

Morphological and aerodynamic characterization of airborne pollen grains captured in a Paul trap.

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ABSTRACT

A novel pollen levitation cell was developed enabling a long-term study of pollen suspended in ambient air under controlled conditions. The experimental design is suitable to determine the settling velocity of individual pollen grains concurrent with a direct microscopic inspection. We describe the design, the operation and first applications of this electrodynamic levitation cell adapted to a light microscope. The pollen are levitated in a so-called Paul trap providing a micro-environment of adjustable atmospheric conditions. Nearly motionless trapped pollen are accessible to high-resolution microscopy. Determined stability boundaries of the trap are used to determine the intrinsic design parameters and the settling velocity of single and agglomerated pollen grains. For single birch pollen a settling velocity of 1.57 ± 0.14 cm/s is determined. This value corresponds to a Stokes diameter of $22.8 \pm 1 \mu\text{m}$. The settling velocity agrees very well with results from settling experiments with a sedimentation cell and may establish an alternative to classical sedimentation cell measurement, in future. The instrument, furthermore, is supposed to provide an appropriate approach to study morphological changes arising from varying atmospheric conditions and/or the impact of air contaminants.

Key words

Airborne pollen, agglomerates of pollen, electro-dynamic levitation, morphological characterization, settling velocity

INTRODUCTION

Only little is known about the shape of airborne pollen as a function of meteorological conditions. In literature, pollen are typically presented in their ideal shape shown by fresh pollen or rehydrated pollen embedded in an aqueous solution. However, there is strong evidence that the shape of airborne pollen differs considerably from the shape of fresh or rehydrated pollen. The pollen settling velocity depends on actual shape and constitutes an important parameter for a reliable modeling of pollen dispersal. Observations reveal that pollen can be

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dispersed over much larger areas than commonly expected (Campbell, 1999). This is particularly important in respect to the discussion about cross-pollination of genetically modified plants (GMO) and the spread-out of neophytes due to climate change.

There is strong evidence that the pollen surface and shape may change during pollen transport due to aging, dehydration, and presence of air contaminants. This will lead to changes of the optical and aerodynamical properties of the pollen grains and furthermore may affect their allergological relevance. Still not completely understood is the intensified discharge of allergens as a consequence of interaction with harmful gases such as NO₂ and O₃, the interaction with particulate matter, *e.g.* soot particles, and reaction of pollen during rainfall.

These topics call for a suitable environment, where pollen can be levitated in an airborne state over periods of hours, exposed to varying atmospheric conditions thus providing the opportunity for an *in situ* study of morphological changes and corresponding variations of aerodynamical parameters. Such an instrument may eventually also enable the localized study of surface properties of pollen.

The present paper describes a novel approach to such a characterization of pollen in airborne state at specified atmospheric conditions. The method is based on numerous previous experiments with electrically-charged and μm -sized objects such as droplets, aerosol particles or fibers (Ataman and Hanson, 1969; Davis and Ray, 1980; Armstrong *et al.*, 1992; Hesse *et al.*, 2002). We realize a pollen levitation cell in an adapted Paul-trap environment and demonstrate a novel way for measuring settling velocities of individual pollen grains as well as the possibility of long term observation of individual pollen, under airborne conditions. The presented method is transferable to other kinds of aerosol particles.

MATERIALS AND METHODS

The focus of this work lies on pollen at the airborne state. To eliminate effects due to the contact to a surface, the pollen should be observed solely surrounded by air. Levitation lends itself to contact-free observation of an object over long periods in airborne state. To levitate small particles different techniques exist such as laser tweezers (Askin, 1986; Burnham and McGloin, 2006), strong magnetic fields (Geim, 1999; Simon and Geim, 2000), ultrasonic levitation (Azzouz *et al.*, 2006) and AC-electric fields (Zheng *et al.*, 2001). Organic materials like pollen are readily charged electrically. This tribocharging occurs when small objects are separated mechanically by wind or physical separation by shaking. This led us to the idea of using a Paul trap (Paul and Steinwedel, 1953; Davis, 1985; Paul, 1990; Laucks, 1999; Major *et al.*, 2005) for the levitation of pollen. In such a trap a limitation to the size of objects which can be suspended

comes from the gravitational force which has to be balanced by a vertical DC electric field, E_v , such that

$$qE_v = mg$$

Here q is the total charge ($q = Ze$, where $e = 1.6 \cdot 10^{-19} C$ is the unit of charge and $Z \in \mathbb{Z}$), m is the mass of the particle and $g = 9.81 m/s^2$ is the gravitational constant. As practical electric fields should be kept in the range below $1000 V/mm$ to avoid electrical breakdown, the mass m should be less than $Z \cdot 1.6 \cdot 10^{-5} ng$ for stable levitation. A purely vertical balance to gravitation does not yet ensure stability of the levitated object however. A localized minimum in the electrical potential is required for trapping. In a Paul trap this is achieved by a time-varying electric quadrupole field from typically 3 electrodes (Paul, 1990) to which an AC-voltage is applied. In this environment a dynamic equilibrium can be established which under atmospheric pressure can result in an over-damped motion, leading a particle to reach a stable equilibrium position. In its idealized version, a Paul-trap consists of two hyperbolic metal electrodes with their foci facing each other and a hyperbolic ring electrode halfway between the other two electrodes.

The choice of geometry is a question of specific demands, for example the possibility to load the trap or the visual access to the trap center. Nearly arbitrary geometries are possible. The condition necessary for stable trapping is to find a configuration which results in time-varying forces whose strengths are in first order proportional to the distance from a central origin, the trap center. We tested traps formed by three or four circular electrodes with inner diameter of $2mm$. The top and bottom electrodes are segmented into four parts each, thus allowing the application of additional anisotropic DC fields, superimposed on the AC field of the Paul trap. The DC-fields serve to apply the gravity balancing field E_v , but may also be used to orient the microscopic object if its charge is in homogeneously distributed over the size of the object. Our adaptation of the Paul trap electrodes is optimized to high-resolution light-microscopy with incident and transmitted illumination. Both, in the vertical and along a horizontal trap axis, two dedicated CCD-cameras are used for image recording. The time-varying force is typically provided by a sinusoidal alternating electric field applied to the center electrode. We use AC amplitudes of the order of $1 kV$ at frequencies in the range of 30 to $500 Hz$ to trap naturally charged pollen which are inserted into the trap by means of a small spoon from the top.

A schematic drawing of our levitation cell is shown in Figure 1 as it is located under the microscope objective. Temperature and humidity are measured next to the centre of the levitation cell by a capacitive sensor module (HYTEMOD from Hygrosens). On the opposing side, a window provides the access to a CCD

camera for lateral examination of the trapping volume. The actual trap is built within a copper block with dimensions of $24\text{mm} \times 26\text{mm} \times 15\text{mm}$ for reasons of temperature stability. The trap volume is protected against ambient air by glass windows. The levitation cell is positioned under a long distance objective (50x) of a Nikon LV100D microscope. The actual trapping volume is approx. 1mm^3 . In it particles with specific charge ratios (q/m) in the range of 1mC/kg are trapped by AC amplitudes of $\sim 1\text{kV}$ at frequencies in the range of $\sim 200\text{Hz}$. The instrument has been tested with a variety of pollen (birch, rye, grass, maize, etc.) with typical sizes ranging from 10 to $80\mu\text{m}$, suspended indefinitely, the micro-motion being damped by the aerodynamic friction in the atmospheric environment.

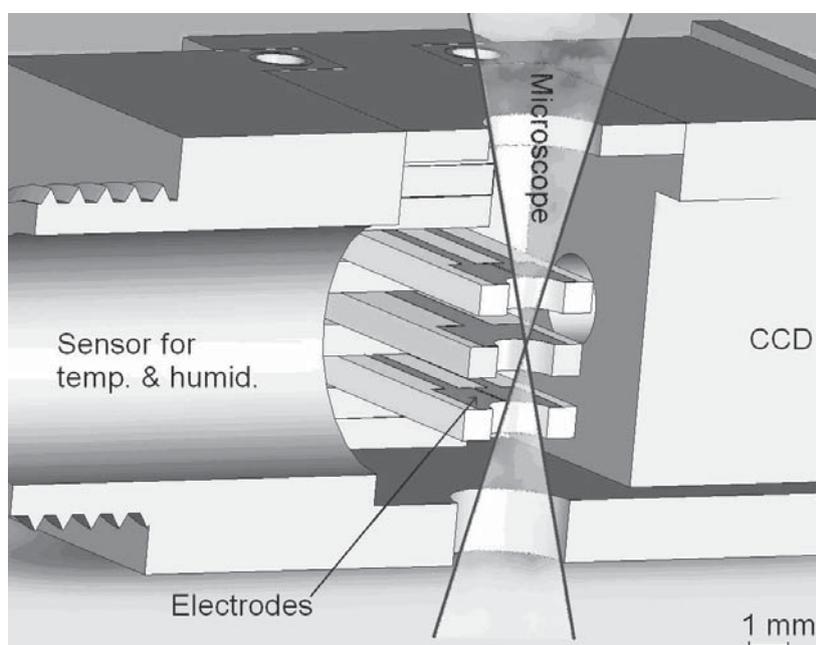


Figure 1. Cross-sectional view of the actual levitation cell. The electrodes and the trapping chamber are designed for incident transmitted light microscopy. The access ports for the humidity and temperature sensors as well as the one for the lateral camera are also visible.

Away from the trap center suspended pollen quivers with the frequency of the applied AC trap voltage. By applying different potentials to different parts of the segmented electrodes and due to a potential difference between the top and the bottom electrode a 3D control of the pollen position in the trap is possible. The pollen can be brought to the center of the trap by adjusting the potentials applied to the individual electrodes. In this configuration the micro-motion of the object can be reduced to amplitudes of the order of below $1\mu\text{m}$. Focused images of levitated pollen can be taken by the camera adapted to the microscope. Images of specific pollen grains recorded in our trap are shown in Figure 2.

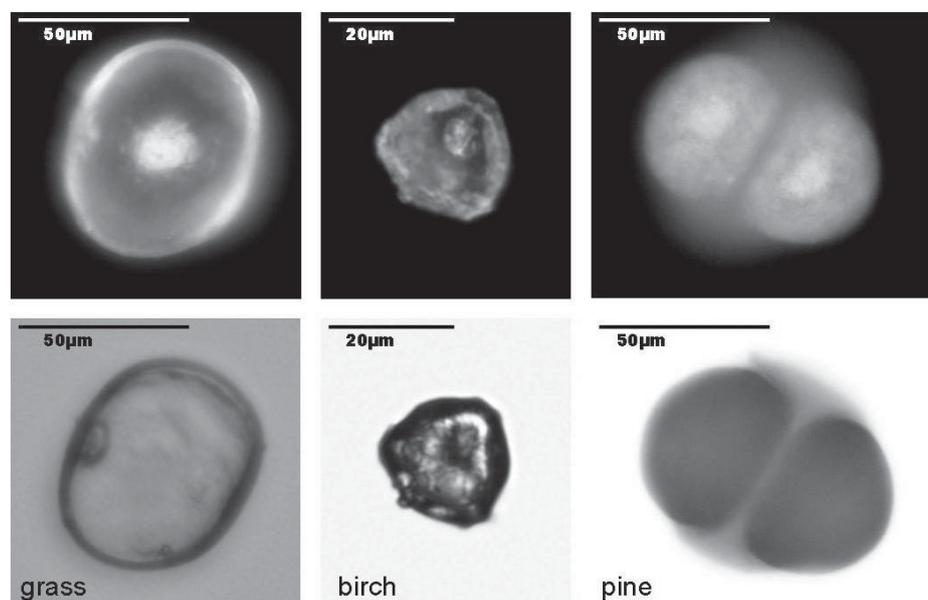


Figure 2. Samples of isolated levitated pollen grains are shown under top-illumination (top row) and under transmission (bottom row). The conditions are 40% RH and 20°C.

The absolute vertical position of the pollen in the trap was determined as a function of the levitating DC-field by focusing the microscope along the vertical direction. This allows determining the charge to mass ratio of the pollen captured in the trap. Typical values for q/m observed in our trap lie in the range of 0.4-4 mC/Kg . This value is consistent with the stability conditions estimated from the frequency and field strength applied (see discussion above). From the observed pollen volume and assuming a pollen specific weight of $0.45-1.4 g/cm^3$ (Jackson and Lyford, 1999) we conclude that a trapped pollen typically carries a net charge of the order of $q = 10^{-12} - 10^{-14} C$, or $Z = 10^3 - 10^5$ electron charge units. This range of values need not necessarily coincide with the typical values of charge carried by airborne pollen but rather reflects the typical charge state of pollen captured in the trap and formed by tribological separation of the pollens. The pollen is introduced into the trap by uncontrolled dispersion of pollen grains from a small wooden spoon.

The capture of a single pollen is favored under normal operating conditions. The presence of two or more pollen in the trap leads to a regular motional state, where pollen separate in space due to their Coulomb repulsion and undergo large amplitude oscillations. The reason is that they repel each other and experience the AC field which rapidly increases with the distance from the trap centre. The sign of charge of the pollen can be determined by the sign of the levitating field. Positive and negative charged pollen can equally be trapped.

RESULTS

Comprehensive reviews of electro-dynamic levitation of microscopic objects exist in the literature (Wuerker *et al.* 1959; Hartung and Avedisian, 1992; Zheng *et al.*, 2000; Shaw *et al.* 2000; Baron and Willeke, 2005), so only a very brief summary of the theory of operation is presented here. The technique is based on the containment of charged particles in a time-varying electric field of a given frequency and amplitude. The leading forces are the electric force $q\vec{E}$, the aerodynamic drag $-K\dot{\vec{r}}$ and the gravity $-mg\vec{e}_z$. Here \vec{r} is the spatial coordinate measured from the trap origin and \vec{e}_z the unit vector in the vertical direction. The motion of the particle is governed by the differential equation:

$$m\ddot{\vec{r}} = q\vec{E} - mg\vec{e}_z - K\dot{\vec{r}}$$

Analytic solutions are possible for small perturbations from the trap center, when the electric field can be approximated by an ideal quadrupole field, $|\vec{E}| \sim r^2$ (Hartung and Avedisian, 1992). The influence of the geometry of the trap electrodes is reduced to two independent parameters which vary with the actual shape of the trap. The electrodynamic levitation problem can then be described by a differential equation of the Mathieu type. The Mathieu equation has stable and unstable solutions depending on operating parameters (Meixner and Schäfke 1954; Guitérrez-Vega and Rodríguez-Dagnino, 2003). For our case it predicts that for a particle of a given charge-to-mass (q/m) ratio, only certain combinations of frequency and AC-amplitude voltage, applied to the cell electrodes, can lead to stable trapping of a single particle. The range of operating parameters for which a particle can be stably trapped is represented in stability diagrams for the vertical and axial motion. In Figure 3 we show the stability diagram for the vertical motion.

Here the reduced coordinate axes ε_{ac} and δ are in units of

$$\varepsilon_{ac} = \frac{-2C_1 q V_{ac}}{m z_0^2 \Omega^2} \quad \text{and} \quad \delta = \frac{K}{m\Omega}$$

where V_{ac} is the amplitude of the AC field with frequency Ω . z_0 is the geometric and C_1 a intrinsic form factor of our trap. K indicates the friction parameter. By varying the frequency and amplitude of the voltage, a prior stable trapped pollen can be moved to the so called "Sprungpunkt" (Hesse *et al.* 2002), the point where the pollen starts to quiver with an exponentially increasing amplitude. This is when the boundary to instability is approached. In Figure 3 we show the theoretical boundary between stable and unstable operation of our trap as predicted from the theoretical treatment discussed above. Shown as black

squares are conditions for the “Sprungpunkt” determined experimentally. The experimental points are scaled to the theoretical prediction using the shape parameter C_1 and the aerodynamic drag parameter over the mass K/m . Note that this calibration is possible only because all data points shown were obtained for the same pollen. Thus the aerodynamic drag parameter and the specific charge are identical for all transitions to instability and the empirical stability boundary defines values for C_1 and K/m . We obtained a value of $C_1 = -0.38$ and a value of $K/m = 650 \text{ s}^{-1}$. Of course the value of K/m is dependent on the specific pollen studied. Since the settling velocity v_s is related to the friction parameter by the relationship we are able to deduce from measurements of a “Sprungpunkt” position values of v_s for any given pollen, irrespective of the number of charges Z it carries.

$$v_s = \frac{mg}{K},$$

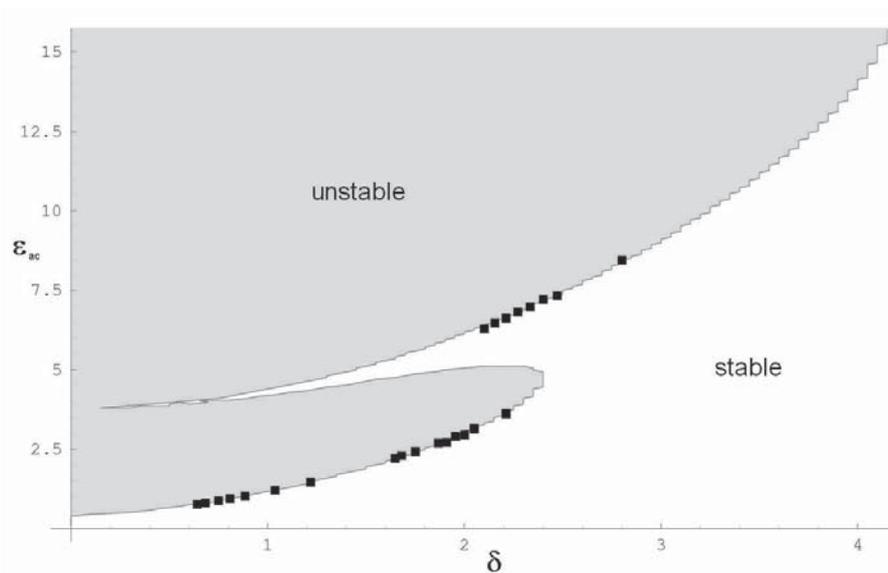


Figure 3. The theoretical stability domain of our trap is shown by the white area. Boundary parameters observed for vertical stability of a single birch pollen are given by the points. The trap geometry parameter C_1 and the friction term K/m are derived from the fit.

As an example in Figure 4 the measured settling velocity of single birch pollen and for agglomerates of birch pollen are shown. As the pollen are observed under the microscope the number of pollen in agglomerate are easily detectable. The data were recorded at a relative humidity of 40 % and a temperature of 23°C at atmospheric pressure. In a series of measurements of 20 different birch pollen

under such conditions we determined the mean settling velocity of a single birch pollen to $1.57 \pm 0.14 \text{ cm/s}$ where the error given is the standard deviation.

It is interesting to see that agglomerates of pollen have slightly higher settling velocities. For spherical particles the Stokes law would predict that the settling velocity v_s is proportional to the squared radius, r^2 . As the mass scales with the radius to the power three ($m \sim r^3$ or $m^{1/3} \sim r$) and at the same time the mass scales with the number of pollen in agglomerate one could deduce that the settling speed of spherical particles should scale as $v_s \sim r^2 \sim m^{2/3} \sim N^{2/3}$ (with N = the number of pollen in agglomerate). The dashed line in Figure 4 gives this relationship, least squares fitted to the observed data. We realize that the agreement here is surprising as one may expect the aerodynamic parameter to be dependent on the actual agglomerate structure.

According to Hinds (Hinds, 1999) the Stokes diameter is given by

$$d_s = \sqrt{\frac{18\eta v_s}{\rho_0 g}},$$

where η is the viscosity of air at 20°C and $\rho_0 = 1 \text{ g/cm}^3$ the unit density. From our measurement data we obtain a value of $22.8 \pm 1 \mu\text{m}$ for the *Stokes diameter* of single birch pollen. Assuming an average density of $\rho_0 = 0.8 \text{ g/cm}^3$ (Sofiev *et al.*, 2006) for birch pollen yields an *aerodynamic diameter*

$$d_{ae} = \sqrt{\frac{18\eta v_s}{\rho_p g}} \text{ of } 25.5 \pm 1.1 \mu\text{m}.$$

The actual aerodynamic drag parameter of the trapped pollen K_p can only be determined by an independent measurement of the absolute weight of the pollen. For the latter only an estimated pollen size is used and an assumed, but plausible specific density. On this basis a mean value of $K_p = 2.09 \cdot 10^{-9} \text{ kg/s}$ was calculated which may be compared with the Stokes value of spherical objects K_s which is given to $K_s = 3.39 \cdot 10^{-9} \text{ kg/s}$. A Shape factor χ (Hinds, 1999) is given by the ratio of K_p to K_s . From the here presented measurements this shape factor can be specified to $\chi = 0.62 \pm 0.05$ for single birch pollen.

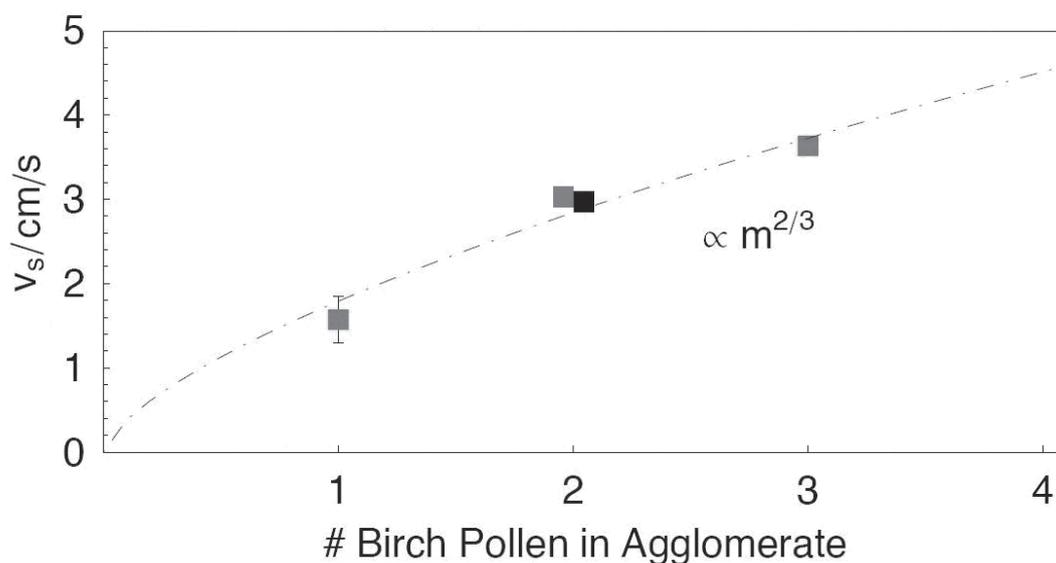


Figure 4. Sinking velocities determined for several single birch pollen and for agglomerates of two and three birch pollen determined from the stability analysis.

To independently validate our approach for determining the settling velocity of pollens we determined the settling velocity in a sedimentation chamber (ASM 1000 from PALAS, Karlsruhe, Germany). Analyzing the trace length and image-to-image motion of the falling objects in the recorded movie sequences of falling pollen. We obtained the distribution of settling velocities for birch pollen shown in Figure 5. The distribution of velocity values observed clearly suggests the presence of at least two groups of birch pollen with substantially different settling velocities. Mean values of 1.58 cm/s and of 2.7 cm/s are obtained by fitting two Gaussian profiles to the observed distribution. On the basis of our pollen experiments in the levitation cell described above (see Fig. 4) we are able to identify the two groups of differing settling velocities. These groups describe single birch pollen and agglomerates containing two pollen each, respectively. The settling velocities obtained in the two rather different experimental approaches are in very good agreement with each other, thus validating the new approach.

DISCUSSION AND CONCLUSIONS

We have demonstrated that a Paul trap can be adapted to a conventional light-microscope. This arrangement enables microscopic studies of pollen grains in an airborne state without any contact with surrounding surfaces. Within the trap an isolated pollen can be kept for days and studied under defined atmospheric conditions. Additionally the position and orientation of the trapped pollen can be

adjusted by applying different electric potentials to different parts of the segmented electrode structure of the trap.

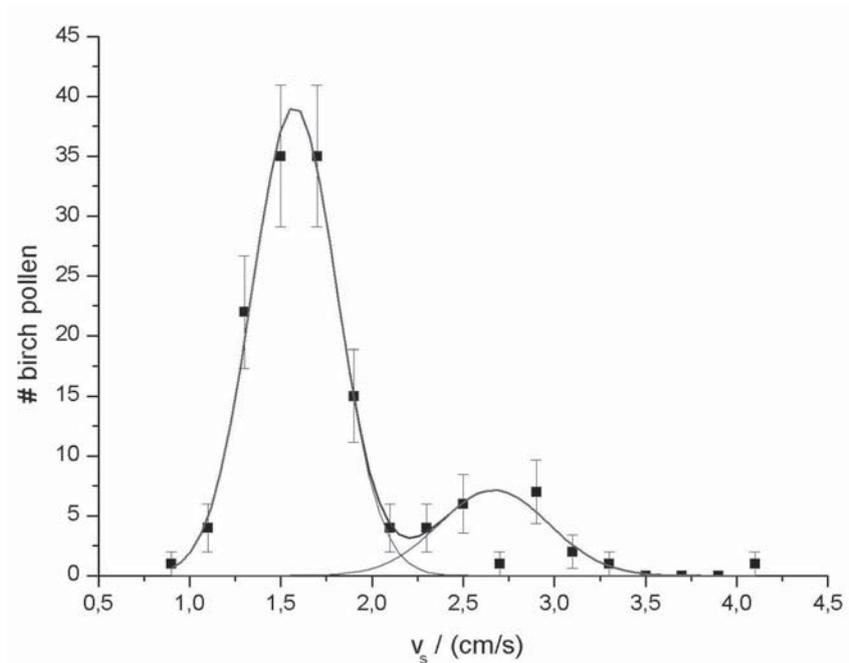


Figure 5. Distribution of sinking velocities of birch pollen as determined it in a classical sedimentation experiment.

The microscopic study of levitated pollen provides the accurate measurement of morphological parameters of airborne pollen as a function of time and atmospheric conditions. Beyond it the incorporated micro-environment is also suitable for a loading of trapped pollen with atmospheric contaminants.

As a major experimental result the settling velocity of pollen was determined in good agreement with conventional settling experiments. These results enable to precisely calculate the aerodynamic shape factor of pollen. It is supposed that modeling of pollen dispersal and pollen forecast will profit from corresponding pollen data considering varying atmospheric conditions and their impact on pollen aerodynamics.

Future research on this field will encompass the following topics:

- 1) Developing a programmed scheme of DC fields applied to the segmented electrodes in pulse sequences in order to allow the full, three-axis positioning of a levitated particle from a joy-stick.
- 2) Studying specific parts of the pollen surface with a laser tweezers and/or laser-induced fluorescence monitor in order to follow the dynamics of exchange of matter between pollen and environment, combined with site-specific bio-chemical analysis.

- 3) Investigating shape and morphology of airborne pollen grains under varying atmospheric conditions by laser scanning microscopy and image analysis in order to continuously record hydrating and dehydrating states of airborne pollen.
- 4) Applying micro-fluid techniques in a miniaturized “wind-tunnel” for an independent and rapid control of trace gas concentration of the environment in which the levitated pollen resides.
- 5) Monitoring the response and the morphological modification of the trapped pollen grain to specific trace gases in the environment. This miniaturized “wind-tunnel” will also allow studying the variation of the specific weight of the pollen owing to water-uptake and drying and enable experiments analogous to the time-honored Millikan experiment.

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