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Effect of Solution Aging Time on Stability of Colorimetric Assay for Degradation Rate Evaluation of Porous Si in Artificial Cerebrospinal Fluid

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Abstract. In order to evaluate the degradation rate of porous Si in artificial cerebrospinal fluid and obtain more accurate results, the effect of solution aging time on the stability of the ammonium molybdate colorimetric assay was investigated. Following the protocol of the colorimetric assay, the blue colored solutions with various silicic acid concentrations were prepared and then aged for different time periods up to 7 h. On the basis of linear regression analyses, the absorbance value of the blue colored solution was directly proportional to the silicic acid concentration. Moreover, the optimal aging time span of the solution maintained until 7 h, depending on the stability of the solution.

Introduction

As a promising biomaterial, porous silicon (PSi) is attracting growing interests in the fields of tissue engineering and drug delivery, owing to several advantages over the existing materials [1, 2]. Firstly, in aqueous solutions, PSi degrades completely into non-toxic silicic acid, one of the most common forms of silicon in human body. Moreover, it was reported that PSi as the scaffold supported and promoted primary osteoblast growth, protein-matrix synthesis, and mineralization [3]. Therefore, it is potential to repair or regenerate damaged tissue with the degradation of PSi matrix and the integration of the cultured cells to the native tissue. Another attractive property of PSi is the tunable porosity and its large surface area (400~1000 m^2/g). In terms of pore size, PSi is divided into three categories, from micropores (<5 nm), mesopores (5~50 nm) to macropores (>50 nm). Depending on the processing parameters, such as anodization current density, time, electrolyte composition, the pore size and porosity can be precisely controlled and highly tunable. Hence, one of the potential medical applications of PSi is to serve as a carrier to load and deliver various biomoleculars and drugs to target issues. It was suggested that PSi particles loaded with the isotope ^{32}P suppressed the growth of cancer cells (HepG2 and 2119), and reduced the volume of tumors [4]. Photoluminescence is also one of well known properties of PSi, and Park *et al.* showed that the dextran-coated degradable PSi nanoparticles were capable of imaging tumors [5].

Since many potential medical applications of PSi arise from its degradation property, in many studies the degradation rate of PSi is one of the critical parameters to be investigated. PSi matrix should degrade in an appropriate matching rate as the tissue cells grow in or drugs are released. Several methods were employed to investigate the degradation rate of PSi, such as interferometric reflectance spectroscopy [1], the ammonium molybdate colorimetric assay [6], inductively coupled plasma optical emission spectrometry [7], scanning electron microscopy [8], etc. Due to its high sensitivity, efficient batch processing, and cost saving, the ammonium molybdate colorimetric assay is regarded as a convenient method to evaluate the concentration of silicic acid, the degradation product of porous Si. However, the effect of solution aging time on the stability of the colorimetric assay has been rarely reported. In the current study, sodium metasilicate pentahydrate ($Na_2SiO_3 \cdot 5H_2O$) was dissolved in artificial cerebrospinal fluid (aCSF) to form silicic acid, and then

silicic acid solutions with various concentrations as the model of porous Si degradation product were mixed with ammonium molybdate for the colorimetric assay. Subsequently, the blended solutions were reduced by sodium sulfite yielding blue colored molybdate, followed by aging the blue colored molybdate solution for different time periods up to 7 h. At each time point, the absorbance of the blue colored solution was recorded and compared to evaluate the stability of the solution. Finally, the optimal time span to measure the absorbance of the solution was obtained in accordance with the slope (B) and coefficient of determination (R^2) from the linear regression equation.

Materials and Methods

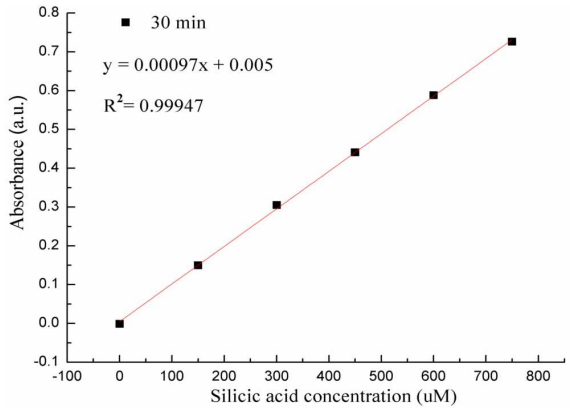
The theory of the molybdate colorimetric assay for silicic acid detection. Porous silicon dissolves in aqueous solution to form monomeric silicic acid ($Si(OH)_4$). In an acid medium (pH < 2.5), the silicic acid reacts with ammonium molybadate to form the yellow colored silicomolybdate complex. The complex is then reduced by sodium solfite to yield the blue colored molybdate. The absorbance value of the blue colored solution is quantified by a spectrophotometer. Within a reasonable certain range, the absorbance is expected to be directly proportional to the Si content of the sample assayed. Therefore, a quantitative measurement of Si content can be established in accordance with the recorded absorbance value.

Standard solution preparation. 2.1214g sodium metasilicate pentahydrate (Molar Mass: 212.14) was dissolved into 100 ml aCFS, and then the 150, 300, 450, 600 and 750 μM silicic acid solutions were prepared by serial dilution of the 100 mM stock solution. For the colorimetric assay, 42 mM ammonium molybdate (Amoly) solution prepared by dissolving ammonium heptamolybdate tetrahydrate salt in distilled water (DIW) was acidified by mixing with 0.3 M HCl in ratio of 1:2 by volume. 1.35 M sodium sulphite solution and 50 mM EDTA solution were prepared by dissolving anhydrous sodium sulphite salt and EDTA disodium salt into DIW, respectively.

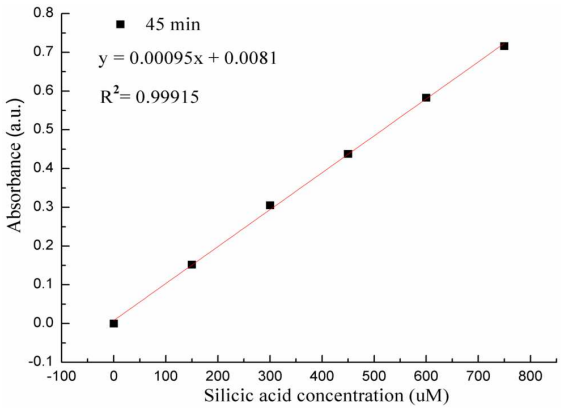
Colorimetric assay. 200 ml standard silicic acid solution was transferred into a clean 0.5 ml polypropylene microtube, followed by mixing with 50 μl Amoly/HCl solution. The mixed solution was vortexed for 3 sec and then incubated at room temperature for 10 min. Subsequently, 25 μL EDTA solution was added, followed by vortex for 3 s and incubation for 5 min. 25 μl sodium sulphite was then blended with the mixed solution via vortex for 3 s. After aging at room temperature for different time span up to 420 min, 250 μl solution was transferred into 96-well microplate and its absorbance was determined using a microplate reader (DTX 800 Series Multimode Detectors, Beckman Coulter, CA) at 600-nm wavelength. At each time point, the measured absorbance was plotted as the function of silicic acid concentration, and the figure was fit via linear regression (y = A + Bx). Accordingly, the intercept (A), the slope (B) and coefficient of determination (R^2) were obtained from the linear regression equation.

Results and discussion

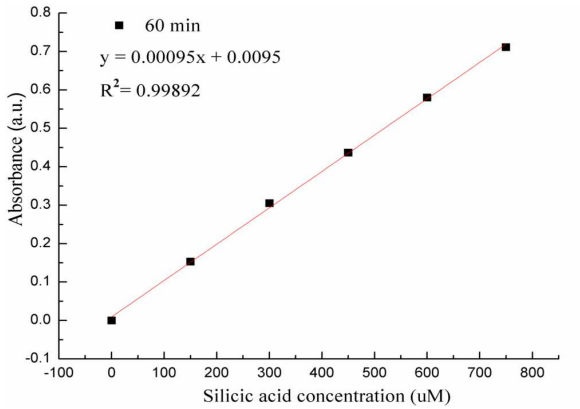
Fig.1 shows the absorbance value as the function of silicic acid concentration at each time point.



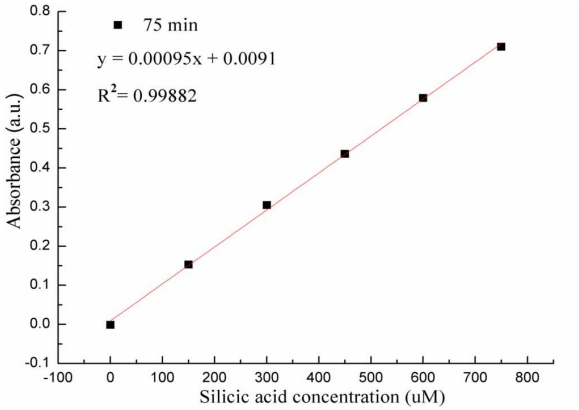
(a)



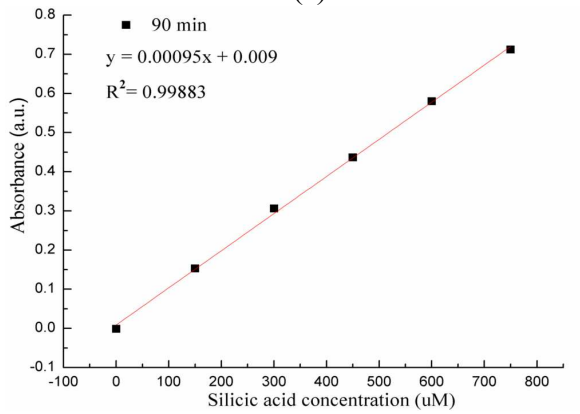
(b)


60 min
y = 0.00095x + 0.0095
R²= 0.99892
Absorbance (a.u.)
Silicic acid concentration (uM)

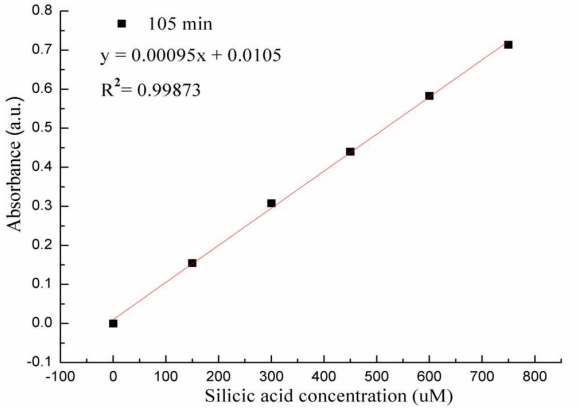
(c)


75 min
y = 0.00095x + 0.0091
R²= 0.99882
Absorbance (a.u.)
Silicic acid concentration (uM)

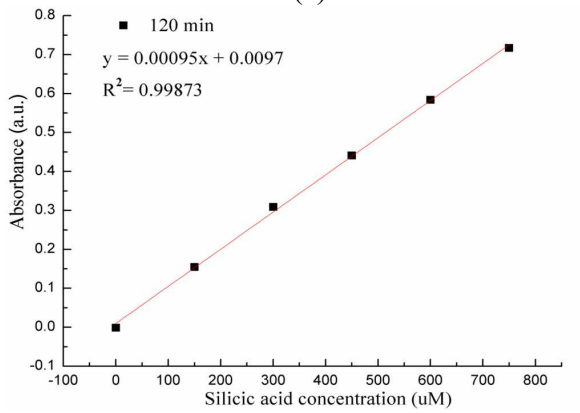
(d)


90 min
y = 0.00095x + 0.009
R²= 0.99883
Absorbance (a.u.)
Silicic acid concentration (uM)

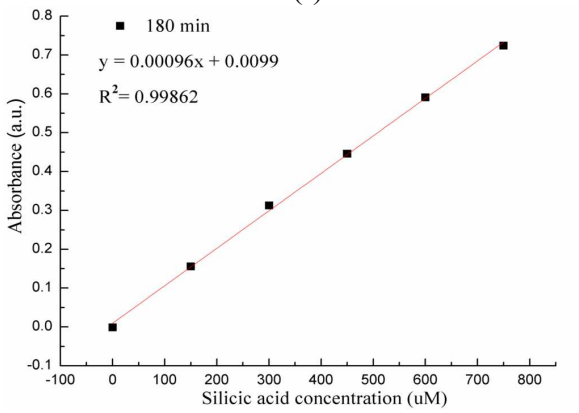
(e)


105 min
y = 0.00095x + 0.0105
R²= 0.99873
Absorbance (a.u.)
Silicic acid concentration (uM)

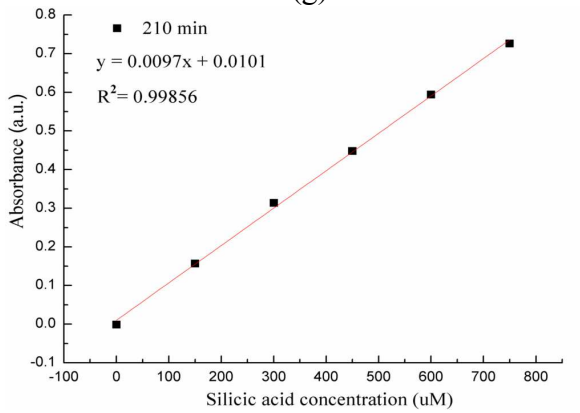
(f)


120 min
y = 0.00095x + 0.0097
R²= 0.99873
Absorbance (a.u.)
Silicic acid concentration (uM)

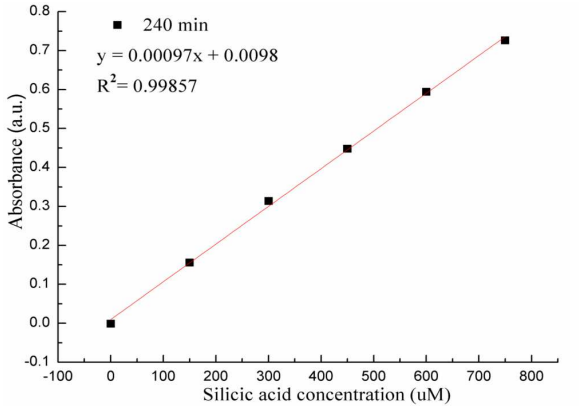
(g)


180 min
y = 0.00096x + 0.0099
R²= 0.99862
Absorbance (a.u.)
Silicic acid concentration (uM)

(h)


210 min
y = 0.0097x + 0.0101
R²= 0.99856
Absorbance (a.u.)
Silicic acid concentration (uM)

(i)


240 min
y = 0.00097x + 0.0098
R²= 0.99857
Absorbance (a.u.)
Silicic acid concentration (uM)

(j)

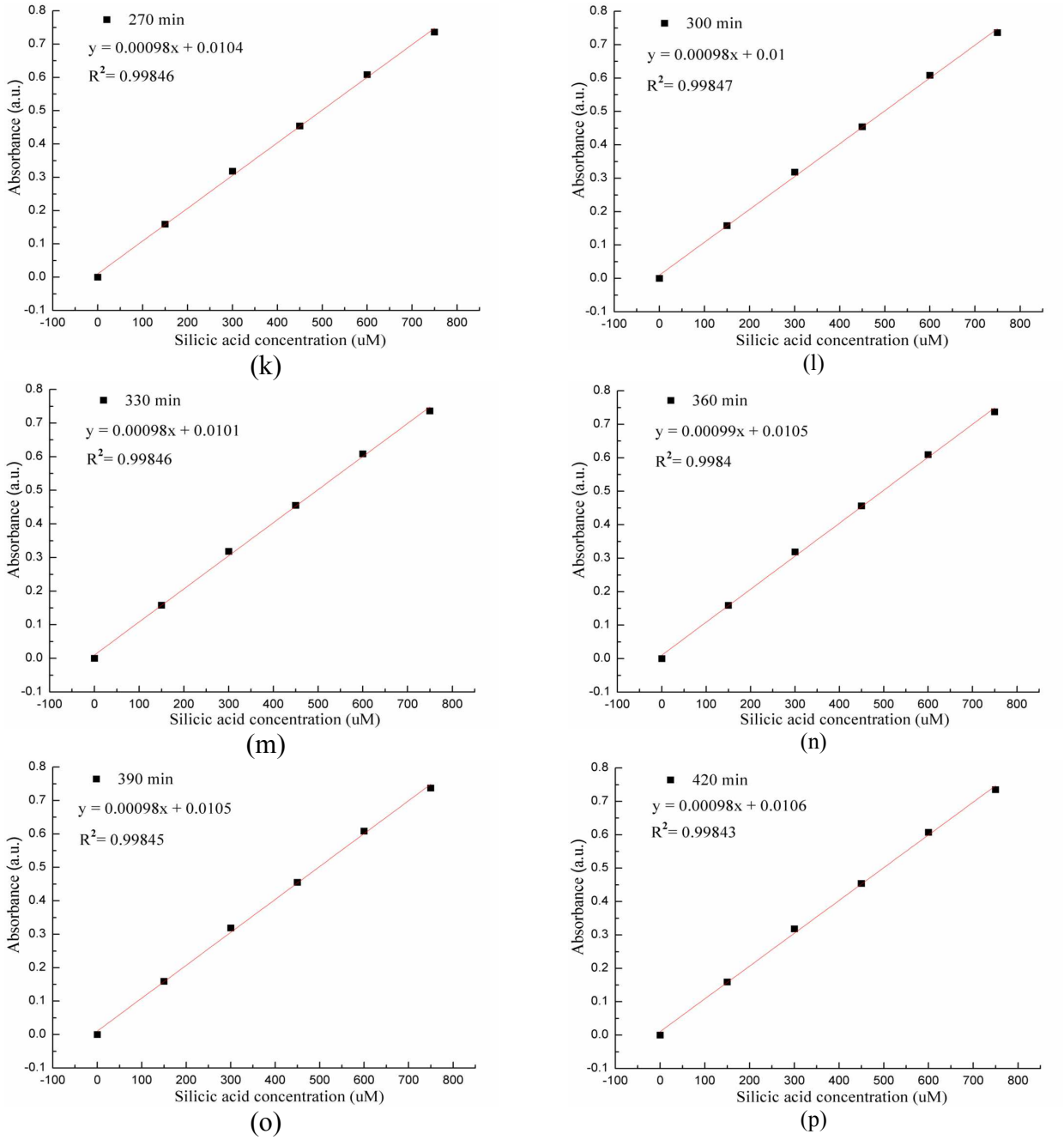


Fig.1. The absorbance value as the function of silicic acid concentration at each time point and its linear regression analyses: (a) 30 min, (b) 45 min, (c) 60 min, (d) 75 min, (e) 90 min, (f) 105 min, (g) 120 min, (h) 180 min, (i) 210 min, (j) 240 min, (k) 270 min, (l) 300 min, (m) 330 min, (n) 360 min, (o) 390 min, (p) 420 min.

In addition, the linear regression fitting was carried out for the data, in order to determine the relationship between the absorbance and silicic acid concentration. On the basis of the results from the fitting analyses, the absorbance value seemed exactly proportional to silicic acid concentration in the range of 0 ~ 750 μM over the whole aging period. Fig.2 displays the variation of slope (B) and coefficient of determination (R^2) and slope (B) with the solution aging time. Although relatively significant variation was observed for R^2 within 75 min, after aging for 120 min R^2 had a trend to become stable and their variation was less significant than that within 75 min. Moreover, the difference between the maximum and minimum R^2 (corresponding to the values at 15 min and 420 min, respectively) was only 0.1% of the mean of R^2_{max} and R^2_{min}. Similarly, the difference between the maximum and minimum B (corresponding to the values at 360 min and 45 min, respectively) was only 4 % of the mean of B_{max} and B_{min}. Therefore, the variation of R^2 and B was not statistically significant over the whole aging period. The optimal aging time span for the measurement lasted for 7 h in the current research. But the maximum optimal solution aging time for the colorimetric assay should be further investigated in the future.

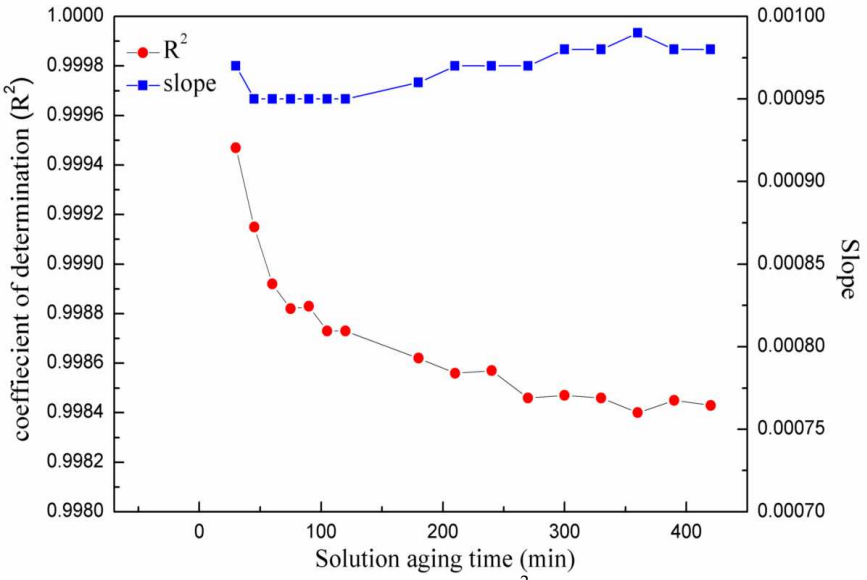


Fig. 2. The variation of coefficient of determination (R^2) and slope (B) with the aging time.

Conclusions

As the stability of the Si-containing blue colored solution influenced the results of the colorimetric assay, the relationship between the solution aging time and the solution stability was investigated in the current research. After aging for different time periods up to 7 h, the absorbance of the solution was recorded, and exactly proportional to the silicic acid concentration in accordance with linear regression analyses. Due to the slight variation of B and R^2 derived from the linear equations, the solution maintained stable at least until 7 h. Therefore, in the current research the optimal aging time span of the colorimetric assay lasted for 7 h.

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