SUPPLEMENTARY MATERIALS

Development of a Simple Reversible-Flow Method for Preparation of Micron-size Chitosan-Cu(II) Catalyst Particles and Their Testing of Activity

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- Supplementary Material B: Measurement of particle size by ImageJ software: Effect of threshold number

Supplementary Material A

Characterization of CS-Cu(II) catalyst particles

Characterization of chitosan-Cu(II) catalyst particles were carried out by: (a) scanning electron microscopy (SEM), (b) Fourier transform spectroscopy (FTIR), (c) x-ray diffraction (XRD), (d) specific surface areas (nitrogen adsorption/desorption isotherms) and (e) measurement of pore size distribution [1].











(e) Pore size distribution



Figure S1 Characterization of chitosan-Cu(II) catalyst particles: (**a**) SEM image; (**b**) FTIR spectrum of chitosan-Cu(II) catalyst showing bands at 3430, 2868, 2364, 1622, 1379, and 1097, 1013 and 599 cm⁻¹, respectively; (**c**) XRD pattern,; (**d**) Nitrogen adsorption/desorption isotherms and (**e**) Pore size distribution.

Supplementary Material B

Measurement of particle size by ImageJ software: Effect of threshold number

Image J software is used for analyzing the size of objects on an image and measuring distance between objects. It is a free software for downloading from http://rsb.info.nih.gov/ij/index.html. Image J software is developed by National Institute of Mental Health (NIMH) in United State. It can measure objects in many units, such as square millimeter or number of pixels.



Figure S2 Overview of steps including: (1) Preparation of CS-Cu(II) catalyst particles, (2) recording of particles image by an optical microscope, and (3) measurement of particles for size by Image J software.

Figure S2 shows the schematic procedure for image analysis. This work uses ImageJ for measurement of particle size of the chitosan-Cu(II) catalyst particles. The operation steps for measurement are shown in Table S1.

Threshold adjustment is a software for segmenting the pixels of an object from the background. Advantages of this software are: 1. reduction of unnecessary pixels of the object, 2. converting image data into bitmap that it is easy to analyze or interpret.

To use the Image J program, the operator has to adjust some parameters before image analysis such as "threshold adjustment".

Step	Description	Screen capture	
1	 Opening a digital image file: Go to File ▶ Open from menu bar to open a digital image of particles file, as shown in picture of the right. 		
2	Setting scale bar in ImageJ: Select a		

Table S1 Step-by-step procedure using Image J software for particle size analysis.

2	Setting scale bar in ImageJ: Select a
	straight line from the menu bar and draw
	a straight from the known distance on
	your digital image file, as shown in
	picture of the right.

Converting color digital image (RGB) 3 into grey scale: If your digital image file is color image, it shoud be converted to grey image. Once the image is in grey scale "Image ► Type ► 8-bit", as shown in in picture of the right.



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Description

Screen capture

- 4 Setting a threshold of digital image file:
 Setting a threshold number to remove the background of image before image analysis (as seen in red color). A threshold number adjustment includes
 "Image Adjust ► Threshold, as shown in picture of the right.
- 5 Measuring area of objects: After finish setting parameter, go to "Analyze ► Analyze Particles", as shown in Figure A.5. The output of software reports the area. The area data (blue color) was converted to diameter (see picture of the right).



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Reference:

Step

[1]. Chutimasakul, T.; Na Nakhonpanom, P.; Tirdtrakool, W.; Intanin, A.; Bunchuay, T.; Chantiwas, R.; Tantirungrotechai. J. Uniform Cu/chitosan beads as green and reusable catalyst for facile synthesis of imines via oxidative coupling reaction. *Unpublished results*. **2020**.