Quantitative reinterpretation of quartz-crystal-microbalance experiments with adsorbed particles using analytical hydrodynamics

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Despite being a fundamental tool in soft matter research, quartz crystal microbalance (QCM) analyses of discrete macromolecules in liquid so far lack a firm theoretical basis. Currently, acoustic signals are qualitatively interpreted using ad-hoc frameworks based on effective electrical circuits, effective springs and trapped-solvent models with abundant fitting parameters. Nevertheless, due to its extreme sensitivity, the QCM technique pledges to become an accurate predictive tool. Using unsteady low Reynolds hydrodynamics we derive analytical expressions for the acoustic impedance of adsorbed discrete spheres. Our theory is successfully validated against 3D simulations and a plethora of experimental results covering more than a decade of research on proteins, viruses, liposomes, massive nanoparticles, with sizes ranging from few to hundreds of nanometers. The excellent agreement without fitting constants clearly indicates that the acoustic response is dominated by the hydrodynamic impedance, thus, deciphering the secondary contribution of physico-chemical forces will first require a hydrodynamic-reinterpretation of QCM.

PACS numbers:

The quartz crystal microbalance (QCM) is one of the most versatile tools to study subtle effects in soft matter, resolving forces in the pico-Newton to nano-Newton range and nano-gram masses. Due to its low operating cost, sensor compactness, real-time data, label-free operation and subnanogram sensitivity, QCM has become a fundamental tool in analytical chemistry and biophysics research. The number of applications (from nanotribology to health care, environmental monitoring \textsuperscript{[1,2]} and even crude oil \textsuperscript{[3,4]}) is huge and cannot be exhaustively listed here. QCM has also become one of the important techniques in biosensing for DNA \textsuperscript{[6,7]} and other biomolecules \textsuperscript{[10]} including virus detection \textsuperscript{[11]}.

These distinct features make QCM competitive with other common analytical and detection tools \textsuperscript{[12]} such as optical DNA detection via fluorescence-labeled oligonucleotides, surface plasmon resonance \textsuperscript{[13]}, or electrochemical assays. Subtle nanometric phenomena such as variations in contact forces, molecular stiffness \textsuperscript{[14,15]}, kinetics of adsorption or bio-molecular interactions \textsuperscript{[16]} are routinely sensed using QCM. However, in these liquid environments, QCM lacks the theoretical foundation required to become a measurement technique.

The idea of using the inverse piezoelectric effect to sense mass, which is in essence the QCM, was born for experiments in a vacuum. The surface of a cut of quartz exposed to an AC potential, oscillates at MHz experiments in a vacuum. The surface of a cut of crys-

\[ m_{\text{QCM}} = -C \Delta f / n \]  

where \( n \) is the overtone of the surface wave (odd integer \( n \leq 13 \)). The mass sensitivity constant, typically \( C = 17.7 \text{ ng} \cdot \text{cm}^{-2} \text{Hz}^{-1} \), reveals an extremely small limit of detection, as \( \Delta f \sim -0.1 \text{ Hz} \) represents 1.7 ng/cm\(^2\).

Interpreting QCM in liquids faced challenges, many of them still unresolved. In a liquid, viscous forces propagate the wall oscillation upwards, moving a layer of fluid of about 3 times the so-called penetration depth \( \delta = (2\eta/\rho \omega)^{1/2} \) (here \( \omega = 2\pi f \), \( \eta \) is the fluid viscosity and \( \rho \) its density). In water, \( \delta \propto \eta^{-1/2} \) typically ranges from 71 to 238 nm. The resulting laminar flow, called Stokes flow, creates wall viscous stress oscillating with a 45° phase lag with respect the surface motion. The out-of-phase component damps the wall motion. Its decay rate \( \Gamma \) is directly measured in “ring-down” sensors (QCM-D) \textsuperscript{[2]} while in forced QCM, this dissipative effect broadens the spectra, with a quality factor \( D^{-1} \). The new actor, “dissipation” \( D = 2\Gamma / f \), introduces another channel of information in liquids. In Newtonian fluids \textsuperscript{[18,19]} \( -\Delta f \) and \( \Delta \Gamma \) are equal and proportional to the mass of moving fluid. Viscoelastic films \textsuperscript{[20,21]} present different contributions which can be traced using 1D laminar flow equations. However, QCM was soon used to investigate all sorts of soft discrete 3D objects, for which an analytical approach has so far been elusive. The QCM technique faced proteins \textsuperscript{[22,23]}, DNA strands \textsuperscript{[24]}, supported lipid bilayers \textsuperscript{[25,26]}, polymers \textsuperscript{[27]}, vesicles \textsuperscript{[28]}, liposomes \textsuperscript{[8,4,30]}, viruses \textsuperscript{[11,31]}, different kinds of nano and microparticles \textsuperscript{[6,7,10,32]}, bacteria \textsuperscript{[13]}, living cells \textsuperscript{[33]}, crude oil \textsuperscript{[34]} and more.

Experiments urgently required ways to rationalize the distinct acoustic features and peculiar behaviors of these

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discrete analytes. The adsorbed mass predicted from \( \Delta f \) using Eq. 1 was seen to significantly differ (usually appearing larger) than other independent measurements of \( m \), e.g. using scanning electron microscopy (SEM) \( [34, 35] \). For more than one decade such a difference, measured by the mass ratio \( H = 1 - m/m_{\text{QCM}} \) \( [34, 32, 57] \), has been explained using the “trapped solvent” model \( [34] \) which assumes that the extra QCM mass is due to solvent molecules being trapped by the analyte and moving concomitantly with it. Despite the reported deficiencies \( [34] \), several versions of this model are still routinely used to interpret experiments \( [37] \).

Another unexplained puzzling phenomenon concerns the frequency inversion. As the analyte size (or QCM frequency) is increased, \( \Delta f \) becomes more and more negative until above a certain size (or frequency) it suddenly becomes positive \( [38] \). Phenomenological models were designed to reproduce such behavior. The coupled-resonator model \( [39] \) is based on a series of masses connected with effective springs representing analyte-wall contacts \( [2, 35, 41] \) placed either in parallel (Kelvin-Voight) or in series (Maxwell model) \( [14] \). This model predicts a transition from “inertial” (\( \Delta f < 0 \)) to “elastic” (\( \Delta f > 0 \)) response at high frequency, when the large contact stiffness overpowers the inertia of deposited mass. Imaginary springs are also added to act as dampers, introducing the concept of “viscous load” (\( \Delta \Gamma > 0 \)) of the adsorbed structure. Tuning the model parameters permits fitting experimental data and gauging different analyte “stiffnesses”, adsorbed “mass” or analyte-wall “interactions”. However, the coupled-resonator model completely neglects the role of the solvent hydrodynamics. These phenomenological pictures very much constitute the basis of present analyses \( [14, 15] \). Quoting Tarnapolsky and Freger \( [11] \), QCM-D has “mainly become a comparative tool in particle adhesion research. Unfortunately, such development lacks an adequate quantitative model”.

About one decade ago, simulations started to highlight the relevance of hydrodynamics in discrete-particle QCM \( [31, 42] \). Coverage effects such as the decrease of the acoustic ratio \(-\Delta D/\Delta f\) with \( \Delta f \) were qualitatively reproduced in 2D simulations \( [31, 42] \) and later in 3D \( [43, 14] \), revealing a hydrodynamic origin, which has not yet been theoretically explained. The relevance of the particle shape \( [45] \) was also analyzed. Recently, it was proved that hydrodynamics lie behind the extreme sensitivity of QCM to how broadly mass is distributed over the resonator \( [1] \) and also that it is responsible for anti-Sauerbrey responses (\( \Delta f > 0 \)) \( [58] \).

Before introducing the concept of hydrodynamic impedance, a comment on the phasor formalism is in order. The resonator position can be expressed as \( \ddot{x}(t) = \text{Re}[x \exp(-i\omega t)] \) where \( \omega \equiv 2\pi f + i \Gamma \) is the complex frequency and \( x \) is its phasor. This complex number determines its phase lag with respect some time reference. The central phasor quantity in QCM is the impedance \( Z = \sigma_{\text{wall}}/v_0 \) which, following the small load approximation (\( \Delta f/f << 1 \)), relates the overall tangential wall-stress \( \sigma_{\text{wall}} \equiv \dot{x} \cdot \sigma_{\text{wall}} \cdot \dot{z} \) with the complex frequency shift \( \Gamma \),

\[
\Delta f + i\Delta \Gamma = if \frac{Z}{\pi Z_Q},
\]

where the impedance of the quartz crystal cut is usually \( Z_Q = 8.8 \times 10^6 \text{ kg/(m}^2\text{s}) \).

The origin of the hydrodynamic impedance is simple \( [3, 38] \): any force acting on the analyte propagates fluid momentum to the resonator, creating extra wall-stress which is measured by the QCM. It is important to note that particle-forces arise not only from molecular linkers, adhesion forces, etc., but they are also induced by the fluid traction itself. In fact, we shall show that the QCM response is dominated by fluid-induced forces. In any case, to understand the QCM response one needs to determine the lag-time required to transmit the analyte-force to the wall. This time crucially depends on the vertical coordinate \( z \) because (as shown below) the fluid-momentum propagator is proportional to \( \exp(-\alpha z) \), with \( \alpha = (1 - i)/\delta \). An oscillatory force (phasor \( F \) acting at some point located at \( z = d \) transfers a stress \( F/A \exp(-\alpha d) \) to the wall (\( A \) is the resonator area). If the force is placed at the wall (\( d = 0 \)) this leads to \( \text{Re}[Z] = 0 \) and \( \text{Im}[Z] > 0 \) (or, from Eq. 2 \( \Delta \Gamma = 0 \) and \( \Delta f < 0 \)). In the QCM jargon this would correspond to an inertial load. But the very same force applied at \( d = (\pi/2)/\delta \) would then be understood as a purely viscous load (\( \Delta D > 0 \) and \( \Delta f = 0 \)), while farther away it would become an elastic load (\( \Delta f > 0 \)). This simple example clearly illustrates the need for a rigorous hydrodynamic reinterpretation of QCM signals.

In general, the values of \( \Delta f \) and \( \Delta \Gamma \) result from summing up the propagation of all forces acting on each point of the ensemble of analytes. This leads to a far-from-trivial convolution expression, which should be derived using zero-Reynolds unsteady hydrodynamics \( [46-48] \). Indeed, the hydrodynamics of QCM gathers all the difficulties one might expect: the semi-bounded unsteady flow lacks spherical symmetry and obtaining the perturbative flow created by the particle (which creates the extra wall stress) requires solving the dynamics of the analyte, which, in turn, is coupled to the fluid-induced forces. While such an intertwined problem can be partially tackled in the case of point particles \( [10] \), many QCM analytes (liposomes, nanoparticles) are far from being “points” and reach the size of the penetration depth \( R \sim \delta \sim 100\text{nm} \). Fortunately, QCM senses the total stress over the surface which simplifies the analytical expressions for the impedance of finite adsorbed particles, derived below. Comparison with 3D simulations and abundant available experimental data proves that our approach is valid up to \( R/\delta < 2 \). Notably, although we just consider free particles (wall-particle forces are absent) the theory shows an excellent agreement with quite a disparate set of experiments, without any fitting constants. This result urgently calls for a quantitative reinterpret-
tion of QCM signals starting from the dominant role of hydrodynamics, adding to the predictive power of QCM and becoming a tool for measuring relevant forces, due to molecular/structural elasticity, adhesion, ionic-strength or other long-ranged physico-chemical interactions with the substrate.

I. THEORY

We consider a sphere of radius \( R \) and density \( \rho_p \) whose center, located at \( \mathbf{r}_c = (0,0,d) \), is at distance \( d \) from the QCM plane \( z = 0 \). The QCM resonator oscillates at angular frequency \( \omega \) in the \( x \) direction with velocity \( v_0 \cos(\omega t) \) and its amplitude \( x_0 \) is small enough (typically around 2nm) to neglect non-linear couplings. The total impedance \( Z \) sums up all the forces (per area) acting on the surface. As customary, the baseline is set at the impedance of the base Stokes flow (equal to \( \eta \alpha \)) so we consider stress in excess of that reference. The forces acting on the wall are either directly due to the particle (impedance noted as \( \mathbf{Z}_{\text{pw}} \)) or to the fluid (hydrodynamic impedance, \( \mathbf{Z}_{\text{hydro}} \)).

\[
\mathbf{Z} = \mathbf{Z}_{\text{pw}} + \mathbf{Z}_{\text{hydro}} \tag{3}
\]

In turn, \( \mathbf{Z}_{\text{pw}} \) has contributions from the particle inertia and from wall-particle forces (adhesion, molecular linkers, etc.). The latter will not be considered hereafter, so as to isolate the hydrodynamic effects. The particle inertia is just the Archimedean force due to the acceleration of the excess particle mass so that \( \mathbf{Z}_{\text{pw}} = i n m v_0 \). Implicitly, we have assumed that the adsorbed particle velocity \( \mathbf{u} \) concomitantly follows that of the resonator \( \mathbf{u} = v_0 \). Here \( n \) is the particle’s surface density and \( m_{\text{e}} = (\rho_p - \rho) V_p \) is the excess in mass with respect to the displaced fluid \( (V_p = 4\pi R^3/3 \) is the particle volume \). In terms of the scaled impedance \( \tilde{Z}_{\text{pw}} \equiv \mathbf{Z}_{\text{pw}}/(6\pi n m R) = -(2/9)(\rho_p/\rho)(\alpha R) \), with \( \rho_p = \rho_p - \rho \). This precisely the Sauerbrey contribution to the impedance, with zero dissipation and negative frequency shift (i.e., \( \text{Re}[Z_{\text{pw}}] = 0 \) and \( \text{Im}[Z_{\text{pw}}] > 0 \), as \( \text{Im}[\alpha^2] < 0 \).

Any force acting on the particle is transferred back to the fluid (Newton’s third law) as a force density field which propagates momentum to the surface and creates extra wall-stress (detected by the QCM device as frequency \( \Delta f \) and dissipation \( \Delta D \) shifts). As stated, here we will only consider fluid-induced forces. The fluid velocity field can be expressed as \( \mathbf{v} = \mathbf{v}_\infty + \mathbf{v}_p \), where \( \mathbf{v}_p \) is the perturbative flow created by the particle presence and the ambient fluid \( v_\infty \) is here ascribed to the base laminar Stokes profile \( \mathbf{v}_\infty = \mathbf{v}_S = v_S(z) \mathbf{x} \). Its phasor satisfies \( v''_S - \alpha^2 v_S = 0 \) (prime denotes spatial derivation) with boundary conditions \( v_S(0) = v_0 \) and \( v_S(\infty) = 0 \). The solution, \( v_S(z) = v_0 \exp(-\alpha z) \), unveils the exponential propagator of momentum mentioned above.

The hydrodynamic impedance requires evaluating the tangential stress due to the perturbative flow at the resonator \( z = 0 \). Such a flow is governed by the Green function tensor field \( \mathbf{G}(\mathbf{r}, \mathbf{r}') \) of the problem (Methods). For instance, a point-particle at \( \mathbf{r}_c \) receiving an oscillatory force (phasor) \(-\mathbf{F}\) creates a flow field \( \mathbf{v}_p(\mathbf{r}) = \mathbf{G}(\mathbf{r}, \mathbf{r}_c) \mathbf{F} \). A finite particle propagates the forces acting on each differential element \( ds' \) on its surface, which (in the absence of wall or external forces) is induced by the fluid pressure at the particle surface, so

\[
\mathbf{v}_p(\mathbf{r}) = \int \mathbf{G}(\mathbf{r}, \mathbf{r}') \mathbf{\Pi}(\mathbf{r}') \cdot \mathbf{n} ds'. \tag{4}
\]

Here \( \mathbf{\Pi}(\mathbf{r}') \) is the local fluid pressure tensor, \( \mathbf{n} \) is the outward surface vector and the integral runs over the particle surface with \( ds' \) centered at \( \mathbf{r}' \). As \( v_S(z = 0) = v_0 \), one has to impose \( \mathbf{v}_p = 0 \) at \( z = 0 \) and at \( z \to \infty \); these boundary conditions are inherited by \( \mathbf{G}(\mathbf{r}, \mathbf{r}') \) (see Methods). In the present setup, however, an explicit derivation of \( \mathbf{v}_p \) faces serious difficulties. The Fixén theorem route consists in integrating the no-slip condition \( \mathbf{v} = \mathbf{u} \) at the particle surface to impose a translational (and in general rotational) constraint \( \mathbf{v} = \mathbf{u} \) onto Eq. 4. Providing \( \mathbf{u} \) should lead to \( \mathbf{\Pi} \). In general, though, \( \mathbf{u} \) for suspended particles has to be determined from the particle equation of motion (for a free particle, \( -im_\omega h = \oint \mathbf{\Pi}(\mathbf{r}') \cdot \mathbf{n} ds' \)). Due to the lack of spherical symmetry this route becomes impracticable and, to complicate matters further, in this setup \( \mathbf{G}(\mathbf{r}, \mathbf{r}') \) has no closed analytical form[47]. A second route, based on hydrodynamic reflections[48, 49], is to expand \( \mathbf{\Pi} \) into ambient and perturbative parts \( \mathbf{\Pi} = \mathbf{\Pi}_S + \mathbf{\Pi}_p \). Introducing this form into Eq 4 leads to a series expansion with operators acting on \( \mathbf{v}_p \) and involving increasing powers of \( \mathbf{G} \). But again, this requires a closed form for \( \mathbf{G} \) in real space. Fortunately, Felderhof[18] demonstrated that it is possible to derive the Fourier transform of \( \mathbf{G}(\mathbf{r}, \mathbf{r}') \) in the xy-plane which, as we will show shortly, suffices for our purposes. The pressure tensor has a viscous stress \( \mathbf{\sigma} \) and a kinetic pressure contribution which create a viscous \( \mathbf{Z}_c \) and kinetic \( \mathbf{Z}_k \) impedance derived below. The kinetic stress is just the virial pressure created by fluid inertial forces relative to the base flow \( \omega(\rho_p \mathbf{u} - \mathbf{v}_S) \) (with \( \mathbf{v}_S = (3/(4\pi R^4)) \int \mathbf{v}(\mathbf{r}) d^3r \) the average fluid velocity over the particle volume). The viscous stress includes a dominant contribution from the Stokes base flow \( \mathbf{\sigma}_S \) and another from the perturbative flow \( \mathbf{\Pi}_p \). The excess pressure tensor at the particle surface can thus be written as

\[
\mathbf{\Pi}(\mathbf{r}') = \mathbf{\sigma}_S(\mathbf{r}') + i \omega \left( \rho_p \mathbf{u} - \mathbf{v}_S \right) \mathbf{r}' + \mathbf{\Pi}_p, \tag{5}
\]

where \( \mathbf{\sigma}_S = \eta_S \mathbf{v}_S(z) \mathbf{\hat{x}} \) and \( \mathbf{\Pi}_p \) is expected to be small for \( R/\delta < O(1) \) and shall be neglected in this analysis. This approximation finds support later in the comparison to simulations and experimental results. We assume that the particle moves in the \( x \) direction \( \mathbf{u} = u \mathbf{x} \) and note that \( \mathbf{\nabla}_S = \mathbf{\nabla}_S(\mathbf{\hat{x}}) \mathbf{\hat{x}} \).

To evaluate the net shear stress at the wall, one needs to integrate over the resonator plane (\( z = 0 \)), the tangential stress \( \eta \partial_z \mathbf{v}_p \) due to the perturbative flow in Eq.
\( \dot{\sigma}_{\text{wall}} = n\eta \int_{z=0} dS \int_{r=a} dS' \hat{x} \cdot [\partial_z \mathbf{G}(r, r')]_{z=0} \Pi \cdot \hat{n} dS' \)  

(6)

Owing to the planar symmetry of the system \( \mathbf{G}(r, r') = \mathbf{G}(s - s', z, z') \) where \( r = s + z \hat{k} \) and \( s \) lies on the xy-plane. This permits the introduction of the Fourier transform on the xy-plane,

\[ \mathbf{G}(s - s', z, z') = \int dq e^{iq(s - s')} \hat{G}(q, z, z') \]

to obtain

\[ \dot{\sigma}_{\text{wall}} = n\eta \int_{z=0} d^2s \int_{r=a} d^2S' \int dq e^{iq(s - s')} \hat{x} \cdot \partial_z \hat{G}(q, z, z') \Pi \cdot \hat{n} dS' \]  

(7)

Using the Dirac delta relation \( \int \exp(-i\mathbf{q} \cdot \mathbf{s}) ds^2 = 4\pi^2\delta(\mathbf{q}) \),

\[ \dot{\sigma}_{\text{wall}} = 4\pi^2 n\eta \int_{r=a} d^2S' \lim_{q \to 0} \hat{x} \cdot [\partial_z \hat{G}(\mathbf{q}, z, z')]_{z=0} \Pi \cdot \hat{n} dS' \]  

(8)

Taking the \( q \to 0 \) limit in the full expression for \( \partial_z \hat{G}(\mathbf{q}, z, z') \) at \( z = 0 \) (see Ref. [50]) leads to a particularly simple expression. For the relevant \( xx \) component,

\[ \lim_{q \to 0} \partial_z \hat{G}_{xx}(\mathbf{q}, z = 0; z') = -\frac{\exp[-\alpha z']}{4\pi\eta} \]

This allows us to integrate \( \dot{\sigma}_{\text{wall}} \) and derive the impedance due to the viscous stress \( \sigma_S \) in [5]

\[ \hat{Z}_v(d, R) = -\frac{Z_v}{6\pi\eta R} \frac{\pi e^{-2\alpha d}}{6} \left( \frac{2\alpha R \cosh(2\alpha R) - \sinh(2\alpha R)}{\alpha R} \right) \]  

(9)

and the kinetic contribution,

\[ \hat{Z}_k(d, R) = \frac{2\pi e^{-\alpha d}}{3 \alpha R} \left( \alpha R \cosh(\alpha R) - \sinh(\alpha R) \right) \left( \frac{\rho_p u - \rho \bar{v}^{(a)}}{\rho v_0} \right) . \]  

(10)

These expressions apply for a particle suspended at a distance \( d \) over the resonator, moving with a velocity \( u \) (in turn, \( u \) needs to be determined from the flow-traction, see SI). To evaluate the impedance of adsorbed particles \( Z^{(ad)} \) we set \( d = R \) and \( u = v_0 \) and add the Sauerbrey contribution, leading to

\[ \hat{Z}^{(ad)}(R) = -\frac{2\rho_p}{9\rho} (\alpha R)^2 + \hat{Z}_v(R, R) + \hat{Z}_k(R, R) + \hat{Z}_p \]

(with \( u = v_0 \)).  

(11)

![FIG. 1: Translational velocity of a spherical particle of radius \( R = 50 \) nm suspended over the QCM surface. The distance \( \Delta = d - R \) is the gap between the particle surface and the QCM surface, and \( \delta \) is the Stokes flow penetration length. Dashed lines correspond to the result of Mazur and Bedeaux relation [51] (see Supplementary Information) taking the Stokes flow as the mean flow.](image)

Recall that we neglect the impedance due to the perturbative flow \( \hat{Z}_p \) and later validate such an approximation. It is interesting to scrutinize the robustness of the “no-slip” condition \( u = v_0 \) to estimate how feasible it is to get a phase lag between \( u \) and \( v_0 \). To this end Fig. 1 illustrates the velocity of a free sphere moving at a gap-distance \( \Delta = d - R \) over the oscillating surface. The case corresponds to \( R = 50 \) nm. Solid lines correspond to the Mazur-Bedeaux relation [51] (see SI), which is valid far away from the surface, as it neglects the reaction field reflected back from the wall. Notably, even in the absence of wall-particle forces, the strong hydrodynamic friction close to the wall (lubrication) leads to \( u \approx v_0 \) as \( \Delta \to 0 \) (we note that particle slip might take place in specific cases, for instance between two smooth hydrophilic surfaces [52]). If the fluid carries along the particle concomitantly with the wall, the amplitude of any (distance-dependent) wall-particle force should be small or even zero, thus creating a small load impedance. This fact partially explains why the present theory reproduces so well a large list of experiments with considerably different colloidal particles and substrates.

II. DISCUSSION: COMPARISON WITH SIMULATIONS AND EXPERIMENTS

In what follows we compare the prediction in Eq. 11 with 3D simulations of spherical rigid particles (see Methods) and published experimental data for a wide range of analytes. We first deal with quasi-neutrally buoyant analytes (proteins, viruses, liposomes, polymer beads, which possess densities \( \rho_p \) that differ from that of the solvent...
by less than 30%) and also treat their mixtures (latex nanoparticles). Secondly, we consider inertial effects in massive particles by comparing our results with experiments with silica nanoparticles in ethanol \(\rho_p \approx 2.42\rho\).

1. Neutrally buoyant particles

Simulations of neutrally buoyant spheres \((\rho_p = \rho\) in Eq. 10 and \(\rho_c = 0\) in Eq. 11) were performed using the immersed boundary method combined with an elastic network model for rigid spheres. Details can be found in [2] and Methods. We measured the impedance as a function of the resonator-particle gap distance \(\Delta\) and here we consider the limit \(\Delta \to 0\), to deal with the case of adsorbed particles. Again, it is important to stress that in these simulations we have not imposed any adhesion force between the particle and the wall, so their impedance arises only from purely hydrodynamic effects.

Figure 2 compares the prediction in Eq. 11 with simulation results. The agreement is excellent, both for the real and the imaginary parts of \(Z\). Figure 2 shows the contributions to the hydrodynamic impedance in Eq. 11. The viscous contribution \(Z_v\) dominates the impedance of small particles \(R/\delta < 0.5\). Contrary to the commonly assumed relation between viscous forces and dissipation, \(Z_v\) determines both \(\Delta f\) and \(\Delta D\) for small \(R\). In turn, for large particles \(R \gtrsim \delta\), the inertia of the displaced fluid \(Z_k\) becomes dominant (although \(\text{Re}[Z_k]\) remains significant). A maximum of \(\text{Im}[Z]\) is found near \(R/\delta \approx 1.5\), which corresponds to the most negative value of \(\Delta f\). For \(R/\delta > 2\) (not shown) a sudden transition to \(\text{Im}[Z] < 0\) (\(\Delta f > 0\)) is expected. Interestingly, Eq. 11 predicts the cross-over, but just for any non-zero gap \(\Delta > 0\). This suggests that the frequency inversion could be consequence of the counter-flow created by the near-field perturbative current and possibly some particle velocity phase-lag induced by slip or rotation about the linker point. The analysis of this range of \(R/\delta\) is left for a future contribution.

Experiments. In QCM, the frequency is usually taken as a proxy to the surface coverage as in most cases \(|\Delta f|\) increases almost linearly with \(n\). However, coverage effects arising from hydrodynamic couplings between analytes often induce non-monotonic relations between the dissipation and \(n\). As a consequence, if the analyte size is typically larger than proteins, the acoustic ratio \(-\Delta D/\Delta f\) decreases with \(\Delta f\). By extrapolating to large \(\Delta f\), up to the intercept \((\Delta D/\Delta f)_0 = 0\), some works found a way to estimate the particle size by assuming that in such a limit, adsorption reaches the close-packed limit, treated as a rigid film via Eq. 2. In many instances the estimated “Sauerbrey height” \(h\) compares quite well with the particle diameter, but the procedure was reported to fail severely in some other cases (e.g. for massive particles). The limit value of the acoustic ratio in the other (dilute) limit \(\Delta f \to 0\), is frequently used to avoid hydrodynamic interactions between analytes (“cross-talk” effects) and compare the “dissipation capacity” of different analytes. This limit acoustic ratio is taken from the offset \(\Delta D/\Delta f_0\) of the linear fit \(-\Delta D/\Delta f = |\Delta D/\Delta f_0 - a|\Delta f\). The present work focuses on this dilute limit, where particles can be treated as discrete isolated elements. We deploy the non-dimensional acoustic ratio \(A_r \equiv f_0|\Delta D/\Delta f_0|\) which can be extracted from the relatively abundant experimental data. Figure 3 shows such comparison between the prediction of Eq. 11 and quite disparate experiments summarized and labeled in Table 1. Data include proteins, viruses, liposomes and latex particles ranging from a few nanometers to a few hundred nanometers adsorbed on different substrates. As a first conclusion, the good agreement with the theory validates our approximation concerning the perturbative stress, at least for \(R/\delta \leq 2\). For \(R/\delta < 1\) all the data collapses onto a quasi-linear relation \(A_r \approx 3R/\delta\). Interestingly, a linear relation (with a smaller prefactor) was also derived from hydrodynamic arguments for the acoustic response of simple fluids to rough walls in the limit of large corrugation lengths. Another point to highlight is the large sensitivity of the impedance to the gap \(\Delta = R - d\) between particle and resonator surfaces. According to Eq. 11 a gap as small as \(\Delta = 0.05R\) (just 5 nm for a 200 nm particle) creates a measurable increase in \(A_r\) (see dashed line in Fig. 3). Such sensitivity becomes particularly important as \(R/\delta > 2\) because \(\Delta f\) gradually vanishes and the acoustic ratio diverges. As shown in the inset of Fig. 3 we estimate that the divergence takes place at \(R/\delta \sim 3\), which is consistent with the experimental data by Sato et al. with micron-sized particles, at the other side of the divergence.

The large disparity of cases included in Fig. 3 deserve some comments. The experiments by Tellechea et al. correspond to colloidal particles on inorganic surfaces:icosahedral cowpea mosaic viruses of 30 nm in diameter (CPMV) and extruded dialmitoyl phosphatidyl choline (DPPC) liposomes, with diameters of 83 nm (DPPC-41) and 114 nm (DPPC-57). These sizes, measured by dynamic light scattering in bulk, coincide with the Sauerbrey height \(h\) thus confirming that these particles do not deform upon adsorption (having a well defined size and spherical morphology and relatively high stiffness). Experimental \(A_r\) for different overtones nicely follow the theoretical curve. Reviakine et al. considered softer liposomes which deform upon adsorption on TiO\(_2\) substrate. They used dimyristoyl phosphatidyl choline (DMPC) liposomes of about 90 nm at temperatures of 10°C and 32°C, which are respectively below and above the lipid gel-to-fluid phase transition (\(T_m \approx 24^\circ\)). DMPC liposomes are rigid at 10°C while for \(T = 32^\circ\)C they substantially soften and deform upon adsorption, exposing a height \(h \approx 65\) nm over the resonator which is significantly smaller than their diameter in solution. Despite such deformation, Fig. 3 shows that the trend
for soft DMPC liposomes agrees with our theory if the
liposome height $h$ is taken as its effective diameter. This
indicates that the hydrodynamic impedance essentially
depends on how far from the resonator the mass is dis-
tributed (especially, if the particle inertial mass is zero).

The case of proteins allows us to further explore the
scope of such a claim and to gauge the relevance of the
substrate. Fig. 3 includes values of $A_r$ for avidin (Av),
streptavidin (SAv) and neutravidin (Nav) over biotynil-
ated supported lipid bilayers (b-SLB) and silica, taken
from Bingen et al. 34 and Wolny et al. 52 (see Table
I). Bingen et al. compare two quite similar proteins (SAv
and Av) whose acoustic response over b-SLB only dif-
fers in their dissipation (SaV is slightly more dissipative
34). Remarkably a purely hydrodynamic theory cor-
correctly captures the response of these proteins with a
radius of about 2.5 nm. Such agreement confirms that
collective modes in fluids persist up to few-nanometer scales
50, 51 which contradicts the hypothesis of trapped solvent mov-
ing in “solid-like” fashion with the analyte 30, 34, 37.
Wolny et al. 52 studied Av, SAv and Nav in b-SVB,
gold and silica substrates. Their data (at 45 MHz) on b-
SLB is consistent with that of Bingen et al. (at 35 MHz).
However, drastic differences are revealed on gold and sil-
ica. On gold, SAv and Av present an extremely small
acoustic ratio $A_r \approx 0.016$ which evidences that these
proteins tightly collapse onto the gold substrate. As re-
ported by Milioni 22 Sav on gold forms an homogeneous
surface with a height ranging in the atomic scale. By con-
trast, SAv presents an extremely large acoustic ratio on
silica ($A_r \approx 1$) which evidences that it is not adsorbed
52, but in suspension. According to Eq. 11 (taking $u$
from Mazur-Bedeaux theory 51, see SI) $A_r \approx 1$ corre-
ponds to SaV suspended about 15 nm from the surface.
By constrast, Av in silica presents $A_r \approx 0.075$, which is
consistent with the hydrodynamics of adsorbed spherical
particles. The response of NAv presents significant
variations with $A_r \leq 0.15$ and $0.25$ on gold and sil-
ica 52. According to our theory, the large values of $A_r$
reported indicate adsorption of small clusters of proteins
(between 6 and 10 nm radius, in agreement with the esti-
mate made by Wolny et al. 52). These authors report
the presence of relatively rigid small aggregates of NAv in
the stock solution 52, 53 and, consistently, they observe
that the acoustic response of NAv decreased if they in-
creased the centrifugation time of freshly thawed aliquots
53. In this vein, more recent experiments performed at
larger fundamental frequency 150 MHz 56 report values of
the acoustic ratio of NAv in gold which are in agree-
ment with the hydrodynamic result for single protein de-
position, as indicated in Fig. 3. In conclusion, our anal-
ysis indicates the leading role of hydrodynamics, even in
the case of proteins. Deviations from the theoretical
hydrodynamic trend trend should help to decipher strong
protein deformations, clustering, substrate-protein and
protein-protein interactions.

A particularly enlightening verification of such state-
ment is offered by the mass ratio $H = 1 - m/m_{QCM}$
routinely measured in many QCM studies. In terms
of impedances, $H = 1 - n\rho_d\omega/Im[Z]$ or $H = 1 -
(4/9)(R/\delta)^2/Im[Z]$ (recall $Z \equiv Z/(6\pi\eta R n)$). Figure 3(b)
shows that the hydrodynamic theory predicts the exper-

| Material                  | Particle radius [nm] | Frequency range [MHz] | Label         | Reference |
|---------------------------|----------------------|-----------------------|---------------|-----------|
| Rigid liposome DPPC       | 57 ± 4               | [15 – 75]             | DPPC-57 2009  | [31]      |
| Rigid liposome DPPC       | 41 ± 2               | [15 – 75]             | DPPC-41 2009  | [31]      |
| Cow Pea Mosaic Virus (CPMV)| 14                   | [15 – 75]             | CPMV-14 2009  | [31]      |
| Rigid liposome DPPC at 25°C| 41.5                 | [15 – 75]             | DPPC-41 2012  | [30]      |
| Rigid liposome DMPC at 10°C| 45                   | [15 – 55]             | DMPC-45 2012  | [30]      |
| Soft* liposome DMPC at 32°C| 33                   | [15 – 55]             | DMPC-33 2012  | [30]      |
| Supported Unimodal Vesicles| 12.5 ± 2.5           | 15, 45                | b-SUV 2008    | [34]      |
| Avidin b-SLB              | 2.5 ± 0.5            | 45                    | Av-SLB 2008   | [34]      |
| Streptavidin on b-SLB     | 2.5 ± 0.5            | 35                    | Av-SLB 2010   | [35]      |
| Avidin b-SLB              | 2.5 ± 0.5            | 35                    | SaV-SLB 2010  | [35]      |
| Streptavidin on b-SLB     | 2.5 ± 0.5            | 35                    | SaV-SLB 2010  | [35]      |
| Neutravidin on b-SLB      | 2.5 ± 0.5            | 35                    | NaV-SLB 2010  | [55]      |
| Neutravidin on silica     | 2.5 ± 0.5            | 35                    | NaV-Si 2010   | [55]      |
| Neutravidin on BSA        | 2.5                  | 150                   | NaV 2020      | [56]      |
| Latex NP mixtures         | 57 and 12            | 35                    | Latex 2013    | [53]      |
| Polymer NP               | 13, 20, 33.5, 70     | 5                     | Polymer 2020  | [37]      |
The work by Olsson et al. 53 offers another interesting validation of the present theory. These authors considered mixtures of latex nanoparticles with nominal diameter of 24 and 110 nm, adsorbed on to either silica- or alumina-coated surfaces. Comparison between the purely hydrodynamic theory and the experiments will illustrate to what extent contact forces affect the acoustic response of adsorbed particles. The acoustic ratio against \( \Delta f \), reported for \( n = 3 \) of a 5 MHz AT cut, \( f_3 = 15 \text{MHz} \) permitted us to extract values of \( A_r \). When adding a mixture of nanoparticles, the Sauerbrey-relation 1 offers an effective particle size, but it does not provide information on the mass fraction of the different types of particles (which in the experiment were known a priori). In order to apply our theoretical result to these mixtures we need a weighted average for the impedance (note that it is incorrect to average acoustic ratios). The impedance is proportional to the wall stress which has to be summed up over the total number of particles. We denote \( N_D \) as the number of particles with diameter \( D \) (in nm). The fraction of \( D = 24 \) particles is \( \phi = N_{24}/(N_{24} + N_{110}) \) and using the simple relation \( m_D \propto N_D \rho D^3 \), we relate \( \phi \) with the mass ratio \( m = m_{24}/m_{110} \),

\[
\phi = \frac{m}{m + r^3},
\]

where we have defined the ratio-of-diameters as \( r = D_{24}/D_{110} \approx 0.218 \). The weighted average for the impedance is simply,

\[
Z_{\text{mix}}(m) = \phi(m)Z(D_{24}) + [1 - \phi(m)]Z(D_{110}).
\] (12)

Theoretical curves are compared with experiments Fig. 4. The agreement is quite good and it indicates that theoretical approaches can be used to disentangle the fraction of nanoparticles size in a mixture. In mixtures with more than two components one might use the extra information from \( \Delta f \) and \( \Delta D \) to fit the mass fractions with the theoretical expressions. This analysis indicates that contact forces have a smaller contribution than hydrodynamics. Therefore unveiling the physical properties of contact forces, wall-induced physico-chemical interactions or any other molecular feature, first require extracting the leading effect of hydrodynamics from the analysis.

3. Massive particles: inertia effect

The experiments of Grunewald et al. 35 allow us to validate our theory against the effect of particle inertia. These experiments studied the acoustic response of amine functionalized porous silica nanoparticles strongly adsorbed on gold surfaces. These nanoparticles, with nominal radius 68.5 nm, were immersed in ethanol at \( T = 25^\circ \text{C} \) (\( \rho = 0.785 \text{g/cm}^3 \)), and were prepared to present a repulsive electrostatic interaction which induced an ordered deposition, reaching a maximum coverage of about 15\%. Values of the frequency and dissipation shifts were obtained for a range of overtones \( n \in [3, 13] \). The kinetic viscosity of ethanol \( \nu = 1.33 \times 10^{-6} \text{m}^2/\text{s} \) yields a penetration length \( \delta_n = \frac{292n^{-1/2}}{nm} \) nm for the \( n^{th} \) overtone (the fundamental resonator frequency being \( f_1 = 4.95 \text{MHz} \). Mesoporous silica nanoparticles were reported to have a void fraction of about 15\% which yields a density in ethanol of about \( \rho_p \approx 1.9 \text{g/cm}^3 \). The authors evaluated the deposited mass \( m_{\text{QCM}} \) using the Sauerbrey relation 1 which resulted to be significantly larger than the
versus the mass ratio (see Fig. 5). However, $R/\delta$ among limit, correspond to acoustic ratio relevant information on the underlying micro-

Alumina versus Silica or to simulations (see text). Filled circles correspond to simulations (color code similar to Fig. 2). The label “CPMV 2008” in (b) indicates data for the cowpea mosaic virus taken from Ref. [34], while in (a) “CPMV-14 2009” is taken from Ref. [31].

![Graph](image)

**FIG. 3:** (a) Non-dimensional acoustic ratio $f|\Delta D/\Delta f|_0$ versus $R/\delta$ for experiments and simulations compared with the theoretical result obtained from Eq. 11. The solid line is the theoretical trend for $d = R$ (adsorbed particle) and the dashed line corresponds to $d = 1.05R$ (small gap between the particle and surface). The acronyms used for the legend are explained in Table I. The inset zooms out the particle size range. Beyond the divergence ($R \approx \delta$), the squares correspond to experimental data by Sato et al. [59] for micron-size particles.

(b) Theoretical (lines) and experimental evaluation of the mass ratio $H = 1 - m/m_{QCM}$ (see text). Incidentally, we found a better agreement using $\rho_p = 1.6 \text{ g/cm}^3$ (see Fig. 5). However, the analysis of the experimental frequency $\Delta f$ revealed an interesting surprise: $\Delta f$ increases sublinearly with the deposited mass $m$. This fact is revealed in Fig. 3(b): in terms of the scaled impedance $\operatorname{Im}[\hat{Z}] \sim m^{-0.18(5)}$, which implies $\Delta f \sim m^{0.81(5)}$. Theoretical predictions for $\operatorname{Im}[\hat{Z}]$ (using $\rho_p = 1.9 \text{ g/cm}^3$, plotted as horizontal lines in Fig. 3(b)) consistently extrapolate the experimental values to the ultra-dilute regime $m \approx 0.2 \text{ng/mm}^2$ which is close to or below the QCM’s limit of detection. In such a limit, $\Delta f$ becomes slightly larger, which explains the theoretical underestimation of $A_r$ in Fig. 3(a). In passing, we note that the sublinear scaling $\Delta f \sim m^{0.815}$ is most probably due to hydrodynamic interaction among silica particles, but this issue is beyond the present contribution.

In summary, the present analytical study on the QCM response of discrete adsorbates shows that the main source of acoustic impedance comes from the hydrodynamic propagation of fluid-induced forces on the analyte. The sensed extra wall stress strongly depends on how mass is distributed over the resonator. And, in turn, such distribution is determined by physico-chemical forces (adhesion, dispersion and electrostatic forces, structural elasticity, etc.). This fact already permits the extraction of relevant information on the underlying microscopic configurations, uniquely invoking fluid-induced response (as done in the present work). However, physico-chemical forces are also transferred to the fluid and hydrodynamically propagate to the surface. An extension of the present theory including these secondary forces (the

![Graph](image)

**FIG. 4:** The acoustic ratio $A_r = -f|\Delta D/\Delta f|_0$ for mixtures of latex particles of diameter 24 and 110nm adsorbed on to Silica or Alumina versus the mass ratio $m \equiv m_{24}/m_{110}$. The experimental data was published in Ref. [33]. The theoretical curve is the weighted impedance (Eq. 12) $Z_{\text{mix}} = \phi Z(R_{24}) + (1 - \phi) Z(R_{110})$, where the particle number fraction is $\phi = m/(m + r^3)$, with $r$ defined as the diameter-ratio,

$$r \equiv R_{24}/R_{110} = 0.218.$$
FIG. 5: (a) Comparison between experimental and theoretical results for the acoustic response of adsorbed Silica particles in ethanol in the low frequency shift limit $\Delta f \approx 0$, reported by Grunewald [54]. (b) The scaled impedance versus the adsorbed mass. Horizontal lines correspond to the theoretical prediction (using the experimental nanoparticle density $\rho_p = 1.9$ g/cm$^3$).

very purpose of QCM research), will allow deciphering and measuring subtle molecular properties, such as the different acoustic response of avidin and streptavidin, the bending rigidity and membrane fluidity of liposomes, or the reason behind the deviation from the purely hydrodynamic trend of the acoustic response of adsorbed virus capsids.

III. METHODS

We have performed three-dimensional simulations of the QCM response of elastic spheres with our own software for Graphical Processors Units FLUAM [63-65]. It uses the immersed boundary method (IBM) to couple the hydrodynamics of compressible flows with the dynamics of immersed molecular structures. The integration scheme is second-order accurate in space and time and the spatial discretization is based on a staggered grid [64] of cell size $h = 3.958$ nm. Simulations were performed in boxes periodic in the resonator plane. Boundary conditions for the top and bottom walls were imposed using a ghost cell to easily impose a tangential velocity $v_0 \cos(\omega t)$ (along the $x$ direction) at the bottom wall [9]. The tangential velocity gradient at the wall $(\partial v_x / \partial z)_{z=0}$ was calculated using a second order spatial interpolation from the upper fluid cells. The fluid traction (stress) at the resonator is measured by averaging $\eta \left(\partial v_x / \partial y\right)_{y=0}$ over all the surface. Using the small load approximation, the complex Fourier amplitude of the average stress directly leads to the impedance. Hollow spheres over the resonator (representing liposomes) were modelled using the elastic network model (ENM). The sphere’s surface is created by an arrangement of IBM markers in close packing, connected to their nearest neighbours (at distance $\ell \approx 2h$) by strong harmonic springs. The bending rigidity of the structure corresponds to the rigid limit ($k_L \ell^2 \sim 10^5 k_B T$ for $T = 300$ K)). The number of beads required to build the hollow sphere increases as $(R/h)^2$ being about 6000 beads for a liposome of radius $R = 50$ nm.

IV. ACKNOWLEDGMENTS

This work was funded by the EU FET-Open Project “CATCH-U-DNA”.

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motion. However, this contribution can be neglected due to the extremely fast QCM oscillation frequency.