Draft Internal Memorandum

To: Linda Black and Will Clodfelter

From: Yeon Choi and Ramesh Rathi

Subject: Recommendations for Optimal Mixing of SutureSeal (SSP)

Date: 14 April, 2017

Introduction

Medicus has developed, tested and patented a synthetic biopolymer ("Suture Seal") that can be used as a "liquid bandage" to control infections in animal wounds that have been sutured or are general open wounds. The SutureSeal™ components are stored in two syringes, a male and a female syringe. The female syringe contains a mixture of pre-polymers. The male syringe contains a aqueous buffer solution. The two syringes are connected and the contents mixed to produce a liquid polymer. The liquid polymer is then applied over the wound using a brush.

The currently recommended mixing directions (both PI and online video) for SutureSeal recommend "slowly" mixing for 10-15 times or a minimum of 10-15 seconds; Medicus has received several user reports that when users follow the instructions (video or PI) they do not achieve consistent gelling (consistency or time). Medicus has generated data in the past showing that ambient temperature does have an effect on gel time, however, when controlled for temperature there is a hypothesis that the "speed" of mixing (strokes/sec) and time of mixing (s) could affect the gel time. The objective of this study was to evaluate the effect of various mixing parameters and to make recommendations for optimal mixing conditions that ensure consistent product performance.

Materials and Methods

Table 1. SutureSeal lots studied

LOT #:	SSP160817	SSP161220
Description:	SutureSeal (blend fill process)	SutureSeal (blend fill process)
Purpose:	Test lot	Reference lot
Quantity:	75 kits	5 kits

Table 2. Mixing parameters investigated

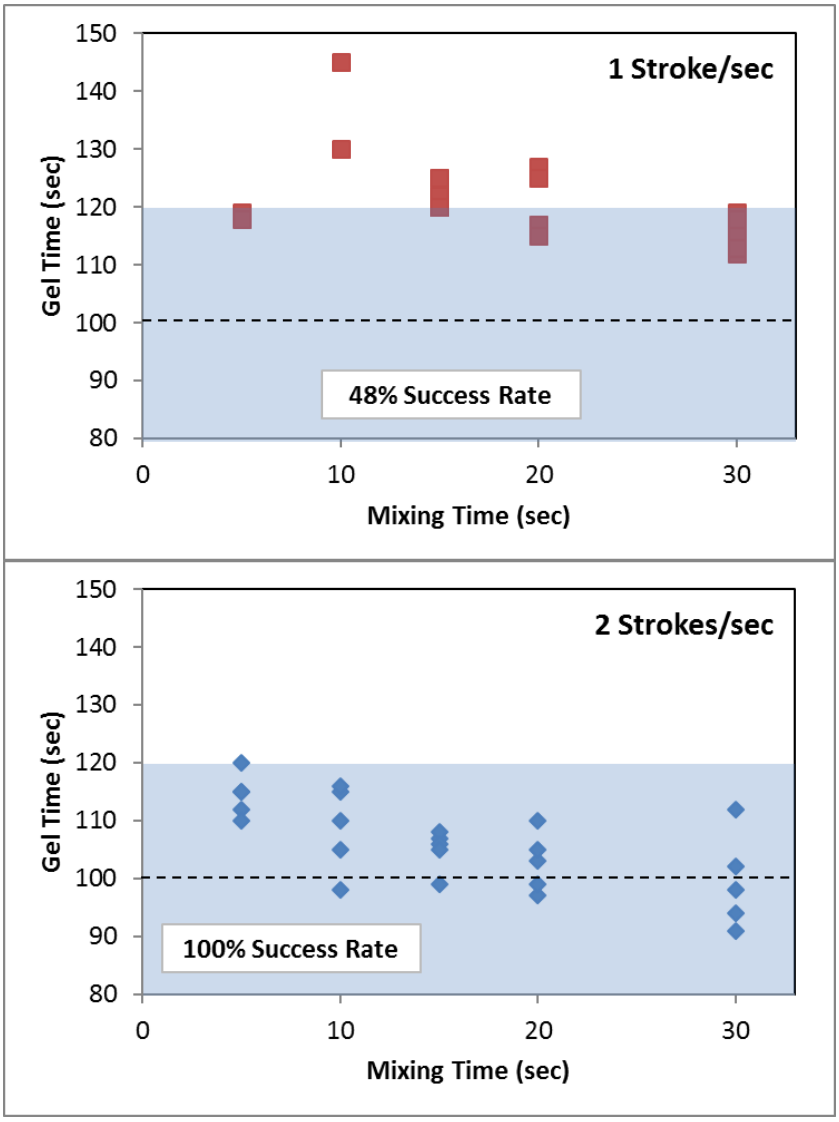
Mixing Speed (Strokes/sec)	**Mixing Time (sec)**				
1	5	10	15	20	30
2					
3					

Test kits were pulled from a SutureSeal lot in commercial circulation (Table 1). Five different mixing times were evaluated at three different mixing speeds (Table 2) and 5 samples (n = 5)

were tested under each variable condition. Reference kits were pulled from a different SutureSeal lot in commercial circulation (Table 1) and mixed under current mixing conditions used by three different lab personnel. Gel times were measured and the appearance of the mixed polymer solution was documented through photos along with written records (degradation time measurements are in progress). The photos were correlated with the gel time data and the optimal mixing parameters were determined by calculating the success rate, which was defined as the percent of samples that had gel times within the product specification of 80 to 120 seconds.

Figures

Figure 1. Scatter plots of gel times vs. mixing times for 1, 2 and 3 strokes/sec. The blue shaded region denotes the gel time specification for SutureSeal. The dashed line denotes the average gel time for the reference lot under current mixing conditions used by lab personnel.



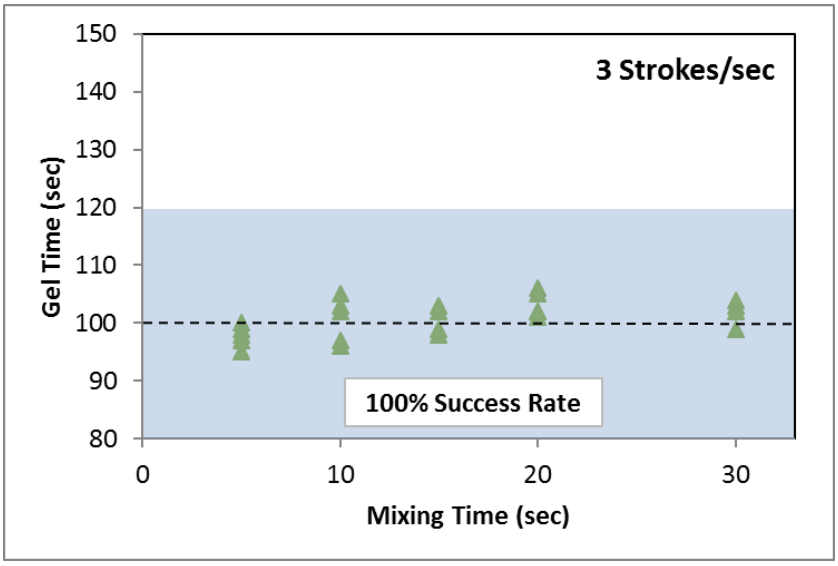
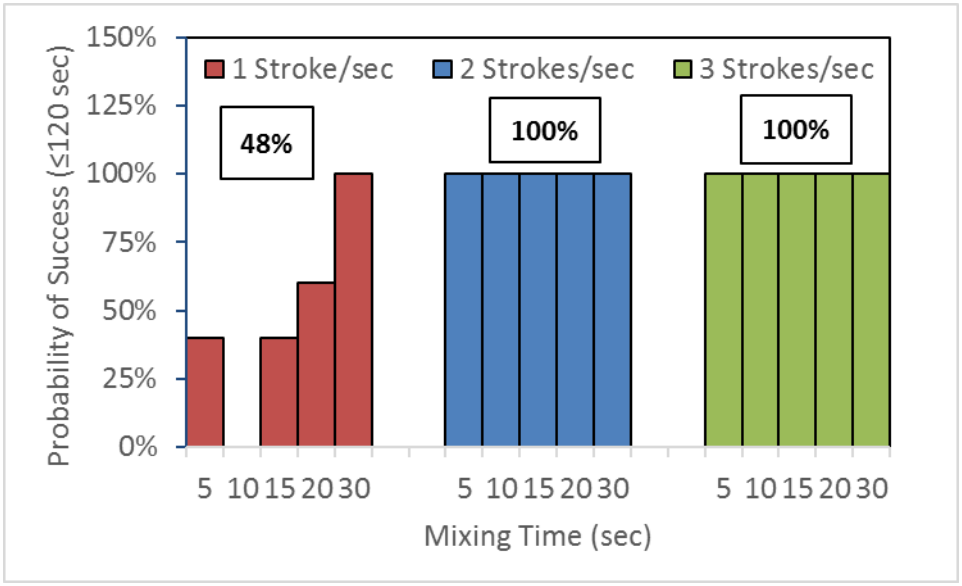
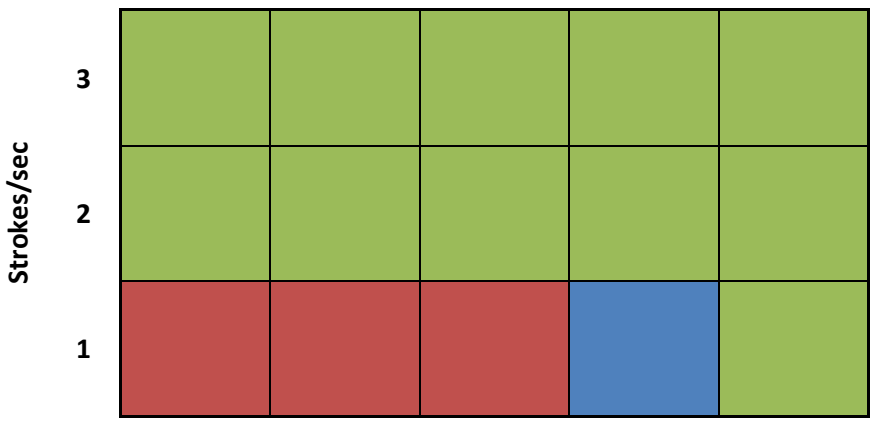


Figure 2. Probability of success at each mixing time for 1, 2 and 3 strokes/sec



*the percent in the box denotes the probability of success in each group

Figure 3. Matrix representation of success rate at or below 120 seconds



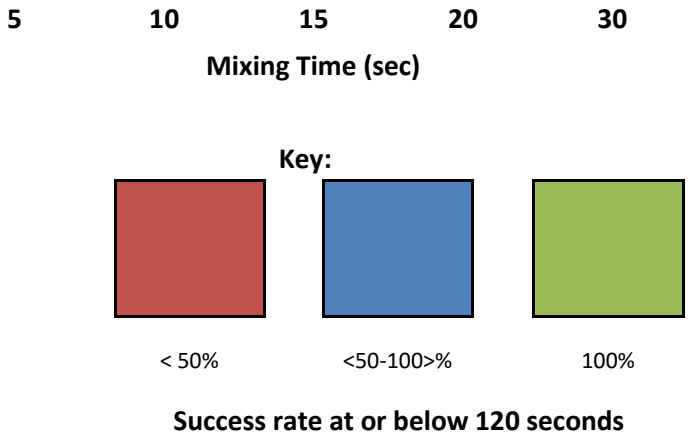
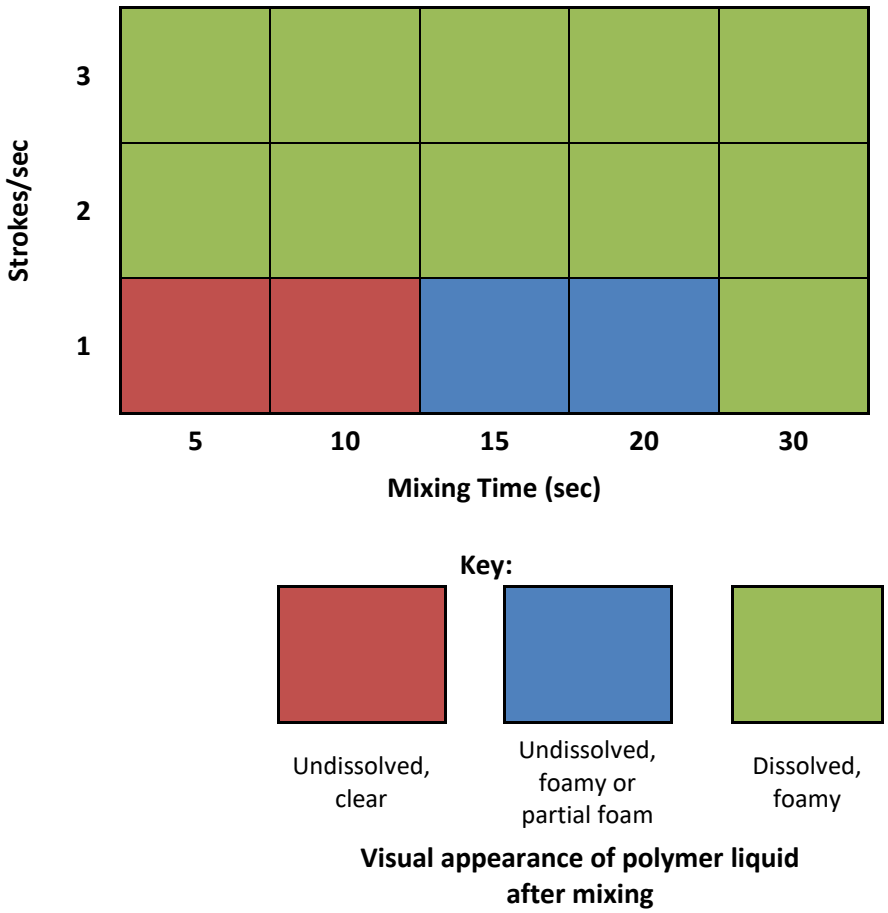


Figure 4. Matrix representation of polymer liquid appearance after mixing



Photos

Photo 1. Representative photo of polymer liquid appearance after mixing and the resultant gel for the appearance description: Undissolved, clear

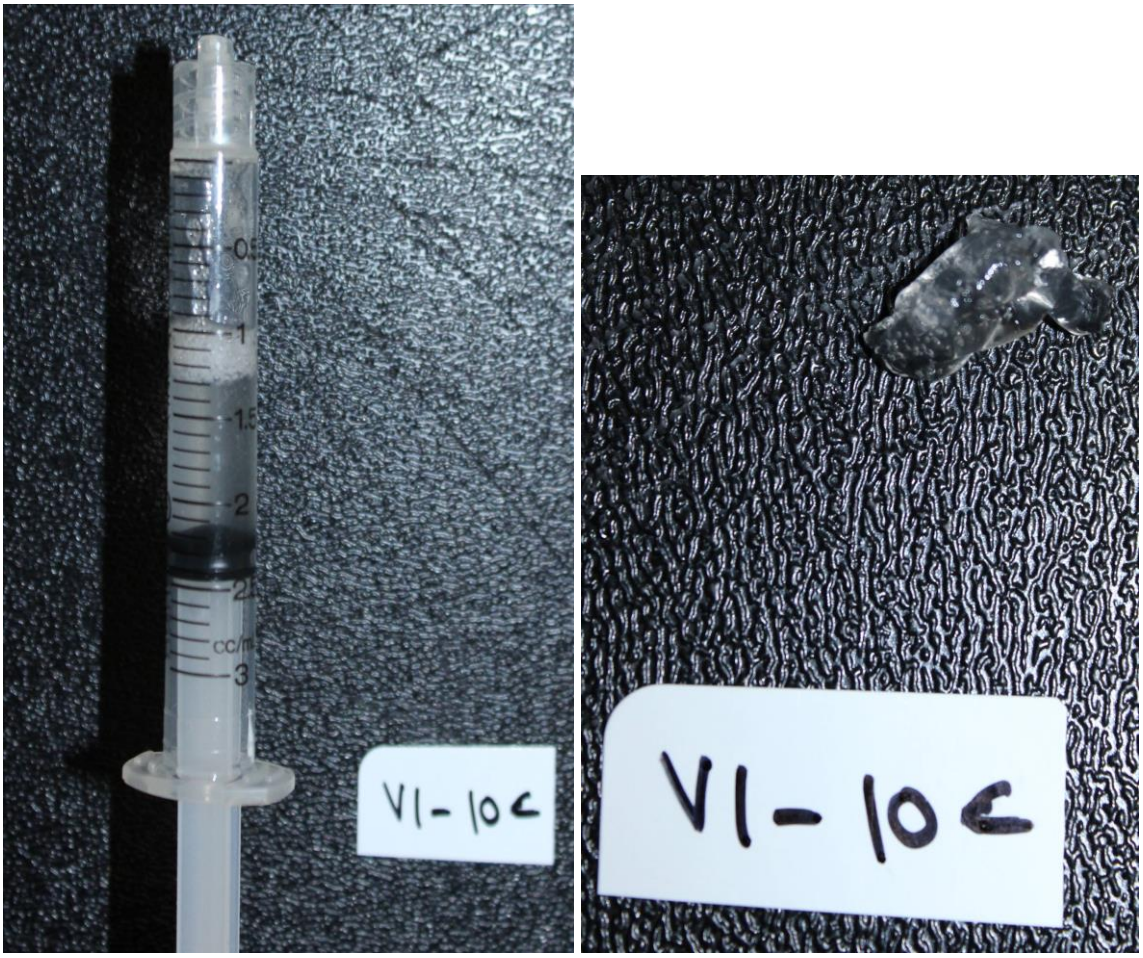


Photo 2. Representative photo of polymer liquid appearance after mixing and the resultant gel for the appearance description: Undissolved, foamy or partial foam.

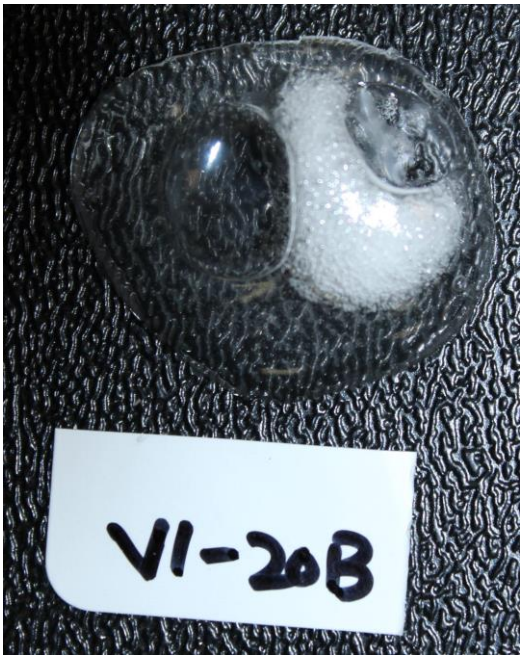
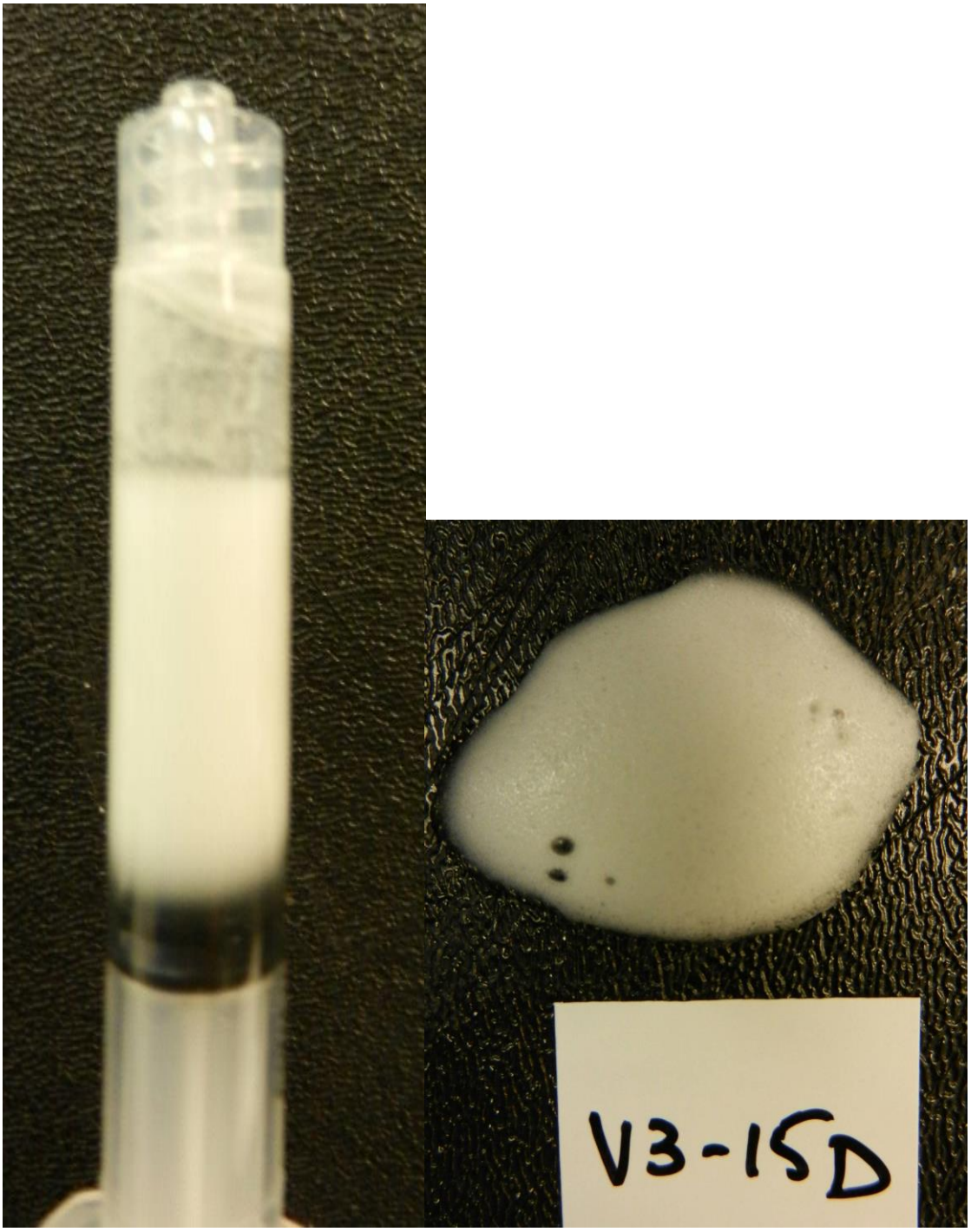


Photo 3. Representative photo of polymer liquid appearance after mixing and the resultant gel for the appearance description: Dissolved, foamy.



Results and Discussions

Gel Time Measurements

The gel time data for mixing speeds of 1, 2 and 3 strokes per second are shown as a function of mixing time in Figure 1. The success rates at various mixing times are depicted in Figure 2 for mixing speeds of 1, 2 and 3 strokes per second. Figure 3 summarizes the success rate at or below 120 seconds as a matrix representation.

At 1 stroke per second, the overall success rate was calculated to be 48%; thus, roughly half of the test samples exhibited gel times greater than 120 seconds. The majority of failures at 1 stroke per second occurred at mixing times of 15 seconds or less, clearly indicating inadequate mixing conditions. When the mixing time at 1 stroke per second was increased to 20 seconds, the success rate slightly improved from below 50% to a value of 60%; a higher mixing time of 30 seconds further improved the success rate to 100%. Thus, at 1 stroke per second a strong mixing time effect was observed with increasing mixing times yielding higher success rates.

At 2 strokes per second, the overall success rate was calculated to be 100%. However, at 5 and 10 seconds of mixing, the observed gel times were slightly higher than the gel times of the remaining samples in the group, indicating a less than adequate level of mixing. Thus, mixing times of less than 15 seconds at 2 strokes per second may present a threshold of acceptability.

At 3 strokes per second, the overall success rate was calculated to be 100%. Mixing times had a negligible effect on gel times and polymer gels were formed consistently under all mixing conditions.

Visual Observations

The visual appearance of the polymer liquid after mixing was described in three distinct categories: 1) undissolved, clear; 2) undissolved, foamy or partial foam; and 3) dissolved, foamy. Representative photos depicting each category and the resultant gels are found in Photos 1, 2 and 3. Figure 4 presents a matrix representation of the visual appearance after mixing.

Photo 1 exhibits an example of an undissolved, clear appearance after mixing; a significant amount of undissolved solid polymer was observed in the spent syringe after mixing and disconnecting the two mixing syringes. The resultant gels typically had grossly failing gel times and were qualitatively weaker and relatively smaller than the gels formed in the groups with 2 and 3 strokes per second. Undissolved, clear appearances were only observed at 1 stroke per second and mixing times of 5 and 10 seconds and corresponded to success rates of less than 50%.

Photo 2 exhibits an example of an undissolved, foamy or partial foam appearance after mixing; a small amount of undissolved solid polymer was observed in the spent syringe after mixing and disconnecting the two mixing syringes. The resultant gels were qualitatively similar in strength to the normal gels, but displayed a blotchy clear/foamy appearance. Undissolved, foamy or partial foam appearances were typically observed at 1 stroke per second and mixing times of 15 and 20 seconds and corresponded to success rates of less than 100%.

Photo 3 exhibits an example of a dissolved, foamy appearance after mixing. The resultant gels were similar to normal gels and displayed a mostly white appearance. Stroke rates of 2 and 3 strokes per second produced dissolved, foamy appearances after mixing at all mixing times studied. At 1 stroke per second, a mixing time of 30 seconds also produced dissolved, foamy appearances after mixing. Dissolved, foamy appearances after mixing typically corresponded to 100% success rates; however, it is possible that in some cases a foamy appearance may mask the appearance of undissolved solids.

Conclusions and Recommendations

Based on the gel time data, a reasonable recommendation for the mixing conditions to achieve a 100% success rate and consistent product performance are: a stroke rate of 2 to 3 strokes per second at a mixing time of 15 to 20 seconds. Although a mixing time of 30 seconds was also successful, it may prove to be too impractical or inconvenient for the end user. Furthermore, an undissolved, clear or partial foam appearance was found to be a reliable indicator of less than 100% success rates.

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and

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