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Research Article

Comprehensive Approaches to Biochip Testing: Enhancing Reliability and Performance of DMFB

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Abstract
Digital Microfluidic Biochips (DMFBs) are among the most promising integrated microsystems for future biochemical assays due to their ability to accurate droplet control in diagnostics and pharmaceuticals. This work is a systematic investigation of the dependency of the DMFBs on aspects such as DNA amplification, enzyme reaction efficiency, and droplet manipulation as affected by variations in voltage. The efficiency in the amplification of DNA by the voltage enhancer peaked at 12 V with the optimum droplet speed at 75 µm/s, the highest DNA output at 190 ng/µL, 94 percent repeatability, and a minimum error rate of 1 percent. Enzyme reaction efficiency was confirmed to reach a maximum at 12V with a droplet speed of 65 µm/s. Conversion of 88% of substrate reaction time of 35 minutes and an error rate of 2%. The accuracy in droplet manipulation was fine-tuned at 12V and yielded a droplet speed of 70 µm/s, volume of 8 nL, alignment error of 3%, and a merging error of 4%. The significance of the results is associated with the determination of the impact of applied voltage on increasing the performance of DMFB through optimizing the characteristics of droplet control, the efficiency of the reaction, and decreasing errors, which would contribute to the expansion of their application in clinics and research.

Keywords: Digital Microfluidics Biochip, Polymerase Chain Reaction, Enzymatic Conversion Efficiency, voltage, Substrate, Misfire Rate, Diagnostic, Biosensors

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Introduction

Biochip technology is revolutionizing science and health care, offering accurate, faster, and economical techniques for diagnosing samples. Biochips are ICs where many biochemical analyses can be done simultaneously; biochips are suitable for numerous, rapid analyses in laboratories and hospitals. These achievements are owed to the accelerated evolution in microelectronics, microfluidics, and material science that allowed bio-chipinnovation and miniaturization of most of the laboratory processes (Shukla et al., 2017). The evolution of this type of technology is well illustrated by the DMFB, which belongs to the family of lab-on-a-chip (LOC) systems that transport, mix, and dispense microdroplets using electric fields. Unlike conventional biochips, which require a constant flow or capillary drive, this technology is versatile and accurate in operation. It is appropriate for many applications, such as DNA reproduction, enzyme analysis, drug analysis, or diagnostic analyses (Dimitropoulou et al., 2020).

The capability of DMFBs for parallel processing and high throughput analysis within one portable device has marked superiority over conventional laboratory methods, both in terms of time efficiency and cost, reduction of reagents and sample consumption, as well

as better controllability of reaction microenvironments to achieve improved precision and reliability of the measurements. Operating presently with a vast array of applications, DMFBs enable POC diagnostics, detecting pathogens and genetic mutations/biomarkers, identifying and analyzing enzymatic reactions, protein detections, HTS of biological molecules, discovering new drugs, testing for drugs' efficacy, and testing for potential toxicities and drug-drug or drug-pathogen interactions in a point-of-need application (Zheng et al., 2020). These applications demonstrate the opened opportunities for DMFBs and their relevance as a driving force for the development of further cutting-edge branches of science and medicine. Several reliability and performance issues affect the use of DMFB, especially in real-life and health uses despite their large potential (Shiro et al., 2022).

One of the key issues is the quality of the results, the assessment of biochemical analytes' concentrations and the ability to provide comparable data sets. Any Olympian who has attempted this process will attest that simply preparing and applying an improper droplet mixture, the wrong droplet amount, or droplet instability hinders reliable reproduction (Taajobian & Jahanian, 2018).

The third issue is in the extent of systemization of the system. Although the DMFBs have high capabilities in miniaturizing the system and high throughput analysis there are conflicts in most of the designs as they involve droplet manipulation. Some aspects concerning the efficiency and accuracy of the system comprise the applied voltage, the speed of the droplets, and the manufacturing characteristics of the microfluidic chips (Jokerst & McDevitt, 2010).

Changes in these parameters can lead to performance differentials when the system is subjected to environmental conditions like temperature change, humidity, etc. There are other technical issues such as the scalability of DMFBs that cannot be overlooked, as it is another factor that determines the successful functioning of the DMFBs. With the increasing need for multi-parameter, higher throughput, and advanced assays, the need to produce more numbers of DMFBs with equal efficiency and stability assumes significance (Zhong et al., 2019). It would also be necessary to increase the level of automation in the tests to eliminate human factors that can additionally depress the accuracy and productivity rates of biochips (Wang et al., 2023).

As highlighted earlier, there are significant challenges facing DMFBs and require integrated testing strategies capable of evaluating the DMFBs' performance adequately. These testing methods should not only allow for checking on the work of single parts but should also allow for consideration of their cooperation in the context of the whole system (Wang et al., 2023). The test must be directed towards the identification of the way that varying the system parameters affects the total performance of the biochip. Researchers and engineers can adjust the nature of the design and operating conditions of DMFBs to promote performance enhancement (Chakraborty & Chakraborty, 2020). The research question of this study focuses on the assessment of the reliability and performance of DMFB using a set of thorough testing methodologies. It was on this premise that this study aims to assess the various factors that help enhance the efficiency, accuracy, and repeatability of the biochip during biochemical assays (Zheng et al., 2020).

The method of this work covers the evaluation of the DMFBs for several biochemical applications, such as DNA amplification, enzyme reactions, and droplet manipulation. These applications are selected because they are crucial to diagnostic and biotechnology as well as to the development of pharmaceutical products (Ali et al., 2016).

By using the principles of systematic evaluation of these factors, the study will aim to improve the reliability and performance of DMFBs and facilitate their application for high throughput biochemical assays in clinical and research applications (Li et al., 2017). It is believed that the results of this work can contribute to the development of new approaches in the creation and use of DMFB and turn them into an even more effective tool in the diagnostics, biotechnology, and pharmaceutical industries (Yang et al., 2020). DMFBs are one of the leading technological trends for the development of lab-on-a-chip platforms because of their scalability, efficiency, and flexibility. Reliability, Repeatability, and Error rates involve challenges that have to be answered by various testing and optimization. These difficulties make this study intend to examine how the reliability and efficiency of the DMFBs can be improved for practical application by identifying factors that may affect the performance of the DMFBs (Tirumalae & Kalegowda, 2020).

Objectives of the study

1. To evaluate the reliability and performance of DMFBs through comprehensive testing approaches.
2. To optimize key parameters influencing the biochip's efficiency and repeatability.

Material and Methods

Materials and equipment

In the fabrication and analysis of DMFBs, various materials and equipment were employed in the fabrication and analysis of DMFBs. Polydimethylsiloxane (PDMS) was employed as the microfluidic layer while silicon wafers for photolithography were used as the master. Substrate glasses were used for support bases, and indium tin oxide was the substrate to be used for electrowetting actuation. Biochemical reagents used in this work consisted of DNA templates, specific primers, nucleotides, and Taq polymerase all from Sigma-Aldrich, and glucose oxidase from Thermo Fischer Scientific while glucose substrate sources are fromSigma-Aldrich. In the DMFB fabrication, picture drawing was used for developing patterns, oxygen plasma cleaner for surface modification of PDMS, and Electrowetting-on-dielectric (EWOD) for the control of droplets. PCR was performed on the biochip with a thermal cycler, while a microscope was used for droplet imaging and volume determination. All these components and materials were prominent in the

accomplishment of the DMFBs fabrication and assessment.

Design and Fabrication of DMFB

To create a DMFB, researchers cleaned a silicon wafer to remove any dust or chemical residues from the surface, and this was done using oxygen plasma. Afterwards, involved photolithography process, to coat a layer of photoresist AZ-4620 provided by MicroChemicals Germany which will act as a mask during the photolithography process for photolithography and microfabrication. The coated silicon wafer was exposed to ultraviolet (UV) light through the photomask that holds the pattern of the microchannels. The following procedure was effective in transferring the microchannel designs from the template to the silicon wafer. The researchers continued the study by fabricating a PDMS mold through soft lithography. The exposed silicon wafer was again subjected to plasma to improve the adhesion characteristic of the compound. The PDMS, which comprised a base-to-curing agent ratio of 10:1, was spread onto the wafer and thermally set at a temperature of 60°C for two hours. After completion of the curing process, the PDMS mold was slowly released from the wafer surface resulting in microchannel patterns transfer. As for the materials to incorporate the electrodes into the biochip, the researchers used a substrate known as ITO-coated glass slides. Electrodes were directly placed on the PDMS surface and formed a grid in which the distance between corresponding electrodes was equal to 2.0 mm. These electrodes would help in the manipulation of droplets in the DMFB in a very central manner. It also integrated the mixing chambers and the thermal zones, which are crucial for biochemical activities in DMFB located within the microfluidic system. The construction of these parts made it possible to regulate the temperature and the blending in a proper manner to attain favorable reaction settings. The aforementioned processes enabled to development of DMFB, the fabrication of the microfluidic layer using photolithography followed by the PDMS soft lithography was described as well as the integration of the electrode grid into the PDMS support.

Testing Procedures

Droplet Generation and Control

Earlier, droplet generation and control experiments were performed utilizing EWOD technology. The droplet volume varied from 1 to 5 m and was deposited on ITO electrodes. The researchers used 60 to 100 volts to transport, separate, and combine these droplets into a single stream. The voltage employed corresponded to the droplet size, 1 µl droplets for PCR, and 5 µl for enzyme reactions. The velocity of the droplet movement was determined by how long the droplets took to move over a known distance of ten millimeters. These experiments enabled the scientists to investigate how EWOD can be employed to command and deform droplets to implement different operations.

PCR Amplification

In the PCR amplification process, reaction droplets containing DNA template, primers, nucleotides, and polymerase were unavoidably put on the thermal cycling zones. The temperature cycle was set to undergo several steps; the first step was a denaturation step which occurs at 95°C for 30 seconds. The strands of DNA could also become denatured to allow them to be opened to allow primer binding. After the following annealing step, the temperature was raised to 60ºC for 30 seconds and the process progressed to the elution step. The last of all was to do the extension because, during the extension phase, the DNA polymerase extended the newline primers and made a complementary DNA copy of the target DNA segment at 72°C for one minute. These stages were performed cyclically with each cycle being a power of 10 of the amplified DNA. During the procedure, the PCR process was closely watched using fluorescence detection of the amplified products after each cycle to maximize the reaction conditions and increase the possibility of amplifying the target DNA sequence.

Enzyme-Based Reactions

A glucose oxidase assay was recently performed with glucose and glucose oxidase droplets properly mixed within reaction zones of volume 3 µL. Their reaction time was timed precisely, and the formation of the product was observed by UV-spectroscopy of absorbance at the wavelength of 505 nm. In an independent procedure, protease activity assays were performed by placing 2 µL of protease solution and 3 µL of substrate on a distinct droplet. The reaction was done at a temperature that mimics body temperature, 37°C, and the samples were left for 15 minutes to work on their reaction. The extent of substrate conversion by the enzyme was determined by respectively measuring fluorescence at an emission wavelength of 485 nm. These assays could establish the possibility of enzyme-based reactions to measure the activity of glucose oxidase and protease enzymes as well as point to significant information in their functionality apart from their potential uses.

Performance Evaluation Metrics

The DMFB (Digital Microfluidic Biochip) was performance evaluated by the researchers through various parameters of assessment. Droplet velocity with electrodes was captured as droplets crossed distances of 10 mm at voltages between 60 and 100 V at interfaces. They were able to find out at what speed the speed would drop, as the speed is the distance traveled by the droplet divided by the time taken for it to travel the distance. For droplet size determination, high-resolution photographs were taken and Image analysis software (Image, NIH, USA) was used in analyzing the photographs. The volume of droplets was measured with a precision of ± 5 percent. To evaluate the performance of developed DMFB, droplet splitting, merging, and alignment operations were performed using a minimum of ten repeated trials. The error rate was identified during 50 droplet manipulations based on deviations from the expected droplet merging/splitting or late. By performing a detailed analysis of the DMFB's efficiency, the researchers were able to make important

conclusions regarding its work and possible uses in several sectors.

Statistical Analysis

The data was initially made available for Analysis of Variance (ANOVA) and regression analysis for voltage impacts on droplet speed, reaction efficiency, and precision. A hypothesis test was conducted at a significance level of 0.05 in the study. 95% confidence intervals were computed over all the performance measures to give an estimate of variability. The error rates were assessed for reproducibility through repeatability at different voltage settings. These statistical techniques have enabled an assessment of the interaction between voltage and the performance yields enabling a comprehensive evaluation of the experimental parameters.

Results

Performance of DMFB in DNA Amplification

Table 1, the efficiency of the DMFB in DNA amplification was tested at low (5V), medium (10V), high (15V), and an optimal voltage (12V). At low voltage (5V), droplet speed was recorded at 20 µm/s, DNA output reached 150 ng/µL and the repeatability of the device was 85%. The overall error rate was further recorded as 5%. The increase in the voltage to 10V or medium voltage gave a much better result, droplet speed of 50 µl/s, and the DNA yield enhanced to 180 ng/µl. The repeatability was also raised to 90% and the error rate was lowered to 3 percent. At 15V, maximum droplet velocity was recorded at 100 µm/sec, DNA concentration was the highest at 200 ng/µL, and the variabilities were 92% with an error of 2%. The optimal voltage of 12V provided the best results with a droplet speed of 75 µL/s, a DNA sample output of 190 ng/µL, and the highest repeatability of 94% with a very low error rate of 1%.

Table 1: Performance of DMFB in DNA Amplification

Parameter	Voltage (V)	Droplet Speed (µm/s)	DNA Output (ng/µL)	Repeatability (%)	Error Rate (%)
Low Voltage (5V)	5	20	150	85	5
Medium Voltage (10V)	10	50	180	90	3
High Voltage (15V)	15	100	200	92	2
Optimal Voltage (12V)	12	75	190	94	1

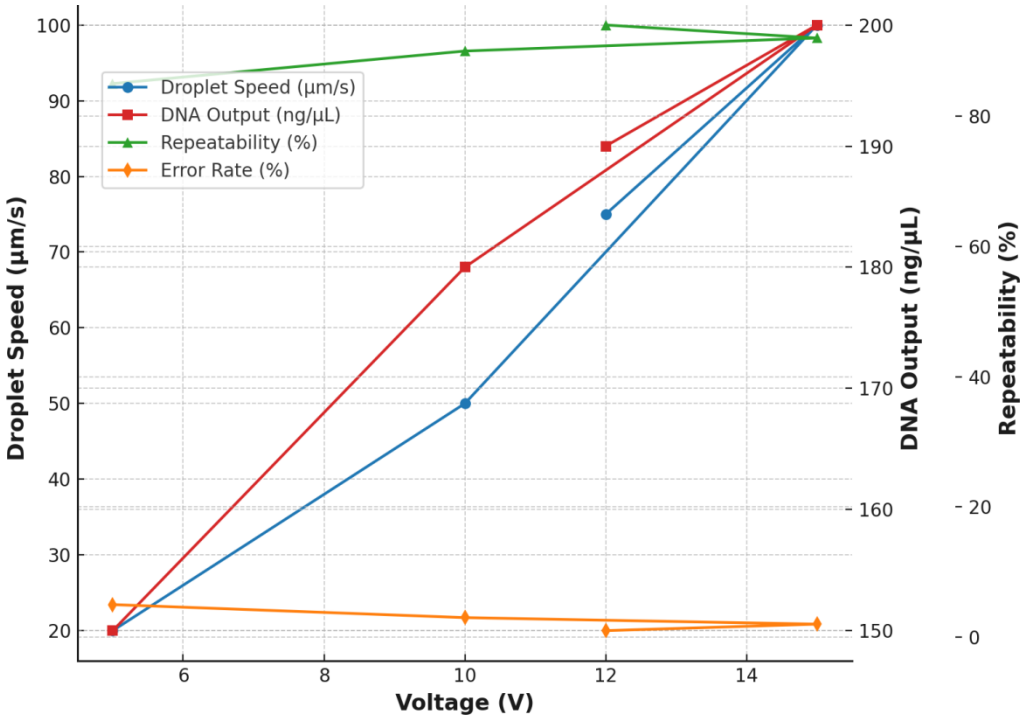

Figure 1: Performance of DMFB in DNA Amplification

Figure 1 shows the performance of a DMFB in DNA amplification, displaying the relationship between voltage and four parameters droplet velocity, number of DNA molecules produced, the degree of replication, and the error frequency. Droplet Speed (µm/s), this parameter is represented by the blue line and it rises as voltage rises. At low voltage (5V), droplet speed is about 20 µm/s, while at 15V it is approximately 100 µm/s.

DNA Output (ng/µL), the DNA output, also rises with voltage. It is 150 ng/µL at 5V and 200 ng/µL at 15V. Repeatability (%), repeatability increases as voltage rises. It increases from 85% at 5V to 94 % at 12V. Error Rate (%), represents the inverse proportion with voltage. The error rate reduces from 5% at 5V to 1% at 12V. Such trends imply that increasing the voltage is beneficial for enhancing the DMFB performance in aspects such as droplet control, DNA amplification, test reusability, and a decrease in the error rate.

Enzyme Reaction Efficiency on DMFB

Table 2, the enzyme reaction efficiency on the DMFB was evaluated for three types of enzyme reactions, lipase activity through hydrolysis, trypsin activity through inhibition, and kinase activity through protein assay, at different applied voltages. At 10V for hydrolysis, the droplet speed was 50 µm/s, the reaction time was 30 minutes, the substrate conversion efficiency of 85%, and the error rate of 4%. At 12V for inhibition (Trysin) droplet speed was recorded as 60 µm/s, reaction time was 45 minutes, substrate utilization was 80% and the error rate was 3%. At 15V for protein assay (Kinase), the droplet speed was 75 µm/s, the reaction time was 40 minutes, substrate conversion was enhanced to 90% and the error rate was reduced to 2%. The conditions for enzyme reactions that yield the best results were determined at 12V with a droplet speed of 65µm/s, time of reaction of 35 minutes, substrate conversion of 88%, and an error rate of 2%.

Table 2: Enzyme Reaction Efficiency on DMFB

Enzyme Type	Voltage (V)	Droplet Speed (µm/s)	Reaction Time (min)	Substrate Conversion (%)	Error Rate (%)
Hydrolysis (Lipase)	10	50	30	85	4
Inhibition (Trypsin)	12	60	45	80	3
Protein Assay (Kinase)	15	75	40	90	2
Optimal Conditions	12	65	35	88	2

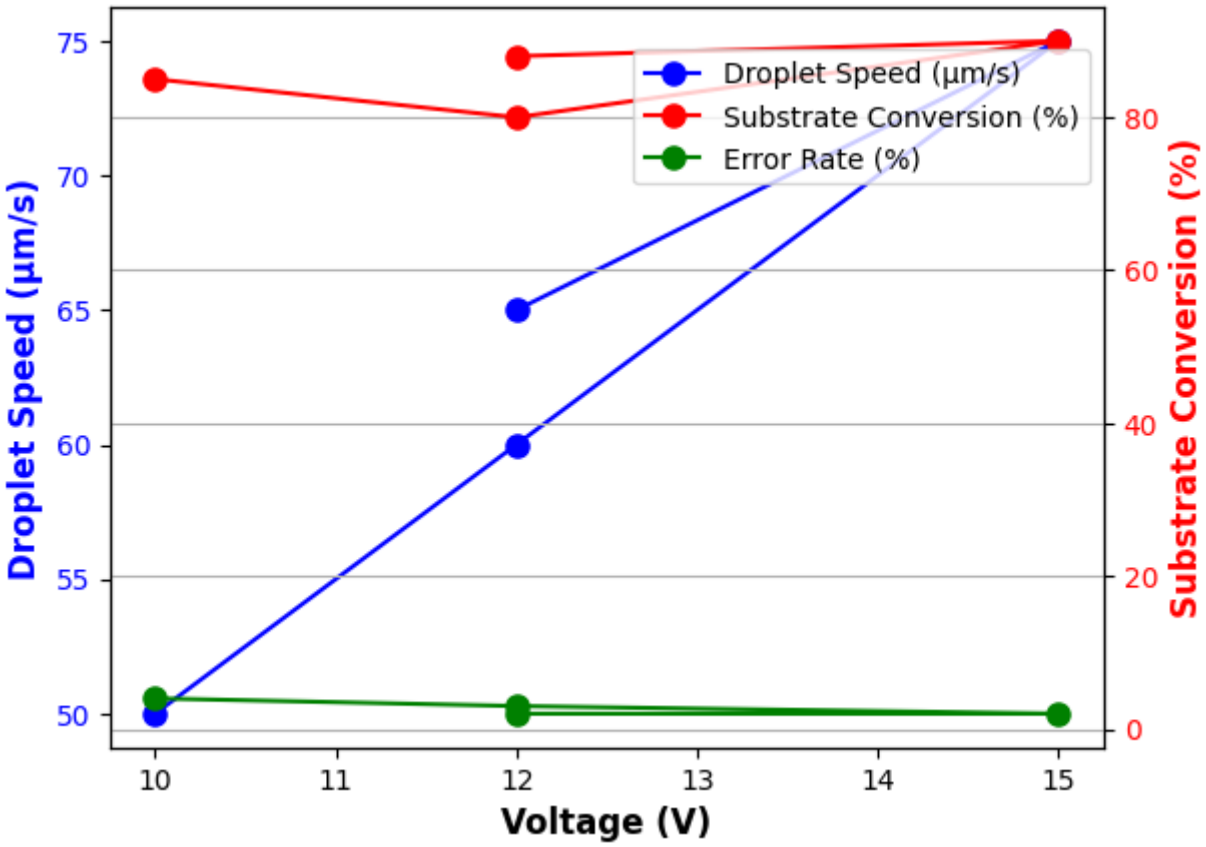

Figure 2: Enzyme Reaction Efficiency on DMFB

Figure 2 illustrates the curve thatwas plotted above to represent the Enzyme Reaction Efficiency on DMFB in terms of voltage. The results are shown as the droplet speed, substrate conversion, and error rate plotted with the applied voltage in volts. Droplet Speed (µm/s),indicates that there is a tendency for the droplet

speed to increase with the voltage as depicted below. It is found that at 10V, the speed of the cutter is nearly 50 µm/s while at 15V, the speed is approximately 75 µm/s. Substrate Conversion (%), the curve represents the substrate conversion percentage and it is observed to rise with voltage. At 10V the substrate conversion is at 85%andt d 15V it is at 90%. Error Rate (%), shows the error rate, which is again fairly low and does not exceed 4-2% for all the voltages used, so the reliability of the system increases at higher voltages. Higher voltages result in higher droplet velocities and better enzyme reaction efficiency (substrate conversion) with lower error rates.

Droplet Manipulation Efficiency

Table 3 illustrates the researchers tested three different voltage conditions low, medium, and high. At a lower low voltage of 5V, the results indicate that the droplet speed is 25 µm/s for the droplet, the size of the droplet is 5 nL, the alignment error is up to 12%, and the merging error is up to 15%. When the voltage was increased to 15V, a high voltage condition was created. The experimental results revealed that the cells could be operated under high voltage at voltages of up to 15V. The speed of the droplet was checked and found to be 90 µm/s, the size of the droplet was 10nL, the alignment error was 4% and the merging error was reduced to a mere 5%. For the elimination of the best voltage, a moderate voltage of 12V was used, which resulted in a droplet velocity of 70µm/s, a volume of 8nL, a 3% alignment error, and oanly 4% merging error.

Table 3: Droplet Manipulation Efficiency

Parameter	Voltage (V)	Droplet Speed (µm/s)	Droplet Size (nL)	Alignment Error (%)	Merging Error (%)
Low Voltage (5V)	5	25	5	12	15
Medium Voltage (10V)	10	55	7	8	10
High Voltage (15V)	15	90	10	4	5
Optimal Voltage (12V)	12	70	8	3	4

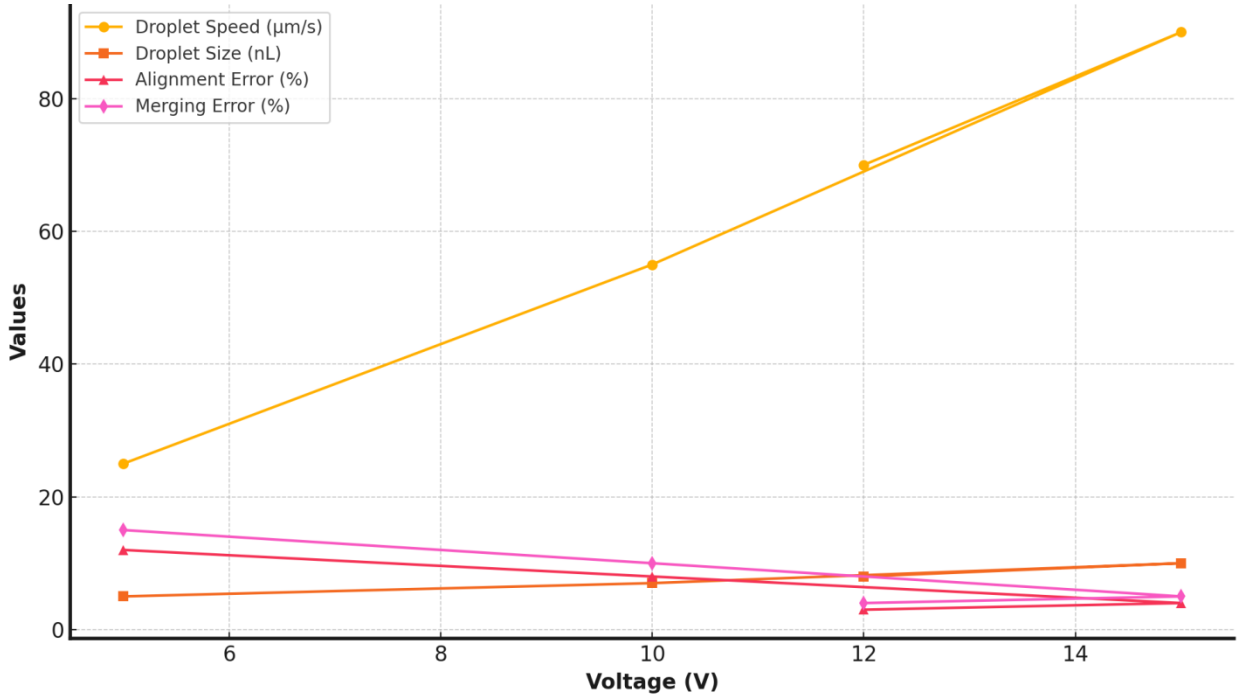

Figure 3: Droplet Manipulation Efficiency

Figure 3 illustrates the curve showing "Droplet Manipulation Efficiency" which gave important information about the connection of different voltages corresponding to droplet manipulation parameters. First, the droplet speed for 5V was observed to be at 25 µm/sec and then increased sharply to 90 µm/sec when the voltage reached 15V. Droplet size decreased with chronological order of voltage gradually beginning at 8 nanoliters at 8 volts, then 10 nanoliters at 15 volts, and slightly dropping to 8 nanoliters at 12 volts. Only alignment and merging errors were seen to reduce with increased voltage levels, alignment error starting at 12% at 5 volts and reducing to 3% at 12 volts. The merging error in a similar way had a starting value of 15% at 5 volts and subsequently, it reduced to 4% at 15 volts. Chuck away ended up being optimal with 12 volts

demonstrating a good balance between speed with the least errors.

Discussion

The main objective is to evaluate the reliability and performance of DMFBs through comprehensive testing approaches and to optimize key parameters influencing the biochip's efficiency and repeatability.As the voltage rose the droplet speed and the DNA generation rate opposite to the one carrying the target sample increased, and the best results were obtained at 12V. At this voltage, parameters such as the DNA yield (190 ng/µL), repeatability (proved to be 94%), and error rate (which has been decreased to 1%) have been reached. These results imply that 12V is the ideal condition to improve the droplet velocity, but at the same time, it minimizes errors, which makes 12V the most appropriate voltage for DNA amplification on DMFBs. The error rate was low and substrate conversion was more efficient at high voltages with the best showing at a 12V voltage speed of 65µm/s, reaction time of 35 minutes, and a substrate conversion of 88%.

This pattern of the droplet manipulation study was also observed in this study. Droplet speed increased with voltage, the maximum value being 90 µm/s at a voltage of 15V while alignment and merging errors were inversely proportional to the voltage. Droplet size was found to increase with increasing voltage which could hamper the efficiency of the system. 12V proved to be the best compromise between droplet size and alignment error and merging error, droplet size 8 nL, alignment error 3%, and merging error 4%.

The influence of droplet speed and the voltage are the most important factors relating to the result that could be identified. Our results deduce that higher droplet speed is beneficial for a faster reaction rate and better efficiency for DNA amplification and enzyme reactions (Dupuy et al., 2005). At voltage above 12V, the high speeds were undesirable because they led to fluctuations in droplet size with an impact on the kinetics of the reaction (Gaudin et al., 2014). The study established the fact that speed and accuracy are critical to assuring the intended biochip performance. The reproducibility and reliability of DMFBs were also tested, with preferred voltage levels showing high concordance for DNA amplified on and enzyme ac on. This finding is critical to building confidence in the DMFBs as dependable devices for biochemical analysis (Lamanna et al., 2020). A comparative analysis of DNA amplification and enzyme-assisted reactions indicated differences in their performance. Despite the improved repeatability and decreased errors in DNA amplification as compared to previous methods, enzyme reactions provided increased efficiency to changes in voltage, especially the conversion of substrate(Jain, 2004). These observations indicate that DMFB performance depends on the assay type and indicates that certain aspects of the operational conditions might need to be fine-tuned for different kinds of assays (Xu & Chakrabarty, 2009).

The findings of the study help to give a practical evaluation of the DMFBs by identifying 12V for these different assays, thus giving an optimal working voltage. The enhancement of droplet speed, high substrate conversion efficiency, and low error rate at this voltage demonstrates the promising ability of DMFBs to accurately execute multiple biochemical assays time and again. Data related to the efficiency of droplet manipulation can enhance the knowledge in the improvement of DMFB technology (Fair, 2009). The decrease in alignment and merging errors discovered with elevated voltages shows that optimal voltage values could improve droplet control, a factor that is crucial to the successful completion of assays in DMFBs. These observations may be used to enhance the design of subsequent DMFB systems that possess increased droplet control capacities. The result also correlateswith the need for assay-specific optimization. 12V was found to be ideal for the two applications of DNA amplification, and enzyme reaction, enzyme reactions also required the assessment of substrate turnover rates and reaction rates. The work presented here indicates that the most effective future research directions lie in creating assay-specific DMFB platforms. From the above analysis, the study proposes the integration of the AVC strategies into DMFB architectures. Such mechanisms would enable a user to dynamically adjust operational parameters so that performance is optimized across numerous assays. Incorporating superior error correction techniques could also improve the reliability and repeatability of DMFBs for high throughput diagnostic and drug discovery applications (Kawakami et al., 2023).

Comparison with Previous Research Industry trends analysis is the method of evaluating the current state and growth path of a particular business sector based on the comparison of the current state of this business sector with the state characterized by maximum and minimum indicators of its activity. The results obtained in this study support the information that has previously been published in scientific sources studying DMFB testing and optimization, underlining the importance of voltage values to predict biochip efficiency. Previous works have revealed the same, with increased droplet velocity and reaction efficiency reported at higher voltage and the error rate coming down (Azizipour et al., 2020). This work advances prior research by comprehensively analyzing the dependence of several performance characteristics on voltage in a variety of assays, DNA synthesis, enzyme activity, and droplet handling. A difference from prior studies is the fact that droplet size was found to increase with higher voltages. Unlike previous research work that has mainly employed speed and error rates, this study explores the possibility of items that could optimize droplet size and efficiency. This result offers a novel view of the DMFB shortcomings, which indicates the importance of fine-tuning appropriate factors for gaining optimal outcomes in terms of connectivity speed, device size, and precision. The second novel contribution of this study is the determination of 12V as the optimal generic voltage for other several assays. In a series of earlier studies, there were attempts to define the ranges of the voltage settings for various applications, and this study reveals that straying from such procedures is beneficial, in that one voltage setting can yield high performance for a rather versatile number of assays. This knowledge is of

immense importance to the design of the DMFBs, implying that if certain voltage values
are set at standard these devices will become easier to use (Choi et al., 2012).

This research has several drawbacks that need to be elaborated on in further investigations. These experiments were performed in laboratory conditions and therefore were questionable whether they encompassed real-world operating environments (Rahimee, I., &Azeemi, M. A., 2020). Possible shorts coming from changes in temperature, contamination, and when working with varying biological samples might affect the overall capability of DMFB, more trials are needed when tested under more conditions. Voltage was the main parameter investigated as an operating condition while other parameters like electrode configuration, coating, and fluid characteristics were examined to a limited extent. Future work should explore the relationship of these factors with voltage to gain a further understanding of DMFB performance. The results are obtained from a limited number of assays such as DNA amplification and enzyme reactions. Despite these biological assays indicating typical usage of the biochip, further studies must be conducted to assess DMFB in other areas of application that are important use, such as cell culture, immunological tests, and synthetic biology.

Conclusion

This work presents the potential of DMFBs in enhancing diagnostic, biotechnological, and pharmaceutical applications through systematic performance enhancement. The results show that the best voltage of 12V increases the droplet speed, decreases the error rate and increases the reproducibility in the assays such as DNA amplification and enzyme reactions. At this voltage, the system was able to produce a droplet speed of 75 µm/s, DNA yield of 190 ng/µL, repeatability of 94% and low alignment and merging errors. These results show that it is crucial to adjust the voltage to achieve optimal performance in terms of different operational characteristics. The study also supports the need for optimization of the assay because biochemical processes have unique needs. For instance, DNA amplification had better speed and fewer errors while enzyme reactions had better substrate conversion rates. The results also show that the use of Adaptive Voltage Control (AVC) and advanced error correction methods can improve the DMFB reliability and achieve high throughput and repeatability in various applications. However, the study has some limitations that are recognized by the authors of the work. The experiments were performed in a controlled environment and the effects of temperature variations, contamination and different types of biological samples have to be tested in the future. Furthermore, since voltage optimization was the main concern, other factors such as electrode arrangement and fluid characteristics should be investigated to gain a more complete picture of the DMFB performance. The study finds that incorporating flexible optimization mechanisms and considering real-world issues can greatly enhance the potential of DMFBs. These developments could make DMFBs a multi-purpose device for diagnostics, drug discovery, and other high-need areas, providing compact, effective, and accurate lab-on-a-chip systems. Further studies should be directed towards the development of the assay-specific platforms and new application areas to enhance the possibilities of this promising approach.

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