Analysis of atomic force microscopy data for deformable materials

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Abstract—A protocol for measuring the interaction, deformation and adhesion of soft polymeric substrates with the atomic force microscope (AFM) is described. The technique obtains the photodiode response of the AFM (constant compliance factor) by independent calibration against the rigid substrate adjacent to the deformable particle or patchy film. The zero of separation is taken as the end-point of the jump into contact. A method is given for correcting the velocity dependence of the piezodrive expansion factor, the neglect of which will cause artefacts in dynamic viscoelastic measurements. It is emphasised that conventional force curve analysis, which uses the apparently linear large force region for calibration, will generate erroneous results for deformable substrates. Results are obtained for cellulose particles and for polystyrene films, and their Young’s moduli are found to be 22 MPa and 100 MPa, respectively. The latter is about a factor of 30 less than for bulk polystyrene, which indicates that the polystyrene surface is in a less glassy state than the bulk.

Keywords: Polystyrene; cellulose; deformable materials; atomic force microscopy; piezo-calibration.

1. INTRODUCTION

The atomic force microscope and other surface force devices have long been used to measure forces between rigid particles and surfaces, and they are being increasingly applied to soft objects (a review of the former is given in Ref. [1] and of the latter in Refs [2, 3]). In the deformable case two experimental issues must be addressed for the quantitative interpretation of the data, namely the determination of the zero of separation and the calibration of the electronic force measurement device. Unlike rigid surfaces, the gradual increase in the repulsive force between the surfaces and the consequent gradual increase in the deformation of the surfaces

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prior to their coming into intimate contact, and also the fact that intimate contact is not marked by a sharp discontinuity in the slope of the force curve, mean that the precise location of the surface cannot be deduced immediately from the force curve. Further, increasing deformation with increasing applied load means that the deflection of the force measuring spring in the contact region is not equal to the distance moved by the driving piezocrystal, and thus it is not possible to convert the electronic signal to deflection or force. Theoretical calculations [4–6] have shown that the deformation is negligible in the large separation, weak force regime, and if the force law is known for rigid particles, then this fact can be used to establish the zero of separation. This procedure has been used to analyse Atomic Force Microscopy (AFM) measurements on poly(dimethylsiloxane) emulsion droplets [7, 8] and oil droplets [9] that interact with an electric double layer repulsion. A number of other proposals for establishing the zero of separation are reviewed in Ref. [7].

This paper reports AFM force measurements on a cellulose particle and on a polystyrene film in air and presents results for their surface elasticity. We explore the utility of using the van der Waals attraction to establish the zero of separation. The steepness of the van der Waals force, in principle, presents an advantage over the relatively slowly decaying electric double-layer repulsion used previously to determine the zero of separation [7–9]. In practice we find that the presence of adsorbed water films in air creates some uncertainty in this quantity. The calibration of the force measuring photodiode is performed here in situ by moving the cantilever off the particle or film and pressing against the adjacent hard substrate, as has been done previously for droplets [8, 10]. The calibration is a significant issue in this context because both cellulose [11–14] and polystyrene [15–18] have previously been the subject of force measurement, and in all these cases the calibration factor was taken to be the rate of change of the force measuring voltage with piezocrystal movement in the pseudo-linear regime. This assumes not only that the force curve is linear, but also that the increase in deformation of the particle is negligible. Here, it will be shown that the apparent linearity of the force curve cannot be used as a guide to the magnitude of the deformation of these materials and that the calibration factor so deduced differs significantly from the actual calibration factor of the device. The force curves produced by such procedures can be in significant error. Furthermore, in those viscoelastic materials where dynamic effects are of interest [2, 3, 6, 8, 18–22], velocity-dependent artefacts in the piezodrive can also lead to a misinterpretation of the results unless they are accounted for, as will also be shown.

2. MATERIALS AND METHODS

The polystyrene surface was prepared under an optical microscope (Olympus BH2) equipped with a heating stage. Pieces of silicon wafer were washed with ethanol (analytical grade), rinsed thoroughly with high-purity MilliQ water (Elga UHQ
purification system) and left to dry in a clean-room environment. On the surface of the silicon wafer was a native oxide layer from exposure to the atmosphere, and henceforth it will be referred to as the silica substrate. A droplet of an aqueous suspension containing 3 $\mu$m polystyrene spheres (Polysciences, USA) was placed on the silica substrate and subsequently heated. The liquid evaporated and the particles initially formed regions of crystalline arrays and subsequently melted to form smooth polystyrene films with well-defined borders. Scanning probe imaging of the film over an area of 1 $\mu$m$^2$ revealed a root mean square roughness of less than 0.4 nm. The force measurements on polystyrene were carried out under ambient conditions of 17°C and 78% relative humidity.

Cellulose particles [13] were attached to fragments of oxidised silicon wafer (approx. 1 cm$^2$, supplied by Okmetic, Finland) with a high-melting-point adhesive (Epikote 100, Shell) as follows. The silica substrate was placed on a heating stage at a temperature above the adhesive melting point on the translation stage of a binocular microscope. Two etched tungsten wires were attached to an x-y-z micromanipulator (Narishige MN-151). With one of the wires tiny spots of adhesive (between $10^{-15}$ and $10^{-17}$ l) were applied to the substrate in a predetermined pattern to aid recognition in the AFM. With the other wire cellulose particles of about 20 $\mu$m in diameter were placed onto the adhesive spots and the wafer was removed from the heating stage. For force measurements, particles were located under the AFM cantilever tip by manipulating the sample stage while monitoring it with a video microscope placed directly above the interaction zone. The force measurements on cellulose were carried out under ambient conditions of 26°C and 31% relative humidity.

Experimental data were acquired using a Nanoscope IIIa AFM (Digital Instruments) employing a V-shaped silicon nitride cantilever. The spring constant was taken to be 0.58 N/m, which is the manufacturer’s nominal value, and no independent calibration was performed. A piezo-tube scanner (type E, Digital Instruments) with vertical (z) range 2.5 $\mu$m was used to drive the sample stage. Characterisation of the sample stage piezocrystal (i.e. movement versus applied voltage) was performed using an optical displacement sensor (model d20, Philtec, Annapolis, MD, USA) mounted directly above the sample piezocrystal [19]. The sensor utilises bundled optical fibres to transmit a divergent beam of light and to receive its reflection, the intensity of which is proportional to the distance between the tip of the fibre bundle and the reflecting substrate, which in this study was a clean, polished piece of oxidised silicon wafer placed on the piezodrive. Conversion of reflected intensity to distance moved was accomplished using the manufacturer’s precalibrated response curve after the maximum response was set to the recommended value by means of the inbuilt gain adjustment. In these piezodrive characterisations, which were independent of the force measurements, the AFM optical head was physically removed to improve displacement sensor access to the piezodrive, although the electrical connections were maintained to facilitate software control of the piezocrystal. Signals from the displacement sensor were logged on an auxil-
A sketch of the experimental geometry is shown in Fig. 1 for the post-contact situation. Note that the radius of curvature of the end of the tip is much greater than the width of the tip. Note also that on this scale the undeformed cellulose particle and polystyrene film appear planar. In each experiment normal forces were obtained by ramping the lower surface towards the cantilever using a triangular wave without scanning in the lateral direction. Rates between 0.03 and 73 $\mu$m/s were employed. In each experiment (cellulose particle and polystyrene), forces were first measured on a proximate region of the silica substrate, which enabled the photodiode voltage response to be calibrated as a cantilever deflection [8, 10]. For reasons discussed in detail below, this constant compliance calibration obtained from the tip–silica interactions was performed at the same rate as the subsequent force measurements on the soft substrate. In order to avoid nanoindentation, (irreversible plastic deformation), only small loads were applied so that the amount of flattening was less than 25 nm. It was confirmed that the forces measured upon approach and upon retraction were approximately coincident in the contact region, which indicates that the deformation was elastic and that no nanoindentation had occurred.

3. RESULTS

3.1. Effect of piezodrive speed

The calibration factor, which converts the AFM photodiode output voltage to the cantilever deflection, is generally obtained in the so-called constant compliance regime, where the cantilever is in hard contact with the substrate that is being moved by the piezodrive. Figure 2 shows measurements of the calibration factor (V/nm) as a function of the drive speed ($\mu$m/s) for a cantilever in hard contact with the silica substrate. Triangular waveforms for the drive signal were used with constant voltage amplitude and various frequencies. The drive speed was obtained by converting the voltage amplitude to a drive distance using the expansion coefficient obtained by
Figure 2. Speed dependence of the calibration factor for a tip in contact with a silica substrate. The same expansion coefficient has been used in all cases to convert the applied voltage to piezodrive displacement.

Figure 3. Measured total displacement of the piezodrive as a function of scan rate for a given applied voltage. The arrow indicates the nominal displacement expected from the original interferometric calibration. The straight line is a least-squares fit ($R^2 = 0.80$).

A prior interferometric calibration (at a single velocity). It may be seen that the calibration factor depends upon the speed of the measurement, and that it decreases in magnitude monotonically by about 10% over the four decades increase in speed.

The displacement of the piezodrive was measured directly using the displacement sensor. Figure 3 shows displacement versus scan rate at constant applied voltage amplitude. It can be seen that the displacement depends upon the frequency (equivalently speed), and that it decreases monotonically with increasing frequency. The change was about 10% over the range of frequencies studied.
3.2. Interaction and deformation of cellulose

AFM measurements were performed for a cantilever tip interacting with a cellulose particle in air (Fig. 4). The particle was mounted on the silica substrate driven by the piezocrystal rather than the more common attachment to the cantilever tip. The force can be obtained by multiplying the deflection of the cantilever by the spring constant, 0.58 N/m. The AFM force measuring photodiode was calibrated by moving the cantilever off the particle and performing a force measurement for the cantilever tip interacting with the silica substrate. The calibration factor was found to be $-0.191 \text{ V/nm}$. This calibration factor makes the linear, constant compliance region of the voltage versus displacement curve for silica vertical in the force versus separation plane (Fig. 4B), and this vertical portion was taken to define the zero of separation for the tip–rigid substrate, as is usual.

The data for the tip interacting with the cellulose particle in Fig. 4 were obtained following the tip–silica measurements by aligning the particle beneath the cantilever using the lateral piezodrive and translator stage (i.e. without altering the optical path.

![Figure 4](image_url)

Figure 4. Deflection of a cantilever (spring constant 0.58 N/m) for a tip in air interacting with a rigid silica substrate (crosses, 0.7 µm/s drive speed) or with a cellulose particle at driving speeds of 0.50 (circles) and 1.51 (triangles) µm/s. (A) Jump into contact (dashed arrow). The curve is the van der Waals force $F$ as a function of separation $h$, $F(h) = AR/12(h - d)^2$, using the water–air–water Hamaker constant, $A = 3.7 \times 10^{-20}$ J [23], a fitted radius of curvature of the tip of 700 nm, and a fitted total water film thickness of $d = 3.2$ nm. (B) Post-contact deformation. The curve is a JKR calculation using a fitted $E/(1 - \nu^2) = 2.2 \times 10^7$ Pa, the radius of curvature $R = 700$ nm, and a fitted surface energy, $\gamma = 1.2 \text{ mJ/m}^2$. The retract data lie slightly below the approach data in each case. For clarity, only every fiftieth datum is plotted.
in the AFM). The cellulose force data were converted from photodiode voltage to deflection, and from displacement to separation, using the tip–silica photodiode calibration factor obtained adjacent to the particle (−0.191 V/nm). In Fig. 4B it can be seen for the cellulose that there is a well-defined constant compliance region (where deflection changes linearly with drive distance). This has a slope of −0.153 V/nm. The zero of separation for the cellulose was defined by shifting horizontally the cellulose–tip force-separation curve so that the first measured datum after the jump-into-contact occurred at zero separation (Fig. 4B). At positive separations there is an attractive force that causes the cantilever to jump into contact (Fig. 4A). The negative nominal separations in Fig. 4B signify the amounts of deformation or flattening of the cellulose, since the nominal separation is the separation between the surfaces as if they were rigid [4, 6]. There is a small amount of hysteresis evident for cellulose in Fig. 4B, with the retract curve lying beneath the approach curve.

The adhesion (not shown), which is the maximum tension measured upon retraction, was 70 nN for both the tip–silica and the tip–cellulose cases. This was approximately independent of the speed of the retraction.

Figure 4A includes the calculated van der Waals attraction prior to contact, $F(h) = AR/12(h-d)^2$, where $F$ is the force, $h$ is the separation, $A = 3.7 \times 10^{-20}$ J is the Hamaker constant for water–air–water [23] and $R$ is the radius of curvature of the tip. Both $R$ and $d$ were used as fitting parameters. This equation assumes that there are water films present on the tip and the substrate of total thickness $d$. Figure 4B includes the JKR theory [24] for the force versus deformation. The radius obtained in the precontact data was used in the JKR theory, and the Young’s modulus, which changes the slope, and the surface energy, which shifts the curve vertically, were used as fitting parameters. The influence of the capillary condensate, which would mainly shift the curve vertically, was not accounted for in the JKR theory (cf. Ref. [25]).

3.3. Interaction and deformation of polystyrene

AFM measurements were made on a tip interacting with a polystyrene film in air (Fig. 5). The photodiode calibration factor (−0.230 V/nm) was measured by pressing the tip against the silica substrate on an adjacent bare patch in the film. This differs from the calibration factor used for cellulose (−0.191 V/nm) due to the experiments being performed on different days with different mirror angles and hence optical paths. The same cantilever as for the data in Fig. 4 was used, and hence the spring constant was again 0.58 N/m. The zero of separation for the polystyrene film was established by horizontally shifting the curve so that the first point after the jump into contact coincided with the zero of separation for the tip–silica curve. Prior to contact an attractive force acts that either is smaller in magnitude or else is shifted to smaller separations for polystyrene than for silica. A linear constant compliance regime can be seen in the polystyrene post-contact data (Fig. 5B), with a slope of −0.212 V/nm.
Figure 5. As Fig. 4, but for approach to silica (crosses, 0.03 µm/s drive speed) and to a polystyrene film at a driving speed of 0.09 (circles) and of 25.2 (triangles) µm/s. (A) Jump into contact. The van der Waals curve has parameters $A = 3.7 \times 10^{-20}$ J [23], a fitted $R = 700$ nm, and fitted $d = 6$ nm (silica) and fitted $d = 4.5$ nm (polystyrene). (B) Post-contact deformation. The JKR curve (obscured) has fitted $E/(1 - \nu^2) = 1 \times 10^8$ Pa, $R = 700$ nm and fitted $\gamma = 1.5$ mJ/m$^2$.

Van der Waals forces were fitted to the pre-contact attraction by fitting a total water film of $d = 6$ nm for silica and $d = 4.5$ nm for polystyrene. The same radius of curvature of the tip as found from the fit in Fig. 4 fitted the pre-contact data when the water–air–water Hamaker constant was used. A JKR fit was also made to the post-contact deformation in the case of polystyrene by fixing the radius of curvature of the tip and fitting Young’s modulus and the surface energy.

In the contact region, the retract data for polystyrene were virtually coincident with the approach data and are not shown in Fig. 5. The adhesion was 244 nN for the tip–silica and 203 nN for the tip–polystyrene experiments.

4. INTERPRETATION AND DISCUSSION

4.1. Speed effects

The speed-dependence of the calibration factor measured in Fig. 2 arises from a change in the expansion (i.e. displacement) of the piezodrive with speed. This
follows because the change in photodiode voltage is linearly proportional to change in deflection of the cantilever tip, which, for the hard substrate, is equal to the distance the piezo-drive moves. Since the data in Fig. 2 were obtained using a constant amplitude voltage applied to the piezo-drive, this indicates that the expansion factor itself must be changing. This conclusion is confirmed and quantified by the direct measurements reported in Fig. 3, which show a change in the total expansion with scan rate (equivalently speed), again for a constant amplitude applied voltage. The speed dependence arises from the nonlinearities and hysteresis in the piezo-drive. In the absence of a displacement sensor such as the one used in Fig. 3, it is difficult to account for these artefacts, and commonly in AFM measurements a single expansion coefficient for the piezo-crystal is used to describe motion along the \( z \)-axis. Even when hysteresis effects are taken into account by the application of the manufacturer’s preprogrammed polynomial curve, which attempts to describe the actual piezo-drive movement, the full expansion is overestimated at high speeds.

The speed dependence of the expansion factor can be substantial; neglecting it can introduce errors, particularly if one is seeking to measure the viscoelastic properties of soft samples. Depending on the stiffness of the sample, this change with speed can be of the same order as the change in slope due to deformation. In the present case of polystyrene, Fig. 5B shows that the results at a speed of 0.09 \( \mu \text{m/s} \) are coincident with those obtained at 25.2 \( \mu \text{m/s} \) when the correct calibration factor and expansion coefficients are used. From this, one can conclude that on this scale the polystyrene deformation is elastic rather than viscoelastic. However, if one were to use a single expansion factor, the force curves in contact would differ from each other and the apparent speed-dependence would be wrongly interpreted as viscoelastic deformation.

The quantitative measurements of the expansion of the piezocrystal in Fig. 3 shows that the amount of expansion decreases with increasing speed (scan rate, Hz). This is consistent with the results in Fig. 2, where the magnitude of the calibration factor decreases with increasing speed. In Fig. 3 the displacement is approximately linear on the logarithmic scale, \((z - z_0)/z_0 = -r \log_{10}(v/v_0)\), where \( z \) is the displacement, \( v \) is the speed, the subscript 0 denotes a canonical case and \( r \) is the proportionality constant. The decrease of about 45 nm for each order of magnitude change in speed corresponds to \( r = 2.4\% \) change in the displacement per decade. The data in Fig. 2 are also approximately linear on the logarithmic scale, corresponding to \( r = 2.7\% \) change in the calibration factor per decade of speed.

On the basis of these results, we have developed a practical protocol for performing dynamic measurements. Most AFM systems do not have a displacement sensor for directly measuring the expansion factor of the piezodrive on the \( z \)-axis. It may be both impractical and tedious to carry out an interferometric calibration as a function of drive speed. Instead the following protocol may be implemented. Let \( \alpha(v) \) be the expansion factor measured (interferometrically) at a single speed \( v \), so that
\( \Delta z(v) = \alpha(v) \Delta V_{pz} \) is the change in piezodrive position due to the change in applied voltage \( \Delta V_{pz} \). Let \( \Delta z_{\text{nom}}(v') = \alpha(v) \Delta V_{pz} \) be the nominal expansion at a different speed \( v' \), and let \( \beta_{\text{nom}}(v') = \Delta V_{pd}/\Delta z_{\text{nom}}(v') \) be the nominal photodiode calibration factor at this speed, which is measured in the usual way by pressing the cantilever tip against the hard substrate. Since the correct calibration factor must be independent of speed, \( \beta(v') = \beta(v) \), the correct expansion factor at the relevant speed can be obtained from the ratio of the measured quantities

\[
\alpha(v') = \frac{\alpha(v) \beta_{\text{nom}}(v')}{\beta(v)}. \tag{1}
\]

This expansion factor is used to obtain the correct displacement of the piezodrive at the speed of the measurement, and this should be used to analyse the data in conjunction with the correct calibration factor \( \beta(v') = \beta(v) \). The nominal calibration factor that is required for this correction must be obtained by pressing the cantilever against the hard substrate adjacent to the soft particle or film and at the same nominal speed as each of the subsequent measurements on the soft matter.

4.2. Water films

The van der Waals attractions fitted in Figs 4 and 5 included a parameter representing the total thickness of the water films assumed present on both surfaces. The data in Fig. 4 were obtained when the relative humidity was 31% and that in Fig. 5 when the relative humidity was 78%. In the case of Fig. 4A, the tip jumped into contact with the silica substrate from a separation of 5.5 nm to a separation of 0.82 nm, a jump of 4.7 nm. This distance is much larger than expected for a van der Waals jump between the solids. However, water is known to condense on hydrophilic substrates [26, 27], and contact with the tip has been shown to facilitate the deposition of water and to lead to long-lived, locally thickened films that can be measured on subsequent approaches; Xu et al. [26] report induced droplets 15 nm in height and 1 \( \mu \)m in radius with lifetimes of the order of minutes. If it is assumed that there is a water film on both surfaces of total thickness 3.2 nm, then quite a good description of the prejump data can be obtained using the water–air–water Hamaker constant, \( A = 3.7 \times 10^{-20} \) J [23], and fitting a radius of curvature of the tip of 700 nm (if the Hamaker constant were doubled, on the grounds that the solids underlying the water films also contribute, then the radius of curvature would be reduced by a factor of two). The coincidence of the prejump data for the tip–silica and tip–cellulose interactions supports the assumption that the van der Waals force is due to the interaction of water films in both cases, since the van der Waals interaction of the bare solids would differ from each other.

A value of \( R = 700 \) nm is not unreasonable for a worn tip, since there is no direct relation between the radius of curvature of the tip and the width of the tip itself. Provided that the interaction pressure becomes negligible by the edge of the curved region of the tip, the Derjaguin approximation will remain quantitatively accurate for the analysis of the forces. For an introduction to surface forces, Hamaker constants and the Derjaguin approximation, see Ref. [28].
The thickness of the water film is not unambiguously determined by the present method that varies the tip radius to make the calculated van der Waals attraction fit the pre-jump force data. A similarly good fit to the data in Fig. 4A can be obtained for a thickness of 2 nm by increasing the product $AR$ by a factor of 2.5, and for a thickness of 4 nm by decreasing the product $AR$ by a factor of 2. These are about the limits on the thickness that can be tolerated as greater or smaller values make it impossible to have both the correct tangent at the jump point and the correct magnitude of the van der Waals force at larger separations.

Following the jump of the tip into contact with the silica substrate, there is a small hook or soft compliance region of width 0.82 nm (Fig. 4B). Assuming a conical tip with bulk elastic properties, it can be shown that the deformation of the tip is negligible under these loads. If one assumes that the deformation of the silica substrate is negligible (JKR theory would give a deformation of 0.2 nm at zero load), then the most likely explanation of this soft compliance is the Reynolds drainage of the water film between the substrate and the approaching tip.

In the case of Fig. 4A, the same thickness water film was used for both silica and for cellulose. This is supported by the coincidence of the forces prior to the jump (i.e. the magnitude of the van der Waals force for the tip–silica and for the tip–cellulose would only be identical if both were coated with a water film) and the identical jump distances that were measured (see also the discussion of the zero of separation below).

For the measurements of the tip–silica interaction in Fig. 5A, the relative humidity at 78% was higher on that particular day than the 31% for the measurements in Fig. 4, which is consistent with the fact that a total water film thickness (tip plus silica) of 6 nm was required to fit the prejump van der Waals force, compared to a total thickness of 3.2 nm fitted in Fig. 4. This is also consistent with the fact that the postjump hydrodynamic drainage hook is larger in Fig. 5B (1.52 nm) than in Fig. 4B (0.82 nm). As discussed in connection with Fig. 4A, the data could still be fitted when the thickness of the film was varied by ±1 nm.

For the tip–polystyrene data in Fig. 5A, a water film of total thickness 4.5 nm has been used in the fit to the van der Waals force. This water presumably adsorbs mainly on the tip, since polystyrene has a hydrophobic character (contact angle 83–89°) [17]. This is consistent with the smaller thickness used for the water film for these data than for the tip–silica data obtained in the same series of measurements.

The pull-off forces (not shown) were 70 nN for both the tip–silica and the tip–cellulose experiments reported in Fig. 4, when the relative humidity was 31%, and were 244 nN for the tip–silica and 203 nN for the tip–polystyrene data reported in Fig. 5, when the relative humidity was 78%. The force due to capillary condensation, $F = 4\pi \gamma R$, where $\gamma = 72$ mN/m is the surface tension of water, and where zero contact angle on both surfaces has been assumed, is 633 nN for $R = 700$ nm. The assumption that the pull-off force is due to capillary condensation is confirmed by the facts that the force is independent of the nature of the solids on any given day, that it is of the same order as the calculated capillary force (this
is much greater than the expected solid–solid adhesion, and roughness accounts
for the fact that the measured values are less than the theoretical ones) and that it
increases with increasing relative humidity. It has been shown theoretically [29],
that going from less than 40% to greater than 60% relative humidity leads to a
large increase in adhesion due to nanoscale roughness. These predictions have been
broadly confirmed experimentally (Refs [26, 29, 30] and data not shown) and are
consistent with the present results. That the measured pull-off forces are consistent
with capillary adhesion confirms the conclusion based on the precontact van der
Waals data that the surfaces are covered with water films.

4.3. Zero of separation

A crucial issue in AFM measurements is defining the zero of separation. For
rigid substrates such as the present tip–silica measurements, the zero is found
quite simply from the location at which the force curve becomes vertical when
plotted against separation. For the case of deformable materials, such as the
present cellulose particles and polystyrene films, determining the zero of separation
is problematic. The zero of separation was here defined by shifting horizontally
the force-separation curve for deformable surfaces, so that the first measured
datum after the jump into contact occurred at zero separation (Figs 4B and 5B).
This procedure ignores the flattening of the cellulose and polystyrene immediately
following the jump. On the basis of the JKR theory [24], the amount of deformation
in the case of cellulose is 2 nm, which suggests that the cellulose data ought to be
shifted to more negative separations by this amount, and which implies that the
water film is 2 nm thinner on cellulose than it is on silica. For polystyrene, the
post-jump flattening is estimated as 0.3 nm.

The procedure for establishing the zero of separation also ignores the slow
drainage of water following the jump, which added 0.82 nm to the separation for
the tip–silica case. This was obtained from the separation relative to zero at the end
of the jump in Fig. 4A. This drainage effect partially cancels the deformation effect.

The uncertainty in the thickness of the water film (±1 nm) creates uncertainty
in the magnitude of the tip radius. It is the uncertainty in the amount of initial
deformation and the film drainage that creates uncertainty in the zero of separation.
We have taken the view that the best procedure is to set the zero of separation as
the first data point after the jump into contact as this is a clearly defined protocol
that avoids having to invoke a JKR fit to the data to obtain the amount of initial
deformation.

4.4. Deformation

It can be seen from the finite slope in the cellulose force data in Fig. 4B that
significant deformation of the cellulose occurs and that at no stage does it become
rigid (i.e. have an elastic modulus comparable to that of the silica substrate).
Rigidity in the cellulose, as for the silica, would be signified by a vertical region
in the force *versus* separation curve. The photodiode was found to change at a rate of $-0.153$ V/nm at the largest force (46 nN) employed on the cellulose particle. It can be seen that the force curve appears linear in this regime and, hence, one concludes that linearity does not signify rigidity.

What we mean by this last remark is that the appearance of a constant compliance region in the force curve in which the photodiode signal appears to increase linearly with drive distance does not mean that the substrate has become rigid. The slope of such a region should not be used to calibrate the photo-diode because the substrate continues to flatten as the load is increased, and the deflection of the cantilever is less than the movement of the piezo-drive. The reason that a number of previous studies on deformable substrates [11–18] have used such erroneous calibration procedures is that they have assumed that it is only the curvature in the contact region that represents deformation. This is not true, as even JKR theory predicts a quasi-linear force curve at high enough loads. The data in Figs 4B and 5B, which use the calibration factor obtained for the silica substrate, confirm that one cannot use the deformable substrate to calibrate the system.

The negative nominal separations that occur for cellulose as the load is increased in Fig. 4B indicate the amount of flattening or deformation of the particle. The nominal separation is the separation that would occur between rigid particles that could interpenetrate; the actual separation (not shown for cellulose) is the physical separation of the surfaces, which is always positive [4]. For the loads shown the amount of deformation of the cellulose is as much as 25 nm. In the post-contact situation the JKR [24] theory can be fitted to the measured data using $E/(1 - \nu^2) = 22$ MPa, where $E$ is Young’s modulus and $\nu$ is Poisson’s ratio. This rather low value is consistent with a wet surface layer of the amorphous cellulose II particle.

The hysteresis evident between the loading and unloading force curves in Fig. 4B, and the decrease in deformation with increase in speed indicate that the cellulose particle is slightly viscoelastic. We have not attempted to analyse the data using the viscoelastic theory of Attard [6, 31], because of the uncertainties regarding the tip curvature, the surface energy and the influence of the capillary adhesion (see Refs [8, 19] for quantitative analyses of AFM measurements on viscoelastic materials).

The polystyrene film appeared more elastic than viscoelastic, since there was little hysteresis between loading and unloading when in contact (force curves not shown). The slightly steeper loading force curve at the higher speed in Fig. 5B is consistent with the increased stiffness on short times scales of viscoelastic theory [6, 31]. The deformation following the jump into contact has been estimated by a fit to the JKR theory, which yields an elasticity parameter, $E/(1 - \nu^2) = 0.1$ GPa. This fitted value depends on the value of the tip radius, but the latter is constrained by physically reasonable values of the Hamaker constant because the product $AR$ must be constant to fit the van der Waals force. The lower bound, which was used here, corresponds to the water–air–water value $A = 3.7 \times 10^{-20}$ J [23], which
gives \( R = 700 \text{ nm} \) and \( E/(1 - \nu^2) = 0.1 \text{ GPa} \). The upper bound corresponds to the silica–air–silica value \( A = 7 \times 10^{-20} \text{ J} \) [23], which gives \( R = 350 \text{ nm} \) and \( E/(1 - \nu^2) = 0.15 \text{ GPa} \).

The value of Young’s modulus found here, 0.1 GPa, is markedly less than the value of bulk polystyrene, \( E = 3 \text{ GPa} \) [32], and is consistent with the fact that the surface of polystyrene films is less glassy than the bulk [33–39]. It is also consistent with the value of 0.1 GPa found by Overney \textit{et al.} [39] for the storage modulus of a polystyrene film in a poor solvent. Although not stated explicitly in Ref. [39], it appears that the AFM photodiode calibration factor was taken from the slope of the force curve at the highest applied load where the tip was thought to have fully penetrated the 5-nm-thick polystyrene film and to have been in contact with the underlying silica substrate. The zero of separation was taken to be the beginning of the saturation of the AC response, and was said to represent contact with the underlying silica substrate. The sample indentation was estimated by assuming that the polystyrene behaved as a simple spring, although it was noted that the spring constant changed with applied load. The value of the zero-shear rate storage modulus was deduced using a Maxwell rheological model from the AC measurements made in water, a poor solvent for polystyrene. The present measurements give the material properties of the surface of the film because the amount of deformation is only 10 nm, and deformation decays away from the point of contact. The thickness of the soft surface layer of polystyrene has been estimated to be greater than 4 nm [39]. Because of the small amount of deformation, the thickness of the film (order of \( \mu \text{m} \)), the rigidity of the underlying substrate, or the bulk properties of the film do not have much influence on the measured data. Previous measurements on sub-micrometer polymer colloids revealed the effect of the substrate at high loads when the deformation was greater than about 10% of the radius of the colloid [8].

In contrast to the present results, the surface force measurements for polystyrene of Schmidt \textit{et al.} [17] were apparently fitted with a value for the combination of Young’s modulus and Poisson’s ratio of \( E/(1 - \nu^2) = 2.2 \text{ GPa} \). They analysed their surface force measurements with a deformation of 17 nm, which they state is consistent with the JKR theory. This is close to the bulk value but it corresponds to a material with a surface more than 20 times more rigid than is found here. The discrepancy appears due to the fact that Schmidt \textit{et al.} [17] have calibrated the force measuring bimorph from the apparently linear region at the highest applied loads. It can be seen in Fig. 5B that the force data for polystyrene are quite linear at large applied loads, but that the slope, \(-0.212 \text{ V/nm}\), is significantly less than the actual calibration factor of the AFM, \(-0.230 \text{ V/nm}\) in this case. Using the wrong calibration factor significantly overestimates the rigidity of the deformable material in any subsequent JKR analysis.

Biggs and Spinks [18] performed AFM force measurements on polystyrene spheres. However, the quantitative values reported by these authors have been viti-ated by the experimental protocols employed. In those experiments the photodidode
was calibrated from the slope of a pseudo-linear regime (and different slopes were used on the approach and on the retraction curves). As pointed out here, this neglects the flattening of the deformable particle with increasing load. The zero of separation was taken as the point where the calibration was made at the highest applied load. This again neglects the post-contact flattening of the particle. Finally, a single expansion coefficient was used for the piezo-drive for all the different drive speeds and the changes in the force curves were attributed to viscoelastic effects. As shown here, the expansion of the piezo-drive is speed dependent and this must be accounted for in a quantitative analysis of the data.

Using the wrong calibration procedure for deformable substrates has serious consequences, as is graphically illustrated in Fig. 6. Here the correct results obtained using the present method of calibration (open symbols) are contrasted with the erroneous results that use the apparently linear, high load part of the force curve to calibrate the electronic force measuring device and to set the zero of separation (filled symbols). This erroneous procedure has been used on a number of previous occasions [11–18]. It can be seen that it gives force curves with wrong shapes and wrong slopes and that it leads to a significant underestimate of the amount of deformation. The amount of error depends upon the relative stiffness of the force measuring spring and the deformable substrate.

5. CONCLUSIONS AND SUMMARY

This study has been concerned with force measurements on deformable particles and substrates using the atomic force microscope. The problem of establishing the zero of separation during force measurements in air was addressed. A common procedure in the past has been to take the zero of separation to be the separation corresponding to the position of largest load, but this is in substantial error for deformable materials. Instead we attempted to use the van der Waals force law
prior to contact. Whilst quite a good fit to the data could be obtained, the presence of water films diminished the utility of this method to establish unambiguously the zero of separation. We concluded that the best approach was to set the zero of separation at the end point of the jump. Whilst this ignores the competing effects of the initial post-jump flattening and the drainage of the water film, it does provide an unambiguous and reproducible protocol for analysing the force curves.

It was pointed out that for deformable materials the photodiode or other electronic force measuring devices must not be calibrated by fitting the linear regions of the force curves because part of the motion of the piezodrive goes into the deformation of the particle or film. Instead, the calibration has to be performed by pressing the cantilever against a rigid substrate.

It is necessary to recalibrate the photodiode for each experiment since the calibration factor varies with the positions of the cantilever and mirror, unless special care is taken in the setup [40]. The calibration must be performed in situ by moving the cantilever off the deformable particle or film and pressing it against a hard substrate. The calibration should also be performed at the same speed as the measurement unless a closed loop piezodrive is used. The expansion coefficient of the piezocrystal must be corrected for its dependence on speed using equation (1). This is particularly important for studies of viscoelastic materials where dynamic effects are of interest, because speed-dependent artefacts in the piezodrive will lead to a misinterpretation of the results unless they are accounted for. For measurement on deformable colloid particles, it is best to mount the deformable particle on the substrate rather than on the cantilever so that the calibration procedure described above can be used. For quantitative force measurements, a rigid colloid probe mounted on the cantilever can be used, and the apex of the two colloids can be located using the imaging facilities of the AFM. In cases where measurements of the interactions of two deformable colloids are required, it appears best to use the procedure of Feiler et al. [40] in which two calibrations are performed (pre- and post-mounting), with the optical path unchanged.

Specific results for the deformation of a cellulose particle and of a polystyrene film were obtained and elasticity parameters $E/(1 - \nu^2) = 22$ and 100 MPa were obtained, respectively. In the case of the polystyrene film, the deformation corresponded to an elasticity parameter that was 30-times less than the bulk value, which indicated that the surface was in a less glassy state than the bulk.

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REFERENCES

Analysis of AFM data for deformable materials