dialysis resumed. Solid and dashed lines represent dialysis through a 3.5K MWCO dialysis membrane; shapes represent dialysis through a 6−8K MWCO membrane. Tartrazine concentrations reported are averages of all sample wells (n = 13) for one dialysis run taken at the designated time point. Error bars represent ± one standard deviation.

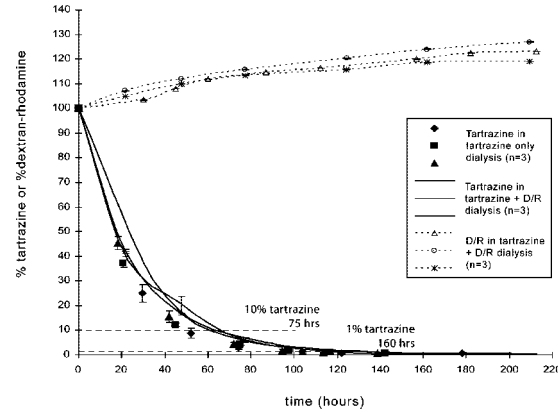

Figure 3. Dialysis profiles of tartrazine from wells initially containing 25 μM dextran-rhodamine (10K g/mol, 1:1 mol ratio of rhodamine:dextran) and 50 μM tartrazine in 0.1 N HCl. Dialysis of tartrazine was slightly retarded in the presence of dextran-rhodamine (D/R) with dialysis to less than 10% occurring after approximately 75 h and less than 1% after approximately 160 h. D/R profiles were also monitored to confirm that leakage did not occur throughout dialysis. Shapes represent tartrazine profiles in tartrazine only dialyses; solid lines represent tartrazine profiles in tartrazine + D/R dialyses; dashed lines with shapes represent D/R profiles in tartrazine + D/R dialyses. Tartrazine and D/R concentrations reported are averages of all sample wells (n = 13) for one dialysis run taken at the designated time point. Error bars represent ± one standard deviation.

include (1) compatibility with automated liquid handling systems, (2) resistance to acidic environments, (3) minimal creation of waste, (4) ease of assembly, (5) the obviation of specialized equipment, and (6) ready integration with in line high-throughput data acquisition technology (e.g., 96-well plate reader).

Results and Discussion. Figure 1a and b shows an exploded and assembled illustration of the dialysis apparatus with the source of all materials summarized in Table 1. To characterize the time course of dialysis from within the reported system, dextran-rhodamine (D/R) (10K g/mol, 1:1 mol ratio of rhodamine:dextran) and tartrazine (534 g/mol) were used as model compounds to represent the high MW retentate and low MW dialysate, respectively. The model compounds were dissolved and dialyzed against 0.1 N HCl from within the wells of the 96-well assay plate as shown in Figure 1c. The reasons for choosing 0.1 N HCl as the dialysis medium are discussed in the Dialysis System Assembly section of the Supporting Information. Sample well positions were assigned accordingly to monitor for cross contamination between wells during dialysis. A detailed assembly procedure of the dialysis system, experimental protocol, data analysis, and drawings for each component of the apparatus are provided in the Supporting Information.

Time Study of Equilibrium Dialysis of Low MW Compound through a 3.5K MWCO and 6−8K MWCO Membrane. To characterize the time course of dialysis from the reported apparatus, dialysis of 50 μM of tartrazine through a 3.5K MWCO and a 6−8K MWCO membrane was quantified daily. Experiments consisted of tartrazine dialysis from wells containing no high MW polymer. On the basis of the dialysis profiles, shown in Figure 2, of three independent experiments at each MWCO, there is little disparity between tartrazine dialysis through a 3.5K and 6−8K MWCO membrane. However, in one of the three 6−8K experiments, a relatively large standard deviation was observed at t = 38 h (arrow in Figure 2). Upon removing the apparatus to obtain the time point reading, we discovered that an air bubble was present in a well of the top plate that corresponded to a tartrazine well in the 96-well assay plate. The bubble prevented dialysis from the well resulting in the relatively large standard deviation. Removing the air bubble for the remainder of the experiment resumed dialysis. The results highlight the importance of expelling all air bubbles before commencing dialysis. Nevertheless, in both experi-

Table 1. Materials for Equilibrium Dialysis System

material	manufacturer	catalog no.
regenerated cellulose dialysis membrane (flat sheet) MWCO = 3.5K Da	Spectra/Por (Rancho Dominguez, CA)	132723
regenerated cellulose dialysis membrane (flat sheet) MWCO = 6−8K Da		132677
clear gasket (silicone rubber)	McMaster-Carr (Elmhurst, IL)	86915K16
orange gasket (silicone rubber)		8608K51
polycarbonate sheet (12 in. × 12 in. × 3/8 in.)		8574K31
polycarbonate sheet (12 in. × 12 in. × 1/4 in.)		8574K28
polycarbonate bar (48 in. × 1 in. × 1/2 in.)		1749K14
96-well assay microplate (flat bottom, polystyrene, clear)	Matrix Technologies (Hudson, NY)	4915
titanium hex nut (10−32)	Small Parts, Inc. (Miramar, FL)	HNTT-1032
titanium threaded rod (10−32)		TRTT-1032

Table 2. Percent Tartrazine Remaining after 160 and 220 h of Uninterrupted Dialysis0

	tartrazine only dialysis(160 h)	tartrazine + dextran-rhodamine dialysis(220 h)	
	% tartrazine	% tartrazine	% dextran-rhodamine
3.5K MWCO	0.53% [a]	0.58% [a]	112.18%
std error	0.003%	0.028%	0.095%
6−8K MWCO	0.62% [a]	0.63% [a]	105.53%
std error	0.008%	0.015%	0.097%

[a] For comparisons between tartrazine only and tartrazine plus dextran-rhodamine dialyzed under the same MWCO conditions, no statistical difference in the final percent tartrazine remaining was observed ($p > 0.05$).

Table 3. Comparison between Reported Dialysis System and Several Commercially Available Systems

type	description	start up cost	cost per 96 samples	notes
equilibrium dialysis system	Spectra/Por 96 well microdialyzer *(#132326)*	$1379		available in 3.5K, 6−8K, 12−14K MWCO membranes
	custom-made 96 well plate (no. 132330)	$ 224		samples must be transferred for high-throughput data acquisition (e.g., microtiter plate reader)
	dialysis sheets (MWCO = 3.5K) (no. 132723)		$ 8	reusable sample plate
	dialysis sheets (MWCO = 6−8K) (no. 132677)		$ 3	minimal assembly required
	Harvard Apparatus 96-Well DispoDIALYZER [a]			available in 1K, 2K, 5K, 10K, 25K MWCO membranes
	MWCO = 2K *(#740901)*		$317	samples must be transferred for high-throughput data acquisition (e.g., microtiter plate reader)
	MWCO = 5K *(#740902)*		$317	new sample plate required for each dialysis
				no assembly required
	Novagen/EMD Biosciences D-Tube96 Dialyzer (no. 71712−2)		$354	available in 6−8K and 12−14K MWCO membranes
				samples must be transferred for high-throughput data acquisition (e.g., microtiter plate reader)
				new dialysis tubes per sample required for each dialysis
ultrafiltration	Millipore MultiScreen Filter Plate with Ultracel-10 Membrane *(#MAUF01010)*		$ 6	centrifuge equipped with a 96-well plate rotor required
				available in 10K MWCO membrane only
	standard 96-well receiver plate		$ 2	samples must be transferred for high-throughput data acquisition (e.g., microtiter plate reader)
	centrifuge			new sample plate required for each dialysis
				no assembly required
plasma protein binding systems	Pierce Biotech Rapid Equilibrium Dialysis (RED) Device			available in 8K MWCO membrane only
				samples must be transferred for high-throughput data acquisition (e.g., microtiter plate reader)
	reusable Base Plate (no. 89811)	$ 320		reusable sample plate
	RED Device Inserts (no. 89809)		$700	minimal assembly required
	HTDialysis, LLC 96-well Micro Equilibrium Dialysis block Complete Set (no. 1006) [b]	$2995		available in 3.5K, 6−8K, 10K, 12−14K, 25K, 50K MWCO membranes
				samples must be transferred for high-throughput data acquisition (e.g., microtiter plate reader)
	dialysis strips (MWCO = 3.5K) (no. 1135)		$ 35	reusable sample plate
	dialysis strips (MWCO = 6−8K) (no. 1103)		$ 30	assembly of dialysis apparatus can be cumbersome
reported dialysis system	polycarbonate housing, titanium screws and hex nuts, gaskets	$ 153		available in 3.5K, 6,194 > 8K, 12−14K MWCO membranes
				compatible with standard 96 well plate (reusability optional)
	96-well plate		$ 2	minimal assembly required
	dialysis sheets (MWCO = 3.5K)		$ 8	
	dialysis sheets (MWCO = 6−8K)		$ 3	

[a] Design similar to the one reported by Banker, et al.[5] [b] Design similar to the one reported by Kariv, et al.[6]

mental setups, tartrazine was dialyzed to less than 10% of the initial concentration after 60 h and less than 1% after 130 h.

The relatively long dialysis times may be attributed to two possible factors: (1) limited mass transport from the retentate side of the dialysis membrane and (2) a convection-free layer that may exist on the dialysate side of the membrane located within each channel of the top plate. Shorter dialysis times may be achieved with a next-generation dialysis system that incorporates a method to agitate the retentate-side dialysis solution (e.g., rotating or shaking the assembled dialysis apparatus throughout dialysis) or by reducing the thickness of the top plate so as to minimize the convection-free layer potentially located on the dialysate side of the dialysis membrane.

Effect of High MW Polymer on Equilibrium Dialysis of Low MW Compound. In the presence of a high MW species, dialysis of a low MW molecule may be retarded because of accumulation of the high MW species at the membrane surface, which may prevent the low MW molecule from freely permeating through. Alternatively, if both the high and low MW species possess aromatic functional groups, $\pi-\pi$ orbital stacking interactions may also affect dialysis. To determine the potential effects on dialysis of such situations, the time course for the dialysis of 50 μM of tartrazine through a 3.5K and 6−8K MWCO membrane from wells containing 25 μM D/R (MW = 10 000 g/mol) was quantified. Similar to the initial experiments of dialyzing tartrazine in the absence of a high MW polymer, there is little disparity between dialyzing through a 3.5K and a 6−8K MWCO membrane (data not shown). However, tartrazine dialysis appeared to be slightly retarded in the presence of D/R with dialysis to less than 10% of the initial concentration occurring after 75 h and to less than 1% after 160 h. (Figure 3) The precise cause for this is not fully understood although

it is likely to be attributed to one or both of the previously mentioned factors.

The presence of D/R was also monitored throughout dialysis to confirm that it was not leaking out either during dialysis or while obtaining the time point readings. The D/R profiles shown in Figure 3 confirm that the concentration of D/R did not appear to decrease during dialysis.

Effect of Membrane Replacement and Evaluation of Leak Resistance Throughout Dialysis. To confirm that replacing a fresh dialysis membrane between each UV−vis absorbance time point reading in the daily experiments did not alter the time course for tartrazine dialysis, the presence of tartrazine was also quantified after 160 and 220 h of uninterrupted dialysis, for the tartrazine only and tartrazine plus D/R experiments, respectively. The results shown in Table 2 confirm that after the prescribed time course, tartrazine had indeed dialyzed out of their respective wells to less than 1% as expected. Further, the presence of D/R at 100% of its initial concentration after the dialysis time course indicates no apparent leakage of the high MW molecule from their respective wells.

In all of the dialysis experiments, the presence of cross contamination of tartrazine or D/R into neighboring wells was monitored by observing increases in absorbance at 430 and 550 nm, respectively, within wells that originally contained the 0.1 N HCl dialyzing media. No such increase was observed, thereby confirming an acid-resistant and leak-free seal that prevented cross contamination between wells.

Comparison of Additional Factors with Commercially Available Dialysis Systems. In addition to dialysis performance, important factors to also consider are the costs per sample plate, range of MWCO membranes available, easy integration with in-line high-throughput data acquisition systems (e.g., microtiter plate reader), minimal creation of waste, and ease of assembling the apparatus. These factors served as additional design criteria for the proposed dialysis system and are compared in Table 3 with several commercially available systems, including those that were designed for protein binding assays, but could likely be adapted for equilibrium dialysis.

A striking distinction between the reported dialysis system and all other systems compared in Table 3 is the use of a standard 96-well microtiter plate as the sample plate from which dialysis occurs. This feature was designed to facilitate seamless integration into a continuous process workflow that may include standard multiwell dispensing systems for sample preparation before dialysis, as well as high-throughput data acquisition systems after dialysis. In addition, the use of a microtiter sample assay plate can potentially reduce the amount of waste generated, especially if the microtiter plate can be reused for multiple dialyses, compared to other dialysis systems where the sample plate and dialysis membrane are manufactured as one conjoined unit.

An additional enhancement of the reported dialysis system is its economical construction compared with the other systems presented in Table 3. The Spectra/Por 96-well microdialyzer, the most similar in design to our apparatus, is costlier by 10-fold. While products such as the MilliPore MultiScreen Filter Plate offer an affordable ultrafiltration system, only a 10K MWCO membrane is currently available and dialysis requires a centrifuge equipped with 96-well plate rotors.

As more areas of research embrace the advantages of high-throughput strategies, simple yet reliable technology will be critical to fully realize the benefits that such strategies can offer. The reported dialysis system offers researchers an improved high-throughput approach to purifying high MW product (e.g., polyelectrolytes, nonionic polymers) from solutions comprising a mixture of low MW byproduct and reagents. Constructed from materials that are commercially available, the apparatus can be affordably and easily fabricated in a standard machine shop. The proposed system also provides important enhancements over both recently reported and commercially available systems. In particular, the system (1) is readily integrated with in-line automated high-throughput technology (e.g., robotic liquid and solid handling systems, 96-well plate readers), (2) is resistant to acidic environments, (3) creates minimal waste, and (4) is easy to assemble.

More importantly, the dialysis apparatus and the validation approach described herein for a 96-well assay platform provides a readily transferable template upon which other multiwell assay platforms can be produced. With all other components of the dialysis apparatus interchangeable, simply modifying the dimensions of the top plate and the gasket sheets can enable high-throughput dialysis on a 6-, 24-, and 384-well plate platform.

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Supporting Information Available. Detailed assembly procedure of the dialysis system, experimental protocol, data analysis, and drawings for each component of the apparatus. This material is available free of charge via the Internet at http://pubs.acs.org.

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