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# An electrical conductivity based method of determining the particle deposition rate in air–liquid interface devices

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## ABSTRACT

A new in-situ method of determining the particle deposition rate onto cell cultures inside air–liquid interface devices is described. It is based on depositing a surrogate aerosol of salt particles onto the water filled wells of a culture plate while measuring the resulting change in electrical conductivity of the solution in situ, in order to derive the accumulated particle mass. For evaluation purposes, the wells of a six-well cell culture plate were equipped with custom designed electrodes and calibrated with a series of commercially available standard solutions. After the necessary corrections prescribed by theory, the calibration resulted in an accuracy and comparability between cells of  $\pm 3\%$  in terms of measured conductivity. The method was then applied to a specific ALI device consisting essentially of the calibrated six-well culture plate inside an electrostatic cross-flow precipitator, and tested with submicron NaCl aerosol of defined size distribution produced by nebulization of a salt solution. 2 h of particle accumulation were sufficient to accumulate between 30 and 10  $\mu\text{g}$  of salt per well, depending on the location in the precipitator. Resulting deposition rates varied narrowly between the wells by about  $2 \text{ ng min}^{-1} \text{ cm}^{-2}$ . Factors affecting the overall accuracy and reproducibility are discussed.

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## 1. Introduction

Air–liquid interfaces (ALI) are the preferred way of exposing cell cultures in vitro to particles under conditions resembling the uptake of aerosols through the respiratory system (Aufderheide, 2008). On the other hand they are also more complex to operate than submerged systems (Seagrave, 2005). Aside from having to provide a suitable environment for the stress-free survival of the cell cultures (BéruBé et al., 2009), one needs to determine the efficiency and rate of particle deposition on the cells (Paur et al., 2011) – which depends on particle size and operating conditions – in order to calculate an exact dose (Lenz et al., 2009). This makes an accurate characterisation of such exposure devices quite challenging (Stevens et al., 2008).

Precipitation efficiencies in ALI devices have typically been inferred from the decrease in aerosol concentration during passage through the device, measured with standard on-line methods such as SMPS (Bitterle et al., 2006; de Bruijne et al., 2009; Savi et al., 2008; Volckens et al., 2009). While this approach is fast and relatively accurate, it is indirect and cannot distinguish between particle deposition onto cell culture surfaces and wall losses in general (Savi et al., 2008; Volckens et al., 2009).

Direct measurements of particle deposition onto the relevant surfaces have mostly relied on the use of fluorescent particles, which were either non-soluble such as monodisperse polystyrene spheres, or contained a fluorescent dye soluble in water or organic solvents (de Bruijne et al., 2009; Savi et al., 2008; Stevens et al., 2008; Volckens et al., 2009; Teeguarden et al., 2007). In both cases the actual measurement took place outside the ALI chamber, either by counting particles on the substrate with a fluorescent optical microscope (Savi et al., 2008), or by dissolving them and subsequently measuring the fluorescence level in the solvent with a spectrophotometer (de Bruijne et al., 2009). The former technique is currently the only practiced method to provide a deposition rate in terms of particle number (de Bruijne et al., 2009), while the latter expresses it in terms of mass (Savi et al., 2008) assuming that particle mass and fluorescent signal are proportional (Burry, 2010; Hoffmann, 2012). The accuracy and reproducibility achieved by fluorescence based studies appear to be limited, although it is not always clear to what extent a specific experimental protocol or the ALI device itself contributed to the overall uncertainty. Spectrophotometric analysis is normally done on the entire sample surface and thus provides a true average of the deposition rate, while microscopy based particle counting on a large sample surface would not be economical. However, averages based on a limited number of randomly chosen locations depend heavily on the uniformity of the deposit. On the other hand, spectrophotometric

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analysis requires solvent extraction that may become a source of potential errors, while particle counting under a microscope is quite accurate, especially when automated.

Another direct approach described in the literature is based on measurements of the deposited particle mass vs. time via the change in resonant frequency of a vibrating piezoelectric crystal (Mülhopt et al., 2009; Lenz et al., 2009). This quartz crystal microbalance (QCM) technique functions in situ, although with a relatively small “field of view” in the order of 40 mm<sup>2</sup>, with a sensitivity range of 3–160 µg cm<sup>-2</sup> and a reported repeatability (i.e. standard deviation of the deposition efficiency divided by the mean deposition efficiency) of 12% (Lenz et al., 2009). The QCM therefore works best with ALI devices that deposit particles in a small spot.

In the following we describe a new, accurate method of characterising the particle deposition rate of ALI type devices in situ, via measurements of the electrical conductivity directly inside the wells of a culture plate. Basically, an inorganic salt aerosol – in our case NaCl – of known particle size distribution and concentration is deposited onto the water filled wells, wherein the particles dissolve rapidly. The resulting increase in electrical conductivity can be used to infer the accumulated particle mass and thus an average mass based deposition rate for each well. Once an ALI exposure chamber is characterised in this way for a range of particle sizes, the deposition rate for arbitrary aerosols of known size distribution and concentration can be calculated. Below we first describe the measurement technique including relevant fundamentals of ion conductivity measurements. As an illustration we then operate it in a model electrostatic precipitator equipped with a six-well culture plate, in order to demonstrate the properties of this technique.

## 2. Materials and methods

### 2.1. The relation between electrical conductivity and electrolyte concentration

The electrical conductivity  $\kappa$  of an ionic solution is basically determined by the concentration and mobility of charge carriers in the liquid. It depends on the viscosity and the dielectric properties of the solvent, the type, charge and activity concentration of the dissolved ions, as well as the geometry of the electrodes between which charge carriers are transported (Czeslik et al., 2009; Atkins and de Paula, 2009).

The conductivity  $\kappa$  of an ionic solution is the product of the molar conductivity  $A_m$  and the electrolyte concentration  $c$

$$\kappa = A_m \cdot c. \quad (1)$$

It is common knowledge that the molar conductivity  $A_m$  is not independent of the concentration  $c$ , i.e. the ratio of  $\kappa/c$  is not a constant, even for strong electrolytes at low concentrations which can be considered fully ionised in solution. This non-ideal behaviour is explained by the relaxation and the electrophoretic phenomena affecting the size and the shape of the hydrate clusters formed around the central ions, as well as by the reciprocal influence of ions moving in opposite directions. For these reasons,  $A_m$  also depends on the frequency of the electrical field (Wright, 2007). In general, the ions move slower than theoretically expected, meaning a lower conductivity must be taken into consideration for an accurate interpretation of data (Czeslik et al., 2009). The molar conductivity  $A_m$  and the electrolyte concentration  $c$  are connected via the “Kohlrausch Square Root Law”,

$$A_m = A_m^0 - (a + b \cdot A_m^0) \cdot \sqrt{c}. \quad (2)$$

The constants  $a$  and  $b$  account for the electrophoresis effect and the relaxation effects described in the Debye–Hückel–Onsager

theory. They depend on the temperature, the dielectric properties and the viscosity of the solvent as well as on the number of electric charges of the ions. Values for  $A_m^0$ ,  $a$  and  $b$  can be found in the literature (Czeslik et al., 2009; Wright, 2007). Even Eq. (2) is only an approximation that works well for certain substances in specific concentration ranges, but requires further corrections in case of weak electrolytes and/or materials with higher or asymmetrical charge numbers (Wright, 2007).

The conductivity  $\kappa$  is typically measured in a conductivity cell as shown in Fig. 1. An external AC voltage  $U$  causes an electrical current flow  $I$  through the solution due to ion oscillation between a pair of immersed electrodes. The electrical resistance  $R$  of the solution, expressed via Ohm’s law, in combination with the electrode distance  $l$  and the cell cross-sectional area  $A$  determines  $\kappa$ :

$$\kappa = \frac{I}{U} \cdot \frac{l}{A} \cdot Z_{field} \cdot k(T). \quad (3)$$

Suitable values for the so called cell constant  $l/A$  depend on the range of conductivities to be measured. For example, low conductivities between about 1 and 100 µS cm<sup>-1</sup> require cell constants of 0.1–0.01 cm<sup>-1</sup> (Heule, 2014). The non-dimensional constant  $Z_{field}$  is a correction factor to adjust the geometrical cell cross-section for distortions of the electrical field around its edges where the field lines are not ideally parallel.  $Z_{field}$  is specific to a certain cell geometry and must be obtained from calibration experiments with solutions of known conductivity (Roloff, 2014). The factor  $k(T)$  accounts for the temperature dependent viscosity (and thus ion mobility) of the solution, by converting the electrical resistance measured at an arbitrary temperature  $T$  to a reference temperature, typically 20 °C or 25 °C. For NaCl a linear temperature compensation is adequate, with suitable factors found in the literature (Flad, 2013).

Finally, the combination of Eqs. (1)–(3) gives an expression in which the electrolyte concentration  $c$  remains the only unknown quantity:

$$[A_m^0 - (a + b \cdot A_m^0) \cdot \sqrt{c}] \cdot c = \frac{I}{U} \cdot \frac{l}{A} \cdot Z_{field} \cdot k. \quad (4)$$

The exact use of this equation for our purposes will be discussed later on, in connection with the cell calibration and the actual concentration measurements.

### 2.2. Experimental setup

The sodium chloride aerosol was generated by dispersing an aqueous sodium chloride solution (3.75 g l<sup>-1</sup>) in air using an

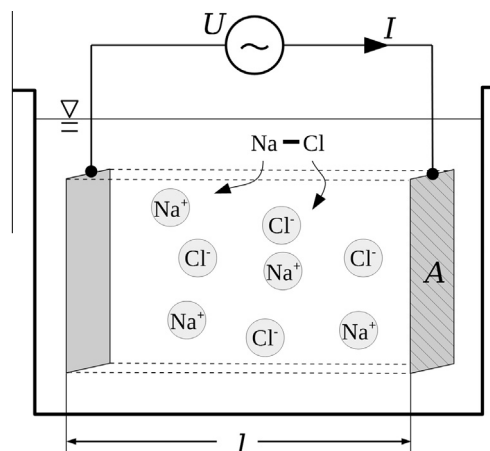


Fig. 1. Schematic diagram of a conductivity cell with electric field lines (dashed) between electrode plates of surface area  $A$ .

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