

---

# Single-Bond Force Measured by Means of Scanning Force Microscopy

M. LEKKA, J. GRYBOŚ, J. LEKKI, Z. STACHURA AND J. STYCZEŃ

Institute of Nuclear Physics, Radzikowskiego 152, 32-341 Cracow, Poland

*(Received January 2, 2002; revised version April 22, 2002)*

The aim of this investigation was to determine the adhesion force as a function of calcium dichloride concentration in water. The studies were performed with two surfaces carrying opposite charge in water solution: mica — as a negatively charged surface and glass covered with poly-L-lysine — as a positively charged surface, the latter due to the presence of amino groups. The surfaces were immersed in a  $\text{CaCl}_2$  solution in the range of concentration varied from 0 to 100 mM. The scanning force microscopy was applied to determine the adhesion force by measurements of the pull-off force. Additionally, the values of the single bond force were estimated basing on the Poisson distribution of the number of binding sites.

PACS numbers: 68.35.Gy, 07.10.Pz

## 1. Introduction

The investigation of intermolecular forces deliver important information about phenomena like adhesion or friction [1] and therefore improve our understanding of the specific interactions between ligand-receptor pairs [2, 3]. Such studies involved various techniques like surface force apparatus (SFA [1]), colloid force microscopy [4], and scanning force microscopy (SFM [5]). Recently, the latter method is widely used because of its good spatial and force resolution, providing the information on the discrete molecule–molecule interactions.

The adhesive force (i.e. pull-off force) measured by SFM can be analysed using the Poisson statistics that allows for estimation of the binding force *both* between two chemical groups *and* two molecules. The basis for this approach is the assumption that the observed adhesion (pull-off) force is a sum of a finite number of discrete, independent, randomly formed chemical bonds of similar value of the interaction force [6], and therefore is governed by the Poisson statistics.

The most important feature of the Poisson distribution is that its variance and mean value are equal. Mean adhesion force is a product of the single-bond force and the mean numbers of bonds active during the contact of the tip with the surface. The variance is a product of a square of single-bond force and of variance of the number of sites. Therefore, the dependence between the variance and the mean adhesion force is linear, its slope determines the single-bond force while the intercept with the  $Y$ -axis describes an additional non-specific force if such is present. The intercept value close to zero means that only specific force for given interaction appears.

The aim of these studies was to make a comparison between two types of surfaces: mica and glass covered with poly-L-lysine. Basing on the measured adhesion force and its distribution, the single-bond force estimate was obtained. The investigations were performed in aqueous solution of calcium dichloride (concentrations up to 100 mM) in order to study the influence of calcium ions on the adhesion and the single-bond forces.

## 2. Materials and experimental method

### 2.1. Sample preparation

The measurements were performed for two surfaces carrying different charge: the mica as a negatively charged surface and the glass covered with poly-L-lysine as a positively charged surface due to the presence of amino groups. The chemical structure of both surfaces is shown in Fig. 1a, b, respectively.

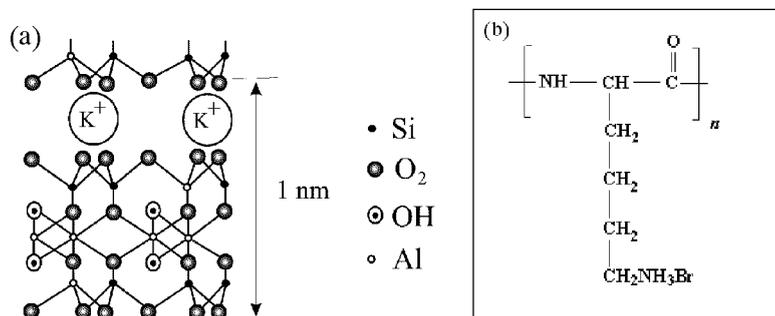


Fig. 1. The chemical structure of (a) muscovite mica,  $K[Al_2(OH)_2AlSi_3O_{10}]$  and (b) poly-L-lysine,  $C_6H_{12}N_2O_2HBr$ .

The cleavage of the muscovite mica was performed along  $K^+$  surface, in deionised water directly prior to use [11]. When mica is immersed in water solutions, its surface has a negative charge [7].

The glass surface was prepared as follows: the drop of 1% poly-L-lysine solution (poly-L-lysine hydrobromide, molecular mass 70–150 kDa [12]) was put onto cleaned glass coverslip for 5 minutes and left for overnight to dry in a clean atmosphere. In the presence of water molecules the amino groups become positively charged. Figure 2 shows the topography of the surface of glass (Fig. 2a) and glass covered with poly-L-lysine (Fig. 2c) obtained in water with the SFM working in contact mode. The corresponding cross-sections (Fig. 2b, d) show the roughness

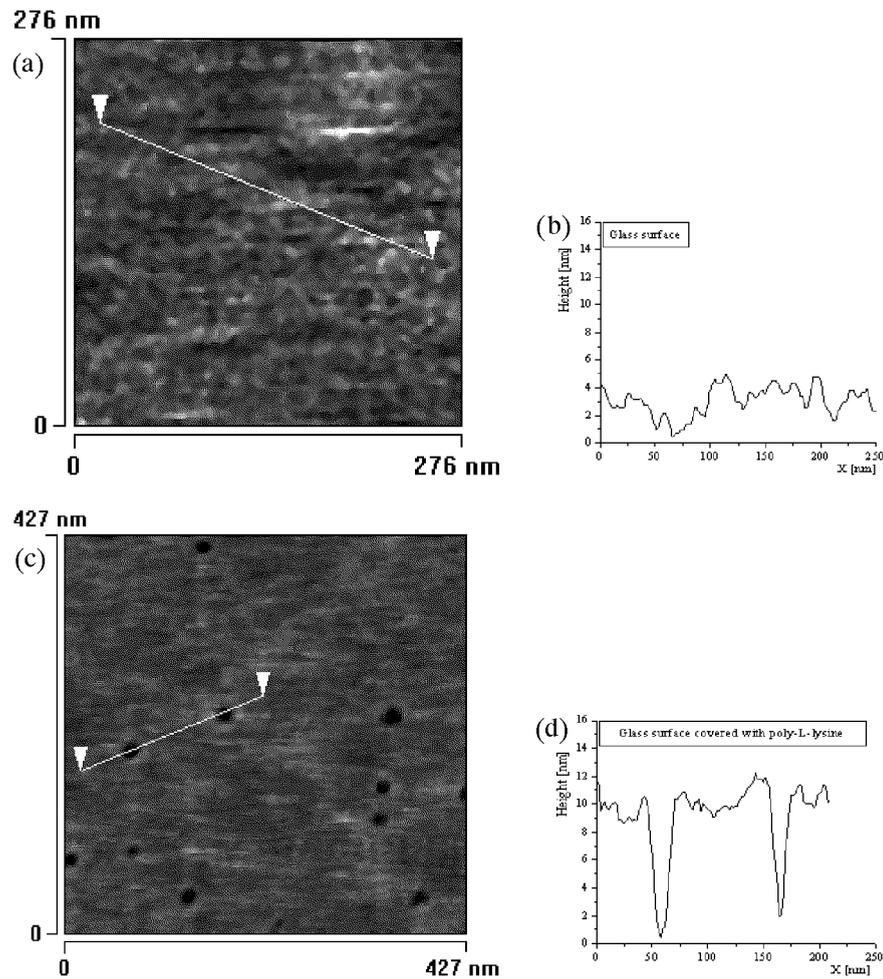


Fig. 2. The SFM images of the topography and corresponding cross-sections of the glass surface (a, b) and the glass covered with poly-L-lysine (c, d). The vertical resolution of the SFM used was about 2 nm.

of both surfaces. The randomly distributed holes in the poly-L-lysine surface have the same depth ( $9 \pm 2$  nm), which is probably a measure of the thickness of the poly-L-lysine layer.

The fluids used in the experiment were solutions of the calcium dichloride (Poch S.A. Gliwice, Poland) prepared using the deionised water (18.2 M $\Omega$  cm, Barnstead water purification system, pH 6.0–6.4). The range of applied concentrations varied from 10 mM to 100 mM. Additionally, the measurements were performed in pure deionised water. All fluids were filtered (directly before using in SFM) applying the 0.2  $\mu$ m filter in order to remove organic contaminations from the liquid.

## 2.2. Scanning force microscope

The scanning force microscope used was a home-built device that was described in details elsewhere [8]. It is equipped with a "liquid cell" setup made of plexiglass. All measurements were recorded at room temperature using commercial Si<sub>3</sub>N<sub>4</sub> cantilevers (MLCT-AUHW; Atos GmbH, Germany). The nominal spring constant of the cantilevers was 0.03 N/m. The measurements of resonant frequency of thermally excited cantilevers allowed for a selection of the cantilevers with similar spring constant. Tip radii were of about 80 nm. They were checked using TGT01 silicon standard (NMDT, Moscow). The velocity of the atomic force microscopy approach/retract cycle was set to 2.6  $\mu$ m/s and kept constant.

The values of the pull-off force were obtained from the retracting part of the force-versus-sample position curves. The adhesion force is defined as a force needed to separate two bodies from each other. Therefore, the pull-off force is a measure of the adhesion force. Due to measurement errors, the value must be averaged over a large number of force-versus-distance curves. Therefore, at each position on the surface a set of 100 force curves was collected. The total number of sets collected for the particular surface at a given calcium chloride solution was 6. Every set was measured using a new tip, a new sample and fresh solution. The final value of adhesion force was determined by calculating the mean value and a standard deviation from all data collected for a given concentration.

## 3. Results

### 3.1. Results of the adhesion measurements

Figure 3 represents the measured adhesion force as a function of calcium dichloride concentration. For every salt concentration, the average value of the adhesion force was determined basing on a histogram of the adhesion force. Two different surface dependent behaviours were observed:

— for the mica surface: the adhesion force increased up to the maximum value at 40 mM concentration of calcium dichloride. Then, its value decreased with the increasing salt concentration;

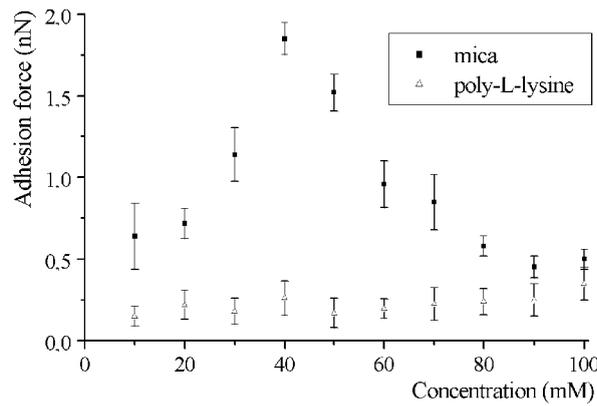


Fig. 3. The adhesion force as a function of the calcium chloride concentration measured using scanning force microscopy between the bare silicon nitride tip and two surfaces: the mica and the glass covered with poly-L-lysine.

— for the poly-L-lysine covered glass surface: the adhesion force was independent of the salt concentration and almost constant in the range of the experimental error.

### 3.2. Results of the single-bond force estimation

Using the distribution of the adhesion measurements and assuming the Poisson statistic, the estimates of the strength of the single chemical bond were performed for both types of surfaces (Fig. 4). The method of the estimation of

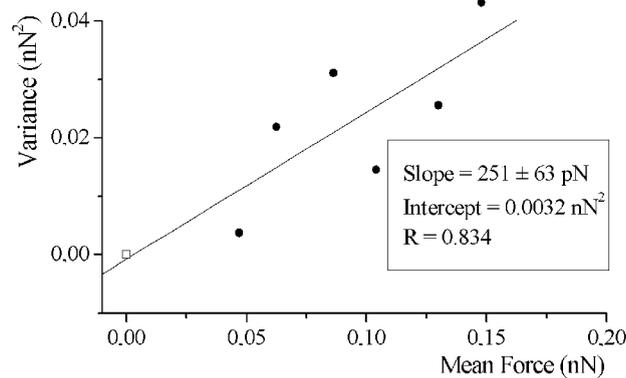


Fig. 4. An example of the relation between a variance and a mean force (black dots) obtained for mica surface and silicon nitride tip immersed in 20 mM aqueous solution of  $\text{CaCl}_2$ . The linear regression line is plotted. The origin is marked (square) but it was not included in the linear fit in order to get information about non-specific forces. The value of slope ( $251 \pm 63$  pN) corresponds to the strength of the single bond.

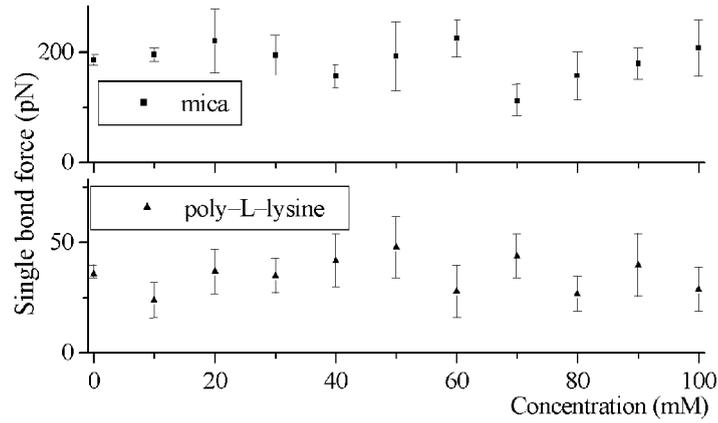


Fig. 5. Single-bond force as a function of the calcium chloride concentration obtained for mica and for the glass covered with poly-L-lysine.

single-bond force was described by Han et al. [6]. In both cases, the single-bond force was independent of ion concentration (Fig. 5), in spite of the adhesion force variation observed for the mica. Therefore, the average value of the single-bond force was obtained by calculation of the mean value for all concentrations (from 0 mM to 100 mM). The single-bond force for the mica surface was  $185 \pm 33$  pN and for the glass covered with poly-L-lysine — the value  $35 \pm 8$  pN was obtained.

#### 4. Discussion

The DeJarguin–Landau–Verwey–Overbeek (DLVO) theory describes the interplay of the attractive van der Waals force and the repulsive electrostatic “double-layer” force between charged surfaces immersed in aqueous salt solutions and screened by electrolyte ions [1]. According to this theory, the observed adhesion force is a result of the competition between repulsion and attraction and its nonzero value indicates the dominant role of the van der Waals attraction during the contact of the surface with the cantilever’s tip. However, during contact another adhesive components may be observed. These are forces that act in certain discrete points on the surface, like forces coming from the chemical bonds formed between two surfaces. If the adhesive sites are so much screened that the force is zero after breaking the contact, the interaction cannot be described by the DLVO theory. The number of adhesive sites during repeated measurements is governed by the Poisson statistics. Assuming equal force for each bond, both the single-bond force and the numbers of active bonds is obtained from statistical analysis.

Therefore the measured pull-off (adhesion) force presented in Fig. 3 may be explained in two ways:

— by the competition of the attractive van der Waals force and the repulsive electrostatic “double-layer” force (DLVO theory). Electrostatic forces may achieve values comparable to the van der Waals interaction and decrease with distance with characteristic Debye length [1];

— by the formation of numerous discrete single chemical bonds. The separation process breaks each single bond. The larger number of chemical bonds is created, the higher is the adhesion value.

#### 4.1. The DLVO approach

Taking into account the DLVO theory, at low salt concentrations the repulsive electrostatic term is stronger than the van der Waals component and the resulting adhesion force is close to zero. In contrast to the van der Waals interactions, weakly dependent on the electrolyte concentration, the electrostatic force is strongly decreasing with the increasing salt concentration. When the salt concentration increases, the repulsion decreases and positive adhesion values are observed. Surprisingly, with further increase in  $\text{CaCl}_2$  concentration, adhesion force vanishes again. This may be explained by introduction of the hydration forces, observed already in similar systems [7–9]. The hydration force originates from binding of water dipoles by counter ions adsorbed at the surfaces and their character is repulsive because of the force needed to remove water dipoles prohibiting the direct contact of the surfaces. The role of the hydration forces increases with salt concentration and they are responsible for the decrease in the adhesion force in  $\text{CaCl}_2$  for the mica surface as seen in Fig. 3.

The almost constant value of the adhesion force in the case when the glass surface was covered with poly-L-lysine indicates that the influence of ions was insignificant. In this case the DLVO approach is not sufficient to explain the small and constant value of the adhesion force. Additional interactions must be taken into account: attractive electrostatic force acting between positively charged poly-L-lysine coated surface and negatively charged silicon nitride SFM tip and two repulsive interactions: steric forces coming from internal structure of polymer (poly-L-lysine) and hydration forces.

#### 4.2. The “discrete” approach

In water solutions, at all investigated surfaces and the SFM tip, certain chemical groups are present that can be involved in the formation of the adhesion force. Also, in any aqueous solution certain number of positively charged hydrogen ions ( $\text{H}^+$ ) and negatively charged hydroxyl ions ( $\text{OH}^-$ ) is created during dissociation of water molecules. In ambient conditions, a layer of surface oxides covers the silicon nitride surface. When immersed in water, the process of the hydrolysis produces silanol groups on the surface [10]. The surface silanols can participate in hydrogen bonding between two surfaces immersed in aqueous solution. Mica has negative charge in water and in electrolyte solutions. A smooth cleavage face of mica is

covered by  $K^+$  ions that neutralise the negative lattice charge due to the periodic replacement of Si atoms by Al [7]. When mica is immersed in water solutions,  $K^+$  ions desorb from the surface and a negative charge on the surface remains coming from the formed silanol groups. Poly-L-lysine covered glass surface has positive charge due to the presence of amino groups.

The dependence between the adhesion force and concentration of calcium dichloride obtained for the mica surface and the silicon nitride tip can be explained using the Poisson statistics, assuming that the adhesion force is a sum of the discrete, single chemical bonds. It has to be mentioned that the higher is the value of the adhesion force, the larger is the number of single chemical bonds formed as it can be deduced from the comparison of Fig. 4 with Fig. 6. It may be assumed that the hydrogen bonds between the mica surface and the SFM silicon nitride tip are created due to the presence of the silanol groups on both surfaces. Additionally, in the Ca solution, a certain amount of hydrogen bonds can be formed due to the presence of hydroxyl groups attached to calcium ions by the strong ionic bonds.

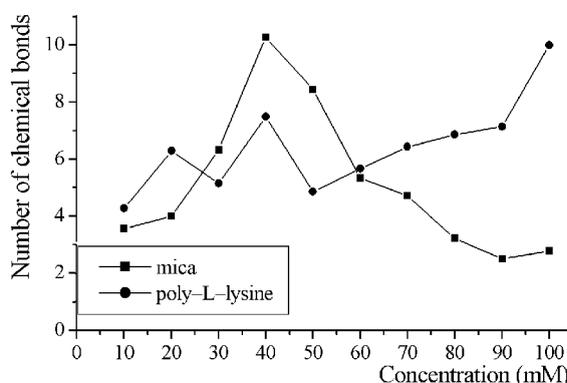


Fig. 6. The number of chemical bonds calculated for a given salt concentration. Squares correspond to mica surface while circles denote poly-L-lysine coated glass.

For smaller concentrations of calcium (below 40 mM) the number of chemical bonds increases with the concentration of Ca ions. Hydroxyl groups attached to Ca can interact with silanols present on both surfaces through hydrogen bond that is weaker than the ionic bond. During separation of the SFM tip from the mica surface, the adhesion force is a result of breaking of hydrogen bonds that were formed either between the silanols on two surfaces or between the silanols on one surface and hydroxyl groups attached to calcium ions. The decrease in the adhesion force for higher salt concentrations (over 40 mM) indicates decreasing number of the hydrogen bonds. This fact can be explained as follows. At low Ca concentrations one calcium ion attaches two hydroxyl groups. At higher Ca concentrations, if there are no free hydroxyl groups, then calcium ion attaches to

the surface involving two silanol groups (either from mica or from silicon nitride surface), thus preventing the formation of hydrogen bonds between surfaces. The obtained value  $186 \pm 9$  pN from the measurements performed in pure water is in a very good agreement with the value reported in the paper by Han et al. ( $181 \pm 35$  pN [6]), and with the value of  $185 \pm 33$  pN obtained as an average for all Ca concentrations.

When the investigated surface was the glass surface covered with the poly-L-lysine, the obtained value for pure water was  $36 \pm 2$  pN and the value averaged over all concentrations was  $35 \pm 8$  pN. Also in this case the most probable chemical bonds were the hydrogen bonds that can be created between the silanol groups of the silicon nitride surface and the amino groups coming from poly-L-lysine molecules.

The energy of the hydrogen bonding between a donor group and an acceptor group depends on the separation and relative orientation of the groups of atoms. The difference between the single-bond force value of the hydrogen bond between the silicon nitride–mica and the silicon nitride–poly-L-lysine surfaces may reflect a different nature of bonds (O–H–O, O–H–N, respectively).

## 5. Conclusions

The adhesion force measured by SFM results from two kinds of interaction: continuous forces that can be described by the DLVO theory and forces that act in certain discrete points on the surface i.e. coming from the chemical bonds formed between two surfaces. The observed dependence of adhesion force and ions concentration can be explained by any of the two approaches, but it is possible to choose a more suitable one if additional information about the contacting surfaces is known. However, a choice of the proposed explanation of experimental findings needs further confirmation.

## Acknowledgments

We would like to thank Dr. B. Kubica from Department of Nuclear Physical Chemistry, Institute of Nuclear Physics (Cracow) for helpful remark and discussions.

This work was partially supported by the grants: No. 6 P04A 038 17 and No. 6 P05A 129 21 of the State Committee for Scientific Research.

## References

- [1] J. Israelachvili, *Intermolecular and Surface Forces*, Academic Press, San Diego 1992.
- [2] E.L. Florin, V.T. Moy, H.E. Gaub, *Science* **264**, 415 (1994).

- [3] P. Hinterdorfer, W. Baumgartner, H.J. Gruber, K. Schilcher, H. Schindler, *Proc. Natl. Acad. Sci.* **93**, 3477 (1996).
- [4] W.A. Ducker, T.J. Senden, R.M. Pashley, *Nature* **353**, 239 (1991).
- [5] B. Cappella, G. Dietler, *Surf. Sci. Rep.* **34**, 1 (1999).
- [6] T. Han, J.M. Williams, T.P. Beebe Jr, *Anal. Chim. Acta* **307**, 365 (1995).
- [7] R.M. Pashley, *J. Coll. Interface Sci.* **80**, 153 (1981).
- [8] M. Lekka, J. Lekki, M. Marszałek, Z. Stachura, B. Cleff, *Acta Phys. Pol. A* **93**, 421 (1998).
- [9] H.J. Butt, *Biophys. J.* **60**, 1438 (1991).
- [10] T.J. Senden, C.J. Drummond, *Coll. Surf. A: Physicochem. Eng. Aspects* **94**, 29 (1995).
- [11] J. Colchero, Ph.D. thesis, Konstanz 1993.
- [12] Sigma Catalogue, Sigma-Aldrich Co, USA, 2152 (1999).