AUTOMATED, ACCURATE, AND INEXPENSIVE SOLUTION-PREPARATION ON A DIGITAL MICROFLUIDIC BIOCHIP*

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Abstract—**Solution-preparation is a basic and repetitive step for many biological and chemical experiments in the laboratory. It uses stock solutions of sample, reagents, and diluents to derive various mixed solutions with the required concentration levels. Manual solution-preparation methods are time-consuming, imprecise, and they require large volumes of liquid. We propose an electrowetting-based "digital" microfluidic biochip design for automated solution-preparation. An efficient solution-preparation algorithm is also presented to generate a preparation plan that lists the intermediate mixing steps needed to generate the target solutions with the required concentrations. It determines the type, concentration, and the number of dispensed droplets of stock solutions. The proposed automated solution-preparation algorithm and biochip platform are evaluated using a protein-crystallization application.**

Index Terms: **Digital microfluidics, droplets, laboratory automation, sample preparation.**

I. INTRODUCTION

Solution-preparation plays an essential role in biological and chemical experiments. For experiments that requires high throughput, such as protein crystallization and DNA sequencing, thousands of solutions need to be prepared. Traditionally, solution preparation in the laboratory has been carried out manually. Unfortunately, this approach not only requires high liquid volumes, but it is also time-consuming and error-prone. Therefore, there is a need for automated solution-preparation techniques that lead to accurate solution concentrations and require low liquid volumes.

Fluid-handling robots have been developed and used for automated solution-preparation [1,2,3]. However, these robots suffer from many drawbacks. First, they rely on high-accuracy microtubes and microtips, which are expensive and fragile [1,2]. Moreover, most fluid-handling robots can only process solutions whose volumes are in the range of microliter or higher. Therefore, this approach still requires a large volume of stock solutions for large-scale bioassays, such as protein crystallization. Finally, it is not easy to re-program a robot for a different set of desired set of solutions and concentration levels, i.e., a robot can only prepare a predetermined set of solutions with fixed concentrations. However, in most biological experiments, the types of solutions and their concentration levels need to be fine-tuned based on intermediate assay outcomes, and new solutions need to be generated in multi-step solution-preparation methods. In this case, manual handling is still necessary to complement fluidic-handling robots.

A promising approach for the automated handling of nanoliter volumes of liquids is based on digital microfluidics. Laboratory experiments for immunoassays, DNA sequencing, and protein crystallization can be easily carried out on miniaturized digital microfluidic biochips [4]. Bioassay protocols are scaled down (in terms of liquid volumes and assay times), and executed on a microfluidic chip by manipulating discrete droplets of nanoliter volume on a two dimensional array of electrodes. Compared to traditional bench-top procedures, microfluidic biochips offer the advantages of low sample and reagent consumption, less likelihood of error due to minimal human intervention, high throughput, and high sensitivity, which make it uniquely suitable for solution preparation.

In this paper, we present the design of a digital microfluidic platform for automated solution-preparation. The chip layout consists of 21 reservoirs for storing various stock solutions as well as intermediate solutions. To reduce solution-preparation time and the consumption of stock solutions, an efficient preparation-planning algorithm is also proposed. In this way, a large number of target solutions can be generated using small volumes of source stock solutions.

II. DIGITAL MICROFLUIDIC BIOCHIPS

The digital microfluidic platform considered here utilizes the electrowetting phenomenon to manipulate and move nanoliter droplets containing biological samples on a two-dimensional electrode array [4, 5]. A unit cell in the array includes a pair of electrodes that acts as two parallel plates. The bottom plate contains a patterned array of individually controlled electrodes, and the top plate is coated with a continuous ground electrode. A droplet rests on a hydrophobic surface over an electrode. It is moved by applying a control voltage to an electrode adjacent to the droplet and, at the same time, deactivating the electrode just under the droplet. This electronic method of wettability control creates interfacial tension gradients that move the droplets to the charged electrode. Using the electrowetting phenomenon, droplets can be moved to any location on a two-dimensional array.

By varying the patterns of control voltage activation, many fluid-handling operations such as droplet merging, splitting, mixing, and dispensing can be executed in a similar manner. For example, mixing can be performed by routing two droplets to the same location and then turning them about some pivot points. Moreover, a surrounding film of silicone oil has been shown to prevent evaporation and cross contamination [4]. The digital microfluidic platform offers the additional advantage of flexibility, referred to as reconfigurability, since fluidic operations can be performed anywhere on the array. Droplet routes and operation scheduling result are programmed into a microcontroller that drives electrodes in the array. In addition to electrodes, optical detectors such as LEDs and photodiodes are also integrated in digital microfluidic arrays to monitor colorimetric bioassays.

To address the need for low-cost, PCB technology has been employed recently to inexpensively mass-fabricate digital microfluidic biochips [6, 7]. Using a copper layer for the electrodes, solder mask as the insulator, and a Teflon AF coating for hydrophobicity, the microfluidic platform can be fabricated by using an existing PCB manufacturing process. This inexpensive process allows us to build disposable PCB-based microfluidic biochips that can be easily plugged into a controller circuit board that can be programmed and powered via a standard USB port.

III. EFFICIENT SOLUTION-PREPARATION PLANNING ALOGRITHM

For a given bioassay, we refer to the set of solutions to be prepared as *target solutions*. In this section, we present an efficient algorithm

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for preparation of the target solutions.

A. Concentration Manipulation using Mixing and Dispensing

Suppose that we have to prepare a target solution of Reagent A with a concentration of 0.2 M. Assume that we only have a stock solution of A with concentration 0.4 M. Therefore, we have to dispense droplets from the stock-solution reservoir and dilute them appropriately, by mixing with equal volumes of diluent in a reservoir. Note that for better mixing, the reservoir must be filled to its capacity. The dilution of one droplet must be followed by the dispensing of a (diluted) droplet of 0.2 M concentration of A from the mixing reservoir. By varying the number of droplets routed from the stock solution reservoir to the mixing reservoir, stock solution droplets can be diluted to different concentrations.

Since digital microfluidic platform can only handle discrete droplets, the number of droplets routed from the solution reservoirs can only be integers. As a result, the stock solution droplets can not be diluted to any arbitrary concentration using one iteration of the dispensing-mixing-and-dispensing sequence. Instead, only a set of discrete (quantized) concentrations are feasible.

For the above example, assume that the dilution is performed in a reservoir whose capacity is four times the volume of a unit droplet. We can only dispense 1, 2, or 3 droplets from the stock solution whose concentration is 0.4 M. The concentrations of the dispensed droplet after the dilution can only be 0.1 M, 0.2 M or 0.3 M. Here we define the difference in the outcome concentrations caused by dispensing one more (or less) droplet into the mixing reservoir as *modulation resolution*.

By definition, the modulation resolution can be determined using the equation:

$$Modulation\ \ resolution = \frac{concentration\ of\ \ stock\ solution \times volume\ of\ \ a\ unit\ droplet}{capacity\ of\ \ mixing\ reservoir} \quad (1)$$

In one iteration of mixing-and-dispensing, the concentrations of the diluted droplets dispensed from the mixing reservoir can only be multiples of the modulation resolution. To obtain other concentrations, extra dilution steps are needed to obtain intermediate stock solutions with reduced concentrations, which in turn yields finer module resolution according to Equation (1).

B. Solution-preparation Algorithm

In this subsection, we focus on the problem of generating target solutions with required sample concentrations using the basic dispense-mix-dispense operation as described above. We refer to this process as "solution-preparation planning".

Given a set of target solutions, the solution-preparation planning algorithm determines:

1. The types of stock solutions that are needed;
2. The concentration of each stock solution;
3. The number of dispensed droplets of the stock solutions;
4. The manner in which these droplets must be mixed so that we can derive the target solutions using smallest number of droplet-manipulation steps (routing, mixing and dilutions).

Next we use an example to illustrate the algorithm. In this example, we plan to generate a set of 24 target solutions for a protein crystallization assay, as shown in Table I. The algorithm first determines the types of stock solutions needed. Here we use one stock solution for each type of reagent included in the set of target solutions. Therefore, the number of stock solutions is the same as the total types of reagents included in the target solutions. For the example in Table I, 17 different types of reagents are included in the target solutions. Thus 17 types of stock solutions are needed, as listed in Table II. These stock solutions are stored in on-chip reservoirs.

Next the algorithm determines the concentration of each type of stock solution. For each type of stock solution, the algorithm

Table I: Target-solution list for protein crystallization

Condition ID	Reagent_ID	*	Condition ID	Reagent_ID	*
MembFac_01	sodium chloride	0.1 M	MembFac_13	polyethylene glycol 4000	12% w/v
MembFac_01	sodium acetate trihydrate	0.1 M	MembFac_13	lithium sulfate monohydrate	0.1 M
MembFac_01	2-methyl-2,4-pentanediol (MPD)	12% v/v	MembFac_13	tri-sodium citrate dihydrate	0.1 M
MembFac_02	zinc acetate dihydrate	0.1 M	MembFac_14	iso-propanol (IPA)	10% v/v
MembFac_02	sodium acetate trihydrate	0.1 M	MembFac_14	tri-sodium citrate dihydrate	0.1 M
MembFac_02	polyethylene glycol 4000	12% w/v	MembFac_14	tri-sodium citrate dihydrate	0.1 M
MembFac_03	ammonium sulfate	0.2 M	MembFac_15	2-methyl-2,4-pentanediol (MPD)	12% v/v
MembFac_03	polyethylene glycol 4000	10% w/v	MembFac_15	sodium chloride	0.1 M
MembFac_03	sodium acetate trihydrate	0.1 M	MembFac_15	tri-sodium citrate dihydrate	0.1 M
MembFac_04	sodium chloride	0.1 M	MembFac_16	magnesium sulfate heptahydrate	1 M
MembFac_04	iso-propanol (IPA)	12% v/v	MembFac_16	tri-sodium citrate dihydrate	0.1 M
MembFac_04	sodium acetate trihydrate	0.1 M	MembFac_17	tri-sodium citrate dihydrate	0.1 M
MembFac_05	sodium acetate trihydrate	0.1 M	MembFac_17	sodium chloride	0.1 M
MembFac_05	polyethylene glycol 4000	12% w/v	MembFac_17	polyethylene glycol 4000	12% w/v
MembFac_06	ammonium sulfate	1 M	MembFac_18	lithium sulfate monohydrate	0.1 M
MembFac_06	sodium acetate trihydrate	0.1 M	MembFac_18	tri-sodium citrate dihydrate	0.1 M
MembFac_07	magnesium sulfate heptahydrate	1 M	MembFac_18	polyethylene glycol 6000	12% w/v
MembFac_07	sodium acetate trihydrate	0.1 M	MembFac_19	magnesium chloride hexahydrate	0.1 M
MembFac_08	sodium acetate trihydrate	0.1 M	MembFac_19	2-methyl-2,4-pentanediol (MPD)	4% v/v
MembFac_08	magnesium chloride hexahydrate	0.1 M	MembFac_19	tri-sodium citrate dihydrate	0.1 M
MembFac_08	polyethylene glycol 400	18% v/v	MembFac_20	sodium chloride	0.1 M
MembFac_09	ammonium dihydrogen phosphate	1 M	MembFac_20	tri-sodium citrate dihydrate	0.1 M
MembFac_09	sodium acetate trihydrate	0.1 M	MembFac_21	polyethylene glycol 400	4% v/v
MembFac_09	lithium sulfate monohydrate	0.1 M	MembFac_21	tri-sodium citrate dihydrate	0.1 M
MembFac_10	polyethylene glycol 6000	12% w/v	MembFac_21	lithium sulfate monohydrate	0.1 M
MembFac_10	sodium chloride	0.1 M	MembFac_22	ADA	0.1 M
MembFac_10	sodium acetate trihydrate	0.1 M	MembFac_22	ammonium sulfate	1 M
MembFac_11	sodium acetate trihydrate	0.1 M	MembFac_23	ADA	0.1 M
MembFac_11	magnesium chloride hexahydrate	0.1 M	MembFac_23	polyethylene glycol 4000	12% w/v
MembFac_11	polyethylene glycol 6000	12% w/v	MembFac_23	lithium sulfate monohydrate	0.1 M
MembFac_12	sodium chloride	0.1 M	MembFac_23	iso-propanol (IPA)	2% v/v
MembFac_12	polyethylene glycol 400	18% v/v	MembFac_24	di-ammonium hydrogen phosphate	1 M

***Reagent concentration**

Table II: Stock solutions needed to prepare the target solutions in Table I.

S1	sodium chloride	1 M	S10	polyethylene glycol 400	40% v/v
S2	sodium acetate trihydrate	1 M	S11	ammonium dihydrogen phosphate	10 M
S3	2-methyl-2,4-pentanediol (MPD)	40% v/v	S12	lithium sulfate monohydrate	1 M
S4	zinc acetate dihydrate	1 M	S13	polyethylene glycol 6000	24% w/v
S5	polyethylene glycol 4000	20% w/v	S14	tri-sodium citrate dihydrate	1 M
S6	ammonium sulfate	2 M	S15	ADA	1 M
S7	iso-propanol (IPA)	20% v/v	S16	Ammonium sulfate	10 M
S8	magnesium sulfate heptahydrate	10 M	S17	di-ammonium hydrogen phosphate	10 M
S9	magnesium chloride hexahydrate	1 M			

Table III. Target solutions containing the reagent polyethylene glycol 4000

Condition ID	Reagent_ID	Reagent concentration		*n*
MembFac_02	polyethylene glycol 4000	12	% w/v	6
MembFac_03	polyethylene glycol 4000	10	% w/v	5
MembFac_05	polyethylene glycol 4000	12	% w/v	6
MembFac_13	polyethylene glycol 4000	12	% w/v	5
MembFac_17	polyethylene glycol 4000	12	% w/v	5
MembFac_23	polyethylene glycol 4000	12	% w/v	6

***n*: # of droplets routed from the stock solution to the mixing reservoir**

Table IV. Preparation plan for target solution MembFac_02

Reagent_ID	Stock solution	Concentration	# of droplets
zinc acetate dihydrate	S4	1 M	1
sodium acetate trihydrate	S2	1 M	1
polyethylene glycol 4000	S3	20% w/v	6
diluent	—	—	2
Note: total number of droplets = 1+1+6+2 = 10 unit droplets = capacity of mixing reservoir			

identifies all the target solutions that contain the corresponding reagent in the stock solution.

For the example shown in Table I and Table II, stock solution S_5 contains the reagent *polyethylene glycol 4000*. There are six target solutions that contain this reagent, i.e., *MembFac_02* and *MembFac_03*, *MembFac_05*, *MembFac_13*, *MembFac_17*, *MembFac_23*, as listed in Table III. The concentration of the reagent *polyethylene glycol 4000* in these target solutions are 12% w/v, 10% w/v, 12% w/v, 12% w/v, 12% w/v, and 12% w/v, respectively. Note that *w/v* refers to "weight per volume", i.e., [Mass of solute (g) / Volume of solution (ml)] × 100. For example, a 10% *NaCl* solution has ten grams of sodium chloride dissolved in 100 ml of solution. The computation in Equation (1) can be carried for both units, i.e., "moles" (M) and w/v.

Recall from Section III.A that these concentrations must be multiples of the modulation resolution, i.e., the module resolution must be a common factor of these concentrations.

In our algorithm, we pick the greatest common divisor (GCD) of these concentrations as the modulation resolution. This is because smaller modulation resolution indicates that more droplets must be routed into the mixing reservoir to obtain the target concentration. As a result, more droplet manipulation steps are needed.

In the above example, the modulation resolution is *GCD*(12, 10, 12, 12, 12, 12), i.e., 2% w/v. Assume that the volume of a unit droplet is 20 nl and the capacity of the mixing reservoir is 200 nl. We use an alternative form of Equation (1) as follows:

$$\text{Concentration of stock solution} = \frac{\text{Modulation resolution} \times \text{capacity of mixing reservoir}}{\text{volume of a unit droplet}} \quad (2)$$

Therefore, we calculate the concentration of stock solution containing reagent *polyethylene glycol 4000* as simply 2 × 200nl / 20nl, i.e., 20% w/v.

Next we calculate the number of droplets that need to be routed from the stock solution to the mixing reservoir in generating a target solution. This number can be obtained using the following equation:

$$\text{\# of droplets routed from stock solution} = \frac{\text{Concentration of the reagent in the tar get solution}}{\text{Modulation resolution}} \quad (3)$$

For the above example, the numbers of droplets *n* from stock solution S_5 for the target solutions are listed in Table III. We can obtain the concentration of all the other stock solutions and the number of droplets to be routed from each stock solution in a similar manner. Now we can generate the preparation plan for a target solution; an example is shown in Table IV.

In Table IV, the total number of droplets from different types of stock solutions is 1+1+6, i.e., 8, which is less than 10 unit droplets (the capacity of the mixing reservoir). Two droplets of diluent are therefore routed to fill up the mixing reservoir.

For other target solutions, the total number of droplets from different stock solutions may exceed the capacity of the mixing reservoir. In this case, we identify the stock solution that dispenses the largest number of droplets and double its concentration. By this means, fewer droplets are needed to generate the target solution. As a tradeoff, the modulation resolution is lowered. To obtain finer resolution, extra dilution iterations are needed.

Next, we enumerate the steps of the solution-preparation planning algorithm.

1. Given a set of target solutions *Ts* (Ts_1, Ts_2, Ts_3, …, Ts_m), identify the types of reagents contained in them, i.e., *R* (R_1, R_2, R_3, …, R_n).
2. Determine the set of stock solutions *Ss* (Ss_1, Ss_2, Ss_3, …, Ss_n)using the mapping: $R_i \leftrightarrow Ss_i$.
3. For each type of stock solution Ss_i, identify the set of target solutions TsR_i that contain the corresponding reagent R_i.
4. Determine the modulation resolution for stock solution Ss_i by equating it to $GCD(TsR_i)$. The well-known Euclidean algorithm is used to compute the *GCD* [8].
5. Determine the concentration for each solution using Equation (2).
6. Calculate the number of droplets dispensed from each stock-solution reservoir for each type of target solution using Equation (3).
7. Check if the total number of droplets dispensed from different stock solution reservoirs exceeds the capacity of the mixing reservoir. If yes, go to Step 8, otherwise the algorithm terminates.
8. Identify the stock solution that dispenses the most droplets into the mixing reservoir during the preparation of the target solution. Double the concentration of that stock solution. Then go to Step 6.

Assume that the total number of target solutions is *m* and *n* types of reagents are contained in them. It can be easily shown the worst-case computational complexity of the proposed algorithm is $O(nm^2)$. The worst-case happens if the total number of droplets dispensed from different stock-solution reservoirs exceeds the capacity of the mixing reservoir and Steps 6-8 are executed for each target solution, i.e., *m* times. In the best case, Step 8 is never reached, and the algorithm takes $O(nm)$ time.

As for the complexity of fluidic operations, in the best case, preparing a single target solution requires only one iteration of mixing-and-dispensing operation. No extra dilution is needed. The entire preparation plan takes *m* mixing-and-dispensing operations. In the worst case, preparing each target solution requires an extra dilution step. The preparation plan requires 2*m* mixing-and-dispensing operations.

IV. SOLUTION-PREPARATION CHIP DESIGN

In this section, we present the design of the biochip used to carry out the solution-preparation plan generated from the algorithm in Section III. The chip layout is shown in Figure 1. It consists of 21 reservoirs. The 18 reservoirs on the right side of the chip are used to store and dispense stock solutions. Two reservoirs on the bottom-left are used for mixing and dilution. One is called Target Solution Generator (TSG), which mixes droplets from different stock solutions with diluents to obtain the target solutions. The other one is called

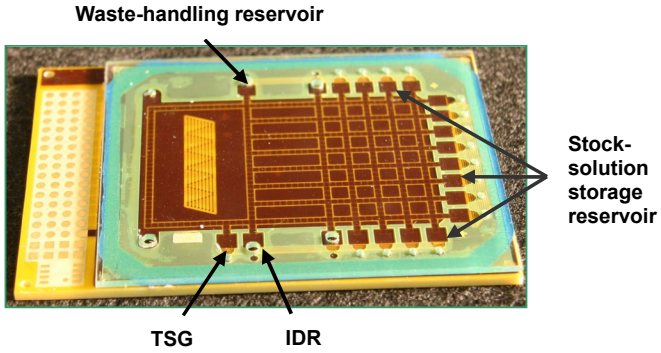

Figure 1: Fabricated chip for automated solution-preparation.

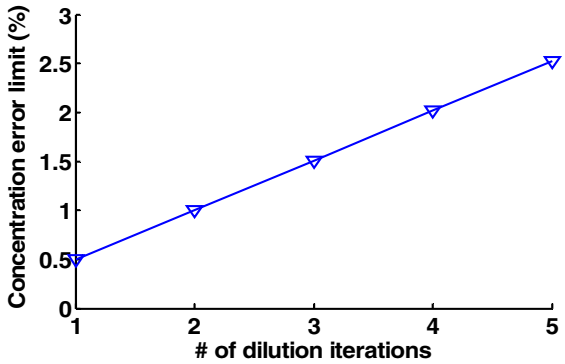

Figure 2: Concentration error limit versus number of mixing-dispensing iterations

Intermediate Dilutions Reservoir (IDR), which is used to dilute the stock solutions for higher modulation resolution. The capacity of both the TSG and IDR are 10 times the volume of a unit droplet. A waste-handling reservoir is positioned as shown in Figure 1.

In the middle of the chip, there are six lanes of electrodes. These electrode lanes, together with the five column lanes and eight row lanes on the right part of the chip are used as routing rails to transport droplets from stock solution storage reservoirs to the IDR and TSG.

We are given a solution-preparation plan generated from the algorithm in Section III. Stock solutions containing the reagents involved in the preparation plan are first injected into the storage reservoirs. Next, based on the preparation plan, target solutions are prepared in an automated manner. For each target solution, the predetermined number of droplets are dispensed from the corresponding stock solution storage reservoirs and transported along the routing lanes to the TSG and mixed there. For target solutions that require extra dilution steps, the droplets from the stock solution reservoir are diluted using the IDP before being routed to the TSG.

After all the required stock solution droplets are routed to the TSG, the diluent droplets are routed into the TSG until the reservoir capacity is reached. Now the target solution with the required concentration has been derived. We then dispense a certain volume of the target solution and store it on the loop of electrodes on the left side of the chip. The rest of the target solutions are then dispensed to the waste-handling reservoir. At this moment, the TSG is empty and ready for the next target solution.

V. Experimental Results and Comparison

Next we apply the proposed chip design and the solution-preparation planning algorithm to carry out solution-preparation for protein crystallization.

Proteins crystallization is a commonly used technique for protein analysis. It predicts the three-dimensional (3D) arrangement of the constituent amino acids, which indicates the specific biological function of a protein [9]. Protein crystallization is a multi-parametric process that involves the steps of nucleation and growth, where molecules are brought into a thermodynamically unstable and a supersaturated state. In order to "hit" upon the correct parameters for the crystallization of proteins, typically a very large number of experiments (10^3 to 10^4) are required and thousands of solutions need to be prepared [10].

For simplicity, we extract 24 target solutions from the thousands of solutions for the experiment as listed in Table I. Table II shows that 17 types of reagents are used. After applying the solution-preparation planning algorithm, 17 source solutions with appropriate concentrations are chosen corresponding to the 17 types of reagents and stored in the 17 reservoirs on the chip in Figure 1.

Next we prepare these target solutions. First, manual operation is used as a baseline case. Pipettes that can handle a minimum volume of 20 µl are used. The preparation of the target solution consumes 22 ml of reagent stock solutions and takes 1.5 hours. Using the digital microfluidic biochip and the solution-preparation planning algorithm, we need only 18 minutes and 12 µl of reagent solutions.

For protein crystallization, reagent concentration is very important. Therefore, we need to guarantee a high level of accuracy while preparing the target solutions. For a digital microfluidic biochip, the key to generating solutions with precise concentrations is to maintain constant volume of the dispensed droplets.

Experiments have shown that our chip design achieves a high level of consistency in the volume of dispensed droplets (variation is less than 0.5%), which indicates high accuracy in the concentration of the prepared target solutions. Note that the accuracy will degrade when multiple iterations of dilution is carried out. However, results also show an error limit of less than 2.5% even when five iterations of mixing-dispensing operations are used in preparing the target solutions; see Figure 2.

VI. Conclusions

We have described a digital-microfluidic biochip platform for automated solution-preparation for laboratory procedures. An efficient solution-preparation planning algorithm has also been presented. Given a set of target solutions, the algorithm determines the type, concentration, and the number of dispensed droplets of the stock solutions. We have used a protein crystallization assay to evaluate the proposed method.

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