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One single, fast and robust capillary electrophoresis method for the direct quantification of intact adenovirus particles in upstream and downstream processing samples

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ABSTRACT

During development of adenovirus-based vaccines, samples have to be analyzed in order to either monitor the production process or control the quality and safety of the product. An important quality attribute is the total concentration of intact adenoviruses, which currently is determined by quantitative polymerase chain reaction (qPCR) or anion exchange-HPLC. Capillary Electrophoresis (CE) was evaluated as alternative to the current methods with the aim to have one single method that allows reliable and fast quantification of adenovirus particles throughout the full process. Intact adenoviruses samples from downstream processing and upstream processing were analyzed directly by CE with UV-detection at 214 nm. Only the samples with high amounts of DNA required a simple sample pretreatment by benzonase. Adenovirus particles were separated from matrix components such as cell debris, residual cell DNA, and/or proteins on a PVA-coated capillary using a BGE consisting of 125 mM Tris, 338 mM tricine and 0.2% v/v polysorbate-20 at pH 7.7. Full factorial design of experiments was used for method optimization as part of the analytical quality by design (AQbD) method development approach. The method was validated for the quantification of adenoviruses on five representative samples from the manufacturing process in the range of 0.5×10^{11}–1.5×10^{11} adenovirus particles per ml (~80 to 250 pmol/l). The CE method showed intermediate precision of 7.8% RSD on concentration and an accuracy (spiked recovery) of 95–110%. CE proved highly useful for process development support and is being implemented for in-process control testing for adenovirus vaccine manufacturing.

1. Introduction

Adenoviruses are non-enveloped viruses with icosahedral symmetry (252 capsomers) and diameters of 70–90 nm [1,2]. The virus particle is built up of ten different structural proteins, with molecular weights ranging from 5 to 120 kDa, which form the capsid. The capsid encloses double stranded linear viral DNA. The total molecular weight of an adenovirus is about 150 MDa. Adenovirus-based vaccines currently under development make use of the ability of the virus to function as a vector for intracellular delivery of DNA. The viral DNA can be modified to encode for e.g. specific proteins that, upon expression, function as vaccine antigens. The Advac® [3,4] vectors are modified by inserting in the viral genome a piece of DNA that encodes for the antigen of interest, e.g. the envelope protein of human immunodeficiency virus (HIV) or the Ebola virus [3]. This insert will be expressed in the host cell upon vaccination and the corresponding protein is expected to

Abbreviations: Ad26, adenovirus serotype 26; Ad35, adenovirus serotype 35; Ad5, adenovirus serotype 5; AEX, anion exchange product; AEX-HPLC, anion exchange HPLC; AQbD, analytical quality by design; ATP, analytical target profile; CH, clarified harvest; CR, crude harvest; DF, diafiltration/ultrafiltration product; DoE, Design of Experiments; DS, drug substance; DSP, downstream processing; HIV, human immunodeficiency virus; LH, lysed harvest; PB, polybrene; qPCR, quantitative polymerase chain reaction; USP, upstream processing; SMIL, successive multiple ionic polymer layer

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induce a protective immune response against HIV or Ebola. The use of adenovirus serotype 26 (Ad26) and serotype 35 (Ad35) as vectors for vaccines is preferred with respect to the more common adenovirus type 5 (Ad5), since most people have neutralizing antibodies against this serotype [5,6]. Ad5, Ad26, and Ad35 differ in protein and DNA composition and, therefore, have different physicochemical properties [7].

During development of adenovirus-based vaccines on and for production of clinical trial material, samples have to be analyzed in order to either monitor the production process or control the quality, safety and efficacy of the product. An important quality attribute is the total concentration of intact adenoviruses, which currently is determined by quantitative polymerase chain reaction (qPCR) or anion-exchange HPLC (AEX-HPLC) [8,9]. The qPCR method consists of DNA amplification of a sequence specific for the adenovirus and subsequent detection of the formed amplicon after fluorescent labelling. An adenovirus reference sample with a known concentration of virus particles is used for calibration. qPCR is an expensive (due to cost price of chemicals) and laborious technique. The time-to-result, which is the total time it takes from sampling until reporting the processed and reviewed analytical result, can be three to four days, since several replicates are required to attain acceptable precision [9].

AEX-HPLC with UV detection yields a charge-based separation of intact adenoviruses from sample matrix components. Quantification is by external calibration using an adenovirus reference standard. The AEX-HPLC method suffers from carry-over and recovery issues for real samples containing cell lysate and/or high salt concentrations, and is therefore not suitable for the accurate and precise determination of the concentration of adenoviruses throughout the entire production process.

In order to support process development for adenovirus-based vaccines, it would be beneficial to have one single method that allows reliable and fast quantification of adenovirus particles throughout the full process. An analytical technique that could meet these targets is CE in which analytes are separated in an electric field based on their charge-to-size ratio. CE can provide high efficiency and resolution for diverse analytes, from small molecules to macromolecules (e.g. peptides and proteins) and even particles [10–12]. CE has proven to be suitable for the characterization of intact poxvirus [13], human rhinovirus [14–16], vesicular stomatitis virus [17], tobacco mosaic virus [18,19] and influenza [20]. Notably, most reported virus concentration analyses by CE involved indirect quantification by measuring viral protein or DNA/RNA content and not the intact virus particles. Mann et al. [21] analyzed intact adenovirus type 5 (Ad5) particles by CE, using a PVA coated capillary with a phosphate buffer of pH 7. Samples were filtrated and buffer exchanged prior to sample analysis. The authors showed the potential of CE for intact Ad5 analysis, however, the accuracy and precision of the method were not assessed. Moreover, the suitability of the CE method for different samples met in the successive stages of the production process was not evaluated. The different matrices may contain, for instance, different levels of cell debris, cellular proteins, DNA, detergents, additives, or buffer components. Oita et al. [22–27] developed a CE method for analysis of the poliovirus in samples from downstream processing. The main challenge in these studies was the matrix effect on the analytical result and Oita concluded that the sample matrix ideally should have a constant composition [27].

In this paper, we describe the development of a CE-based method which should allow precise and accurate analysis of adenovirus samples containing variable amounts of cell debris, cell lysate, host cell proteins, host cell DNA, salts, detergents, and/or additives. An analytical Quality by Design (AQbD) methodology was used for method development. QbD is a scientific, risk-based, holistic and proactive approach originally defined for product development. QbD works with predefined objectives and emphasizes product and process understanding and process control. This is just as valid for analytical method development and AQbB has been adapted for several years in the (bio) pharma industry. The objective of the current method development was to obtain a reliable, efficient and robust cost-saving method for the quantification of intact adenovirus types Ad26 and Ad35 particles in upstream processing (USP) and downstream processing (DSP) samples. The analytical target profile (ATP) compromised precision < 10% RSD on intact adenovirus particle concentration, accuracy (as spiked recovery) between 90 – 110% over a range of 0.5×10^{11}–1.5×10^{11} adenovirus particles per ml (~80 – 250 pmol/l), with a time-to-result of less than 1 day. A representative set of samples was selected from USP and DSP and was used to optimize separation between the intact adenovirus particles and the matrix components. The BGE buffer, pH and additives, and capillary temperature, coating and conditioning were studied to prevent virus adsorption to the capillary wall. The sample pretreatment was investigated for samples containing high amounts of cellular DNA. The CE method development was finalized with a validation study including a comparison with qPCR.

2. Materials and methods

2.1. Chemicals and materials

Extended light path PVA capillaries (PN G1600-61239) of 50 μm id with a total length of 64.5 cm and bare-fused silica capillaries (PN G1600-63211) of 50 μm id with a total length of 33 cm were from Agilent technologies (Waldbronn, Germany), and eCAP neutral capillary 50 μm id with 67 cm total length (PN 477441) from Beckman Coulter (Woerden, the Netherlands). Tris(hydroxymethyl)aminomethane (PN 1083861000), ortho-Phosphoric acid 85% (PN 100573), Polysorbate-20 (PN 655204), and Benzonase (PN 1.01697) were from Merck Millipore (Amsterdam-Zuidoost, The Netherlands). Tricine (PN 93356), Triton X-100 (PN X100), polybrene (PN 107689), dextran sulfate sodium salt from Leuconostoc spp. (PN D8906), 2-mercaptoethanol (PN 63689), and phthalic acid (PN P39303) were obtained from Sigma Aldrich (Zwijndrecht, the Netherlands). SDS 10% solution (PN 24730-020) was obtained from Invitrogen (Bleiswijk, the Netherlands). Adenovirus Type 5 Reference Material (PN VR-1516) from ATCC (Wesel, Germany). Formulation buffer, MgCl 1 M solution, and Ad26 crude harvest, Ad26 lysed harvest, Ad26 clarified harvest, Ad26 anion-exchange product, Ad26 diafiltration/ultrafiltration product, Ad26 drug substance, Ad35 crude harvest, Ad35 reference material and Ad26 reference material were from Crucell Holland (Leiden, the Netherlands). Forward primer (CMV, MGB) 5′-TGGGCGGTAGGCGTGTA-3′ (PN 4304972), reverse primer (CMV, MGB) 5′-CGATCTGACGGTTCACTAAACG-3′ (PN 4304972), and probe (CMV-2, MGB) 5′-VIC-TGGGAGGTCTATATAAGC-MGB-NFQ-3 (PN 4316032) were obtained from Applied Biosystems (Gent, Belgium).

2.2. Instrumentation and CE method

All CE experiments were performed using an Agilent 7100 Capillary Electrophoresis system comprising a UV–Visible diode-array detector (Waldbronn, Germany). Data processing was performed on Chemstation software (B.04.03). In the final method extended light path PVA-coated capillaries of 50 μm id (375 μm od) were used, cut to a total length of 33 cm. Capillary preconditioning was performed prior to each run with 10 mM phosphoric acid and BGE at 2.5 bar for 1 min each. The BGE consisted of 125 mM Tris – 338 mM tricine (pH 7.7), and 0.2% polysorbate-20. Sample injection was at 50 mbar for 5 s at the short end of the capillary (8.5 cm effective length). The separation voltage was −25 kV and the capillary was thermostated at 15 °C. UV-absorbance detection was at 214 nm (4 nm bandwidth).

All samples were diluted with proprietary formulation buffer to an adenovirus concentration of 1×10^{11} vp/ml.