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Surface etching of methacrylic microparticles via basic hydrolysis and introduction of functional groups for click chemistry

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ABSTRACT

Controlled basic hydrolysis of poly(methyl methacrylate-co-ethylene glycol dimethacrylate) P(MMA-co-EGDMA) microparticles with a diameter d_{50} = 6 μm led to high densities of carboxylic groups at the particles' surface of up to 1.288 μeq g^{-1} (equivalent to 1.277 μmol m^{-2}). The microparticles' core has not been altered by this surface activation procedure as seen by fluorescent staining. The kinetics of the hydrolysis reaction was investigated via electrophoretic light scattering and particle charge detection employing polycation titration under shear condition. The activated microparticle's surface was subsequently exploited in carbodiimide-mediated coupling reactions using a variety of molecular reactants, that is, 11-azido-3,6,9-trioxaundecan-1-amine, cysteamine, propargylamine, and fluoresceinamine, thus enabling the introduction of chemically reactive moieties such as azides, thiols, and alkynes. Fluorescent staining of the particles' surface successfully demonstrated the versatile applications of surface functionalized microparticles via copper-catalyzed huisgen cycloaddition. Carrying on this two-step procedure in a controlled manner provides an excellent way for relatively simple but highly effective surface functionalization.

1. Introduction

Microparticles have acquired significant impact in many areas during the past decades. They are commonly used for controlled drug delivery applications [1,2] and have been applied as solid support for the immobilization of various catalytic moieties in chemical synthesis [3–6]. The required degree of swelling as well as their porosity can be tailored for analytical application in chromatography [7,8], while non-porous microparticles have been utilized as toners in electro photographic printing [9–11]. In contrast to nanoparticles, the bigger size allows for their simple large scale processing (i.e., filtering and agglomeration-free drying), while their high surface to volume ratio causes specific advantages in comparison with the bulk materials [3].

Specifically, methacrylic microparticles provide many useful features due to their commercial availability, numerous well investigated synthesis routes (i.e., suspension polymerization [12], precipitation polymerization [13], dispersion polymerization [14]), and their high biocompatibility [15]. Furthermore, the mechanical properties of methacrylates can be altered over a wide range by copolymerization [16] and formulation [17,18].

The surface functionalization of microparticles gives rise to further interesting applications [19], although the methacrylic ester group does not allow for binding of most functional groups. This disadvantage is often overcome by copolymerization with functional monomers (e.g., hydroxyethylmethacrylate), which implement anchor groups at the particles' surface [20]. However, the functional surface density strongly depends on the copolymer composition and affects the bulk properties in comparison with the unfunctionalized poly(methacrylates) [21].

The free carboxylic derivative, poly(methacrylic acid), would enable the coupling of numerous available amines [22] as well as alcohols [23] in addition to the opportunity for electrostatic modifications [24]. However, copolymerization of most acrylic monomers with acrylic acid and methacrylic acid will lead to submicron particles due to an emulgator free emulsion polymerization process, which bases on the in situ generation of an oligomeric macrosurfactant [25,26].

In contrast, hydrolysis of poly(methacrylates) provides a simple route to highly surface functionalized microparticles via post-synthesis modification without altering their bulk properties. After Baumann et al. had reported about the saponification of poly(methyl methacrylate), P(MMA), for the first time [27,28], Sandner

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and Bischof studied the basic hydrolysis of dissolved and swollen poly(methacrylates) in different organic solvents [27], while Semen and Lando investigated the reaction of homogeneously dissolved P(MMA) in concentrated sulfuric acid [29]. Interestingly, the aqueous approach for the functionalization of methacrylic surfaces has only been mentioned briefly in some publications [30–33]. It has never been explored regarding its temperature dependent kinetics and the impact of different alkaline concentrations.

In this article, we provide a detailed kinetic investigation about the controlled surface functionalization of acrylic microparticles in respect to the hydrolysis time, temperature, and the sodium hydroxide concentration. Furthermore, we have studied the subsequent carbodiimide-mediated coupling of numerous functional amines onto the generated carboxylic group. Various chemically valuable functionalities, comprising of thiol, alkyne, and azide, were bound onto the particles's surface and allow for further versatile modifications via huisgen cycloadditions as well as thiol-ene reaction [34–36].

2. Materials and methods

Propargylamine (98%), cysteamine (95%), 11-azido-3,6,9-trioxaundecan-1-amine (90%), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 98%), fluoresceinamine ($\geqslant$75%, mixture of isomers), copper (II) sulfate pentahydrate (98%), l-ascorbic acid (99%), N-hydroxy succinimide (98%), and poly(methyl methacrylate-co-ethylene glycol dimethacrylate) particles were purchased from Sigma–Aldrich GmbH, Steinheim, Germany and used without further purification. Sodium hydroxide (>99%, AppliChem GmbH, Darmstadt, Germany), poly(diallyldimethylammonium chloride) (BTG Instruments GmbH, Herrsching, Germany), and the coumarin dye 3-azido-7-hydroxy-chromen-2-one (iON Blue™, A&Q NanoDesigns LLC, Columbia, USA) were used as received.

2.1. Surface hydrolysis

Hydrolysis of the acrylic particles (5.0 g) was performed in an Oak Ridge polypropylene copolymer (PPCO)-centrifuge tube by adding the dispersion medium (25 mL). The particles were fully wetted for 20 s using a vortex mixer (Uzusio VTX-3000L, LMS Co., Ltd., Tokyo, Japan) and dispersed afterward in a heated laboratory shaker (Inkubator 1000, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Hydrolysis was stopped by filtration and redispersion in phosphate buffer (c = 100 mM, pH = 7.0) for three times followed by washing the particles in H_2O for three more times. The particles were dried under reduced pressure (p < 10 mbar) for 48 h. Aqueous sodium hydroxide solutions were created from solid sodium hydroxide pellets in pure water (Milli-Q®, Millipore GmbH, Schwalbach, Germany).

The sodium hydroxide/potassium chloride-buffer (pH = 13.3) was created by adding an aqueous sodium hydroxide solution (3 M, 725.3 mL) to an aqueous potassium chloride solution (3 M, 274.7 mL). The hydrochloride acid/potassium chloride-buffer (pH = 0.7) was created by adding an aqueous hydrochloride acid solution (3 M, 728.3 mL) to an aqueous potassium chloride solution (3 M, 271.7 mL).

2.2. Carbodiimide-mediated coupling

The carbodiimide-mediated reaction of functional amines was performed at the surface of particles, which had been hydrolyzed with a sodium hydroxide concentration c_{NaOH} of 10 M at 37 °C for 72 h. The hydrolyzed particles (3.0 g) were placed into an Oak Ridge PPCO-centrifuge tube and dispersed in 2-(N-morpholino)ethanesulfonic acid (MES)-buffer (15 mL, 100 mM, pH = 5.0). In relation to the titrated carboxylic groups at the particles' surface (3.86 μmol), a tenfold molar excess of the functionalizing reagents dissolved in dimethyl sulfoxide (DMSO) was added, for example, 2.47 μL (39 μmol) of propargylamine. The coupling was started by the addition of a hundredfold excess of EDC, for example, 74.1 mg (386 μmol) and a fortyfold excess of N-hydroxy succinimide, for example, 17.8 mg (155 μmol). The particles were dispersed for 48 h at room temperature and subsequently cleaned by filtration and redispersion in phosphate buffer (c = 100 mM, pH = 7.0) for three times followed by washing in water for three more times. The particles were dried under reduced pressure (p < 10 mbar) for 48 h.

2.3. Coupling of the coumarin dye

The copper-catalyzed reaction of the coumarin dye, 3-azido-7-hydroxy-chromen-2-one, was performed at the surface of propargylamine-functionalized particles. The amount of functional groups at the particles' surface (1.260 μmol g^{-1}) was determined by titration of the remaining carboxylic groups after the modification. Stock solutions of the coumarin dye 3-azido-7-hydroxy-chromen-2-one (2.95 mM in dimethyl sulfoxide, DMSO), copper sulfate pentahydrate (4.0 mM in H_2O) as well as l-ascorbic acid (7.95 mM in DMSO) were prepared. Afterward, propargylamine-functionalized particles (37.5 mg) were dispersed in 1242 μL DMSO and 80 μL of the coumarin dye solution (236 nmol, 5.0 equivalents in relation to the titrated functional groups), 59 μL of copper solution (236 nmol, 5.0 equivalents in relation to the titrated functional groups) as well as 119 μL (946 nmol, 20 equivalents in relation to the titrated functional groups) were added. The particles were dispersed for 24 h at room temperature and subsequently cleaned by centrifugation (15,000 rpm, 21,380g, 10 min, Microcentrifuge Z 216 MK, Hermle Labortechnik GmbH, Wehingen, Germany) and redispersion in DMSO for three times followed by washing in H_2O for three more times.

2.4. Particle charge detector (PCD)

Specific surface charge was determined potentiometrically with the particle charge detector Mütek PCD-03pH (BTG Instruments GmbH, Herrsching, Germany) connected to a Titrino 702SM (Metrohm AG, Herisau, Switzerland) at room temperature. Dispersion of 400 mg particles in 15 mL phosphate buffer (c = 10 mM, pH = 7.0) was titrated with an aqueous poly(diallyldimethylammonium chloride) solution (PDADMAC, c = 1.60 mM, molecular weight 1.07 x 10^5 g mol^{-1}).

2.5. Electrophoretic light scattering

Zeta potentials were determined with a Zetasizer Nano ZS (Malvern Instruments GmbH, Herrenberg) at 25 °C. 10 mg of particles was dispersed in 4 mL of phosphate buffer solution (c = 10 mM, pH = 7.0). Buffers with the same ionic strength (I = 10 mM) were prepared according to a procedure, which had been reported previously by Perrin [37].

2.6. Laser confocal microscopy

Microscope images were taken with a laser scanning microscope LSM 710 (Carl Zeiss Microscopy GmbH, Jena) at the excitation wavelength λ = 405 nm for images of coumarin functionalized particles, which were suspended in phosphate buffer at pH 7.0. Images of fluoresceinamine functionalized particles were taken at the excitation wavelength λ = 488 nm in phosphate buffer at pH 7.4.