Development and validation of a low cost blood filtration element separating plasma from undiluted whole blood

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Clinical point of care testing often needs plasma instead of whole blood. As centrifugation is labor intensive and not always accessible, filtration is a more appropriate separation technique. The complexity of whole blood is such that there is still no commercially available filtration system capable of separating small sample volumes (10–100 μ l) at the point of care. The microfluidics research in blood filtration is very active but to date nobody has validated a low cost device that simultaneously filtrates small samples of whole blood and reproducibly recovers clinically relevant biomarkers, and all this in a limited amount of time with undiluted raw samples. In this paper, we show first that plasma filtration from undiluted whole blood is feasible and reproducible in a low-cost microfluidic device. This novel microfluidic blood filtration element (BFE) extracts 12 μ l of plasma from 100 μ l of whole blood in less than 10 min. Then, we demonstrate that our device is valid for clinical studies by measuring the adsorption of interleukins through our system. This adsorption is reproducible for interleukins IL6, IL8, and IL10 but not for TNF α . Hence, our BFE is valid for clinical diagnostics with simple calibration prior to performing any measurement. © 2012 American Institute of Physics. [doi:10.1063/1.3672188]

I. INTRODUCTION

Clinical tests are commonly performed on cell-free samples, since particle inclusion and possible cell lysis affect the reproducibility and standardization negatively.¹ Blood plasma is the liquid phase of whole blood in which the blood cells are suspended. Filtration is the preferred method for blood cells separation in point of care testing (POCT) devices. Although centrifugation is the classical bench top technique, it is time and labor intensive and, therefore, not suitable. Filtration, although more adapted for this purpose, is not commercially available, which triggered the research and industrial communities to investigate the major challenges¹ of plasma filtration. Furthermore, our European FP6 project “CARE-MAN” (Ref. 2) envisages to build a next-generation diagnostic device based on biosensor technology to address the most common diagnostic problems like cardiovascular diseases. In the framework of this project, our task was to reproducibly filter 10 μ l of plasma out of 100 μ l of undiluted whole blood in less than 10 min in a disposable microfluidic chip. In addition, this device had to be clinically relevant and interconnectable with the diagnostics system performing fluorescence and microarray-based sandwich immunoassay.² The use of plasma in a point of care setting is not compulsory in every situation. However, in our case, the sensitivity and reliability of the fluorescence measurement requested the use of plasma. The ability to integrate plasma filtration from whole blood within a compact system for clinical diagnostics will enable less sample manipulation,

high degree of automation, fast testing times, compactness, and cost efficiency, which are all important criteria for POCT devices.³

The researchers in microfluidics are developing numerous techniques for separation and concentration of the different components in whole blood. For example, they separate blood cells by liquid-liquid extraction,³ deterministic lateral displacement,⁴ acoustophoresis,⁵ cross-flow filtration,⁶ centrifugal forces,⁷ or gravitational sedimentation.⁸ Some groups integrate microfabricated filter structures in microchannel designs^{9,10} and commercially available filter membranes in polydimethylsiloxane (PDMS) microfluidic devices.^{11,12} Amongst these systems, some methods enable the filtration of small volumes of whole blood (few μl) within seconds^{5,7} or minutes.^{6,8} However, most systems use diluted blood in order to avoid clogging.^{4-6,9-11} In addition, there is an increasing effort in the development of low cost^{7,8} and hybrid^{11,12} systems for blood separation and analysis. A recent study has shown the clinical relevance of integrating commercially available polycarbonate (PC) membranes into PDMS microfluidic devices.¹² The study tested hemodiluted ($\sim 27\%$ – 30%) and heparinized (up to ~ 5000 IU/ ~ 500 ml blood bag) human whole blood. High plasma protein recovery (over 80%) and no indication of hemolysis during the experimental period (over 4 h) were observed. However, there is no clear information about the volumes of whole blood tested and plasma recovered. The basic design of our plasma separation system is based on Ref. 11, where a commercial cross-flow blood filtration membrane was positioned on the top of PDMS microfluidic channels for capillary-driven filtration of plasma from whole blood. In this study, the plasma was extracted from a small quantity of diluted whole blood (20–300 μl). Unfortunately, blood cells leakage was observed and hemolysis occurred when the hematocrit was increased above 20%. There is no information about either the volume of plasma recovered from the original whole blood volume or the filtration time. Here, we improve this existing concept¹¹ by changing the design of the chip and by choosing different materials^{13–15} to suit our final project objective.² As a summary, except for Refs. 7 and 8, no microfluidic system demonstrates a reproducible filtration of small volumes of plasma (10 μl) from raw, undiluted whole blood (100 μl) in a limited amount of time (maximum 10 min). To the best of our knowledge, except for Refs. 11 and 12, nobody performed recovery experiments of relevant biomarkers on the microfluidic systems to assess the clinical relevance of the test devices.

Here, we demonstrate the fabrication and use of a simple, low-cost, and hybrid sample preparation system to separate plasma from raw, undiluted whole blood. The proposed system shows low adsorption of interleukins, making it suitable in clinical diagnostics. Our sample preparation setup is very stable and saves labor costs as it requires a minimum of training. We manufactured our device with low cost techniques and materials, taking advantage of methods like plastic machining, UV-curable polymer molding,¹³ and the integration of a commercial blood filter membrane.¹⁴

II. EXPERIMENTAL

A. Device design, fabrication, and assembly

The blood filtration element (BFE) is shown in Fig. 1. It consists of three parts: (1) the microchannels, fabricated by soft-embossing of thiolene-based UV-curable adhesive (Norland Optical Adhesive 81, Norland Products Inc., Cranbury, NJ, USA), (2) the coverplate, milled in PC, and (3) a commercially available blood filter membrane (VividTM Plasma separation membrane).

1. Microchannels

The microchannels are designed for plasma collection, and they consist of an array of parallel capillaries (50 μm wide, 20 μm deep, and 300 μm apart) and a collection channel (450 μm wide, 150 μm deep, and 17 μl total volume). The collection channel is chosen to be meandering, instead of a large free standing volume,¹¹ to ensure a collapse-free sealing of the parts when pressed together. This meander channel design also enables the use of an additional pressurized

FIG. 1. Schematic representation and photograph of the BFE. (a) Exploded 3D view. Microchannels (yellow): molded in UV Glue; coverplate (transparent-white): milled in polycarbonate; filter membrane (red): Pall "Vivid Plasma Separation" membrane. (b) Cross-sectional view of the BFE. (c) Design of the microfluidic part: the parallel capillaries are $50\ \mu\text{m}$ wide, $20\ \mu\text{m}$ deep, and $300\ \mu\text{m}$ apart. The collection microchannel is $450\ \mu\text{m}$ wide and $150\ \mu\text{m}$ deep. The total volume of the collection microchannel is $17\ \mu\text{l}$. (d) Top view of the BFE: the venting inlet and plasma outlet allow easy connection to standard 1/4-28 UNF fittings. The venting inlet allows flushing of the filtered plasma at the end of the experiment.

inlet for sample delivery after filtration. We chose NOA 81 as the material because of the following properties: hydrophilicity,¹³ ability to be patterned by soft embossing,¹⁶ and biocompatibility.¹⁷ We use a two stage embossing process: first, a master is fabricated by etching two different depths of microchannels in Pyrex glass following the process described elsewhere.¹⁸ Next, we fabricate a second master in PDMS (Sylgard 184, Dow Corning, USA) using the first master. This second master is used for replication with NOA 81. NOA 81 is casted and, subsequently, cured under a UV lamp on the structured PDMS master. Details about the molding procedure are found in Refs. 13 and 16.

2. Coverplate

We designed the polycarbonate coverplate with a recess for the filter membrane and threaded holes for fluidic inlet and outlet connections. Our use of the standard 1/4-28 UNF thread ensures easy and reliable connections. Polycarbonate was chosen for its transparency, easy tooling, and good mechanical strength. The recess is 20 mm wide and 200 μm deep, and the blood inlet hole is 15 mm wide.

3. Filter membrane

The commercially available Pall blood filter, fabricated from asymmetric polysulfone, is designed to have low non-specific binding of common diagnostic biomarkers and target analytes. We chose the Pall GR Vivid membrane grade, as it has a plasma recovery of at least 80% which is higher than the other available commercial membranes. Further, it is designed for larger blood volume applications with a high blood volume per surface area of 40-50 $\mu\text{l}/\text{cm}^2$. With a diameter of 20 mm (3.14 cm^2), it enables the filtration of at least 125 μl of whole blood.

Before the first assembly, we treated the surfaces of the PC coverplate and the NOA 81 microchannels with oxygen plasma. Though the NOA is hydrophilic by nature, this additional treatment was found beneficial for the reproducibility and ensures that the surfaces in contact with the filtered plasma remain hydrophilic. This surface treatment is stable over time¹³ and is done once, just after the fabrication. We seal all three parts by pressing them together in a chip-to-world interface with embedded excenter shaft (see Fig. 2(b)). The excenter shaft, which is positioned at the bottom of the device, serves as an easy way of clamping without having to tighten the screws at the top. The seal is hermetic due to the elastic properties of NOA 81. The NOA 81 is a hard and at the same time elastic material. The elasticity helps to get a good seal when pressing the different elements together. We noticed that after use in our BFE the material is slightly deformed and relaxes only slowly to the original shape. Also, the “stiffness” of the material changes in time when they are stored. Heating up to a temperature of 100 °C, probably close to or over the glass transition temperature, relaxes the structure and makes it go back to its original shape. We decided to do this treatment as a standard procedure so that the starting point is the same every time.

B. Whole blood filtration

We collected the blood samples from healthy donors in heparinized test tubes (Vacuette Lithium Heparin, 2 ml, Greiner, Germany). All blood samples were handled without any dilution.

In a typical blood filtration experiment, we first put the microchannel part in an oven at 100 °C to restore to its original layout. We place the membrane between the microchannel and the coverplate as depicted in Fig. 1 and then press them all in the chip-to-world interface. We screw Teflon tubing along with gripper fittings from Omnifit into the venting inlet and plasma outlet. With the help of a micropipette, we introduce 100 μl of whole blood in the blood inlet without touching the fragile filter surface. We then close this blood inlet with a homemade Teflon Luer adaptor to allow connection to standard fluidic fittings (Upchurch P655-01: male Luer to 1/4-28 UNF). As described later, it is possible to apply an external pressure from the blood inlet to the plasma outlet with the help of a pressure source (microfluidic flow control system,

FIG. 2. Interleukin adsorption study. (a) Flow chart of the experimental conditions: whole blood from each human subject is simultaneously centrifuged and filtered through two BFE in parallel. The interleukin content of the resulting plasma is analyzed with the standard Luminex device. (b) Two BFEs are enclosed in chip holders for good sealing. BFE 1 and BFE 2 are tested in parallel during the study. The red arrows show where blood samples are introduced before the pressure pump is connected to accelerate plasma extraction. The yellow arrows indicate the plasma outlet port. Three channels (P1–P3) of the pressure pump are connected to BFE 1, and three other channels (P4–P6) are connected to BFE 2. Each channel allows for the setting of a different pressure value, the pump's software enables the automation of the pressure control during the experiment. In this particular experiment, P1 = P4, P2 = P5, and P3 = P6. The scale bar denotes 10 mm.

MFC8-8 C, Fluigent, France). This pressure source allows applying and varying positive pressure on up to eight individual channels independently. Its channels must be connected to every fluidic inlet/outlet of the device, and the liquids are pushed through the device by setting a pressure difference between individual channels. After finishing the filtration, we push the plasma towards a low binding tube (Protein LoBind Tubes, Eppendorf AG Hamburg, Germany) by applying a positive pressure between the venting inlet and the plasma outlet. We discard the filter membranes after each experiment. We rinse every part of the BFE in contact with blood and plasma (tubing, fittings, PC coverplate, microchannels) first with an alkaline cleaning solution (S5332, Radiometer Medical Aps, Denmark), then with ultrapure water, and finally, we dry them with nitrogen.

C. Recovery study

We waited maximum 8 h between the sample collection and the experiments and stored the blood at 4 °C until use. We collected the blood samples from healthy donors in heparinized test tubes as previously described. Fig. 2(a) shows the design of the experiment. From the blood sample, we analyzed 1/4th in a central laboratory for base line characteristics. We spiked every remaining 1/4th with interleukins (R&D systems Inc., Minneapolis, USA) at low, mid, and high concentrations corresponding to those shown in Table I in a relatively high range compared to physiologic ranges. We spiked the samples with a deliberately high concentration to allow not only the filtrated sample to be diluted (if filtrated volume was too low for the cytokine measurement kit) but also the retrieval of cytokines (if the recovery rate of the BFE was found to be low).

We processed these aliquots either conventionally by centrifugation (reference sample) or through two BFEs in parallel. Both BFEs were connected to the same pressure source, allowing the filtration process to be automatized. Fig. 2(b) shows two BFEs connected to the pressure source and operating in parallel during the experiment. We separated the plasma simultaneously with both methods, keeping the same experimental procedures throughout the study.

After filtration, we stored both types of plasma samples at −75 °C. We analyzed the samples with a cytokine immunoassay kit from R&D Systems together with a Luminex[®] analyzer (Bio-Plex[®] 200 System, Bio-Rad Laboratories, Vienna, Austria). We prepared samples according to the manufacturer's instructions where a pre-dilution of 1:4 is described. We had to dilute our filtered sample if the collected volume was less than 12.5 μl to fit the volume requirements of the cytokine kit.

We evaluate the BFEs adsorption behavior by calculating its recovery rate (Eq. (1)). We define this recovery rate as the ratio of analytes concentration in the filtered sample to analytes concentration in the reference sample in percent,

$$\text{Recovery rate} = \frac{\text{Interleukin concentration from chip}}{\text{Interleukin concentration from origin matrix}} \cdot 100\%. \quad (1)$$

Both reference sample and filtered sample originate from the same human subject, as depicted in Fig. 2(a).

III. RESULTS AND DISCUSSION

A. Whole blood filtration

Filtration starts immediately after we drop 100 μl of whole blood on the blood inlet. The asymmetric nature of the filter membrane¹⁴ (pore size from ~17 μm to ~1.3 μm) captures the cellular components of the blood in the pores while the plasma flows through the membrane into the microfluidic capillaries. The plasma flows along the parallel microchannels by capillary force and into the collection channel. Without applying pressure this process lasts over 30 min.

Therefore, we decided to speed up the filtration process by applying a pressure drop between the sample port (where the drop of blood is introduced) and the plasma outlet port.

TABLE I. Interleukin type and concentration used in this study. The concentration was kept deliberately high in case the filtrate sample volume had to be diluted for the analysis with the Luminex device.

Range	Interleukin type			
	IL6 pg/ml	IL8 pg/ml	IL10 pg/ml	TNFα pg/ml
Low	1800	1325	1112.5	1940
Mid	3600	2650	2225	3880
High	7200	5300	4450	7760

However, hemolysis occurs if the pressure applied on the blood cells is higher than 50 mmHg (Ref. 19; ~ 66.5 mBar). Hence, we studied the behavior of our blood filtration device as a function of the intensity of the pressure drop applied between the sample port and the outlet. We observed that the plasma collected in the microchannel changed color from yellow to red when the pressure drop was higher than 66.5 mBar. We did not observe this red color for pressure drop less than 60 mBar. By introducing pressure control, we reduced the blood filtration time to 7 min.

According to the manufacturers' information, the Pall GR membrane ensures a blood volume per surface capacity of $40\text{--}50\ \mu\text{l}/\text{cm}^2$. We observed hemolysis if the blood input volume was higher than the volume per surface recommended by the manufacturer, which is $125\text{--}157\ \mu\text{l}$ with an area of $3.14\ \text{cm}^2$. No hemolysis was observed when the sample volume was less than the recommended volume.

On-chip blood filtration kinetics always shows the same trend: most of the filtration occurs within the first 1–3 min. However, we collected reproducible plasma volumes when we waited for longer times, i.e., 7 min. On-chip blood filtration is a trade-off between total filtration time and total volume of extracted plasma. To filter $10\ \mu\text{l}$ of plasma out of $100\ \mu\text{l}$ of whole blood, the most stable and reproducible filtration conditions are by applying an external pressure of 40 mBar for 7 min. For the chemical analysis done upstream of our filtration unit by our project partners,² the total volume of plasma needs to be around $10\ \mu\text{l}$.

This simple hybrid microfluidic chip enables us to efficiently remove cellular components from whole blood. We did not observe any leakage of blood cells. The hybrid nature of the chip enables us to apply enough clamping pressure between the components to prevent unwanted leakage, which (Ref. 11) could not achieve with 100% PDMS devices. The assembly process is rather easy and straightforward as we ran our experiments at least 100 times without failure on two different chip holders, on various times of the year and geographical locations. Ultimately, these hybrid chips will be fabricated by injection molding of thermoplastic material, with the commercial filter embedded in between. This will allow a real point-of-care handling of the chips, with all the chips permanently bonded and disposable. The reason why we re-used the chips in these experiments was the simplicity to open and close the chips and overall versatility to allow, for example, the testing of various filter materials.

B. Recovery study

In this study, we always extracted $12\ \mu\text{l} \pm 3\ \mu\text{l}$ of plasma from $100\ \mu\text{l}$ of whole blood following the procedure explained in Sec. II and pictured in Fig. 2. To avoid hemolysis, the applied pressure is limited to 40 mBar leading to a filtration time of 7 min. We performed two distinct sets of experiments to study the adsorption behavior of the BFE.

In the first experiment, we analyzed the blood from a single human subject. For each spiked interleukin concentration, we repeated the blood filtration experiment three times through centrifugation and two BFEs (see Fig. 2). Calculated recovery rates in the low, mid, and high concentration range of interleukins are shown in Table II. This first evaluation shows

TABLE II. Recovery rates for the first adsorption study experiment performed on one human subject. Mean recovery (mean) and SD are given in % as a function of the type and concentration range (see Table I) of interleukin which flowed through our BFE. Recovery was calculated using Eq. (1).

Range	Interleukin type							
	IL6		IL8		IL10		TNF α	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low	58	9	23	3	59	9	65	15
Mid	65	1	37	2	66	6	66	2
High	74	6	37	2	50	4	71	4

TABLE III. Recovery rates in the second adsorption study experiment performed on three separate human subjects. Mean recovery (mean), SD, and CV given in % as a function of the type and concentration range (see Table I) of interleukin which flowed through our BFE. Recovery was calculated using Eq. (1).

Range	Interleukin type											
	IL6			IL8			IL10			TNF α		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Low	70	5	7	30	7	25	71	9	12	54	19	34
Mid	74	7	9	37	6	16	77	5	6	54	15	29
High	79	9	11	43	8	18	81	6	8	62	15	24

stable recovery rates with standard deviation (SD) always below 10%, except for low concentrations of TNF α . However, the adsorption on the surfaces of the BFE is high as the recovery values were always found below 100%.

To assess the reproducibility of the observed adsorption, we performed a second set of experiments. This time we compared the blood from three human subjects. Recovery rates in the low, mid, and high concentration range of interleukins are shown in Table III and in Fig. 3. These recovery rates are stable with a standard deviation below 10% for all interleukins, except again for TNF α .

The recovery rates are reproducible, but always below 100%. Ideally, a device not retaining any analytes will show a recovery of 100%. This means the interleukins adsorb in the BFE (polymer parts, tubing, and Pall filter membrane). As the commercially available membrane is told to have low non-specific binding, it is possible that some adsorption occurs on the membrane and some on the Teflon tubes and polymer parts. The recovery rates of IL6 and IL10 are similar with a mean value for all concentrations of 75%. The recovery rates of IL8 are much lower with a mean value for all concentrations of 37%. This indicates that IL8 adsorbs much more to the surface of the BFEs. For TNF α , the reproducibility is at the limits of the recommended guidelines²⁰ and needs to be investigated further. The origins of this variation are still

FIG. 3. Recovery rate of plasma filtered through our BFE. The whole blood was donated by three different human subjects. The plasma was studied and analyzed following the procedure described in Fig. 2. The interleukin concentrations are detailed in Table I. Recovery was calculated using Eq. (1). The error on the measurements is detailed in Table III.

unclear; there could be an influence of hemolysis, hydrophobicity of the protein, or *pH* values of the sample. Extended validation experiments are necessary to study the adsorption behavior in more details.

For IL6, 8, and 10, the adsorption is reproducible through different human subjects, as the standard deviation is below 10% and coefficient of variation (CV) below 15%. The U.S. food and drug administration (FDA) guidelines for industry²⁰ recommend evaluating a minimum of three concentrations in the range of expected concentrations. The precision determined at each detection level should not exceed 15% of the CV except for the lower limit of quantification (LLOQ) where it should not exceed 20% of the CV.²⁰ This confirms that the inter- and intra-subjects reproducibility we demonstrate here for interleukin adsorption allows an additional calibration step if clinical validation with the device is wanted. This also demonstrates that our blood filtration elements are suitable for clinical studies.

IV. CONCLUSION

We demonstrated the usability of a novel hybrid point of care blood filtration element to extract reproducibly 12 μ l of plasma from undiluted whole blood within 7 min. We measured the adsorption of interleukins from four separate human subjects and we observed a constant recovery for every tested interleukin, except TNF α . This constant recovery makes it possible to use our devices at the point-of-care with calibration prior to any measurement by connecting them to the compact system for clinical diagnostics of our project partners. The very simple and hybrid nature of our BFE enables its production by mass fabrication and subsequent commercialization. Future improvements should include a study of the adsorption at the LLOQ level, diminution of the standard deviation under 5%, a better reproducibility for TNF α , and an improvement of the inherent interleukins adsorption on the BFE.

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- ¹M. Toner and D. Irimia, *Annu. Rev. Biomed. Eng.* **7**, 77 (2005).
- ²M. Kemmler, B. Koger, G. Sulz, U. Sauer, E. Schleicher, C. Preininger, and A. Brandenburg, *Sens. Actuators B* **139**, 44 (2009).
- ³P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam, and B. H. Weigl, *Nature* **442**, 412 (2006).
- ⁴N. Li, D. T. Kamei, and C.-M. Ho, in Proceedings of the 2nd IEEE Conference on Nano/Micro Engineered and Molecular Systems, Bangkok, Thailand, January 16–19, 2007.
- ⁵A. Lenshof, A. Ahmad-Tajudin, K. Järås, A.-M. Swärd-Nilsson, L. Åberg, G. Marko-Varga, J. Malm, H. Lilja, and T. Laurell, *Anal. Chem.* **81**, 6030 (2009).
- ⁶T. Tachi, N. Kaji, M. Tokeshi, and Y. Baba, *Anal. Chem.* **81**, 3194 (2009).
- ⁷S. Haeberle, T. Brenner, R. Zengerle, and J. Ducrée, *Lab Chip* **6**, 776 (2006).
- ⁸I. K. Dimov, L. Basabe-Desmonts, J. L. Garcia-Cordero, B. M. Ross, A. J. Riccoa, and L. P. Lee, *Lab Chip* **11**, 845 (2011).
- ⁹T. A. Crowley and V. Pizziconi, *Lab Chip* **5**, 922 (2005).
- ¹⁰A. J. de Mello and N. Beard, *Lab Chip* **3**, 11N (2003).
- ¹¹S. Thorslund, O. Klett, F. Nikolajeff, K. Markides, and J. Bergquist, *Biomed. Microdevices* **8**, 73 (2006).
- ¹²K. Aran, A. Fok, L. A. Sasso, N. Kamdar, Y. Guan, Q. Sun, A. Ündar, and J. D. Zahn, *Lab Chip* **11**, 2858 (2011).
- ¹³Ph. Wägli, A. Homsy, N.F. de Rooij, *Sens. Actuators B* **156**, 994 (2011).
- ¹⁴See http://www.pall.com/life_sciences_oem_46962.asp for information about Vivid™ Separation Membranes from Pall Corporation.
- ¹⁵H. Becker, *Lab Chip* **11**, 1571 (2011).
- ¹⁶D. Bartolo, G. Degre, P. Nghe, and V. Studer, *Lab Chip* **8**, 274 (2008).
- ¹⁷L. H. Hung, R. Lin, and A. P. Lee, *Lab Chip* **8**, 983 (2008).
- ¹⁸A. Homsy, S. Koster, J. C. T. Eijkel, A. van den Berg, F. Lucklum, E. Verpoorte, and N. F. de Rooij, *Lab Chip* **5**, 466 (2005).
- ¹⁹P. S. Malchesky, in *Biomaterials Science: An Introduction to Materials in Medicine*, 1st ed., edited by B. D. Ratner, A. S. Hoffman, F. J. Schoen, and J. E. Lemons (Academic, San Diego, 1996), pp. 405–406.
- ²⁰See www.fda.gov for Guidance for industry: Bioanalytical method validation, U.S. Department of health and human services, 2001.