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Alginate Microsphere Fabrication Using Bipolar Wave-Based Drop-on-Demand Jetting

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Abstract

Scale-up microsphere fabrication with controllable microsphere size has always been an exciting manufacturing challenge. The objective of this study is to experimentally study the effects of material properties and operating conditions on the formability of alginate microspheres and the microsphere size during drop-on-demand (DOD)-based single nozzle jetting. Alginate microspheres have been fabricated using bipolar wave-based drop-on-demand jetting, and its formability and size have been studied especially as a function of sodium alginate and calcium chloride concentrations, voltage rise/fall times, dwell and echo times, excitation voltage amplitudes, and frequency. It is found that 1) the formability is sensitive to the sodium alginate and calcium chloride concentrations, dwell and echo voltages, and voltage dwell time; and the formability decreases with the sodium alginate concentration but increases with the calcium chloride concentration, dwell and echo voltages, and voltage dwell time; 2) the size is not sensitive to the sodium alginate and calcium chloride concentrations but increases first with the dwell time and then decreases; and 3) the size increases with the dwell and absolute echo voltage amplitudes.

Keywords

Bipolar wave; drop-on-demand jetting; microsphere formability; microsphere size; sodium alginate

1. INTRODUCTION

Microspheres, small spherical droplets with or without second phase particles embedded or encapsulated, are increasingly used in various biomedical applications such as controlled drug delivery [1], cell encapsulation [2], and cellular spheroid-based tissue engineering such as multicellular spheroids (MCS) [3]. Monodisperse microspheres both within and between batches are generally expected for many biomedical applications. For example, for drug delivery applications, the microsphere size distribution critically impacts the allowable routes of administration, biodistribution, and release rate of encapsulated compounds; for MCS research, uniformly sized cellular spheroids are easy to fuse together to be a functional construct. Thus, precise control of the microsphere size and size variation in a scale-up robust fabrication is of great interest in order to guarantee microsphere monodispersity.

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The control of microsphere size can be achieved by better understanding different microsphere fabrication processes such as nozzle-based jetting, which has been long favored in the fabrication of microspheres due to its good size distribution controllability. For its size distribution controllability and scale-up production potential, nozzle jetting is of interest in this study. As the first step towards robust scale-up fabrication of monodisperse microspheres, the effects of material properties and operating conditions on the formability of microspheres and the microsphere size must be elucidated first.

The objective of this study is to systematically study the effects of material properties and operating conditions on the formability of alginate microspheres and the microsphere size during drop-on-demand (DOD)-based single nozzle jetting based on a preliminary study [4]. DOD-based fabrication of alginate microspheres is of interest in this study due to its great potential of size control and commercial scale-up production. This study provides a new understanding of the effects of DOD operating conditions on the microsphere formability, which is of biofabrication interest but still largely not available.

This study is presented as follows. Firstly, the state-of-the-art in alginate microsphere fabrication is reviewed. Then the DOD-based single nozzle jetting process is explained and the experimental setup is introduced. The experimental results regarding the microsphere formability and size are presented and discussed. Finally, some conclusions are summarized and future work is discussed.

2. BACKGROUND

Among different biomaterials used to encapsulate biological materials, alginate hydrogels are often favored due to their resemblance of natural extracellular matrix (ECM) [5] and property as an immunoisolation barrier for cells. Additional advantageous characteristics of alginate microspheres are their surface smoothness and mechanically stable nature. In addition, the high viscosity of alginate solution also brings interesting manufacturing challenges in making alginate microspheres. As cell encapsulation and drug delivery are of great interest in future studies, sodium alginate ($\text{NaC}_6\text{H}_7\text{O}_6$) has been selected as the microsphere matrix material in this study. Calcium chloride (CaCl_2) has been used to help the formation of gel structure as the calcium ions displace the sodium ions to form a three-dimensional, insoluble gel network. While sodium alginate has been selected as a model material in this study, the resulting knowledge is applicable to other microsphere fabrication using other materials.

Alginate microspheres have been fabricated using various approaches such as microfluidic-based fabrication [5,6], mechanical dispersion [7], electrostatic spraying [8], jet cutting [9], spinning disk atomization [10], and ink jetting [11] to name a few. Thus far, all the above approaches are still limited by their scale-up production potential and/or size variation of fabrication microsphere. Due to its good size distribution controllability, the nozzle jetting-based approach (continuous jetting) [1,12] has been implemented to effectively and efficiently fabricate microspheres using materials such as poly(D,L-lactide-co-glycolide) (PLG). However, the fabrication knowledge in making alginate microspheres is still largely missing partially due to the challenge brought by the high viscosity property of sodium alginate solution.

3. DOD-BASED FABRICATION PROCESS

3.1. Nozzle jetting mechanisms

Nozzle jetting can be implemented in either drop-on-demand (DOD) or continuous jetting (also called continuous ink jetting, CIJ) with (or without) external excitation as shown in

Fig. 1. In the DOD mode, droplets are only formed as needed, and the droplet volume is directly proportional to the applied external excitation pressure pulse due to thermal bubble expansion or piezoelectric disk/sleeve excitation. This pressure pulse is further propagated into the fluid reservoir, generating microspheres as demanded. In the CIJ mode, the liquid is continuously forced through an orifice under an external pressure. At the same time, a high-frequency external excitation is always introduced to break the jet into many droplets.

While continuous jetting provides a fast microsphere fabrication method, DOD jetting is recently favored due to its ease in microsphere size control and satellite droplet elimination by optimizing operating conditions such as the excitation pressure pulse. As precise control of microsphere size distribution is still of great manufacturing interest, DOD jetting has been studied herein to explore its application challenges in making alginate microspheres.

3.2. Materials and methods

The DOD experimental setup in this study is shown in Fig. 1(a), and sodium alginate (Sigma-Aldrich, St. Louis, MO) was the microsphere material. The sodium alginate solution was jetted out of the liquid chamber/reservoir through a 120 μm nozzle orifice (MicroFab MJ-ABL-01-120-6MX dispense head, driven by a sleeve piezoactuator) to a receiving container with calcium chloride dihydrate (Sigma-Aldrich, St. Louis, MO). The DOD pulse was controlled using a MicroFab Jet Driver, and different pulse waveforms are discussed in the following sections. A pneumatic controller was used first to adjust the backpressure of the liquid chamber/reservoir by decreasing at an interval of 0.2 kPa to obtain an ideal meniscus for good droplet formation [13]. Once an ideal meniscus was formed, the DOD fabrication process started. During the experiments, the backpressure was controlled ranging from -3.0 to -1.2 kPa to guarantee a good droplet formation condition. There was an air gap between the orifice and the liquid surface of the receiving container. All the alginate microspheres were printed into a 3 ml calcium chloride solution-filled well of a 24-well plate. The receiving container also had a magnetic stir rod (200 rpm) to avoid any possible microsphere agglomeration. The fabricated microspheres were classified into three categories: 1 if no microsphere is formed, 2 for deformed microsphere formation, and 3 for good microsphere formation. The microsphere size was determined by averaging the diameters of 20 good microspheres, if have, using ImageJ.

3.3. Excitation waveforms in DOD

The formed microsphere size in DOD can be controlled by changing the orifice diameter and/or operating conditions for given materials. For most application scenarios, the orifice diameter cannot be changed easily while the operating conditions, especially the excitation forms, are usually optimized to generate microspheres as designed.

The external excitation in this study was applied as voltages, which were converted into pressure pulses through the piezoelectric device. A simple function can be used to capture the relationship between the driving voltage and the resulting pressure [14]:

$$p_w(t) = k \frac{dV(t)}{dt} \quad (1)$$

where $p_w(t)$ is the resulting pressure, t is the time, $V(t)$ is the applied voltage, and k is a constant coefficient relating the resulting pressure to the applied voltage. The applied voltage resulted in a pressure wave to help form a sodium alginate droplet, which further gelled into a microsphere. Generally, each excitation period is responsible for printing one droplet in the setup of this study [15].

Fig. 2 shows a basic voltage waveform and its corresponding pressure pulse used in this DOD study. The piezoelectric device can be simply driven by a single positive voltage wave (“on-off” pulse), and the piezoelectric sleeve displaces first then returns to a rest state. However, it is difficult to control satellite formation using this simple waveform [16]. Fortunately, some complicated waves have also been studied and applied to the piezoelectric device such as bipolar (with a consecutively positive/negative as shown in Fig. 3 or negative/positive excitation pulse) and tripolar (with a consecutively negative/positive/negative or positive/negative/positive excitation pulse).

As the first step, this study aims to investigate the performance of bipolar waveforms in controlling the formability of microspheres. A bipolar waveform consists of a succession of two square-wave pulses (consecutively positive/negative or negative/positive) followed by a relatively long idle duration. The second wave part is used to cancel some of residual acoustic oscillations that remain in the DOD jetting device after drop ejection [17]. As shown in Fig. 3, t_{p1} , t_{p2} , and t_{p3} are the voltage rise or fall times, which also are the pressure pulse durations as seen from Fig. 3(b), t_1 is the dwell time, t_2 is the echo time, V_1 and V_2 are the excitation voltages, t_{idle1} and t_{idle2} are the idle durations when the voltage dwells at zero, and p_1 , p_2 , and p_3 are the pressure magnitudes and can be determined using Eq. 1 for given k .

While Fig. 3 shows a typical positive/negative bipolar waveform, a negative/positive bipolar waveform has also been used to reduce the drop size [15,16]. As the formability is of main interest, the study has only investigated the effect of the positive/negative bipolar waveform. The resulting knowledge is also applicable to the application with a negative/positive bipolar waveform, which may have a smaller drop size [13].

4. EXPERIMENTAL RESULTS AND DISCUSSION

4.1. Experimental design

As the positive/negative bipolar waveform formability is of interest herein, ten variables have been identified to investigate the alginate microsphere formability. There are two material properties: concentrations of sodium alginate and calcium chloride, respectively, and operating conditions (per Fig. 3(a)): voltage rise/fall/final rise times (t_{p1} , t_{p2} , and t_{p3}), idle durations (t_{idle1} and t_{idle2}), dwell time t_1 , echo time t_2 , and the excitation dwell and echo voltage amplitudes (V_1 and V_2). Usually, t_{idle1} is set as zero, then t_{idle2} can be indirectly determined for a given frequency (f). As a result, there were ten control variables in this study: sodium alginate concentration, calcium chloride concentration, t_{p1} , t_{p2} , t_{p3} , t_1 , t_2 , V_1 , V_2 , and f .

A three-level ten-variable experimental design was formulated and implemented as shown in Table 1. Rather than doing a full factorial experimental design, an efficient and robust design of experiments (DOE) methodology provides a simple and systematic approach to improve performance, quality and cost. In the design, three levels of sodium alginate concentration were taken as 1%, 1.5%, and 2% (w/v) to avoid possible nozzle clogging. The calcium chloride concentrations were 1%, 1.5%, and 2% (w/v) as in other similar studies [18–21]. The levels for waveform variables were: 3, 4 and 5 μ s for the voltage rise/fall/final rise times, 10, 20 and 30 μ s for the dwell and echo times, 100, 500 and 1,000 Hz for the frequency, and 50, 60, 70, and 80 V as the dwell and/or echo voltage amplitudes as frequently adopted in similar studies [14, 22–24].

Herein spherical droplets are considered good for a spherical microsphere by visual inspection under a microscope, deformed for an irregular shaped microsphere by visual inspection under a microscope, or unsuccessful if microspheres did not form under the given

parameters. Ordinal logistic regression [25] is ideal for studying logic type statements such as the formability statement in this study, so it was applied to analyze the formability results. Based on the logistic regression conclusion, a systematic full factorial study was further performed to study the microsphere size as a function of the significant parameters by fixing other conditions.

4.2. Experimental results

The p -value from the ordinal logistic regression study indicates the statistical significance of the result, and a low p -value for the formability probability under a specific variable means that the analysis result regarding this variable is statistically significant. The probability ratio

$\left(\frac{p_i}{1 - p_i}\right)$ is also called the odds and the logarithm of this odds is called the logit. This logit can also be calculated as follows:

$$\ln\left(\frac{p_i}{1 - p_i}\right) = \alpha_i - \beta x \quad (2)$$

where p_i is the probability to form a microsphere, α_i is the intercept, β is the slope, and x is the variable of interest. The importance of any variable on the microsphere formability is measured by β . A larger β means that the formability is sensitive to this variable. If β is zero or very close to zero for a variable, it means that this variable has very little or even no effect on the formability. The β value depends on the units of the parameter. In order to compare parameters, the normalized β coefficient will be studied (β_{norm}), which is nondimensional. This can be done by dividing β by the standard error of β . Table 2 shows the 95% confidence interval-based ordinal logistic regression results using Minitab 15.

It is found from Table 2 that all of the estimated p -values are close to or less than 5% except for t_{p1} (13.7%), t_{p2} (70.6%), t_{p3} (24.5%), t_2 (36.2%), and f (69.8%), meaning that the conclusions regarding these low p -value variables are statistically significant and can be accepted. The sodium alginate concentration has the most significant effect on the microsphere formability with a β_{norm} value of 5.928. Since this β value is positive, it means that the probability of formation decreases with the sodium alginate concentration.

The dwell time has a very significant effect on the formability of microspheres with a β_{norm} value of -3.630 . As the dwell time increases, the probability of microsphere formation also increases. Although the effect is about half as much as the aforementioned parameters, the dwell voltage also has a significant effect on microsphere formability with a β_{norm} value of -2.330 indicating as V_I increases the probability of microsphere formation increases. Calcium chloride concentration has an effect on formability based on a β_{norm} value of -2.313 indicating that increasing the concentration will increase the probability of microsphere formability. The echo voltage amplitude is found to have some effect on the formability based on β_{norm} values of 1.715. As the echo voltage amplitude increases the probability of formability decreases. As the p -values for the rise, fall, final rise and echo times and the excitation frequency are all well above 5%, no conclusive statement can be drawn regarding the dependence of the formability on these variables based on this study. It should be noted that only the main effects have been investigated here and the possible interaction effects will be further investigated in a future study. The following sections are devoted to discussing the formability and size as functions of significant material properties and operation conditions as identified. If not specified, the size dependence study was conducted by fixing other conditions as in Case 43. In the following diameter plots (Figs. 6, 8 and 11), the error bars represent the standard deviation ($\pm$ one sigma) of diameter. For each formability study, some good and deformed are also illustrated for better visualization.

4.2.1 Effect of sodium alginate and calcium chloride concentrations—The effects of the sodium alginate concentration on droplet formation have long been of interest and it is found that there is a concentration range for good droplet formation [8,19]. As found from Fig. 4, as the sodium alginate concentration increases, the likelihood of microsphere formation decreases. This is attributed to the significantly increased viscosity of high concentration sodium alginate solutions: the viscosity of 2% sodium alginate solution may be around 10 times higher than that of 1% counterpart (190 mPa·s vs. 1800 mPa·s) [26]. Generally, a low sodium alginate concentration such as 1 to 1.5% is recommended for nozzle-based jetting applications. However, it should be pointed out that if the sodium alginate concentration is much lower than 1%, gelation between the sodium alginate droplet and the calcium chloride solution may not be well executed and no microsphere can be formed.

Fig. 5 shows the effect of calcium chloride concentration on the microsphere formability. It can be seen that a relatively high calcium chloride concentration facilitates the alginate microsphere formation; however, there is a good probability with no microsphere formed at a high concentration such as 2%. Overall, 1.5% is recommended as a good concentration value for effective microsphere fabrication.

To better appreciate the effect of sodium alginate concentration on the microsphere size, sodium alginate solutions with different concentrations (0.75%, 1%, 1.25%, 1.5%, and 1.75%) was further used to fabricate microspheres. Since the 2% sodium alginate concentration was in general unsuccessful in making microspheres, it was not used for the microsphere size testing. Fig. 6 shows that sodium alginate concentration does not have a significant effect on microsphere size, which agrees well with another study done by Zhang et al. [8]. The microsphere size as a function of the calcium chloride concentration is also shown in Fig. 6, which shows that the calcium chloride does not have much effect on the microsphere size. Although microsphere stability depends on the calcium chloride concentration, the microsphere size is generally unaltered with the calcium chloride concentration [27,28].

4.2.2 Effect of dwell time—Fig. 7 shows the effect of the dwell time on the microsphere formability. It can be seen that a relatively long dwell time facilitates the alginate microsphere formation; however, there is still a good probability to form good microspheres using a short dwell time.

The microsphere size as a function of the dwell time is shown in Fig. 8, which demonstrates that the dwell time does not have a simple monotonic relationship with the size but has a maximum droplet diameter around 25 μ s. The size increases first with the dwell time and then decreases. This observation agrees well with the result by Gan et al. [29] who also found that the dwell time has a non-monotonic relationship with the droplet size.

4.2.3 Effect of voltage amplitudes—Fig. 9 shows the effect of dwell voltage on the alginate microsphere formability. It can be seen that a high dwell voltage better facilitates the alginate microsphere formation than for a low dwell voltage. Fig. 10 shows the effect of echo voltage (in terms of absolute value) on the alginate microsphere formability. It can be seen that a high echo voltage facilitates microsphere formation. Although small echo voltages can still form microspheres there is a good probability that deformed even no microsphere can be formed.

The dependence of microsphere size as a function of the dwell and echo voltages is shown in Fig. 11. It is found that the size increases with the dwell voltage as found by Reis et al. [30] who have used a simple voltage square wave pulse. Some oscillation may be observed

as seen in Fig. 11 and in a study by Gan et al. [29]. It can be seen that with the extreme dwell voltage amplitudes more error is introduced due to some polydispersity. Although well shaped microspheres could be formed under all dwell voltage amplitudes investigated, it is recommended that values greater than 45 V and less than 75 V be used for monodispersity. It is also found that the size increases with the echo voltage (absolute value). The echo voltage is usually implemented in order to suppress satellite droplets. The suction introduced by the echo voltage actually helps to suppress the satellite droplet formation progress [29].

4.2.4 Importance of dwell time on droplet formation—As seen from the previous sections, the microsphere formability is a function of the excitation waveform parameters for given materials. The voltage amplitude and rise/fall time (t_{pi}) all directly affect the resulting pressure amplitude as seen from Eqn. 1. This equation can be further represented in a discretized form during the rise and fall periods as follows:

$$p_w(t) = k \frac{\Delta V}{t_{pi}} \quad (3)$$

where ΔV is the voltage change during the rise/fall periods, and k is a coefficient dependent on the material properties and nozzle geometry. As so, p_w is proportional to the ratio of the voltage change over its rise/fall duration. Any voltage change leads to a pressure wave, so three pressure waves are generated for a typical given bipolar waveform, one for each voltage slope. The pressure amplitude determines the amount of deformation of the piezoactuator, which in turns determines the volume of fluid displaced and therefore the microsphere volume. The dwell time, defining the duration between each pressure variation, has been extensively studied [31–33] and used to control two pressure wave phenomena: 1) damping oscillation and 2) reflecting wave.

Damping oscillation follows the resulting pressure peak and is a function of the nozzle geometry and material properties. Each oscillation is a damped sinusoidal wave until it finally dies out. As the voltage rises or falls, the sleeve piezoactuator either radially expands or retracts depending on its polarity, resulting in a volume change of chamber. This volume change generates a pressure wave inside the nozzle that travels through the fluid towards both the upstream reservoir and the orifice. Considering the second part (negative excitation) of the bipolar waveform as shown in Fig. 12, there is a negative pressure pulse (corresponding to the negative slope), followed by a positive pressure pulse (corresponding to the positive slope). There is a time lapse between the two pulses, which is the dwell time. This dwell time must be chosen carefully in order to match the damping oscillating pressure waves from the negative and positive pressure pulse respectively, making them in phase. Fig. 12(b) illustrates an ideal scenario where the pressure waves are in phase. This can be achieved by carefully selecting the dwell time to start the positive pulse (dashed line) when the negative pulse wave (solid line) oscillates to a positive peak [32], making an enhanced positive pressure wave. Such an enhanced pressure wave better facilitates the droplet formation process at the orifice.

On the other hand, reflecting wave also play an important role during the droplet formation process. As the piezoactuator expands or retracts, it causes a negative or positive pressure wave that travels both towards the nozzle orifice and the reservoir until it hits a boundary. Since the orifice diameter is usually much smaller than that of the chamber diameter, it can be approximated as a closed end. The wave reflects and travels towards the reservoir without any phase change (still negative or positive). However, the reservoir instantly opens into a relatively large area and is usually considered an open end boundary condition. When a wave hits the open end, it is reflected with a changed phase (to be positive or negative) [31].

In order to align these reflected waves instead of canceling out with each other, the dwell time must be properly selected based on the wave travel distance and the wave speed inside the chamber medium. The resulting reflected waves of each pressure wave should be in phase, helping the formation of good droplets. This mechanism can also be explained using Fig. 12 while considering each line (solid or dash) of Fig. 12(b) as an in-phase pressure profile, which is due to two reflected waves from each original pressure wave. As so, the reflected waves and the wave oscillations can be all aligned to enhance pressure amplitude peaks and help form droplets by choosing an optimal dwell time.

5. CONCLUSIONS

Alginate microspheres have been fabricated using bipolar wave-based DOD jetting, and its formability and size has been studied especially as a function of sodium alginate and calcium chloride concentrations, voltage rise/fall times, dwell and echo times, excitation voltage amplitudes and frequency. Main conclusions are drawn as follows:

1. The formability is very sensitive to the sodium alginate concentration and the voltage dwell time; and the formability decreases with the sodium alginate concentration but increases with the voltage dwell time;
2. The formability is moderately sensitive to and increases with the calcium chloride concentration, the dwell voltage, and the echo voltage; and the ideal calcium chloride concentration is found to be 1.5%;
3. The size is not sensitive to the sodium alginate and calcium chloride concentrations under the investigated ranges;
4. The size increases first with the dwell time and then decreases; and
5. The size increases with the dwell and echo (absolute value) voltage amplitudes.

While there are some conclusions regarding the effects of sodium alginate and calcium chloride concentrations, dwell time, and dwell and echo voltages, there are no conclusions regarding the effects of the rise, fall, final rise and echo times and the excitation frequency based on this study and more experimental and theoretical studies [34] are expected to elucidate these effects. In addition, future work shall use a scale analysis or computational approach to understand the physical principle governing droplet characteristics as a function of material properties and operating conditions.

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Biographies

Leigh Herran is a doctoral student in the department of mechanical engineering and researches the formation of droplets, jet instability, viscoelastic fluid printability and manufacturing processes ideal for microsphere fabrication. She graduated with her B.S.M.E. in 2008 from Clemson University, South Carolina and continued her graduate study at Clemson University. Her study, in the area of cell printing, is currently supported by the National Science Foundation Graduate Research Fellowship Program.

Yong Huang received his B.S. degree in Mechatronics Engineering from Xidian University, China in 1993, his M.S. degrees in Mechanical Engineering from Zhejiang University, China and the University of Alabama, in 1996 and 1999 respectively, and his M.S. in Electrical and Computer Engineering and Ph.D. in Mechanical Engineering from the Georgia Institute of Technology, Georgia in 2002. As professor of Mechanical Engineering at Clemson University, South Carolina since 2003, his research addresses various challenges in advanced manufacturing. His current research interests are advanced tissue fabrication, precision engineering, and manufacturing process monitoring. He is the recipient of the 2005 ASME Blackall Machine Tool and Gage Award, the 2006 SME Outstanding Young Manufacturing Engineer Award, the 2008 NSF CAREER Award, and the 2008 International Symposium on Flexible Automation Outstanding Young Investigator Award.

Figure 1.
Schematic of nozzle jetting: (a) DOD and (b) CIJ jetting

Figure 2.

(a) Basic voltage excitation waveform and (b) its corresponding pressure pulse

(a)

(b)

Figure 3.

(a) Bipolar voltage excitation waveform and (b) its corresponding pressure pulse

Figure 4.

Effect of sodium alginate concentration (Good microspheres using a 1.5% sodium alginate concentration (Case 43) and deformed microspheres with long tailed structure using a 2% sodium alginate concentration (Case 21) for the insets)

Figure 5. Effect of calcium chloride concentration (Good microspheres using 1.5% CaCl_2 concentration (Case 11) and deformed microspheres with short tailed structure using 1% CaCl_2 concentration (Case 21) for the insets)

Figure 6. Microsphere diameter as a function of sodium alginate concentration and calcium chloride concentration

Figure 7.

Effect of dwell time (Good microspheres using 30 μs dwell time (Case 43) and deformed microspheres using 10 μs dwell time (Case 12) for the insets)

Figure 8.
Microsphere diameter as a function of dwell time

Figure 9. Effect of dwell voltage (Good microspheres using 70 V dwell voltage (Case 46) and deformed microspheres using 50 V dwell voltage (Case 17) for the insets)

Figure 10.

Effect of echo voltage (Good microspheres using -70 V echo voltage (Case 43) and deformed microspheres using -50 V echo voltage (Case 23) for the insets)

Figure 11. Microsphere diameter as a function of dwell voltage and echo voltage (echo voltage was negative but shown in its absolute value here)

Figure 12.

(a) Excitation voltage waveform and the resulting pressure pulse with an optimal dwell time, (b) the resulting pressure waves near the orifice (the solid line corresponding to the negative pressure pulse and the dashed line corresponding to the positive pressure pulse), and (c) the resulting composite pressure wave if the pressure waves inside the nozzle chamber are in phase

Table 1

Experimental design and results based on bipolar waveforms

Case index	Concentration		Waveform information								Formability 1 = unsuccessful 2 = deformed 3 = good
	Sodium alginate	Calcium chloride	t_{p1} (μ s)	t_{p2} (μ s)	t_{p3} (μ s)	t_1 (μ s)	V_1 (V)	t_2 (μ s)	V_2 (V)	f (Hz)	
1	1.0	1.0	3	4	4	20	60	20	-60	500	3
2	1.0	1.0	3	5	5	30	70	30	-70	1000	3
3	1.0	1.5	4	4	4	20	70	30	-70	100	2
4	1.0	1.5	4	5	5	30	50	10	-50	500	3
5	1.0	2.0	5	3	3	10	70	30	-70	500	3
6	1.0	2.0	5	4	4	20	50	10	-50	1000	2
7	1.0	2.0	5	5	5	30	60	20	-50	100	3
8	1.5	1.0	5	3	4	30	50	20	-70	100	3
9	1.5	1.0	5	4	5	10	60	30	-50	500	2
10	1.5	1.0	5	5	3	20	70	10	-60	1000	3
11	1.5	1.5	3	3	4	30	60	30	-50	1000	3
12	1.5	1.5	3	4	5	10	70	10	-60	100	2
13	1.5	1.5	3	5	3	20	50	20	-70	500	3
14	1.5	2.0	4	3	4	30	70	10	-60	500	3
15	1.5	2.0	4	4	5	10	50	20	-70	1000	3
16	1.5	2.0	4	5	3	20	60	30	-50	100	3
17	2.0	1.0	4	3	5	20	50	30	-60	100	2
18	2.0	1.0	4	4	3	30	60	10	-70	500	1
19	2.0	1.0	4	5	4	10	70	20	-50	1000	1
20	2.0	1.0	5	3	5	20	60	10	-70	1000	1
21	2.0	1.0	5	4	3	30	70	20	-50	100	2
22	2.0	1.0	5	5	4	10	50	30	-60	500	1
23	2.0	2.0	3	3	5	20	70	20	-50	500	3
24	2.0	2.0	3	4	3	30	50	30	-60	1000	1
25	2.0	2.0	3	5	4	10	60	10	-70	100	1

Case index	Concentration		Waveform information							Formability
	Sodium alginate	Calcium chloride	t_{p1} (μ s)	t_{p2} (μ s)	t_{p3} (μ s)	t_1 (μ s)	V_1 (V)	t_2 (μ s)	V_2 (V)	
26	1.5	1.5	3	5	5	30	70	30	-70	100
27	1.5	1.5	4	5	5	30	70	30	-70	100
28	1.5	1.5	5	5	5	30	70	30	-70	100
29	1.5	1.5	3	5	5	30	70	10	-70	100
30	1.5	1.5	3	5	5	30	70	20	-70	100
31	1.5	1.5	3	5	5	30	70	30	-70	100
32	1.5	1.5	3	5	5	30	70	30	-50	100
33	1.5	1.5	3	5	5	30	70	30	-60	100
34	1.5	1.5	3	5	5	30	70	30	-70	100
35	1.5	1.5	3	5	5	30	50	30	-70	100
36	1.5	1.5	3	5	5	30	60	30	-70	100
37	1.5	1.5	3	5	5	30	80	30	-70	100
38	1.5	1.0	3	5	5	30	70	30	-70	100
39	1.5	1.5	3	5	5	30	70	30	-70	100
40	1.5	2.0	3	5	5	30	70	30	-70	100
41	1.5	1.5	3	3	5	30	70	30	-70	100
42	1.5	1.5	3	4	5	30	70	30	-70	100
43	1.5	1.5	3	6	5	30	70	30	-70	100
44	1.5	1.0	4	4	3	30	60	10	-70	500
45	1.5	1.0	4	5	4	10	70	20	-50	1000
46	1.5	1.0	5	3	5	20	60	10	-70	1000
47	1.5	1.0	5	5	4	10	50	30	-60	500
48	1.5	2.0	3	4	3	30	50	30	-60	1000

1 = unsuccessful
2 = deformed
3 = good

Table 2

Ordinal logistic regression results

Variables	β	β_{norm}	p
Sodium alginate concentration	5.051	5.928	0
Calcium chloride concentration	-1.078	-2.313	0.024
t_{p1}	0.291	1.477	0.137
t_{p2}	-0.079	-0.386	0.706
t_{p3}	-0.250	-1.204	0.245
t_1	-0.077	-3.630	0
V_1	-0.046	-2.330	0.018
t_2	-0.019	-0.940	0.362
V_2	0.034	1.715	0.084
f	0.000	0.404	0.698