

**NIST Special Publication 1200-12**

**Reconstitution of 2 nm diameter Silicon  
Nanoparticles (RM8027) into aqueous solvents**

Version 1.0

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May 2015 -



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**National Institute of Standards and Technology Special Publication 1200-12**  
**Natl. Inst. Stand. Technol. Spec. Publ. 1200-12, 23 pages (May 2015)**  
**CODEN: NSPUE2 -**

**This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.SP.1200-12>**

## FOREWORD

This special publication is one in a series stemming from the National Nanotechnology Initiative (NNI) Nano-Environmental, Health, and Safety (EHS) Research Strategy which identified Nanomaterial Measurement Infrastructure as one of the essential areas of research needed in order to develop an effective risk assessment and management plan regarding various aspects of nanotechnology in consumer products as it pertains to human health, exposure and the environment. The National Institute of Standards and Technology (NIST) was identified as a lead agency in the development of measurement strategies for the robust development to assess the potential effects of engineered nanomaterials and their fate in the environment. One important factor in these measurements is having a set of reference materials to evaluate and qualify methodology and/or instrument performance related to the physical/dimensional characterization of nanoscale particles. Reference materials may also be useful in the development of *in vitro* assays designed to evaluate the biological response of nanomaterials and for use in inter-laboratory test comparisons.

The current protocol presents a method to reconstitute the Reference Material (RM) 8027 (Si Nanocrystals, 2 nm nominal diameter) into aqueous solvents using Ultraviolet (UV)-assisted hydroxylation. Updates to this protocol may be released in the future. Visit <http://nist.gov/mml/np-measurement-protocols.cfm> to check for revisions of this protocol, or new protocols in the series. We also encourage users to report citations to published work in which this protocol has been applied.

## 1. Introduction

Nano-crystalline silicon has been extensively investigated for numerous applications including optoelectronic devices, optical biosensing, biomolecular screening [1-3]. The inherent material biocompatibility facilitates Si Nanoparticle (SiNP) use for *in vitro* biological tagging [4, 5] and as a platform for drug delivery [6]. National Institute of Standards and Technology (NIST) recently has released a SiNP reference material RM8027 that consists of five hermetically sealed pre-scored glass ampoules, each containing nominally 1 ml of cyclohexane-stabilized SiNP's suspended in toluene. The suspension contains particles (monomers) and a small percentage of clusters of primary particles. Biomolecular studies typically necessitate the water-dispersable NP's. This document describes a procedure of Reference Material 8027 reconstitution into aqueous solvents using UV- assisted hydrosilylation and phase-transfer with triblock copolymer Pluronic F127. Hydrosilylation method allows to exchange of the hydrophobic surface coating with positively (amine) or negatively (carboxyl) charged moieties, rendering SiNP's water soluble [7]. The triblock copolymer phase transfer results in incorporation of the hydrophobic core into the hydrophobic NP shell, exposing the hydrophilic polyethylene oxide chain resulting in water solubility.

## 2. Principles and Scope

These protocols describe the preparation of water-dispersable SiNP using RM8027 as a stock. The resulting aqueous NP suspensions were characterized using Dynamic Light Scattering (DLS), Zeta Potential (ZP) measurement, Ultraviolet-Visible (UV-VIS) absorbance and Photoluminescence (PL) spectroscopy.

## 3. Terminology

This protocol complies with definitions relevant to nanotechnology as set forth in the ASTM International standard E2456 [8] and is consistent with the draft standard ISO TS 80004-1:2010

[9].

*nanoparticle*—sub-classification of ultrafine particle that is characterized by dimensions in the nanoscale (i.e., between approximately 1 nm and 100 nm) in at least two dimensions; also referred to as “nano-object” in ISO TS 80004-1:2010 [8].

*primary particle* — the smallest discrete identifiable entity associated with a particle system; in this context, larger particle structures (e.g., aggregates and agglomerates) may be composed of primary particles.

*aggregate* — a discrete assemblage of primary particles strongly bonded together (i.e., fused, sintered, or metallicly bonded).

*Note*—The adjective “primary”, when used in conjunction with the term *aggregate*, is employed in the present context to indicate the smallest achievable dispersed particle entity.

*agglomerate*—assemblage of particles (including primary particles and/or smaller aggregates) held together by relatively weak forces (e.g., van der Waals, capillary, or electrostatic), that may break apart into smaller particles upon further processing.

*Note*—Although we define them as distinct entities, the terms *aggregate* and *agglomerate* have often been used interchangeably to denote particle assemblies.

*dispersion*—used in the present context to denote a liquid (aqueous) in which particles are homogeneously suspended, or the process of creating a suspension in which discrete particles are homogeneously distributed throughout a continuous fluid phase; implies the intention to break down agglomerates into their principal components (i.e., primary particles and/or aggregates).

## **4. Materials and equipment**

## 4.1 Reagents

### 4.1.1 RM 8027 (SiNP, 2 nm nominal diameter).

- Packaged in amber glass ampoules, each containing 1 mL SiNP suspension in toluene

### 4.1.2 Buffer solutions : Sigma-Aldrich as ACS reagent grade

- Acetic acid/sodium acetate solution for pH buffer in the range pH 5<sup>2222</sup> 6 :
- Sodium phosphate monobasic/dibasic solution for pH buffer in the range pH 6<sup>2222</sup> 8
- Sodium carbonate/sodium bicarbonate solution for pH buffer in the range pH 9<sup>2222</sup> 11
- Buffer concentration range :0.01 to 0.1 mol/L

## 4.2 Materials

- 4.2.1 Glass ware – 2 mL to 20 mL capacity, transparent to light at the wavelengths > 250 nm
- 4.2.2 1 mL to 5 mL capacity glass syringe
- 4.2.3 Centrifugation vials
- 4.2.4 Cuvettes for UV/VIS and PL measurements
- 4.2.5 Cuvettes for DLS and ZP measurements

### 4.1.3 Allylamine: Sigma-Aldrich, 98 %

### 4.1.4 Acrylic Acid: Aldrich, 99 %

### 4.1.5 Compressed inert gas (Ar or N<sub>2</sub>): at least 99 % pure

### 4.1.6 Nonionic triblock copolymer Pluronic F-127:Sigma-Aldrich

## 4.3 Equipment

- 4.3.1 Ultrasonicator : Branson 2510 *or equivalent*
- 4.3.2 UV A source: Light Emitting Diode (LED) LZ1-00U600 (Mouser electronics or similar, 365 nm or 385 nm emission, 400 mW radiant flux), portable long wavelength (360 nm) UV lamp or equivalent
- 4.3.3 UV B source: portable short wavelength (250 nm) UV lamp or equivalent
- 4.3.4 Inert gas tank with a flow regulator
- 4.3.5 0.2  $\mu\text{m}$  pore size syringe filter, Pall Acrodisc or equivalent
- 4.3.6 0.02  $\mu\text{m}$  pore size syringe filter, Whatman Anotop 25 Plus or equivalent
- 4.3.7 DLS particle size analyzer : Nicomp Model380 *or equivalent*
- 4.3.8 ZP analyzer : Nicomp Model380 *or equivalent*
- 4.3.9 pH meter: MiniLab IQ120 *or equivalent*

## **5. Solvent exchange procedure**

### **5.1 Sample preparation**

- 5.1.1. Hold the glass ampoules of RM 8027 vertically and open by flipping the top section with the thumb. Wrap the ampoule in the paper tissue to prevent spilling the contents.
- 5.1.2. Transfer ampoule contents into a small clear glass container, held by a chemical clamp.
- 5.1.3. Insert a 1 mm Internal Diameter (ID) teflon gas tubing into the glass vessel all the way to the container bottom.
- 5.1.4. Open gas valve and purge the vessel contents for 30 min. Purging rate (5 to 10) gas bubbles/s. Reduce gas flow if splashing occurs.

### **5.2 Photoassisted hydrosilylation and solvent evaporation**

- 5.2.1. Following 30 min initial purging add 40  $\mu\text{L}$  of allylamine per 1 mL of RM8027 (for amine terminated SiNP's) or 10  $\mu\text{L}$  of acrylic acid ( for carboxyl terminated SiNP's).
- 5.2.2. Continue purging for 10 min, then place the UV source adjacent to the sample vial and turn on the UV radiation. Use UV A illumination for the allylamine terminated



preparations and UV B for the acrylic acid passivated particles. Note: **Avoid exposure to UV light.**

5.2.3. Continue the inert gas flow under UV illumination until the complete evaporation of the solvent. Typically it takes from (4 to 6) h for full solvent evaporation, depending on the inert gas flow, sample volume and ambient temperature.

5.2.4. Turn off the UV source and the gas flow. Cover the vial with an appropriate cover or parafilm. The yellowish residue should be visible on the bottom of the vial.

### **5.3 Particle redispersion in an aqueous solvent**

5.3.1. Add 1 mL of the appropriate buffer or Deionized (DI) water to the glass vial. Note: the unbuffered allylamine terminated Si NP solution after reconstitution in DI water will be basic (pH = 10 to 11) and slightly acidic (pH = 3.5 to 4.5) for acrylic acid terminated SiNP's.

5.3.2. Cover the vial and place in a sonicator bath.

5.3.3. Turn on the sonicator and sonicate the sample for 15 min. The sample at this stage may acquire a milky appearance.

### **5.4 Filtering**

5.4.1 Open the vial and draw its contents into the glass syringe with an appropriate volume.

5.4.2 Mount the 0.2  $\mu\text{m}$  pore size syringe filter and slowly push the syringe contents through the filter into the clean container.

5.4.3 Repeat steps 5.4.1. and 5.4.2. using clean containers, syringes and 0.1  $\mu\text{m}$  and 0.02  $\mu\text{m}$  pore size syringe filters.

5.4.4 Following filtering, the sample should be colorless and transparent.

5.4.5 Check sample pH with an appropriate pH meter.

### **5.5 Reconstitution using Pluronic f127 block copolymer**

- 5.5.1. Hold the glass ampoules of RM 8027 vertically and open by flipping the top section with the thumb. Wrap the ampoule in the paper tissue to prevent spilling the contents.
- 5.5.2. Transfer ampoule contents into a small clear glass container, held by a chemical clamp.
- 5.5.3. Add 0.5 mg of Pluronic F127 to the sample
- 5.5.4. Open gas valve and purge the vessel contents until full evaporation of toluene. Purging rate (5 to 10) gas bubbles/s. Reduce gas flow if splashing occurs.
- 5.5.5. Add 1 mL of the appropriate buffer or DI water to the glass vial.
- 5.5.6. Cover the vial and place in a sonicator bath.
- 5.5.7. Turn on the sonicator and sonicate the sample for 15 min. The sample at this stage may have a milky appearance.
- 5.5.8. Perform filtering as prescribed in 5.4.

**Safety precautions.**

**The describe procedure requires use of hazardous and flammable chemicals. Please consult MSDS for toluene, allylamine, acrylic acid and Pluronic F127. It should be performed under adequate ventilation in the chemical hood. Wear protective clothing and nitrile gloves. Avoid exposure to the UV light.**

## **6 Abbreviations**

NIST	National Institute of Standards and Technology
RM	reference material
EHS	environmental, health, and safety
SiNP	silicon nanoparticle
DLS	dynamic light scattering
UV/VIS	ultraviolet-visible
UV	ultraviolet

NP	nanoparticle
LED	light emitting diode
PL	photoluminescence
ZP	$\zeta$ - potential
MSDS	Materials safety data sheet

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## **Appendix A. Properties of the RM802 reconstituted in the aqueous solvent**

### **A1. Particle size measurement using DLS**

A1.1. Blow dust from the Kimble-Chase 6x50 mm disposable glass culture tube with compressed air.

A1.2. Load 300 microliters of the filtered sample into the culture tube.

A1.3. Cover the tube top with parafilm and centrifuge for 5 min at 14,000 rpm to remove residual dust and larger particulates.

A1.4. Place the sample containing tube in the DLS instrument sample mount and allow temperature to equilibrate for 5 min.

A1.5. Set the detector at the backscattering mode (angle 173 degrees relative to the exciting laser beam).

A1.6. Enter solvent viscosity and index of refraction data. Viscosity of pure water  $\rho = 0.933$  cpoise and index of refraction  $n = 1.333$ .

A1.7. Perform data acquisition for 3 min.

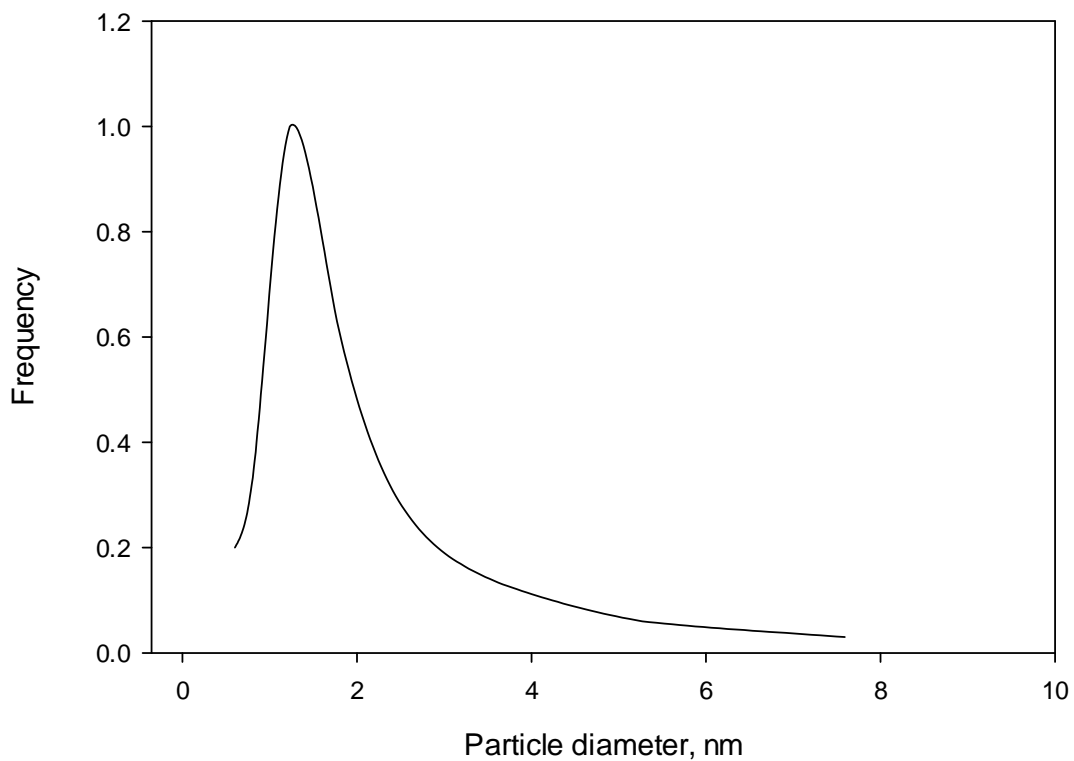


Figure A1 A typical particle size distribution of the RM8027(allylamine modified) in aqueous solvent (DLS data), produced by averaging three sample measurements. Mean particle diameter  $d = 1.9 \text{ nm} \pm 0.3 \text{ nm}$ , where errors indicate one standard deviation.

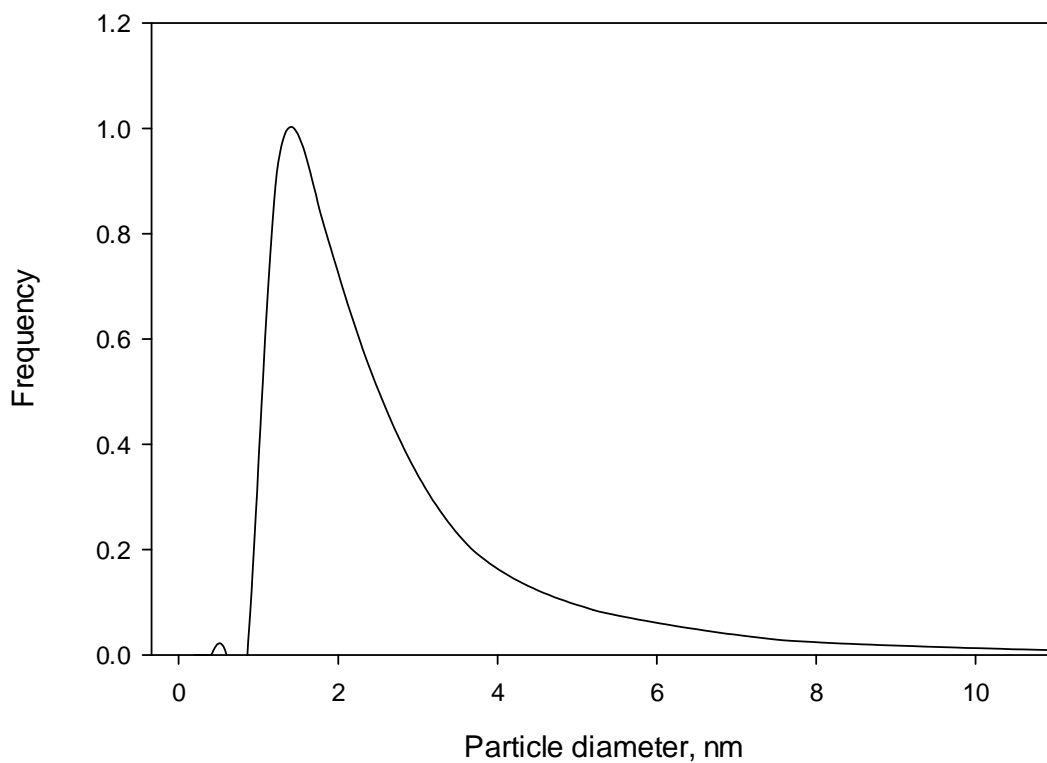


Figure A2 A typical particle size distribution of the RM8027 (acrylic acid modified) in aqueous solvent (DLS data) produced by averaging three sample measurements. Mean particle diameter  $d = 1.8 \text{ nm} \pm 0.4 \text{ nm}$ , where errors indicate one standard deviation.

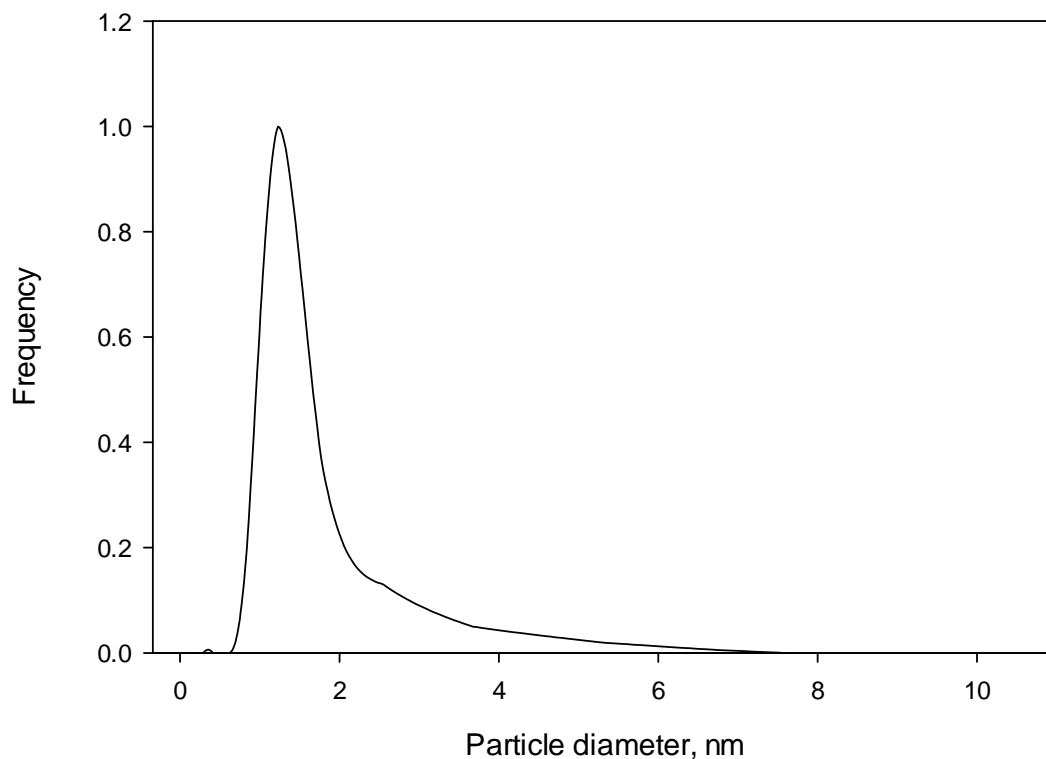


Figure A3 A typical particle size distribution of the RM8027 (reconstituted using Pluronic F127) in aqueous solvent (DLS data) produced by averaging three sample measurements. Mean particle diameter  $d = 1.6 \text{ nm} \pm 0.2 \text{ nm}$ , where errors indicate one standard deviation

## A2. UV-VIS Absorbance

A2.1. Dilute sample x5 with DI water or appropriate media

A2.2. Record reference absorbance using pure DI or media using 1cm optical path length quartz cuvette (Starna Cat# 1-Q-10 or equivalent)

A2.3. Record sample absorbance

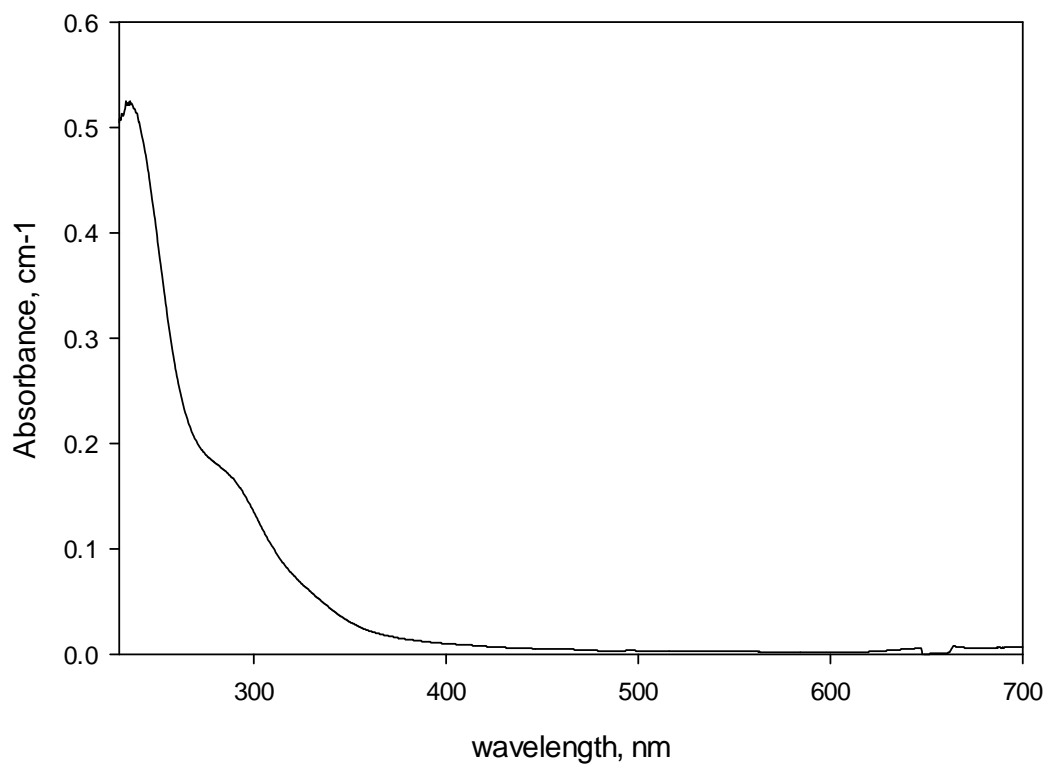


Figure A4 A typical UV/VIS absorbance spectrum of RM8027 reconstituted in the aqueous media using allylamine.



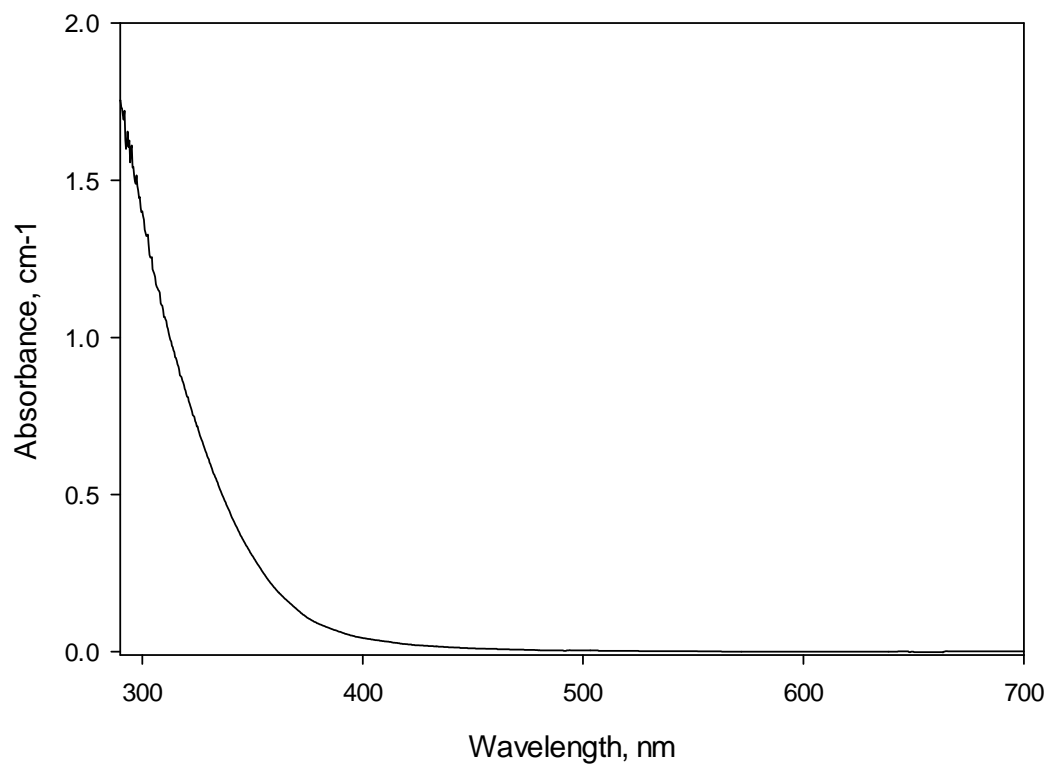


Figure A5 A typical UV/VIS absorbance spectrum of the RM8027 reconstituted in water using Acrylic acid.

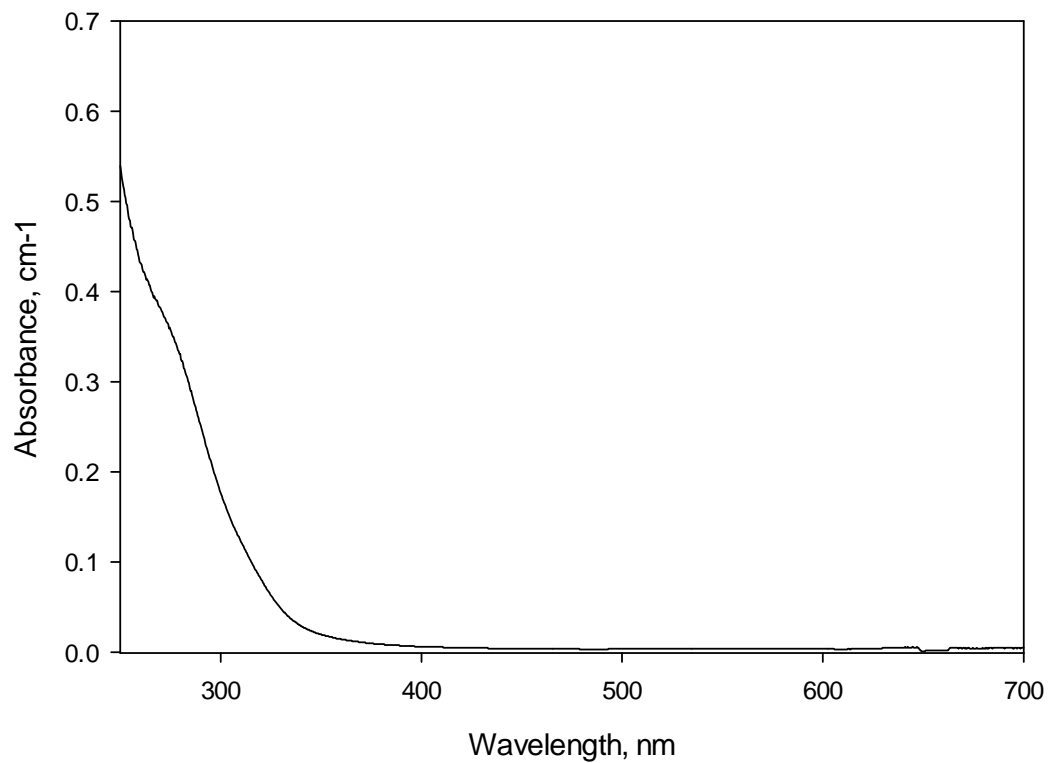


Figure A6 A typical UV/VIS absorbance spectrum of the RM8027 reconstituted in water using Pluronic F127.

### A3. PL spectra

A3.1. Load the reconstituted sample into 1 cm quartz fluorescence cuvette (Starna Cat# 1-Q-10 or equivalent)

A3.2. Record the emission spectrum in the range from 350 nm to 600 nm using 340 nm excitation

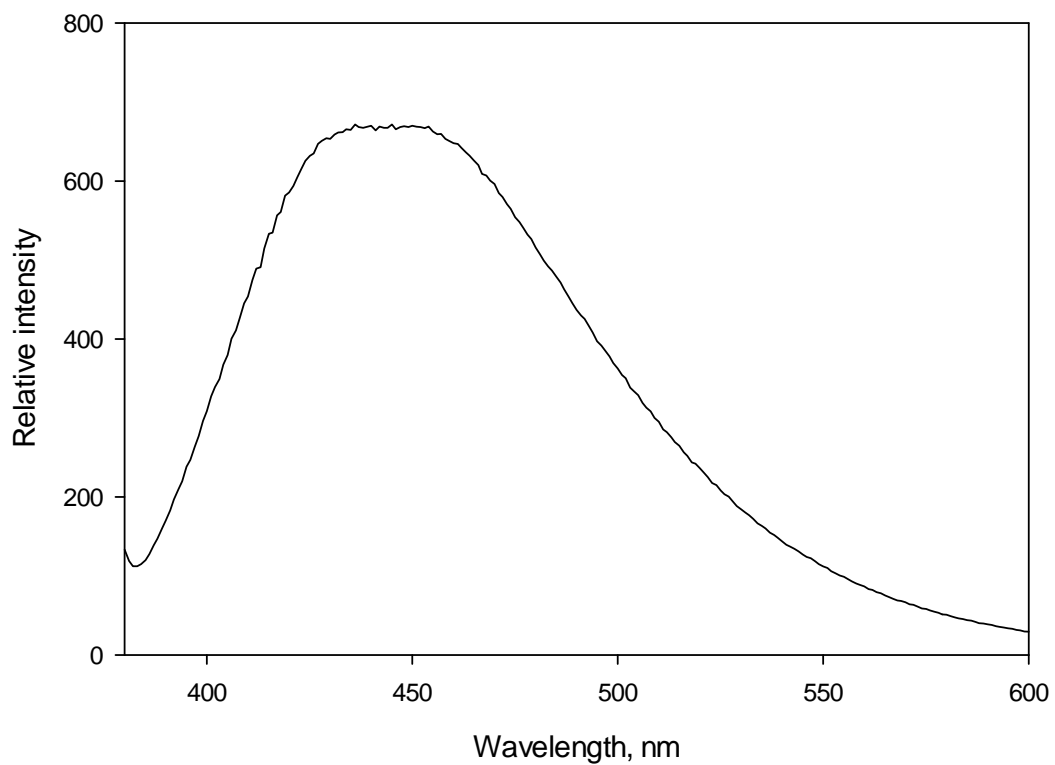


Figure A7 A typical PL (corrected) spectrum of RM8027 in the aqueous media. Allylamine modification.  $\lambda_{exc}=370$  nm

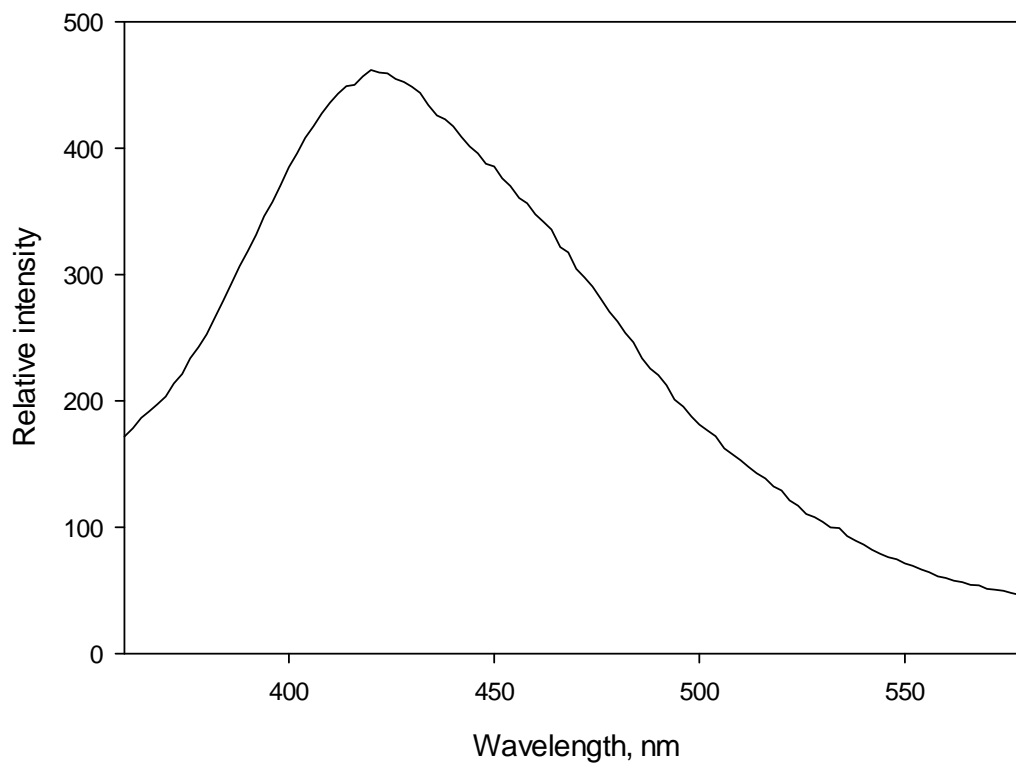


Figure A8 A typical PL (corrected) spectrum of RM8027 in the aqueous media. Acrylic acid modification  $\lambda_{exc}=340$  nm

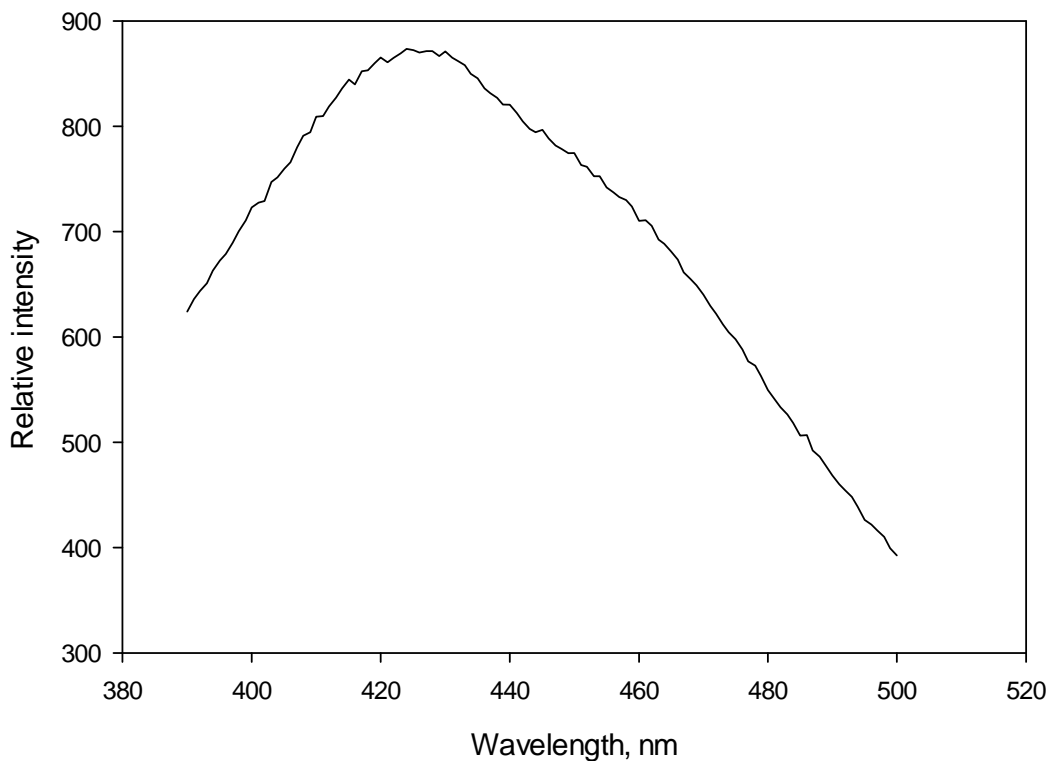


Figure A9 A typical PL (corrected) spectrum of the RM8027 reconstituted in water using Pluronic F127.  $\lambda_{exc}=370$  nm

#### **A4. ZP measurement**

A4.1. Load 3 mL of reconstituted sample into 1 cm pathlength clear plastic disposable cuvette

A4.2. Insert the electrode assembly into the plastic cuvette.

A4.3. Connect the electrode assembly to the instrument insert the sample cuvette into the temperature controlled holder and select  $\zeta$  potential in the instrument software menu.

A4.4. Set electric field strength at 3V/cm.

A4.5. Wait 15 min for the temperature equilibration.

A4.6. Acquire data for at least 1 min.

Representative ZP distributions are provided in Figure A10

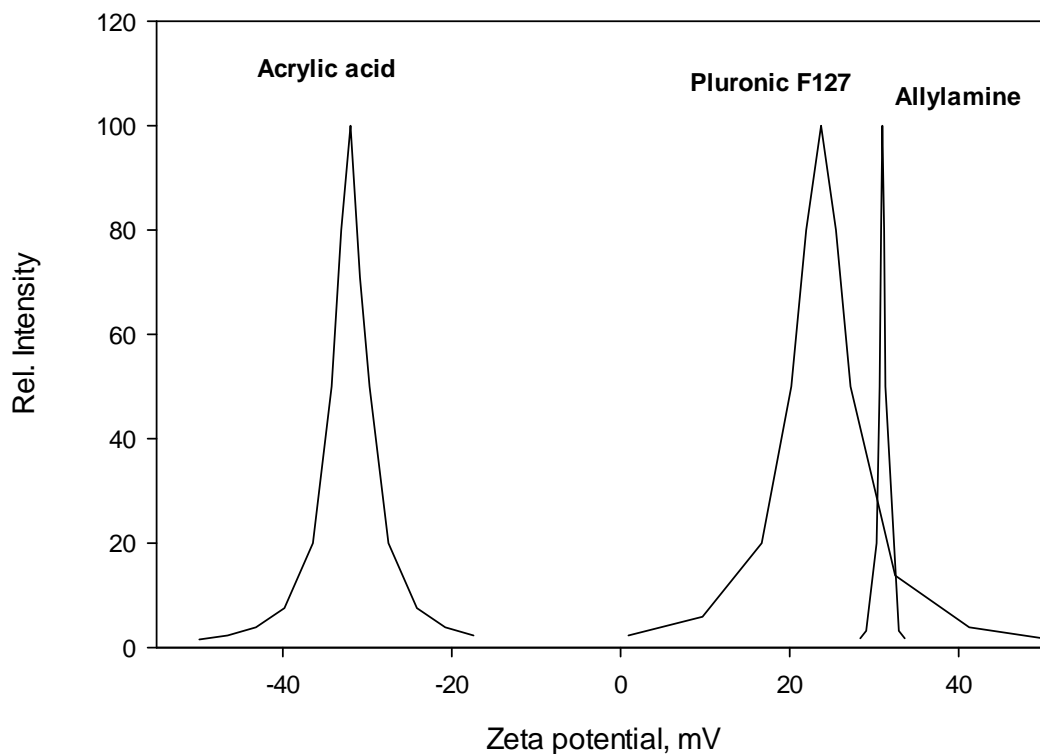


Figure A10 Typical ZP distribution profiles for acrylic acid, Pluronic F127 and Allylamine modified RM8027 samples in DI water, produced by averaging three sample measurements. Average values: Allylamine modified sample  $\zeta = (30.9 \pm 1.2)$  mV; Acrylic acid modified sample  $\zeta = -(21.9 \pm 4.5)$  mV; Pluronic F127 modified particles  $\zeta = (23.7 \pm 7)$  mV. Errors indicate one standard deviation.