Volume-Oriented Sample Preparation for Reactant Minimization on Flow-Based Microfluidic Biochips with Multi-Segment Mixers

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***Abstract*—Sample preparation is one of essential processes in most biochemical reactions. In this process, raw reactants are diluted to achieve given target concentrations. So far, most of existing sample preparation techniques only consider mixing of two source solutions under the (1:1) mixing model. In this paper, we propose the first sample preparation algorithm VOSPA that not only blends several (≥ 2) solutions in a dilution operation but also allows various mixing models on flow-based microfluidic biochips with multi-segment mixers. VOSPA is a volume-oriented sample preparation algorithm that enables segment-based intermediate solution reuse for better reactant minimization. Experimental results show that VOSPA can lower the reactant consumption and operation count by 72% and 59% as compared to the baseline bit-scanning method if an 8-segment mixer is used. Moreover, VOSPA outperforms an optimal algorithm, which merely allows the use of (1:1) mixing model; the reactant usage and operation count can be further reduced by 37% and 76%.**

Keywords—Biochip, flow-based microfluidic biochip, dilution, mixing model, reactant minimization, sample preparation

I. Introduction

Lab-on-a-chip (*LoC*) is emerging as one of the most attractive research topics in the bioelectronics field [1]–[4]. An LoC integrates variety of laboratory functions in a small chip, which offers numerous benefits over conventional biochemical analysis systems, like portability, reactant usage reduction, low power consumption, fast analysis time, increased automation, high throughput, and compatibility with mass production [5]. It is particularly adequate for replacing those conventional bulky and expensive on-site biochemical analysis systems located in laboratories, hospitals, research centers, or even at home. *Flow-based microfluidic biochip* (*FMFB*) is generally considered as a very promising type of LoCs. Many biochemical applications, like protein analysis, drug discovery, and point-of-care disease diagnosis [6]–[8], have been successfully demonstrated on FMFBs. Unlike droplet-based biochips [9], an FMFB utilizes a large number of microchannels, valves, and chambers to create a versatile network. Complex devices can be equipped on-chip by combining valves and channels, and variety of reactions can thus be simultaneously executed. An FMFB consisting of thousands of integrated microchannels and valves is also called as microfluidic very-large-scale integration (mVLSI) [10][11], and the design automation for FMFBs has been becoming one of the emerging research topics nowadays.

Sample preparation is an essential processing step in most biochemical applications. In molecular diagnosis, 90% of cost and 95% of time is associated with sample collection, transportation, as well as preparation [12]. Raw reactants (e.g., bio-sample or reagent) are mixed and diluted to give a product solution with a specified concentration value (CV), called target concentration, during sample preparation [13]–[15]. In general, there are three common optimization objectives for a dilution process: 1) the consumption of valuable reactants; 2) the total number of dilution operations, and 3) the amount of wastes. The valuable reactants such as limited amount of bio-samples (e.g., infant's blood [16] and DNA evidence from a crime scene [17]) or expensive reagents mostly determine the overall cost. Besides, the number of dilution operations is highly related to the sample preparation time. In some urgent clinical incidents, a long preparation time is simply fatal and thus unacceptable. Lastly, a large amount of wastes implies a huge burden for successive waste treatments. Moreover, it also affects the overall sample preparation time due to limited waste reservoirs available on a biochip. Therefore, the above three minimization goals should be carefully considered during sample preparation.

Several approaches aiming at the sample preparation problem on LoCs have been proposed in recent years [18]–[33]. Most of them deal with the sample preparation problem under only the (1:1) mixing model on *digital microfluidic biochips* (*DMFBs*). However, there is still no method focusing on the sample preparation problem on FMFBs with multi-segment mixers so far. Even if those algorithms designed for DMFBs are applicable to the sample preparation process on FMFBs, where various mixing models are available, the overall performance is generally poor because only the (1:1) mixing model is adopted. It is apparent that the results can be dramatically improved if all available mixing models are explored in a dilution operation. Therefore, an alternative that concentrates on the sample preparation problem for FMFBs with multi-segment mixers should be further developed.

Hence, in this paper, we present a *volume-oriented sample preparation algorithm*, VOSPA, aiming at this problem. To the best of our knowledge, VOSPA is the first sample preparation algorithm that allows blending several solutions (≥ 2) with different concentrations together in a single dilution operation. To produce a target solution, VOSPA adopts a volume-oriented strategy, which achieves the target reactant volume segment by segment. With the help of the *CV Bank*, which keeps track of available intermediate solutions, VOSPA can effectively reuse those existing intermediate solutions to achieve better reactant minimization. Compared to the baseline *bit-scanning* method (BS) [20], our experimental results demonstrate that VOSPA can reduce the overall reactant usage

978-3-9815370-4-8/DATE15/©2015 EDAA

and operation count by 72% and 59% respectively in an 8-segment mixer (Mixer-8). Moreover, VOSPA can even outperform a reactant-minimal algorithm [32], which merely adopts the (1:1) mixing model; the reactant usage and operation count can be further decreased by 37% and 76% in a Mixer-8. The results clearly suggest that VOSPA is a promising reactant-minimization technique for sample preparation on FMFBs equipped with multi-segment mixers.

The rest of this paper is organized as follows. Section II briefly describes the dilution process and the mixing models. Section III gives our motivation and problem formulation. Section IV presents our volume-oriented sample preparation algorithm, VOSPA, in more detail. Experimental results are then reported and discussed in Section V. Finally, Section VI concludes this paper.

II. Sample Preparation and Mixing Models

During sample preparation, a specified target concentration value (C_t) is achieved by a series of dilution operations. In each operation, several solutions with various concentration values (CVs) are mixed together to produce a product solution with a new concentration value. On a droplet-based DMFB [9], fluids are dispensed as discrete droplets. Therefore, only two droplets are mixed together in a single dilution operation. The resultant concentration value (C_r) after dilution under the (1:1) mixing model can be calculated as $C_r = (C_1+C_2)/2$, where C_1 and C_2 represent the CVs of two source droplets. However, for other biochip designs such as FMFBs with multi-segment mixers, mixing models in additional to the (1:1) model are available. An *N*-segment mixer (Mixer-*N*) is divided into *N* independent segments of same size, and each segment can keep a solution with a specific CV. Therefore, for a dilution operation in a Mixer-*N*, each segment *i* can be filled with a different solution of the concentration value C_i. Hence, the relationship between the input concentration set $\{C_1, C_2, \ldots, C_N\}$ and the resultant C_r of a dilution operation in a Mixer-*N* can be expressed as:

$$C_r = \frac{\sum_{i=1}^{N} C_i}{N}, \text{where } 0 \le C_i \le 1. \quad (1)$$

For example, a 4-segment rotary mixer (Mixer-4) illustrated in Fig. 1(a), which has been broadly used on FMFB. It can blend at most 4 different solutions in a mixing operation. The CV of the resultant solution, C_r, after a dilution operation in a Mixer-4 can be calculated as $C_r = (C_1+C_2+C_3+C_4)/4$, where C_1, C_2, C_3 and C_4 represent the CVs of four source solutions before mixing.

The CV of a solution decays exponentially if the solution is repeatedly diluted with buffer solution, which is commonly referred to as exponential dilution [25]. For DMFBs under the (1:1) mixing model, one of the two input solutions must be a buffer solution. That is, the resultant CV is always half of the original CV after an exponential dilution operation. However, for a mixer-*N*, the volume ratio between two input solutions is adjustable. According to (1), the possible resultant CVs after an exponential dilution operation can be represented as:

$$C_r = \left\{ \frac{x}{N} \times C_i \middle| 1 \le x < N, x \in \mathbb{N} \right\}, \quad (2)$$

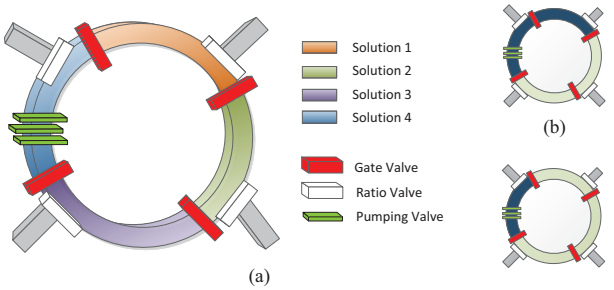

Fig. 1. (a) A 4-segment rotary mixer, and mixing operations under (b) the (1:1), and (c) the (1:3) mixing models.

where *x* is the number of segments filled with the solution of concentration C_i. That is, a Mixer-*N* can provide several valid mixing models even in an exponential dilution operation. For instance, a Mixer-4 can perform not only the (1:1) mixing operation but also the (1:3) mixing operation for two different source solutions, as depicted in Fig. 1(b)(c). Furthermore, a Mixer-4 can actually blend up to four various input solutions, as shown in Fig. 1(a). Despite of those existing techniques always mixing two solutions under the (1:1) mixing model, a new sample preparation method that can fully exploit the advantages of multi-segment mixers is surely demanded to achieve better quality of result.

A dilution graph is commonly used to guide the dilution process, as shown in Fig. 2. In a dilution graph, a node with in-degree of 0 is associated with either raw reactant or buffer solution; a node with out-degree of 0 indicates the product solution with the target CV; every other node implies an intermediate solution. A weight *k* labeled beside an edge *e*, *k*(*e*), indicates the required *dosing volume*, which specifies the ratio to the whole volume of a mixer. The sum of weights of input nodes should be 1 (i.e., fully fill the entire mixer). For a node with nonzero in-degree, its C_r can be expressed as:

$$C_r = \sum_{v \in input\ node} k(v) \times C_v \quad (3)$$

In a sample preparation process on biochips, error between the ideal target concentration and the implementable one is inevitable due to the limitation induced by mixing models. For example, given C_t=1/3, the error is unavoidable if only the (1:1)

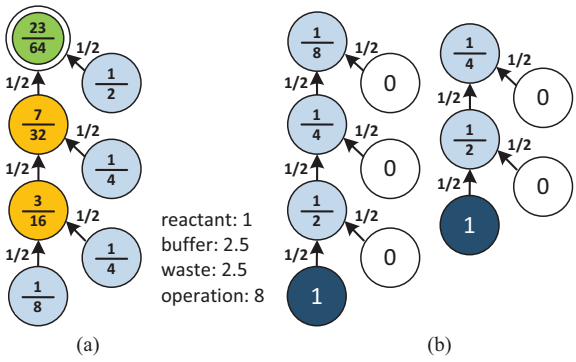

Fig. 2. The dilution process for C_t = 23/64, (a) the mixing tree, and (b) the exponential dilution process by REMIA.

mixing model is used. However, this error can usually be tolerated if it is lower than a certain value, called *tolerance* (δ). Designers can carefully choose what the tolerance is to ensure a correct assay outcome [20][21]. For instance, if the target CV is specified as 0.346 with a tolerance of ±0.0005, it means that the smallest acceptable CV is 0.3455, while the largest one is 0.3465.

III. Motivation and Problem Formulation

Most existing algorithms, such as BS[20], REMIA[25], and OPT[32], only considers mixing two solutions with the (1:1) mixing model. REMIA, a well-known method for reactant minimization during single target sample preparation on DMFBs, divides the whole dilution process into two individual phases, the interpolated dilution and the exponential dilution. REMIA adopts an effective top-down decomposition strategy to create a mixing tree with low reactant consumption for a given target, as shown in Fig. 2(a). Fig. 2(b) illustrates that the required leaf nodes of the mixing tree depicted in Fig. 2(a) are produced through an optimal exponential dilution process. However, as aforementioned, a multi-segment mixer with more available mixing models can provide better reactant-minimized outcomes. For example, Fig. 3 demonstrates the dilution process achieving the same target CV but utilizing more mixing models available in a Mixer-4. Compared with the dilution process sticking to only one mixing model in Fig. 2, it is obvious that both the reactant usage and operation count can be significantly reduced.

Reusing intermediate solutions as many as possible in a dilution process is an effective way for reactant minimization. In REMIA, the CV of every leaf node in the mixing tree is always $1/2^k$, and is named *prime concentration value* (*PCV*). Since only the (1:1) mixing model is in use, PCVs can be easily produced through exponential dilution operations with minimal reactant consumption, as shown in Fig. 2. However, for a Mixer-*N*, more mixing models in additional to the (1:1) mixing one are available for exponential dilution, which implies a much richer set of intermediate solution candidates can be produced. It is actually nontrivial to determine which intermediate solutions should be produced in exponential dilution as well as to reuse them as many as possible for reactant minimization. Hence, in this paper, we present a new algorithm, VOSPA, which works on not only achieving the target reactant volume as fast as possible but also maximizing the reuse of intermediate solutions segment by segment. Besides, further optimization is possible if the given tolerance is big enough. For instance, if C_t is 0.281 and δ is 0.05 and a Mixer-4 is in use, which implies any concentration value between 0.231 and 0.331 is valid. In this case, it is best to set the target value as 0.25 so that the reactant usage and operation count can both be minimized. VOSPA can perform such kind of optimization implicitly.

In this work, the single-target sample preparation problem on FMFBs equipped with multi-segment mixers is formally formulated as follows. Given a target concentration C_t, a tolerance δ, and a Mixer-*N*, determine a dilution process such that the reactant consumption can be minimized. As mentioned, we have developed an algorithm VOSPA to solve this problem and elaborate on it in the next section.

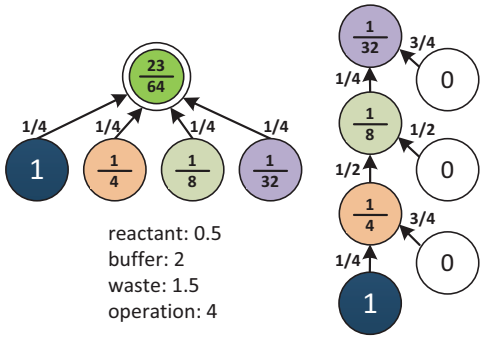

Fig. 3. The dilution process for C_t = 23/64 by VOSPA.

IV. Proposed Algorithm

A. Algorithm Overview

VOSPA consists of two major parts: a *master process* and a *subsidiary process*. The former process aggressively reuses existing intermediate solutions to accumulate the required reactant amount segment by segment to achieve the anticipated volume within the given tolerance, while the latter one is responsible to decide then produce appropriate intermediate solutions for the use of the master process. The details of VOSPA are discussed in the following subsections.

B. Master Process and Volume Selection

The kernel of VOSPA is the master process, which properly selects an intermediate solution to fill into the segment of a Mixer-*N* one by one. A table, named *CV bank*, is created to keep track of how many various intermediate solutions are currently available and their associated amounts. Initially, there is only one entry in the CV bank – raw reactant (i.e., CV = 1) with an infinite supply. Besides, if the capacity of a mixer is regarded as one unit, C_t actually specifies the target volume of reactant in a mixer, or the *expected volume* (*EV*). Hence, the goal of the master process is to accumulate the reactant volume segment by segment and achieves the expected volume at last. As the master process starts, the *accumulated volume* (*AV*) is initially 0 due to an empty mixer. Besides, the volume of reactant contributed by a solution with CV=C_i filled in a segment *i* is C_i/N, denoted as $Vol(C_i)$. Hence, the current *AV* can be calculated by summing up the reactant volume of every solution already filled in a segment of the mixer:

$$AV = \sum_{i \in filled\ segment} V(C_i). \quad (4)$$

Once the difference between *AV* and *EV* is within the tolerance δ, the master process fills remaining empty segments (if any) with buffer solution and then a valid dilution process is successfully created.

The master process adopts a greedy strategy, which tries to accumulate the reactant volume as fast as possible. It implies that solutions are filled into segments in a non-increasing order of their CVs. Hence, two constraints are imposed as the master process selects a solution to fill into the next empty segment. First, the candidate solution should contribute a volume of

reactant no less than the *lower bound of segment* (*LBS*), which can be expressed as:

$$LBS = \frac{EV - AV - \delta}{\#empty\_segment}. \quad (5)$$

Second, the candidate solution should contribute a volume of reactant no more than the *upper bound of segment* (*UBS*), which can be represented as:

$$UBS = EV - AV + \delta. \quad (6)$$

That is, the valid range of $Vol(C)$ can be given as:

$$LBS \le Vol(C) \le UBS, \quad (7)$$

where C is the CV of a candidate input solution. The master process then tries to find a qualified solution from the CV bank. If a qualified solution with CV≠1 is found, it implies the identified solution is successfully reused to fill this segment and no additional raw reactant is actually consumed. If several solutions are qualified, the one with the largest CV would be chosen to achieve the target EV as soon as possible. Take C_t=0.51, δ=0.02 and N=4 as an example, where EV is 0.51 and AV is 0 initially. According to (5) and (6), *LBS*/*UBS* should be 0.1225/0.53 accordingly. According to (7), raw reactant with $Vol(1)$=1/4=0.25 is selected for the first segment. After that, AV is increased to 0.25, and *LBS*/*UBS* is updated as 0.08/0.28, respectively. Again, the raw reactant is selected for the second segment. At this point, AV becomes 0.5 and the difference between AV and EV is smaller than the given δ. Therefore, the master process just fills the remaining two empty segments with buffer solution to accomplish the dilution process.

C. Subsidiary Process

Unfortunately, the master process cannot always find a qualified solution in the CV bank for reuse. For instance, consider the example shown in Fig. 4, where C_t =0.329, δ=0.001, and N=4. The master possess begins with selecting raw reactant for the first segment, as illustrated in Fig. 4(a). For the second run, *LBS*/*UBS* is 0.026/0.08, respectively. Since $Vol(1)$=0.25 is larger than *UBS*, there is no qualified solution in the CV bank for the second segment. If such situation occurs, a subsidiary process is invoked to produce an additional qualified solution through applying exponential dilution on an existing solution (i.e., solution reuse again). Under multiple available mixing models, several qualified candidate solutions may be identified, and the one with the smallest CV is selected to minimize the use of the existing solution. For example, Fig. 4(b) shows the subsidiary process generates a new solution with CV=1/4 from raw reactant and the master process picks it for the second segment. Besides, since a solution with higher CV is regarded more valuable than the one with smaller CV, the subsidiary process always starts with an existing solution with the lowest CV. For instance, at the third run shown in Fig. 4, the subsidiary process tries to get a new solution with CV=1/16 from a solution with CV=1/4 instead of 1 in the CV bank. Again, if a solution with CV≠1 in the CV bank is found to make a new solution, it implies a successful intermediate solution reuse and no additional raw reactant is actually consumed. Fig. 4(c) illustrates the entire dilution process for C_t =0.329 and δ =0.001. If the CV of a required solution produced is extremely low, the subsidiary process calls itself recursively until a qualified solution is produced.

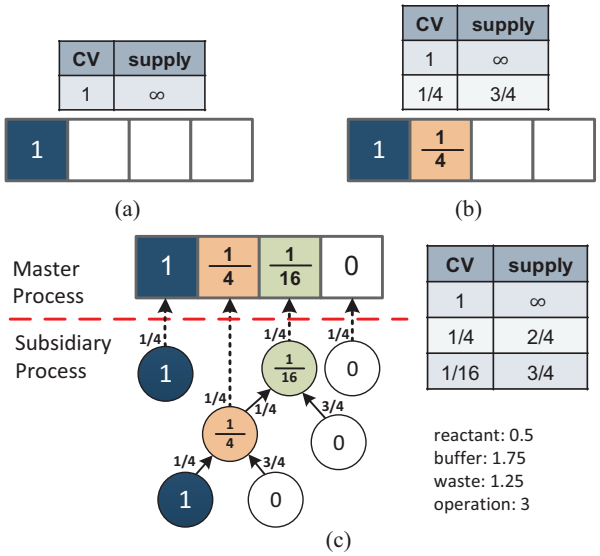

Fig. 4. Volume selection for (a) Segment 1, (b) Segment 2, and (c) the dilution process of VOSPA for C_t=0.329 and δ=0.001.

D. Recursion of the Master Process

For the last segment, similarly, the master process tries to find and then reuse a qualified solution from the CV bank first. If the attempt fails, the master process then tries to invoke the subsidiary process to find a new qualified solution. However, the attempt of the subsidiary process can still fail if *LBS* is larger than $Vol((N-1)/N)$ because there is no way for the subsidiary process to produce a qualified solution since the largest possible CV can be created through exponential dilution, is $(N-1)/N$.

In such circumstance, to produce a qualified solution for the last segment, the master process makes a recursive call to itself with a properly updated set of arguments (i.e., EV' and δ'). Since the resultant solution of the recursive call is merely used to fill the last segment of the current master process, the magnitude of both EV and δ should be amplified. That is, for the recursive call, EV' should be set to $N \times (EV - AV)$, while δ' should be set to $N \times \delta$. For example, in Fig. 5, EV' is set to $(0.907–0.75) \times 4 = 0.628$ and δ' is set to $0.001 \times 4 = 0.004$ for the second call of the master process. Since the tolerance is always amplified by N times for each of recursion call, the termination of the recursive call to the master process is certainly guaranteed because the tolerance δ' would eventually be larger than $Vol(1)$.

In general, the larger the tolerance is, the better reactant minimization outcome VOSPA is likely to produce. Given a large tolerance, VOSPA has a larger degree of freedom to determine what the actual final CV is for the resultant solution. For instance, given C_t =0.748, δ =0.001 and a Mixer-4, the reactant consumption of VOSPA is 1.75 (final CV = 0.74804). However, if the tolerance is 0.005, the reactant usage can be drastically reduced to 0.75 (final CV = 0.75). In the end of this section, the pseudo code of VOSPA is outlined in Fig. 6.

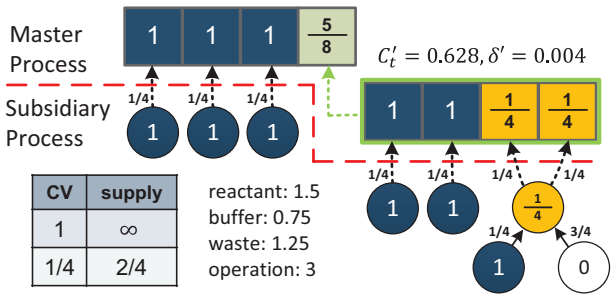

Fig. 5. The dilution process of VOSPA for C_t=0.907 and δ=0.001.

V. XPERMENTAL RESULTS

We have conducted two experiments for evaluation. In the first experiment, we compare VOSPA against three state-of-the-art sample preparation algorithms that merely use the (1:1) mixing model, including BS [20], REMIA [25], and a reactant-minimal network-flow-based approach (OPT) [32]. Since the primary objective is reactant minimization, the cost of buffer is set to 0 in OPT. We also adopt exactly the same experimental environment setup used in both [25] and [32], where all 1023 various target CVs in the range between 1/1024 and 1023/1024 are tested, while the tolerance is set to 1/2048. The results of the first experiment are reported in Table I, in which the volume of a mixer is regarded as a unit of reactant and waste. Each value in the table represents the average result of all 1023 test cases. Table I shows that OPT provides the best reactant minimization outcome under the (1:1) mixing model. However, VOSPA always outperforms OPT as long as N is larger than 2. Specifically, VOSPA achieves a reactant reduction by 22% and 37% as compared to OPT if a Mixer-4 and Mixer-8 is utilized, respectively. Compared to the baseline BS, the reactant reduction can be up to 65% and 72%, respectively. Moreover, though VOSPA is not developed for the minimization of waste and mixing operations, it still does much better than OPT. Besides, VOSPA is actually degenerated to REMIA if a Mixer-2 is in use. It is evident that the combination of VOSPA and a multi-segment mixer can do better reactant minimization than the prior arts. At last, VOSPA is also very time-efficient – it finishes all 1023 test cases in one second.

As mentioned, the master process may call itself recursively. Table II shows the recursive depth of the master process for all 1023 test cases. It clearly indicates that every test case can be finished in 5/4 calls of the master process in a Mixer-4/8. Besides, over 90% of test cases can be finished in 3/2 calls if a Mixer-4/8 is used. It is conclusive that the master process converges faster as N grows since more mixing models

TABLE I. OVERALL COMPARISONS AMONG VARIOUS METHODS.

	BS	REMIA/ VOSPA_ Mixer-2	OPT	VOSPA_ Mixer-4	VOSPA_ Mixer-8
Reactant	2.50 (2.25)	1.21 (1.09)	1.11 (1.00)	0.87 (0.78)	0.70 (0.63)
Waste	4.00 (0.73)	3.27 (0.60)	5.45 (1.00)	2.26 (0.41)	1.69 (0.31)
Operation	9.01 (0.57)	10.13 (0.64)	15.8 (1.00)	5.36 (0.34)	3.71 (0.24)

*Numbers in parentheses: ratios between the specified methods and OPT.

VOSPA(C_t, δ, N)
// C_t: target concentration; δ: tolerance; N: #segment
1. B.supply(1) ← ∞; // B: CV bank
2. MASTER_PROCESS(C_t, δ, B)

MASTER_PROCESS(EV, δ, B)
1. $M \leftarrow \emptyset$; $AV \leftarrow 0$; // M: a Mixer-N
2. **while** ($|EV - AV| > \delta$)
3. $LBS \leftarrow \frac{EV-AV-\delta}{\#empty\_segment}$; $UBS \leftarrow EV - AV + \delta$;
4. Find maximum x from B, where $LBS \le Vol(x) \le UBS$
5. **if** x exists **then**
6. M.fill_segment(x); $AV \leftarrow AV + Vol(x)$
7. B.supply(x)← B.supply(x) − $\frac{1}{N}$
8. **else**
9. **if** $\#empty\_segment > 1$ **then**
10. B ←SUBSIDIARY_PROCESS(LBS, UBS, B)
11. **else**
12. x ← MASTER_PROCESS($N \times (EV - AV)$, $N \times \delta$, B)
13. M.fill_segment(x); $AV \leftarrow AV + Vol(x)$
14. **if** $\#empty\_segment > 0$
15. Fill buffer into all empty segments in M and update AV;
16. **return** AV

SUBSIDIARY_PROCESS(LBS, UBS, B)
1. **for_each** concentration $C \in B$ in increasing order
2. Find minimum i, where $LBS \le C \times \frac{i}{N}$ and $\frac{i}{N} \le B$.supply(C)
3. **if** i exists **then**
4. B.supply(C)← B.supply(C) − $\frac{i}{N}$
5. B.supply($C \times \frac{i}{N}$) ← 1
6. **if** $C \times \frac{i}{N} > UBS$ **then**
7. B ←SUBSIDIARY_PROCESS(LBS, UBS, B)
8. break
9. **return** B

Fig. 6. The pseudo code of VOSPA.

are available for use. As a result, Table I also reports the consistent results – the operation count of Mixer-8 is lower than that of Mixer-4.

In the second experiment, the impact of the tolerance is investigated and Table III presents the related results. The table clearly suggests that the results are getting better as the given tolerance increases since a larger δ generally makes VOSPA terminate earlier, which usually leads to a better outcome.

TABLE II. RECURSIVE CALL DEPTH FOR 1023 TEST CASES.

call depth	1	2	3	4	5
Mixer-4	85	443	402	88	5
Mixer-8	358	586	78	1	0

TABLE III. COMPARISONS AMONG VARIOUS CHOICES OF TOLERANCE δ.

	VOSPA_Mixer-6			
tolerance	1/2048	0.01	0.05	0.10
Reactant	0.76 (1.00)	0.67 (0.88)	0.54 (0.72)	0.48 (0.64)
Waste	1.89 (1.00)	0.79 (0.42)	0.29 (0.16)	0.00 (0.00)
Operation	4.28 (1.00)	2.24 (0.52)	1.40 (0.33)	1.00 (0.23)

*Numbers in parentheses: ratios between the specified tolerance and δ=1/2048.

VI. Conclusion

Sample preparation is one of essential operations in most biochemical reactions. A dozen of approaches have been proposed to address this problem in past few years. However, to the best of our knowledge, there is still no algorithm aiming at the problem on FMFBs equipped with multi-segment mixers. In this paper, we propose the first sample preparation algorithm VOSPA for reactant minimization on FMFBs with multi-segment mixers. VOSPA is an efficient volume-oriented method that maximizes the segment-based reuse of available intermediate solutions with the help of the CV bank. The experimental results demonstrate that VOSPA outperforms all the existing algorithms, including a reactant-minimal one, that merely support the (1:1) mixing model in not only reactant usage but operation count and waste amount as the number of segments is larger than 2. Hence, it is convincing that VOSPA is a very promising algorithm dedicated for single-target sample preparation on FMFBs with multi-segment mixers.

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