Accurate non-invasive determination of pK_a of surface functionalised ion exchange monoliths using capacitively coupled contactless conductivity detection

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Reagents and instrumentation.

Butyl methacrylate (99%, BuMA), ethylene dimethacrylate (98%, EDMA), benzophenone (99+%, BP), 3-(trimethoxysilyl)-propyl methacrylate (98%), *m*-aminophenylboronic acid hemisulfate salt (APBA), poly-(ethylene glycol) methacrylate (PEGMA), decanol, 2,2-dimethoxy-2-phenylacetophenone (99%, DAP), 1,4-butanediol, tert-butyl alcohol (99.5%), sodium hydroxide. and all buffers were purchased from Sigma Aldrich (Dublin, Ireland). Vinyl azlactone (4,4-dimethyl-2-vinylazlactone, VAL) was purchased from TCI Europe. Hydrochloric acid was purchased from Fluka. Methanol, acetone, and 1-propanol were purchased from Labscan (Stillorgan, Dublin, Ireland). All other reagents were of the highest available purity and used as received without additional purification or distillation before use.

The pump used for washing and equilibration of the monolithic capillary column was a quaternary gradient HP 1050 pump operated in isocratic mode at all times. The pump flow was split by the use of a T-piece connector with a section of 50 μm fused silica capillary (Polymicro Technologies, Phoenix, AZ) on the waste side to create sufficient back pressure such that the required split flow was achieved. The operational flow rate through the monolith was 1 μL min^{-1}. The pump was also used to fill the pores of pre-formed monoliths with reactive functional monomers for subsequent photo-grafting, by placing a section of wide bore PEEK tubing (~ 350 μL) filled with the monomer, between the pump and the column. Deionised water was produced with a Millipore Direct-Q 5 (Millipore, Bedford, MA, USA).

The UV source was a Spectrolinker XL-1000 UV crosslinker (Spectronics Corp., Westbury, NY). The operational wavelength of this lamp was 254 nm with an irradiation intensity of 1 J cm^{-2} per dose incident on the capillary/monolith. Monoliths were produced in UV-transparent Teflon-coated fused silica capillary (Polymicro Technologies, Phoenix, , AZ) with sections of PEEK micro-tight sleeves used as photomasks. The UV dose used for the photoinitiated polymerisation of the monolith was 2 J cm^{-2} and 3 J cm^{-2} for photografting of isolated VAL zones onto the pre-formed monolith.

Prior to the photografting of VAL, the column was rendered hydrophilic by the photografting of polyethylene glycol methacrylate onto the surface of the monolith using the two-step photografting procedure described by Stachowiak *et al.*[9]. Briefly, benzophenone (50 mg mL^{-1} in methanol) was grafted onto the entire length of the monolith without the use of a photomask using a total UV dose of 3 J cm^{-2}. The excess benzophenone was washed from the column with methanol for 30 minutes at 1 μL min^{-1}. A solution of 36 μL mL^{-1} polyethylene glycol methacrylate (PEGMA) in water was pumped into the column and photografted using a total UV dose of 3 J cm^{-}

[2]. Excess PEGMA was washed from the column with water at 1 μL min^{-1} for 30 minutes.

Simplified synthetic approach to boronic acid functionalisation

Covalent bonding of APBA to poly(glycidyl methacrylate-co-ethyleneglycol dimethacrylate), monolithic capillary columns has previously been reported by Potter *et al.*[7] using nucleophilic attack of the epoxide with p-hydroxyphenylboronic acid. In an effort to increase the surface coverage of boronic acid groups on the monolith, Potter also grafted chains of glycidyl methacrylate groups onto the surface of the monolith prior to reaction with the p-hydroxyphenylboronic acid. In this paper however, the approach of using VAL chemistry to covalently bind the APBA to the monolith surface is exploited. Vinyl azlactone chemistry has previously been utilized for the immobilization of proteins in the past via the amine group on lysine[5]; in this work VAL is use to immobilize APBA onto a monolithic stationary phase via the free amine group on the APBA molecule. Potter's immobilization strategy is quite lengthy, taking 20 hours at 60 ^{0}C, whereas the VAL method takes only 3 hours at room temperature. In addition, Potter et al. used triethylamine as a base in the reaction (the reaction required alkaline conditions to occur), but found that a positive surface charge was obtained since the monolith exhibited an anodic EOF. Potter et al. postulated that the triethylamine had also acted as a nucleophile and had attacked the epoxy groups resulting in immobilized charged ammonium groups on the monolith in addition to the immobilized boronic acid groups. The immobilization strategy proposed herein (VAL) results in no such unwanted immobilization of other functional groups, since the APBA is presented to the azlactone grafts in water alone.