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Tuning finely the packing density of heavy microparticles in a microfluidic channel.

Pablo E. Guevara-Pantoja and Gabriel A. Caballero-Robledo

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The packing of granulates inside microfluidic channels is important for applications of liquid chromatography and bead based immunoassays. In such applications, the granular packing acts as a porous medium, and properties like pours size, homogeneity, and regularity determine the performance of the device. In this work we show that the packing density of iron micrometric particles can be finely tuned inside a microfluidic channel by applying a vibration protocol originally developed for macroscopic, dry granular systems. Our results suggest that much of what is known about the physics of compaction in macroscopic granular matter could be applied to better control packings inside microchannels with important implications for applications.

Micrometric particles packed inside a microfluidic channel can be useful for applications like liquid chromatography[1], bead based immunoassays[2–5], and multiphase flow research[6]. In these applications, the granulate is usually jammed forming a porous medium through which a fluid can flow. The shape and size of grains, the geometry of the channel, and the packing protocol determine the properties of the porous medium and the performance of the devices depending on the application.

In liquid chromatography the packing is prepared and controlled using a technique based on the use of a slurry[7]. This technique is mostly empirical while the performance of chromatography devices is extremely sensitive to the packing properties[1]. More recently, an interesting phenomenon was reported that could be useful to pack grains in a controlled manner inside micro channels: when a sediment is created by centrifugating a colloidal suspension, the packing properties of the solid sediment depend on the concentration of the initial liquid suspension[8,9].

On the other hand, the physics of compaction of macroscopic granular materials has been studied by physicists and engineers in recent years resulting in an important advancement on the understanding of the subject[10–15]. In particular, the work by E. R. Nowak et al.[16] is a landmark on the study of compaction because it finds an experimental protocol to reversibly adjust the density of a packing through controlled vertical taps: under this protocol, the density of the packing is a monotonous function of the tapping intensity. The vibration protocol is analog to an annealing process typically applied to thermal materials: the granular system must be first strongly shaken, above a threshold intensity, before gradually decreasing the shaking. This allows the packing to reach optimal configurations and therefore high densities. If the packing is initially shaken at an intensity smaller than the threshold, it gets trapped in a metastable state in the same way a thermal material can get trapped in a glassy state. The authors explain this behaviour arguing that the shaking activates two competing mechanisms: one that creates voids and another that suppresses them. In a later work[17] the same group manages to capture much of the phenomenology of the experiments with a simple parking lot model. Interestingly, the same model was previously studied in the context of chemisorption and protein binding. This impressive result supports the idea of describing static packings through a theoretical framework analog to thermodynamics, proposed originally by Edwards and co workers[18,19] and that is still under development[14,20].

Further experiments showed that the packing density of non-Brownian particles submerged in water inside millimetric tubes can also be reversibly varied using flow pulses instead of taps[21,22]. There, as in the works cited above, the packings are disordered, even if the grains are regular spheres. However, agitation protocols also help ordering macroscopic grains in crystal-like lattices, using vibration[23–26], or shearing the sample[27].

Concerning applications that involve particles packed inside micrometric channels it would be ideal to apply all these results to better control the packings, but it is not clear to what extent this macroscopic phenomenology works at the micro scale. In this work we address this question by applying a vibration protocol to micrometric iron particles submerged in water and confined in a vertical microfluidic channel. We find that the packing density follows very closely the behaviour reported by E. R. Nowak et al.[16]. This provides a method to

Cinvestav-Monterrey, PIIT, Nuevo León 66600, México; E-mail: g.a.caballero.robledo@gmail.com

finely adjust the packing density in a chip without the need to open or destroy it and, more importantly, it suggests that much of the phenomenology observed in macroscopic systems can be applied to microfluidic channels.

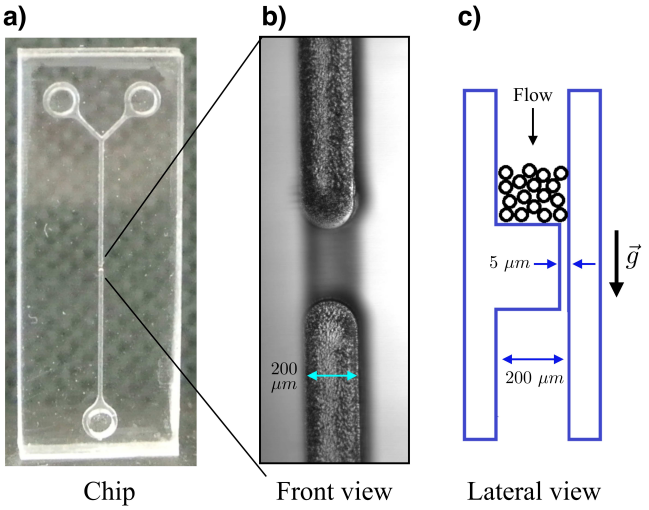

Fig. 1 Chip. a) Picture of the channel. b) Front view of the channel around the restriction using an optical microscope. The restriction is out of focus. c) Schematic representation of a lateral view of the channel. The restriction prevents the 8μm particles from continue falling while it allows the liquid to flow through; the particles form a porous medium.

The microfluidic chip consists of a vertical, 2 cm long channel with a square cross section of 200 μm per side (see Fig. 1). The channel is made on a 1.5 mm thick, PMMA sheet using a 3D Milling Machine (Roland MDX-40A) with a 200 μm drill bit. Once the channel is etched on the PMMA sheet, the system is closed by gluing on top of it a similar sheet without etching. The closing sheet has openings to allow access to the upper and lower extremes of the channel through small plastic tubes.

The upper and lower parts of the channel are separated by an obstruction that blocks almost all the cross section except for a 5 μm gap that allows liquid to flow through it but prevents micrometric particles from passing (see Fig. 1(c)). Iron micro particles of irregular shape and grain size between 6 and 9 μm (puriss. p.a., carbonyl-Iron powder 44890 ALDRICH) form a porous packing above the restriction when introduced through the upper part of the channel. The surface of the particles is coated with citrate anions to avoid adhesion and cohesion (a method for coating magnetic nanoparticles[28] was adapted to microparticles: 2 gm of particles in ultra pure water from a Milli-Q Biocel with 4 ml of citric acid at 2M and sonicated for 60 minutes with an ultrasonic liquid processor Q-sonica Q500 at 25% of the maximum power). The dimensions and parameters of the experiment reported here were adapted from the chip design in B. Teste et al.[5,32], except that their channel is shallower (30 μm).

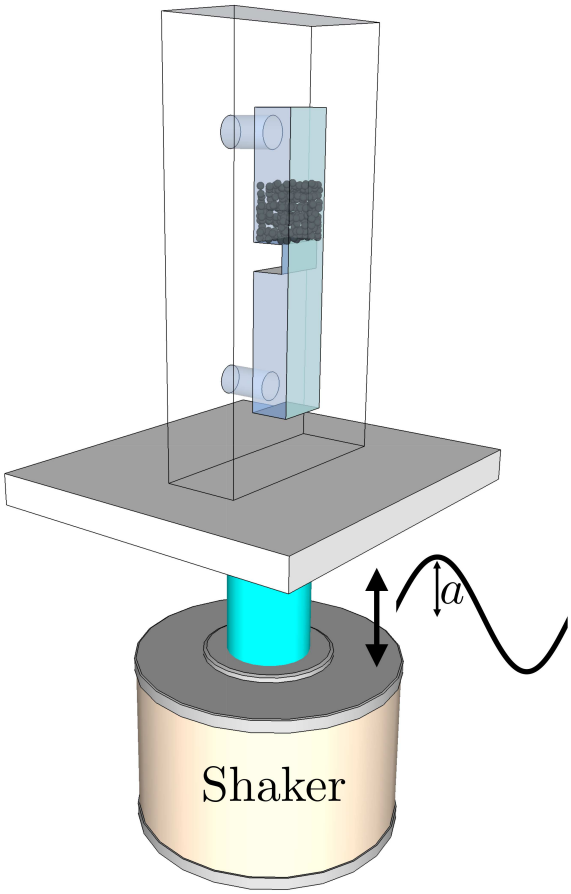

Fig. 2 Schematic representation of the experimental set up (not to scale). The chip is vertically mounted on a shaker and is vibrated sinusoidally at a frequency $f = 645$ Hz. The amplitude a is approximately 20 μm at the maximum shaking strength. A 10 cm rod separates the chip to prevent the magnetic field from the shaker to affect the experiment.

Figure 2 shows a schematic representation of the experimental set up where the microfluidic chip is mounted on a permanent magnet vertical shaker (LDS V201) controlled by a function generator that allows precise control of amplitud and frequency of the vibration. The fixation mechanism that attaches the channel to the shaker has a long rod that separates the channel from the shaker by 10 cm to prevent the magnetic field from the shaker to interfere with the experiment.

The channel is first filled with ultra pure water from the Milli-Q leaving no bubbles inside. Then, with the channel in a horizontal position, the particles are introduced in a tube connected to the extreme of the channel using a syringe needle. The particles sediment to the bottom of the tube into the

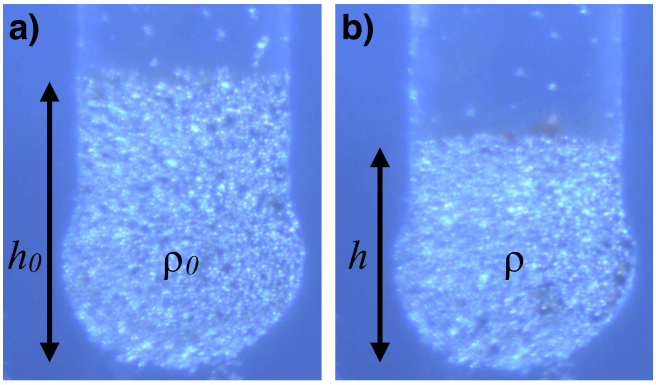

Fig. 3 Particles inside the channel at two different densities: a) initial density, and b) after vibration. Since the depth of the channel and the number of particles are the same, the frontal area of the packing determines the packing density. The density of the initial packing is ρ_0 .

channel. The channel is then put vertically and the particles sediment until reaching the restriction. Approximately 50 μg of particles are introduced into the channel using this method. The initial configuration of the experiment corresponds to the most expanded packing which is obtained by turning the channel upside-down for 30 seconds and then turning it back again. This makes the particles to gently fall at terminal velocity forming a loose packing on top of the restriction. The height of this expanded packing is $h_0 = 267 \pm 4\ \mu m$ (see Fig. 3(a)). The channel is visualised with an inverted microscope Nikon Eclipse TS100 which is turned 90 degrees from its original orientation to be able to be focused on the vertical channel. Three white LED's were adapted around the microscope objective to observe the sample by reflected light, which we found to be the best for our measurements. A colour CCD camera (IDS UI-2230SE) is adapted to the microscope to record images like those shown in figure 3.

From these images we obtain the frontal area, A, of the volume occupied by the static packing. The density of the packing can be calculated as $\rho = m_p/Aw$, where m_p is the mass of particles and w is the depth of the channel. Since m_p and w are constant during the experiments, and since the precise determination of both quantities at such small scales is difficult, the best way to present our results is through the ratio

$$\frac{\rho}{\rho_0} = \frac{A_0}{A}, \tag{1}$$

where ρ_0 and A_0 are the density and frontal area of the (loosest) initial packing.

The packing density is varied by sinusoidally shaking the sample at different shaking intensities, which is characterised by the maximum acceleration of the shaker normalised by the acceleration of gravity:

$$\Gamma = \frac{a(2\pi f)^2}{g}, \tag{2}$$

where a and f are the amplitude and frequency of the sinusoidal movement of the shaker. We shake the sample at constant frequency, $f = 645$ Hz, so the shaking intensity Γ is varied solely by changing the amplitude a. For $\Gamma = 30$, our maximum value, the shaking amplitude a is of the order of 20 μm. The value of the frequency was chosen because we found that at this value the fluidisation of the particles was most effective. The reason of this is not clear for us and it rests as an interesting open question to explore in the future, being the first task to quantitatively characterise the fluidisation of the particles. So far, what we mean by better fluidisation is an apparent homogeneity in the agitation of the particles all along the column. At other frequencies is common to observe adjacent regions with very different agitation.

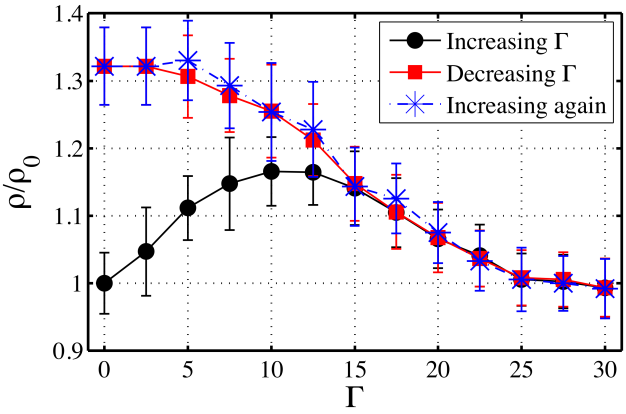

Fig. 4 Density of the packing, ρ, normalised by the initial density ρ_0 as a function of the intensity of vibration, characterised by the non dimensional peak acceleration Γ (eq. 2). Each point is obtained by measuring the frontal area occupied by the static packing after 10 seconds of vibration with an intensity Γ. After an initial irreversible state (circles), the system goes into a reversible situation (squares and stars) where the density is a monotonous function of the vibration intensity.

Figure 4 shows relative variations of the packing density with respect to the initial, loosest state as a function of shaking intensity Γ. Each measurement is obtained by shaking the sample for ten seconds with an intensity Γ, stoping the shaking and waiting for ten seconds to let the sample settle down, taking the picture of the static packing, and calculating the density using equation 1. Each experiment was repeated three times. The shaking protocol that we follow is similar to the one used in [16]: we begin with the loosest packing which we prepare by turning upside down the sample for 30 seconds, turning it back and letting it settle down; then we shake the

sample at different intensities, gradually increasing Γ from 0 to 30 (black circles). Afterwards, we gradually decrease Γ to 0 again (red squares). Note how the density does not follow the same path this time, ending up in a much more compact state (30% more compact) than the loosest packing. Moreover, when Γ is increased again, the density follows the same path as before (blue stars), i.e. the behaviour is reversible.

The results presented in figure 4 are qualitatively similar to the density variations in a dry macroscopic system reported by Nowak et al.[16], and studied later in more detail by J.A. Dijksman and M. van Hecke[29]. The path that follows the density when the shaking strength is increased for the first time is irreversible for $\Gamma < 15$. But when the system reaches the reversible path defined by the two other set of data, the density becomes a monotonous function of Γ. This result is extremely relevant for applications in microfluidics, because it gives the possibility to finely tune the packing density inside a micro channel without opening or destroying it.

One important difference between our results shown in figure 4 and the compaction of dry macroscopic systems reported previously is the range of density that can be reversibly explored by the vibration protocol. Nowak et al.[16] report a density variation range of 4%, but their system is very narrow and they use glass monodisperse spheres. On the other hand, J.A. Dijksman and M. van Hecke[29] obtain a 16% of density variation working with rough, irregular bronze beads. In contrast, the density of our micrometric system can be increased from its loosest value by an impressive 30%. The reason for this we believe resides mainly on the irregularity of the particles: in dry, macroscopic, and non cohesive granular packings it is possible to create extremely loose mechanically stable structures with an occupied volume fraction as low as 40% due only to the irregularity and roughness of the grains[30,31]. The iron powder that we use is much more irregular and rough than the typical grains used in macroscopic experiments as can be seen in Fig. 1(a) of the Electronic Supplementary Material, where we dispersed the particles in 1,2,3-Triacetoxypropane and observe them in an inverted microscope. In addition, the powder has a broad particle size distribution, which we quantify with the histogram of the sizes of the major axes of ellipses fitted to particles through image analysis (Fig. 1(b) of the Electronic Supplementary Material). Such polydispersity certainly allows the system to form very dense packings. Irregularity, roughness, and polydispersity are probably enough ingredients to account for the extreme variation of packing density observed. However, there are probably also cohesive forces which the functionalization of the nano particles reduce but not eliminate, although we would need to quantify the efficiency of the functionalization to estimate this contribution to density variations. Finally, the fact that the particles are submerged in water certainly play also a role.

The validity of the vibration protocol described here could appear to be limited exclusively to heavy microparticles like iron powder. However, the experiments by M. Schröter et al.[21] performed with glass particles as small as 100 μm submerged in water, where the packing density was varied using flow pulses instead of taps, suggest that such agitation method could be used inside microchannels. This would allow to control the density of microparticles of other materials like glass or polystyrene.

The motivation of our work comes from the interest in testing how sensible to the packing density would be a bead-based microfluidic devise for immunoassays developed by B. Teste et al.[5,32]. The chip consists of a nanoparticles magnetic trap based on iron beads packed against a restriction. In the presence of an external magnet, the iron beads concentrate and enhance the magnetic field several orders of magnitude around particles contacts, enough to trap magnetic nanoparticles. Grafting the nanoparticles as immunosupport for allergy detection allows the chip to combine the advantages of homogeneous and heterogeneous immunoassays, considerably outperforming traditional methods. But if functional point-of-care devices are to be developed based on bead-based chips, precise and reproducible control over the granulate packings is needed. The fine control of packing density that gives our vibration protocol results twofold important in this context. First, it gives possible pathways to the mass production of reproducible and reliable devices. Second, it allows to study the role of density on the chip efficiency to trap nanoparticles. Preliminary results point out to a big sensitivity on this parameter (see Fig. 5).

Fluorescent Magnetic Core Shell Nanoparticles (FMCSN) of 53 *nm* in size were trapped at different packing densities of the micrometric iron particles. The trapping was done by approaching a magnet to the iron particles in the restriction as proposed by B. Teste et al.[5,32]. When the magnet is removed the trapped nanoparticles are liberated. Fig. 5 shows an estimation of the number of nanoparticles trapped as a function of packing density. The details of the trapping experiments can be found in the Electronic Supplementary Material. The data shown in Fig. 5 is preliminary and is too noisy to make any conclusions. However, it suggests that the efficiency of the magnetic trap is sensitive to the packing density of the granulate, which supports the idea that care must be taken of this parameter if reliable and reproducible bead-based devices are to be designed. Noteworthy, the lowest packing density that can be achieved by shaking when the micrometric iron particles have already been exposed to the magnet is much lower than ρ_0 and, more importantly, the amount of trapped nanoparticles doubles. Besides, assuming that the nanoparticles are trapped in the contacts between microparticles, it is expected to be more nanoparticles trapped at high densities, but our data shows the opposite behaviour. The noise in the data impedes us to study in detail these findings, but we are working in a

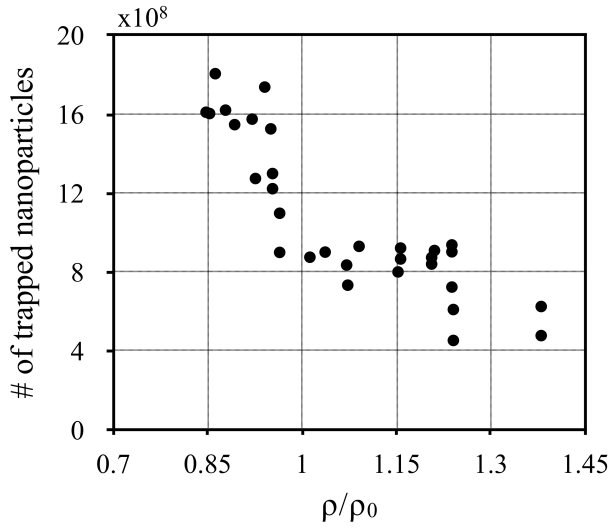

Fig. 5 Preliminary results of the magnetic trap efficiency to trap Fluorescent Magnetic Core Shell Nanoparticles (FMCSN) as a function of packing density. The nanoparticles mean size is 53 *nm*. The normalisation density ρ_0 corresponds to the most expanded configuration when the microparticles had not been exposed to a magnet.

new version of the chip that will allow us to answer many interesting questions that arise from these experiments.

Another consequence with an important potential impact that our vibration protocol has is that it suggests that other phenomena already observed and studied in macroscopic granular materials would be also valid in microchannels. In particular, it is very likely that the vibration protocols that allow to create perfectly ordered packings of macroscopic monodisperse spheres[23–26] will also work in microfluidic channels. As mentioned above, this can have important consequences for applications concerning liquid chromatography, where the properties of the porous medium completely determine the performance of devices. A porous medium with some regularity can be useful to improve the separation efficiency in these devices and, so far, it can only be achieved using complicated and expensive fabrication methods[1]. Our vibration protocols would represent an attractive alternative, easy and cheap, for fabrication of liquid chromatography devices.

It is important to emphasise that the results presented here are the first that show that packing density can be finely and reversibly varied in a micro channel. However, there is still much work to do concerning the characterisation of the phenomenon. Particularly relevant is the study of the homogeneity of the packing density along the column as well as size segregation processes that could occur during shaking since they could sensibly affect the performance of bead-based devices in applications.

In conclusion, we have shown that it is possible to finely and reversibly control the packing density of heavy grains inside a microfluidic channel. It is the first time that vibration protocols originally developed for macroscopic systems are applied at such small scales. It was not clear *a priori* that this could be possible, and it suggests that much more of the current understanding on macroscopic granular systems could be useful to design and fabricate bead-based microfluidic devices.

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Tuning finely the packing density of heavy microparticles in a microfluidic channel

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The packing density of heavy microparticles is finely tuned inside a microfluidic channel by applying a vibration protocol with important implications for applications.