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Effect of Chemical Composition on the Response of Zwitterionic Glucose Sensitive Hydrogels Studied by Design of Experiments

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ABSTRACT: A stimuli-responsive hydrogel that contains the anionic monomer 3-acrylamidophenylboronic acid and the cationic monomer *N*-[3-(dimethylamino)propyl]acrylamide binds with cis-diol groups of glucose molecules selectively and reversibly. Even though such hydrogels have good selectivity for glucose, there are still remaining thresholds that should be overcome to enhance the sensitivity (swelling pressure response magnitude) and to reduce the response time (inverse of 1st order rate constant). In this study, the sensitivity and response time of zwitterionic glucose sensitive hydrogels (GSHs) were studied with three factor DOE analysis. The DOE results show that the molar ratio of 3-acrylamidophenylboronic acid/*N*-[3-(dimethylamino)propyl]acrylamide and the wt % of monomer in the pregel solution are the most important factors for enhancing the hydrogel sensitivity. In addition, fast response times can best be achieved by decreasing the molar ratio of cross-linker. The results of this study will be useful as guidelines for the optimal synthesis of glucose sensitive hydrogels. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40667.

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INTRODUCTION

A glucose sensor that responds reversibly and selectively to glucose has good biocompatibility, sufficient durability, and is small enough to be subcutaneously implanted can be used for real time continuous glucose monitoring. To satisfy these requirements, continuous glucose sensing (CGS) devices have been developed for both noninvasive and invasive systems. Noninvasive systems or semi-noninvasive systems use optical sensing methods such as scattering spectroscopy,[1] polarimetry,[2] Raman spectroscopy,[3] or fluorescence measurements.[4] In an invasive CGS system, a glucose sensor is implanted in certain areas of a body, for example, subcutaneously or intravenously. An implantable glucose sensor is capable of continuously measuring glucose concentration changes in any body fluid in real time.

Currently, one of the most promising recognition elements for use in an implantable glucose sensor is a glucose sensitive hydrogel (GSH) containing phenylboronic acid moieties because of its high biocompatibility, nontoxicity, and chemical stability. Pioneering studies on GSHs containing phenylboronic acid were performed by Kataoka et al.[5] This research group developed on/off insulin regulation systems that employed GSHs that swelled in response to increases in glucose concentrations in media solution.[5] However, the selectivity of the GSHs employed for binding of glucose relative to other simple sugars such as fructose, galactose, and mannose was poor.[6,7] In order to enhance selectivity to glucose, zwitterionic GSHs containing phenylboronic acid groups were developed.[8–11] When a zwitterionic GSH containing phenylboronic acid is exposed to an increase of glucose concentration in a media solution, it responds by shrinking rather than swelling. The explanation for this may be that glucose binds to two 3-acrylamidophenylboronic acid (3-APB) moieties within the GSH, thereby acting as a reversible cross-link,[10,12] or that glucose binding changes the structure of water hydrogen bonding in the solution surrounding the hydrogel.[13] In any case, zwitterionic GSHs shrink in response to an increase in glucose concentration and swell in response to an increase in fructose concentration.[10,12]

Although it is now clear that the most selective GSHs should be zwitterionic and contain both anionic 3-acrylamidophenylboronic acid (3-APB) and a cationic tertiary amine such as *N*-[3-(dimethylamino)propyl]acrylamide (DMAPAA), the precise ratio of the charged monomers that will optimize sensitivity and response time needs to be determined. Thus in this article, a statistics-based DOE technique was used to determine the best composition for a zwitterionic GSH with the minimum number of experimental samples. Statistical methods such as DOE have long been used in the industrial field to enhance reliability of products and to increase product

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Table I. Comparison of Hydrogel Studies Utilizing DOE Methods

DOE study	Method	Response	Analysis Method
Stanojevic et al.[14]	2 factors and 3 levels Full factorial	Water absorption rate	RSM[18]
Pourjavadi et al.[16]	3 factors and 4 levels Taguchi method	Water absorption rate	ANOVA
Rodrigues et al.[17]	4 factors and 2 levels FFD method	Water absorption rate	ANOVA
Current study	1 factor with 2 levels, 2 factors with continuous levels	Pressure responseInverse of 1st order rate constant	ANOVA RSM[18]

yield subject to limitations in cost and time. However, there are only few papers that report the use of DOE techniques for the optimization of hydrogel/polymer synthesis in the research field.[14–17] Stanojevic et al. used a DOE technique to show that the water absorption rate of pH-sensitive hydrogels increases with increase in the amount of itaconic acid content, and with decrease in the amount of cross-linker.[14] Stanojevic et al. set up a full factorial experimental model with two factors and three levels, and analyzed the response, which in this case was the water absorption rate of the hydrogel, with response surface methodology. Response surface methodology (RSM) is used to determine the tendency of responses using a function which can be achieved from experimental data within the continuous range of variables.[18] DOE using the full factorial method is the best methodology for obtaining reliable results when the system has a small number of factors and levels, but the number of required experiments is excessively large when the system has many input factors and levels. Thus, for analysis of cases having many factors, abbreviated DOE methods have been used to reduce the total number of experiments without sacrifice of accuracy. For example, Pourjavadi et al. optimized the chemical composition of a superabsorbent hydrogel utilizing the Taguchi DOE method.[15,16] Through use of the Taguchi method, these authors greatly reduced the total number of samples. For example, they set up a system with three factors, and four levels. If the DOE had been run by full factorial analysis, the total number of experimental samples would have been 64. However, through use of the Taguchi method, the number of samples was reduced to 16, and the data was still reliable.[15,16] Rodrigues et al. used fractional factorial design (FFD) to find the main factors for composition optimization of an acrylic acid/chitosan base superabsorbent hydrogel.[17] Using FFD, they picked 10 cases from the 16 needed for full factorial analysis, and confirmed that the cross-linker concentration in the pregel solution was the most important factor for determining the water absorption rate.

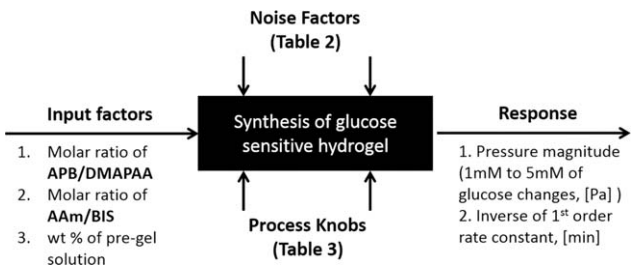

Figure 1. Schematic diagram of the DOE set up with factors that can affect the two outputs.

In this study, DOE was performed to study two types of responses such as swelling pressure and response time using three factors. Two levels were used for one of the factors, and continuously varying levels were used for the two remaining factors. In addition, process knobs and noise factors were strictly controlled in order to reduce errors due to unexpected environment factors. All hydrogel samples were synthesized by a UV polymerization method that is suitable for *in situ* synthesis on small devices. In Table I the different statistical methods are compared for published DOE studies of stimuli-responsive hydrogel composition.

EXPERIMENTAL

Materials

The monomers used for preparation of the gels and solvents were obtained as follows: acrylamide (AAM, Fisher Scientific), *N,N*-methylenebisacrylamide (BIS, Sigma-Aldrich), 3-acrylamidophenylboronic acid (3-APB, Frontier Scientific, Logan, UT), *N*-[3-(dimethylamino)propyl]acrylamide (DMAPAA, Polysciences), 2-hydroxy-4'-(2-hydroxyethoxy)−2-methyl propiophenone (HHMP, Sigma-Aldrich), 1-vinyl-2-pyrrolidinone (V-pyrol, Sigma-Aldrich), D(+)-glucose (Mallinckrodt Chemicals), dimethyl sulfoxide (DMSO, Sigma-Aldrich), 4-(2-Hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES, Sigma-Aldrich), and Dulbecco's phosphate-buffered saline (1X PBS, Sigma-Aldrich).

Equipment

Equipment used included a UV lamp (BIB-150P 365nm, Spectroline®), a piezoresistive pressure transducer (EPX series,

Table II. Control Method of Noise Factors

Noise factor	Control method
Transducer variation	All test was performed with one sensor
Glucose concentration changes during experiment due to water evaporation	Test system was sealed to minimize water evaporation
Material contamination	Use fresh monomers for hydrogel synthesis
Synthesis module contamination	Use a fresh parylene coated glass module for each synthesis
Engineering variation	All experiments were obtained by one engineer

Table III. Controlled Value of Process Knobs

Process knob	Value
Synthesis environment	Ar
UV exposure time	3 minutes
UV intensity	10 mW/cm^2
Hydrogel mold thickness	400 μm
Hydrogel conditioning	After washing with DIW, GSH was washed by 1X PBS and 1/3X PBS three times. Sample was stocked in 1X PBS at room temperature
Initial loading pressure	20K Pa

Table IV. Design Factors

Factors	Level	Minimum limitation	Maximum limitation
APB/DMAPAA	Continuous	0.4	5
AAm/BIS	Continuous	7	160
Wt %	discrete	13 (1st level)	30 (2nd level)

Measurement Specialties™), and a data acquisition/switch unit (34970A, Agilent Technologies). Infrared spectroscopy was performed using an ATR-IR spectrometer (iS10, Thermo Scientific), and the DOE software used was JMP 11[24,25] (design of experiment software, SAS Institute, Cary, NC).

DOE

As shown schematically in Figure 1, experiments were designed with consideration of input factors, noise factors, and process knobs that could affect the output. Synthesized GSHs were evaluated by two types of outputs, namely the swelling response magnitude (Pa), and the inverse of 1st order rate constant (min). The chosen input factors were the monomer concentration of the pregel solution, the molar ratio of the two charged monomers 3-APB and DMAPAA, and the mole ratio of the uncharged monomer (acrylamide, AAm) to the cross-linker (*N,N*-methylenebisacrylamide, BIS). Anticipated noise factors were controlled to minimize environment interferences (Table II). Process knobs were also fixed and controlled (Table III).

Factors that affect the sensitivity, selectivity, and response time of glucose sensitive hydrogels are monomer concentration of the pregel solution and copolymer hydrogel composition. Lin et al. previously optimized the swelling response magnitude of GSHs with respect to the mole ratios of the monomers AAm, 3-APB, DMAPAA, and BIS (cross-linker) prepared by free radical polymerization method with redox initiator.[7] Unfortunately, GSH response time was so long that pressure transducer baseline drift may have been substantial. To address this issue, design of experiment (DOE) was set up for GSHs prepared with the same monomers but with both response magnitude and response time used as DOE outputs. The upper and lower limits of the input factors was empirically determined (Table IV). For example, the molar ratio of AAm/BIS used cannot be greater than 160 because this gives a synthesized hydrogel that is too mechanically weak. After choosing the input factor limits, DOE software (JMP 11[24,25]) was used to choose the 12 different GSH samples synthesized (Table V).

Synthesis of Glucose Sensitive Hydrogels

Glucose sensitive hydrogels were synthesized by a UV activated free-radical crosslinking copolymerization process that can be adapted to *in situ* polymerization on micro-fabricated sensors. The free radical initiator used was HHMP/V-pyrol, which can be activated by UV at 365 nm. All monomers were dissolved in the 10 wt % DMSO/HEPES solvent, after which the solution was purged with Ar gas for 10 min. The synthesis mold was also purged with Ar gas for 10 min. Mold thickness was controlled with a 400 μm Teflon spacer. The pregel solution was injected into the synthesis mold and then the transparent mold was exposed to UV at 365 nm for 3 min (Table III). Synthesized

Table V. List of Synthesized Hydrogel Samples Chosen by DOE and Responses

Sample	APB/DMAPAA (mole/mole)	AAm/BIS (mole/mole)	wt % Pregel solution	Magnitude (Pa)	Inverse of 1st order rate constant (min)
G041613	0.4	160	13	1527 (±35)	35 (±2)
G042513	0.4	25	13	6149 (±230)	71 (±8)
G081630	0.8	160	30	3051 (±220)	42 (±7)
G080713	0.8	7	13	2736 (±240)	163 (±16)
G082013	0.8	20	13	2994 (±130)	147 (±5)
G082530	0.8	25	30	17043 (±1670)	153 (±3)
G084013	0.8	40	13	5006 (±380)	73(±3)
G134013	1.25	40	13	5402 (±740)	114 (±13)
G161213	1.6	12	13	4310 (±450)	76 (±12)
G251613	2.5	160	13	0	none
G254013	2.5	40	13	6934 (±580)	69 (±2)
G502530	5	25	30	2491 (±150)	104 (±20)

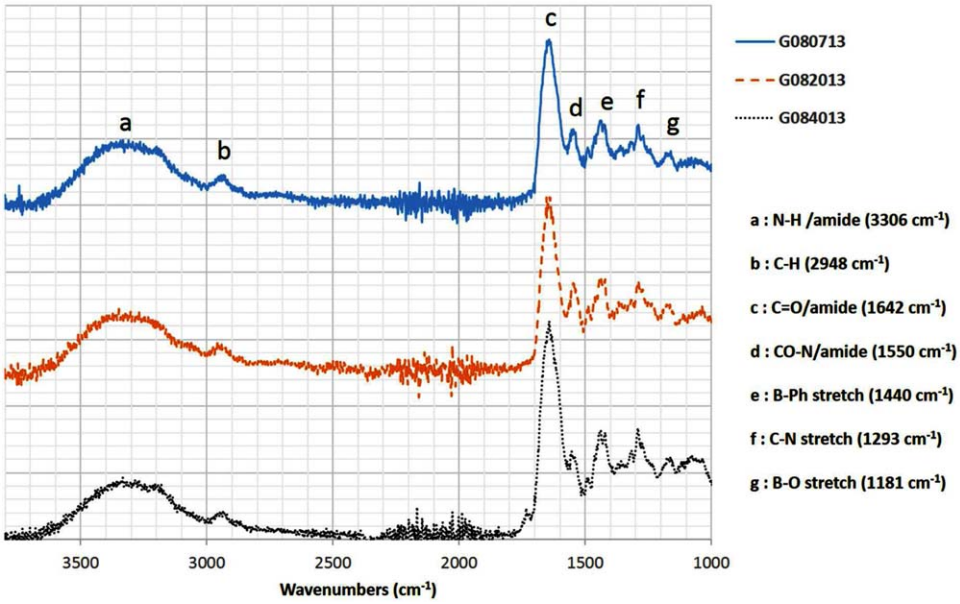

Figure 2. ATR-IR spectra of dried glucose sensitive hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hydrogels were washed first with distilled water, and then they were washed three times with PBS buffer (Table III).

Response Measurement

Hydrogel swelling pressure response was measured for a glucose concentration change between 1 m*M* and 5 m*M* in 1X PBS buffer, because the normal fasting blood glucose level is ≈3.8 to 5.5 m*M*. Swelling pressure response was measured with a piezoresistive pressure transducer.[7,11,19] Glucose responsive hydrogels react to glucose in the surrounding environment by either swelling or deswelling. If the hydrogel is in a confined space in between a rigid porous membrane and a piezoresistive diaphragm, the swelling pressure change results in a mechanical pressure change which is transduced into a measurable voltage. The time-dependent value of this voltage was captured on a PC using the Agilent data acquisition system.

Reaction Kinetics of Glucose Sensitive Hydrogels

As in previous studies,[7] it was found that the time-dependent swelling response of the stimuli-responsive hydrogels could be fit with the first-order kinetic model of Quintana,[26] as given below:

$$\frac{d[P]}{dt} = k_1[P_\infty - P] \quad (1)$$

P: Time-dependent pressure measured with a piezoresistive pressure transducer ≡ [Pa]

P_∞: Pressure measured with a piezoresistive pressure transducer after equilibrium is reached after a glucose concentration change ≡ [Pa]

k_1: Proportionality constant between swelling rate and the swelling capacity ≡ (time^{-1})

The inverse of the first order rate constant was used as a measure of response time.

RESULTS AND DISCUSSION

Characterization of Hydrogels by ATR-IR

Glucose sensitive hydrogels were synthesized using eleven different pregel solution compositions (Table V), and then were characterized by ATR-IR in order to confirm the actual chemical composition within the hydrogels after

Table VI. Comparison of Estimated Ratio and IR Absorbance Ratio of Functional Groups

	C—N stretch from amide groups		B—O stretch from APB		CO-N from AAm, and BIS amides		C=O	
Sample	Pregel	IR[a]	Pregel	IR[b]	Pregel	IR[c]	Pregel	IR[d]
G080713	1.34	1.39	2.00	2.00	1.00	1.00	1.00	1.00
G082013	1.04	1.06	1.00	1.24	1.19	1.16	1.19	1.08
G084013	1.00	1.00	1.00	1.18	1.17	1.15	1.17	1.04

Pregel: estimated value from monomer composition of pregel solution.
[a] C—N stretch from amide and amine groups (relative absorbance ratio of *f*).
[b] B—O stretch from boronic acid group (relative absorbance ratio of *g*/*e*).
[c] CO—N from amide groups (AAm, BIS) (relative absorbance ratio of *d*/*b*).
[d] C=O (relative absorbance ratio of *c*/*b*).

Table VII. Evaluation of Input Factors

	P-values	
Parameters	Response magnitude	Inverse of 1st order rate constant
The ratio of APB/DMAPAA	0.037	0.23
The ratio of AAm/BIS	0.081	0.012
wt % Pregel solution	0.002	0.18

polymerization. Figure 2 shows the ATR-IR spectra of samples G080713, G082013, and G084013. The absorption peaks were identified using Ref. 22. According to Beer's Law, the chemical composition can be estimated by comparison of the absorbance ratio of the functional groups' peaks.[20,21,23] By comparison of IR absorbance ratio of functional group peaks from DMAPAA, APB, and BIS, the chemical composition of the hydrogel can be compared with the chemical composition of the pregel solution. Table VI shows that IR absorbance ratio exhibits the same trends as the molar ratio of pregel solution.

Determination of Contributive Factors by ANOVA

For each of the eleven different GSH compositions, experimental results for the swelling response magnitude (P_a) and the inverse of 1st order rate constant (min) are given in Table V. The main contributive factors for these two responses were determined from *P*-values that were estimated by Analysis of Variance (ANOVA, Table VII), with significant factors corresponding to *P*-values < 0.05 (ANOVA stats with null hypothesis. A value for *P* less than 0.05 implies that the response tendency depends on the variable studied). The results in Table VII show that the molar ratio of 3-APB/*N*-[3-(dimethylamino)propyl]acrylamide and the wt % of monomer in the pregel solution are the most important factors for enhancing the hydrogel sensitivity, and that a fast response times can best be achieved by decreasing the molar ratio of cross-linker. The results of the statistical analysis are summarized by the following equations for predicting response magnitude and response time obtained using JMP 11[24,25] [constant A: (APB/DMAPAA-2.7)/2.3, B: (AAm/BIS-83.5)/76.5, & C: (wt % −21.5)/8.5].

Magnitude estimation equation:

$$Y = 3286 - 4577A - 6906B - 571C - 2661(A \times B) - 5426(A \times C) - 3221(B \times C) \quad (2)$$

Inverse of 1st order rate constant estimation equation:

$$Y = 490 + 436A + 556B + 23C + 681(A \times B) + 57(A \times C) - 56(B \times C) \quad (3)$$

In leverage plots (Figures 3 and 4), the distance from dots and the blue dash corresponds to expected residual value when the effect is removed. Red dash lines are showing significance at *P*-value < 0.05 level. Thus, when two dash lines are cross each other, the data points should be meaningful.

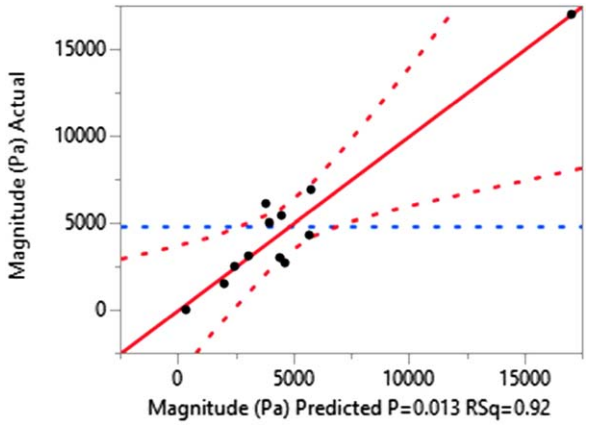

Figure 3. Leverage plot of response magnitude (Rsquared value: 0.92). Red line: predicted value (eq. (2)), black points: actual value, red dash: significance, and blue dash: anticipated residual when the effect is removed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Sensitivity Control Factor

The surface plot from statistical analysis (Figure 5) shows that enhanced sensitivity of zwitterionic GSHs was achieved by increase of either the molar ratio of 3-APB/DMAPAA or of the pregel solution monomer concentration. The best sensitivity of zwitterionic GSHs in the DOE set was achieved when the ratio of 3-APB/DMAPAA was 0.8 (with fixed AAm/BIS = 25, and pregel monomer wt % = 30, sample G082530) in 1X PBS (pH = 7.4). However, a drop in sensitivity is observed in Figure 5 at the highest values of the molar ratio of 3-APB/DMAPAA studied (above 2.5). This can probably be explained as follows. An increase in the ratio initially increases the sensitivity of the GSH by increasing the number of boronic acid groups that reversibly bind to glucose. However, if the ratio becomes too large, then the fraction of boronic acid groups that are charged probably decreases because the mole ratio of cationic tertiary amines becomes too small. The function of the

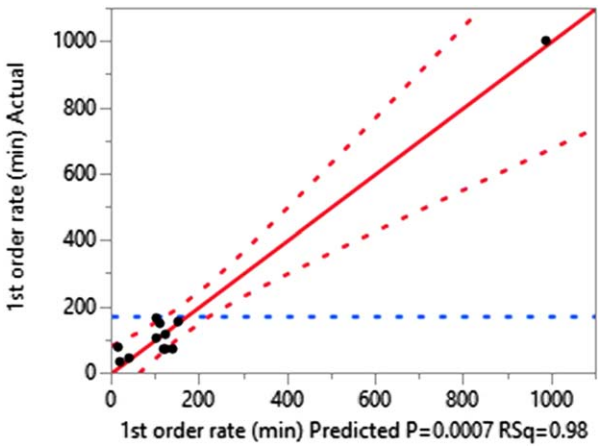

Figure 4. Leverage plot of inverse of 1st order rate constant (Rsquared value: 0.98). Red line: predicted value (eq. (3)), black points: actual value, red dash: significance, and blue dash: anticipated residual when the effect is removed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

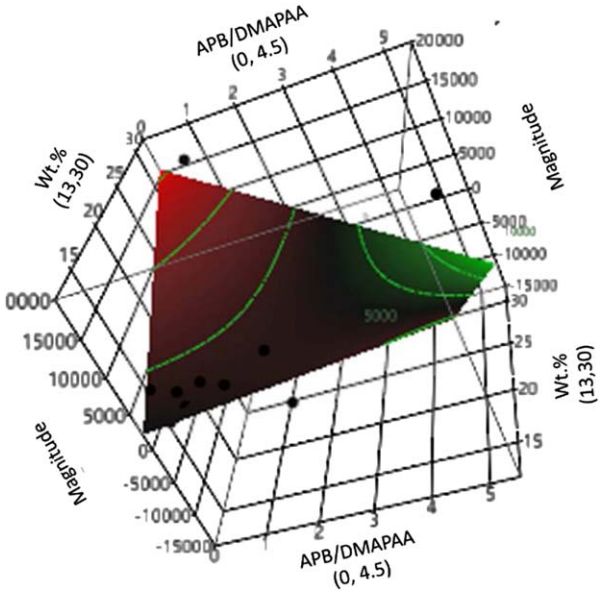

Figure 5. Surface plot of response magnitude to the glucose concentration change in 1X PBS buffer (from 1 to 5 m*M*) vs. the molar ratio of 3-APB/DMAPAA and wt % of pregel solution (dot: actual data points). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

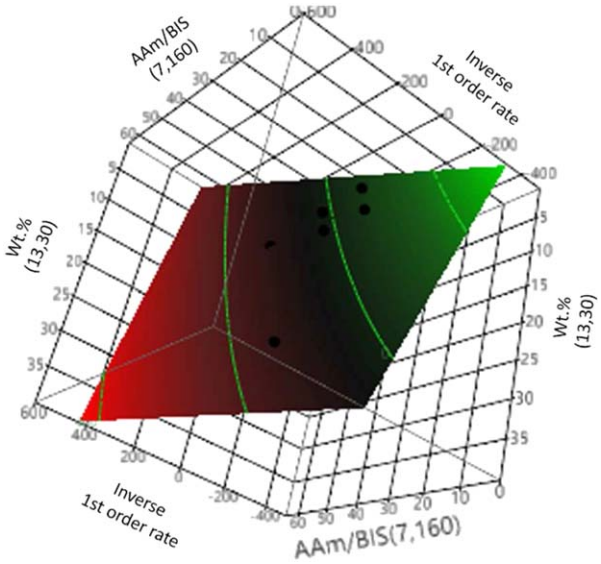

Figure 7. Surface plot of inverse of 1st order rate constant to the glucose concentration change in 1X PBS buffer (from 1 to 5 m*M*) vs. the molar ratio of AAm/DMAPAA and wt % of pregel solution (dot: actual data points). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cationic tertiary amines is to increase the acidity of neighboring boronic acid groups. In addition, an increase in the monomer concentration in the solution used to synthesize GSHs from 13 wt % to 30 wt % increases the glucose sensitivity of the GSH when the chemical composition is fixed. This can be explained by the increase that occurs in the amount of glucose-binding moieties per unit volume in gels synthesized from pregel solutions containing higher monomer concentrations. In support of this idea, Figure 6 shows that the water content of GSHs synthesized from pregel solutions containing 30 wt % of monomer is lower than the water content of GSHs synthesized from pre-solutions containing 13 wt % of monomer, which implies that the polymer weight fraction and the number of glucose-binding moieties per unit volume in the synthesized gel is larger. This explains why the glucose response magnitude is larger for GSHs synthesized from solutions containing 30 wt % monomer than from GSHs synthesized from 13 wt % monomer.

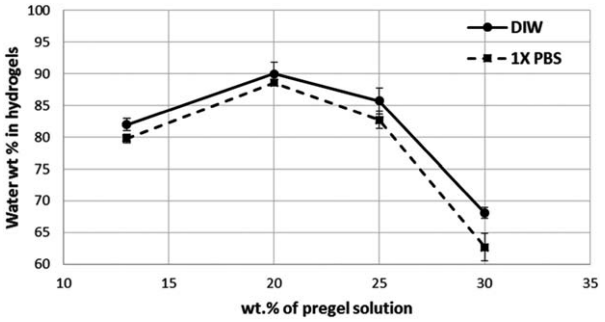

Figure 6. Water wt % of synthesized hydrogels vs. wt % of pregel solution (circles: gel swollen in DIW, squares: gel swollen in 1X PBS).

Response Time Control Factor

The surface plot from statistical analysis (Figure 7) shows that a reduced response time for zwitterionic GSHs can be achieved by decreasing the crosslinker mole ratio AAm/BIS. This is reasonable because the entanglement spacing of the hydrogel network increases with decrease in the density of crosslinks, making it easier for glucose to diffuse into the gel, and also reducing the viscoelastic response time. However, if the chosen amount of cross-linker is too small, the hydrogel is likely to lose mechanical integrity in long-term applications. For our sensor design, in which the hydrogel was confined between a porous steel mesh and a piezoresistive diaphragm, we found that a mole ratio of AAm/Bis of 40 or less was necessary to obtain sufficient mechanical strength for a reversible response.

CONCLUSIONS

Experiments chosen and analyzed using DOE methods show that the molar ratio of 3-APB/DMAPAA and the monomer wt % of the pregel solution are the dominant factors that determine the magnitude of response of zwitterionic GSHs to an increase in glucose concentration from 1 to 5 m*M*. The best glucose sensitivity was achieved when the ratio of 3-APB/DMAPAA was increased from 0.4 to 2.5 in 1X PBS with fixed values of other factors. In addition, GSHs synthesized from 30 wt % pregel solutions show better glucose sensitivity than GSHs synthesized from pregel solutions containing 13 wt % monomer. DOE statistical analysis of experiments also shows that the molar ratio of AAM/BIS is the primary factor for determining the value of the inverse of 1st order rate constant in this study. Faster glucose responses can be obtained by decreasing the amount of cross-linker (BIS). The results of this study will be useful for the design of an implantable continuous glucose sensor that employs zwitterionic GSHs.

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