

Characterization of the mechanical properties of polymeric chromatographic particles by micromanipulation

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Abstract

Chromatographic particles should not possess only desirable surface chemical properties but also mechanical properties. The latter determine the deformation of the particles under hydrodynamic conditions of packed beds and further the pressure drop. Understanding the mechanical properties of chromatographic particles is essential to successful design and operation of such processing equipment. The mechanical properties of single chromatographic particles made of different formulations and with different surface modifications were characterized by a novel micromanipulation technique. The principle of this technique is to compress single particles between two parallel surfaces. The force being imposed on the particles and their deformation under compression are measured simultaneously. The particles range from 30 to 300 μm , depending on their formulation. 10–30 particles from each sample were compressed up to a particle deformation of 70% in order to get statistically representative data. Particles for hydrophobic interaction chromatography were proved more stable than resins for ion exchange or gel filtration. The difference is remarkable in dry state and is less in wet state. Water seems to “level out” the differences in mechanical stability of resins. The stability seems to be also dependent on the speed of water release out of the resins.

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1. Introduction

In downstream processing of biologics, which are mainly proteins and polynucleotides, chromatographic resins are used for their purification. Support materials for the chromatographic particles include silica gel, hydroxyapatite, controlled pore glass, ceramics and polymeric resins. These materials are additionally functionalised with ionic-, hydrophobic or affinity ligands. Because of the required caustic stability most of the resins are from polymeric nature. The most prominent polymers used in production processes are carbohydrate-, methacrylate- and polystyrene/divinylbenzene based resins [1]. Some resins can be used in more than 2000 purification cycles. The stress to the polymeric material is manifold. The pH, solvent, salt and protein content and viscosity change periodically many times. In the past, when chromatography was not a unit operation in down-

stream processing life time of a column was not troublesome. Today the production of protein becomes more and more under economic pressure and the life time of a chromatographic column is an important parameter to the overall process costs. The life time of a packed bed column is also a function of the mechanical stability of the resin. Usually a mechanically stable particle is easy to pack and does not shrink or swell during the chromatographic cycles. These requirements are nearly perfectly achieved by silica gel but not by polymeric resins. A pressure increase during operation indicates changed mechanical properties. The individual reasons are explained by fouling processes or rupture of the beads [2]. To estimate the mechanical stability of a packed chromatographic column usually a pressure-flow curve is recorded which is linear at the beginning. Above a certain velocity there are deviations from the linearity until at a collapsing point. Unclear in this situation is the actual state of the particles. Are they crushed? The problem can be solved by microscopic inspection but the uncertainty remains about the limiting stability of the resin.

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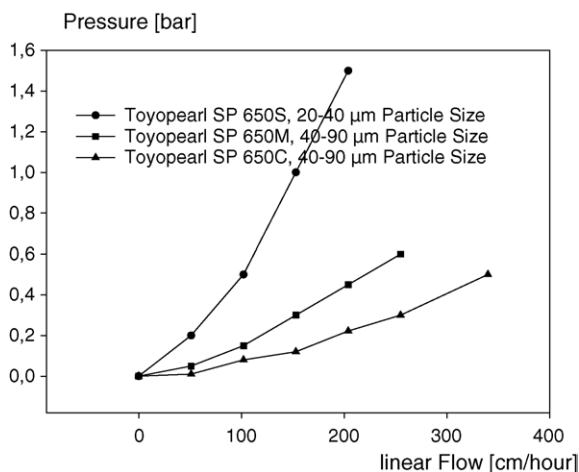


Fig. 1. Pressure-flow curves for Toyopearl SP ion exchanger with different particle grades (1 cm I.D. × 10 cm length, Omnifit column, water 20 °C) [4].

The functional relationship between pressure drop and flow velocity is described by the Blake–Koczeny equation [3]:

$$\frac{\Delta p}{L} = \eta \frac{K_0 (1 - \varepsilon_B)^2}{d_P^2 \varepsilon_B^3} u \quad (1)$$

The Blake–Koczeny equation does not count for the material properties of individual resins, but include the particle size (d_P) and the bed porosity (ε). Fig. 1 shows no clear linear relationship between pressure drop and flow velocity. The pressure drop significantly increases with decreasing particle size but is not inversely proportional to square power of particle diameter. Therefore, the significance of conclusions drawn from pressure-flow curves is of limited value. Many other factors influence the mechanical stability of packed bed resins. Extensive work has been reported on conventional compression measurements (pressure-flow curves) of macroscopic gels [5]. Less work has been reported for entities with dimensions in the microscopic range [6]. In this context some important questions remain to be addressed:

1. Does the particle shape change under the applied pressure in chromatographic processes and to which extend?
2. What is the breaking stress or irreversible point of deformation for resin particles?
3. Do the particles recover their original shape and how long does it take?
4. Is the mechanical stability dependent on the nature of the polymer and surface modification?
5. What are favourable properties for polymer materials used in biochromatography?
6. Is the particle stability a function of the particle size?

These questions cannot be answered by conducting simple pressure-flow curves. Not all resins with an acceptable pressure-flow curve do last long. The paper introduces an experimental micromanipulation technique to measure the mechanical properties of micro-polymeric chromatographic particles. This technique was extensively used for determining of mechani-

cal properties of single biological and non-biological particles [7–9]. The study of individual particles provides a measure of intrinsic properties which are free from the influences of neighbouring particles in their assemblies. Polymeric resins with different functionalities have been investigated in order to understand and predict their stability in chromatographic columns.

2. Theoretical background

2.1. Mechanical properties of polymers

A material is deformed by applying a stress on it. The stress (σ) can be defined as [10]:

$$\sigma = \frac{F}{A_0} \quad (2)$$

where F is the applied force and A_0 is the cross sectional area of the material.

The resulted deformation (strain) ε for the material is described as:

$$\varepsilon = \frac{l_0 - l}{l_0} = \frac{\Delta l}{l_0} \quad (3)$$

where l_0 and l are the length of the material before and after the deformation. Very often the deformation is used to characterize changes in length.

There are two distinct models which can be employed to describe mechanical behaviour of materials. Hook's law and the definition of a Newtonian liquid:

2.2. Hook's law

$$\sigma = E\varepsilon \quad (4)$$

σ is usually the tensile strength which cannot be measured for spherical particles. For particles the compression instead of tensile strength is measured. The compression is a function of the shape of the elastic body. The stress is proportional to the strain and not time dependent as shown in Fig. 2. For liquids the strain is continuously changing by applying a stress so that Eq. (4) cannot longer be applied.

2.3. Newtonian liquid

Since an ideal fluid has no elasticity the fluid will obey Newton's law and the fluid is said to be Newtonian.

$$\sigma = \eta \frac{\partial \varepsilon}{\partial t} \quad (5)$$

The stress is proportional to the speed of change in the deformation. For a liquid the stress is a function of time (see Fig. 2).

Hook's law can be symbolized by a spring whereas a Newtonian liquid by a dash pot.

A piece of synthetic nonporous polymer is mechanically considered as an intermediate between a solid material and a viscous liquid. Typically polymeric materials show a delayed recovery of their shape. Certain combinations of springs and pots describe

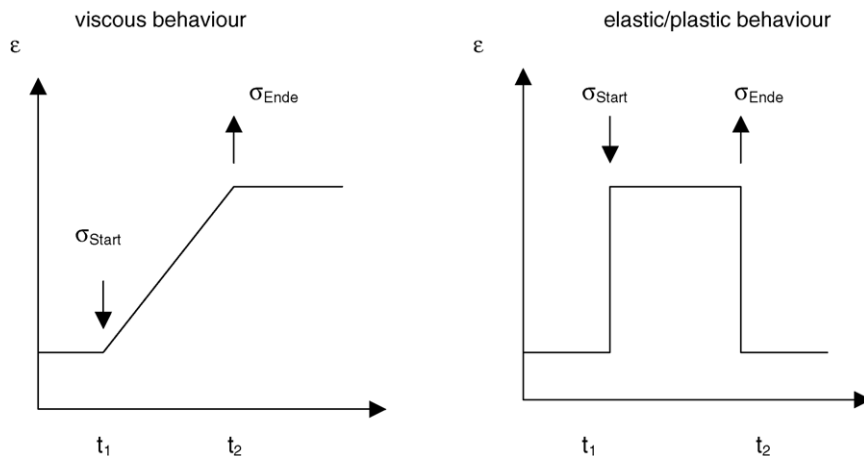


Fig. 2. Time dependent deformation for a Newtonian liquid (left) and time independence for an elastic material according to Hook's law (right).

the time dependencies of visco-elastic properties of polymers in the linear range. Most useful is the Burgers model [11].

Chromatographic particles are not simple polymeric irregular shaped material pieces. They are porous and spherical in shape and consist in general of 50–90% of water and 50–10% polymer backbone. As a result of this the visco-elastic behaviour could be expected, which is different from a simple plastic piece.

As already discussed Hook's law cannot be directly applied to spherical elastic entities.

The theory for entirely (nonporous) elastic spheres compressed between two flat rigid surfaces was first presented by Hertz [12]. The equation is valid only until 10% strain:

The functional relationship between force, F and displacement h can be described as:

$$F = \left[\frac{4 R_p^{0.5} E}{3 \cdot 2^{3/2} (1 - \mu^2)} \right] h^{3/2} \quad (6)$$

At higher levels of deformation the Hertz theory fails. An extension of the Hertz theory has been developed by Tataru [13]. The functional relationship allows the description of up to 60% particle deformation:

$$F = ah^{3/2} + bh^3 + ch^5 \quad (7)$$

where b and c are arbitrary constants and a is equal to the coefficient of the Hertz equation:

$$a = \left[\frac{4 R_p^{0.5} E}{3 \cdot 2^{3/2} (1 - \mu^2)} \right] \quad (8)$$

The Hertz theory is thus the limiting value for small particle deformations.

The stress strain curve is terminated by a point of rupture or irreversible deformation (Fig. 3).

The point of rupture or irreversible deformation called failure strength in material science [10] is dependent on the base matrix, the solvent, the modification and temperature. This point gives valuable information about the limiting stability of resins. In case the breaking stress is high the material can be considered as highly mechanically stable and easy to pack in a

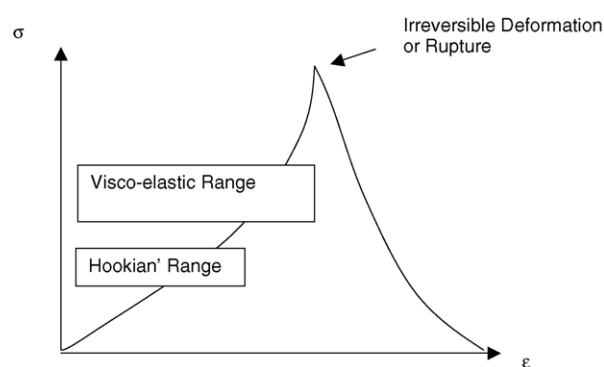


Fig. 3. Schematical drawing of a compression–strain curve for a polymer linear Hookian range, visco-elastic range and irreversible deformation or particle rupture.

column. Chromatographic materials can be classified into rigid-plastic materials, e.g. silica gel, hydroxy apatite, polystyrene and elastic/visco-elastic materials like methacrylates, agaroses and acrylamides.

3. Materials and methods

3.1. Materials

WFI was from Millipore equipment, ammonium sulfate was purchased from Aldrich and the resins were from TOSOH BIOSCIENCE GmbH listed in Table 1. For drying the resins were washed with water, acetone and *t*-butyl ether and dried at 60 °C in a vacuum oven for 48 h until constant weight.

3.2. Micromanipulation

The force being imposed on single resin particles was measured by a micromanipulation rig shown in Fig. 4. The details of the technique is described elsewhere in detail [7].

Briefly, single particles are placed on a glass slide and compressed between a probe connected to a force transducer (Aurora Scientific Inc., Canada) and the slide. The slide is mounted on the stage of an inverted microscope (Microinstruments Ltd., Oxon,

Table 1
Chromatographic resins used throughout the Investigations

| Resin | Base matrix | Modification | Pore size (nm) | Particle size (μm) |
|------------------------|-----------------------------------|--------------------------|----------------|---------------------------------|
| Toyopearl HW 650M | Methacrylate | –OH groups | 100 | 40–90 |
| Toyopearl Butyl 650M | Methacrylate | Butyl groups | 100 | 40–90 |
| TSK Phenyl 5PW | Methacrylate (higher crosslinked) | Phenyl groups | 100 | 30 and 20 |
| Toyopearl DEAE 650M | Methacrylate | Diethylaminoethyl-groups | 100 | 40–90 |
| Toyopearl AF Protein A | Methacrylate | Protein A | 100 | 40–90 |
| TSK Gel Super HZM-H | Polystyrene/divinylbenzene | Nonmodified | 300 | 13 |

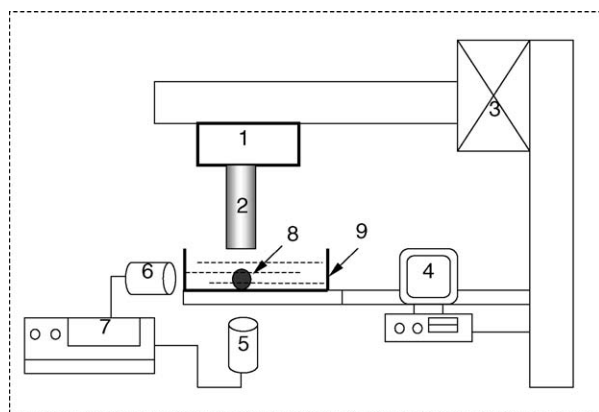


Fig. 4. Schematic diagram of the micromanipulation rig: (1) force transducer; (2) probe; (3) stepping motor; (4) computer with a data acquisition board; (5) bottom-view microscope; (6) side-view microscope; (7) video recorder; (8) resin particle in water and (9) glass chamber.

UK). When a particle is squeezed at a pre-set speed, the force being imposed on it is measured simultaneously by sampling the voltage signal from the forces transducer by using a PC-30D data acquisition board (Amplicon Liveline, Brighton, UK) fitted to a personal computer. The diameter of particles were measured from their image under microscope. The experiments were conducted at room temperature $20 \pm 1^\circ\text{C}$. From the data of force versus probe displacement, the pseudo stress (force divided by the initial particle cross sectional area) and deformation (displacement divided by the particle diameter) were determined.

4. Results and discussion

4.1. Particle size distribution for small resin samples

The resin mechanical stability was determined in dry and in wet state. Chromatographic particles are not-monosized. Hence, the resultant force–displacement values is expected to be a function of the particle size. To guarantee representative values it is required to employ a certain number of resin particles. According to the t-distribution a minimum of 10 particles has to be investigated to get statistical representative data (close to a Gaussian distribution, $\alpha = 95\%$) [14]. Approximately 20 particles were chosen, and a representative particle size distribution is shown in Fig. 5, which satisfies Gaussian with 95% confidence. The descriptive statistical values for wet and dry Toyopearl 650M

Probability Density

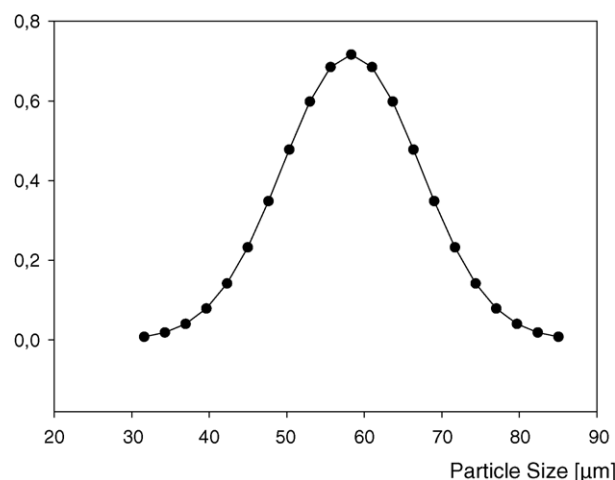


Fig. 5. Particle size–probability density plot for wet non-modified Toyopearl HW 650M (20 randomly selected particles).

are summarized in Table 2. The particle size distributions of the other resin types were similar to the non modified type with deviation of about 10% and are not shown here.

The particle size should be with 90% probability in the range of 40–90 μm which is in accordance with the certificate of analysis of the supplier. As a conclusion small sized samples can properly represent the particle size distribution of a large sample. The dried particles are smaller than the wet particles, 46.4 μm versus 58.3 μm . The swelling ratio can be calculated as:

$$\text{swelling ratio} = \frac{\text{particle diameter in swollen state}}{\text{particle diameter in dry state}} \quad (9)$$

which is for Toyopearl HW 650M equal 1.26

Table 2
Descriptive statistical values of dry and wet Toyopearl 650M

| | Wet Toyopearl HW 650M | Dry Toyopearl HW 650M |
|---|-----------------------|-----------------------|
| Particle number | 21 | 20 |
| Minimum particle size (μm) | 31.7 | 32.5 |
| Maximum particle size (μm) | 86.2 | 59.1 |
| Mean value (μm) | 58.3 | 46.4 |
| Standard deviation (μm) | 8.9 | 4.8 |

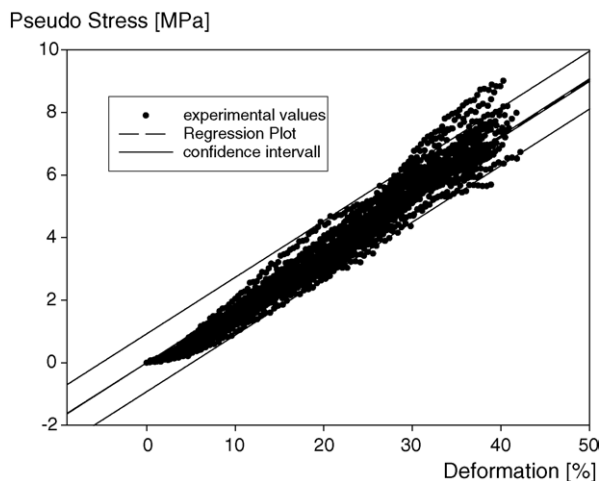


Fig. 6. Pressure-deformation plot for 20 dry non-modified Toyopearl 650M particles (constant compression speed 11 $\mu\text{m/s}$).

4.2. Mechanical stability of non-modified Toyopearl particles

For the above described two particle populations the mechanical properties were investigated by the micromanipulation method. For all measurements a constant compression velocity of 11 $\mu\text{m/s}$ was chosen.

The experimental values of pseudo stress versus deformation for the 20 non-modified, dry Toyopearl HW 650M particles are plotted in Fig. 6.

The relative standard deviation of the method is in the range of 10–15%. The standard deviation is a function of the deformation and increases with deformation.

For dry and wet non-modified Toyopearl HW 650M the Young's modulus was calculated by using the Hertz equation in the deformation range below 10% and with a Poisson ratio of 0.5. The Young's modulus as a function of particle size for the dry Toyopearl HW 650M resin is shown in Fig. 7.

The deviation from the mean value is large but independent to the particle size. The mean Young's modulus for both materials

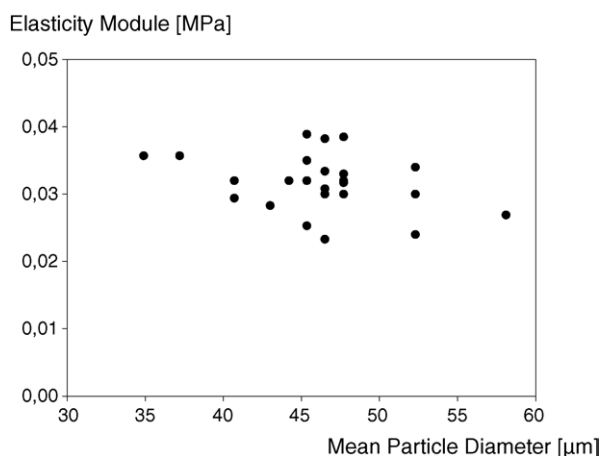


Fig. 7. Elasticity modules for dry Toyopearl 650M calculated with Eq. (7) (Hertz theory).

are:

$$E = 93.3 \pm 8.8 \text{ MPa (dry Toyopearl HW 650M)}$$

$$E = 8.5 \pm 0.78 \text{ MPa (wet Toyopearl HW 650M)}$$

The fluctuations for the same type of particles are random, which reflects the intrinsic inter-particle variation of the mechanical property parameter.

The dry Toyopearl is nearly 10 times as rigid as the wet. In the dry state, the particles behave as glassy entities, and upon immersion in water they become highly plasticised and the Young's modulus decreases significantly in the absolute value. This means water do softens polymeric porous materials. The water content in a resin seem to play an important role on the mechanical stability.

4.3. Rupture force and point of irreversible deformation

A material can be deformed until a point where it is irreversibly damaged [10]. For particles it is again a statistical value and we considered the point as statistically relevant if more than 80% of all particles were crushed or irreversibly deformed. The force corresponding to the particle being irreversibly changed was called rupture force (in case the particle was crushed) or point of irreversible deformation (if the particle remained in a pancake shape).

4.4. The rupture force or point for irreversible deformation was measured for different materials (see Table 3)

The most stable chromatographic material is the inorganic ceramic hydroxyapatite. Nonporous materials are more stable than the porous ones but the limiting deformations are very small. This is different to the porous materials (the data was obtained from [10]). A deformation more than 90% is possible before the point of irreversible deformation is reached. There are also differences in base materials like polystyrene and methacrylate. The polystyrene material is not as stable as the methacrylate and crushes at 50% deformation at a lower rupture force. A higher crosslinking degree as for Toyopearl TSK Phenyl 5PW does remarkable increase the mechanical stability (Table 3).

The total value of the rupture force seems to be out of practical interest because the highest deformation in a chromatographic process column caused by the flow rate is not greater then 10–15% deformation. But the pressure changes many times during the purification cycles and so a higher rupture force assures that a resin last longer.

4.5. Influence of surface modification on the mechanical stability

The mechanical stability of three different resins prepared from the same base matrix Toyopearl HW 650M were compared. One weak anion exchanger Toyopearl DEAE 650M, a resin for hydrophobic interaction chromatography Toyopearl Butyl 650M and the non-modified Toyopearl HW 650M were

Table 3
Limiting stability values for some selected materials

| Resin | Particle size (μm) | Breaking stress (B) or irreversible deformation (ID) (bar) | Compression (%) |
|------------------------------------|---------------------------------|--|-----------------|
| Hydroxyapatite | 30 | 1700 (B) | 50 |
| Polymethylmethacrylate (nonporous) | – | 600–800 (B) | 2–6 |
| Polystyrene | – | 330–550 (B) | 1.5–3 |
| Toyopearl TSK Phenyl 5PW | 20 | 290 (ID) | 94 |
| Toyopearl Butyl 650M | 65 | 180 (ID) | 81 |
| Toyopearl DEAE 650M | 65 | 110 (ID) | 70 |
| TSK Gel Super HZM-H (PS/DVB) | 10 | 110 (B) | 45 |

used. The mechanical stability were determined in dry and in wet state. The data is presented in Figs. 8 and 9.

The experimental data are fitted to the Tataru equation Eq. (7) by using the program routines from SIGMAPLOT[®] (comp. SPSS SCIENCE). The fit was excellent for each data set. The coefficient of variation was almost in the range of 10%. From these parameter an elasticity constant was calculated by using Eq. (8). The Young's modules are listed in Table 4.

The Young's module for nonmodified Toyopearl HW 650M calculated with the Tataru equation is very close to those derived from the Hertz theory in the linear range. The Tataru equation is applicable to describe higher deformations of resin particles.

In dry state the most stable resin is clearly the hydrophobic version, followed by the non-modified and the anion exchanger. Remarkable is the big difference between the resins by nearly a factor of 10 from Toyopearl DEAE to Toyopearl Butyl. A tentative explanation could be the increased chain interaction by the hydrophobic butyl groups which increases the overall rigidity of the resin.

In wet state the same ranking of stability is observed but not as significant as in the dry state. The curves for the dry state are sigmoidal shaped where as the curves for the wet materials

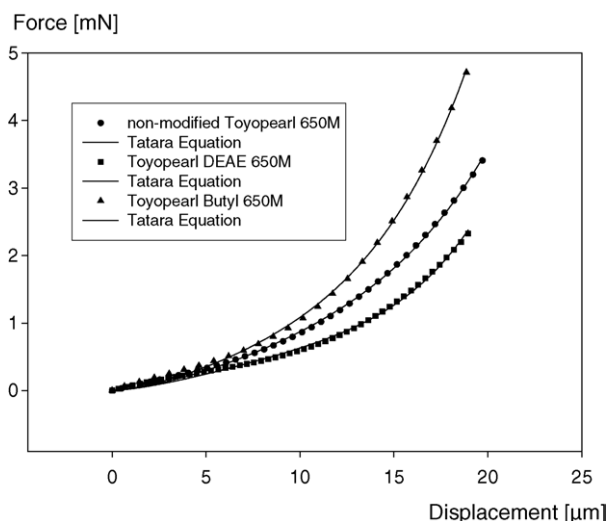


Fig. 8. Mechanical stability of different Toyopearl modifications in water at 20 °C.

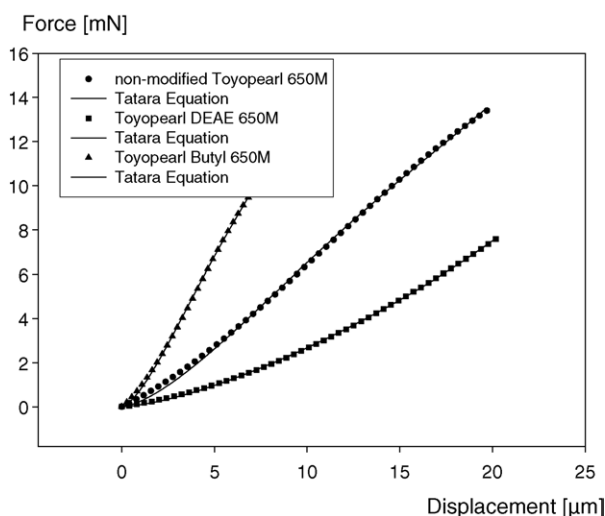


Fig. 9. Mechanical stability of different Toyopearl modifications in dry state.

are concave. This is the result of the porosity of the resins as described from Adams and Lawrence [19]. Obviously the big differences in mechanical stability measured in dry state are “levelled out” in the wet state. The most destabilized resin in water is the hydrophobic. Here the elasticity constant differs nearly 26 times.

All conclusions about the effect of surface modifications on mechanical stability can only be drawn with great care, because the preparation of the functionalised resins may result in different degrees of post cross linking of the resins with an increased mechanical stability. This influence remains to be investigated.

Table 4
Young's modules for different Toyopearl modifications calculated with Eq. (8) (Tataru equation)

| Resin | E (dry state) (MPa) | E (wet state) (MPa) |
|--------------------------------|-----------------------|-----------------------|
| Non modified Toyopearl HW 650M | 82.0 | 9.3 |
| Toyopearl DEAE 650M | 29.4 | 7.9 |
| Toyopearl Butyl 650M | 262 | 10.2 |

4.6. Role of water to the mechanical stability

As already shown, water destabilizes polymeric resins. Hydrophilic resins seem to be not as stable as the hydrophobic ones. The dilemma is that very hydrophobic materials cannot be used for protein separations because of their denaturing character. Therefore, at least a balance between hydrophilic and hydrophobic forces is required.

To investigate the contribution of hydrophobic forces to the mechanical stability of resins the force–displacement curve was recorded for Toyopearl Butyl 650M particles in pure water and in 3 M ammonium sulfate at 20 °C (see Fig. 10).

The difference in the mechanical stability is remarkable. The calculated elasticity modules are:

$E = 3.6 \text{ MPa}$ for Toyopearl Butyl 650M in pure water

$E = 1.8 \text{ MPa}$ for Toyopearl Butyl 650M in 3 M ammonium sulfate

The material treated with 3 M ammonium sulfate is by the factor 2 less stable as the material in pure water. The reason for this behaviour is not totally clear at the moment and a rather simple working hypothesis is proposed as follows:

The viscosity for 3 M ammonium sulfate is 2.5 N s/m^2 instead of 1 N s/m^2 for pure water at 20 °C [16]. Assuming the pores of the system and all other parameter are constant, the volume flow \dot{V} out of the pore system is according to the Hagen–Poiseuille equation [15]:

$$\dot{V} = \frac{\pi \Delta P}{8l_p \eta} r^4 \quad (10)$$

The volume flow is inverse proportional to the viscosity, which means the volume flow for the resin in 3 M ammonium sulphate is about 40% compared to that in water. This factor is close to the difference in mechanical stability. This implies that the water content in the resin treated with 3 M ammonium was

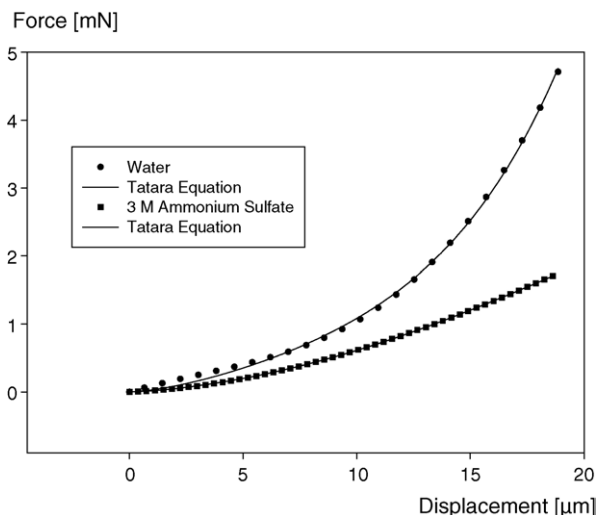


Fig. 10. Mechanical stability of Toyopearl Butyl 650M in water and in 3 M ammonium sulfate.

higher after the compression than that with water. Therefore, the mechanical stability of the resin was less. This explanation is rather a kinetic one and would mean that the stress modulus is rate dependent which was already described for non modified Sephadex® particles by Andrei et al. [9]. The equilibrium explanation, as the result of the different hydrophobicity of the resins is as follow: the starting water content differs for resins in water and in 3 M ammonium sulphate. It takes longer time to extrude the water out of the particles. The above hypotheses are subject to future validation.

4.7. Packed bed behaviour derived from single particle measurements

It is not easy to predict the packed bed properties from single particle measurements.

Less has been reported on the investigations of the influences of neighbouring particles in a random packed bed [17]. The compression of a particle bed can be divided into three different possibilities: (1) rearrangement of particles in the packed bed under the applied stress, (2) deformation of particles in the packed bed under the applied stress and (3) complex case when both effects take place in the packed bed. In random packed deformable particle bed with increase in load the contact area and the coordination area between the particle increases. The specific surface area ratio and the porosity is decreasing by compression as shown in Fig. 11.

The relationship between specific surface area ratio and porosity can be described by the following equation [18]:

$$1 - \left(\frac{A_C}{A_0} \right) = \frac{1 - \exp(-B\varepsilon)}{1 + A \exp(-B\varepsilon)} \quad (11)$$

The constants A and B can be fitted to the stress–strain curves. According to the findings of Lu et al. [18] A and B are decreasing with increasing mechanical stability of particles. The compression of low strength particles bed shows a sigmoid relation between specific surface area ratio and porosity. This indicates that the particles are deformed at the initiate of compression. No rearrangement of particles occurs in compressing a low strength particles bed. They behave like a monolith. In contrast, the free surface in a high strength particles bed will not diminish until the compression transits from rearrangement stage to particle deformation stage. Once all the inter-particles spaces have been lost, that is, the porosity of the bed has been reduced to zero, the resulting solid matrix of deformed particles is much more resistant for further compression.

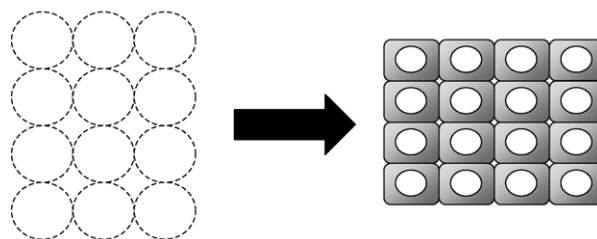


Fig. 11. Bead deformation by applied pressure.

Because the contact area is much more less with increasing pressure for more mechanical stable particles it can be concluded that the prediction of mechanical bed stability might be easier performed from single particle measurement for less deformable particles consisting of synthetic polymers like methacrylates or polystyrene/divinylbenzene.

A quantitative description of the expected bed stability can thus for Toyopearl® be derived from the above calculated Young's modules. The tendency to create fines and the overall bed stability is dependent from this parameter. Resins with high Young's modules, like resins for hydrophobic interaction chromatography, are more stable then resins with other ligand types which is in accordance with our experience.

5. Conclusion

A micro manipulation method was used for the determination of the mechanical stability of polymeric resins with different surface functionalities. The mean elasticity module in each sample was calculated based on approximately 20 single particles using the three-parameter Tatara equation for the deformation up to 50%, which seems to be representative. We found that the hydrophobic Toyopearl Butyl 650M resin is much more stable than non-modified Toyopearl HW 650M the weak anion exchanger gel Toyopearl DEAE 650M. The observed large difference in dry state is "levelled out" in water which weakens the elasticity by a factor of 26 times. The mechanical stability of polymeric resins in wet state is not only a function of the initial water content but also a function of the speed of water release under the applied pressure.

6. Nomenclature

| | |
|-------|---|
| a | arbitrary constant |
| A | adjustable constant |
| A_0 | initial area (initial particle area) (μm^2) |
| A_C | contact area of a compressed particle (μm^2) |
| b | arbitrary constant |
| B | adjustable constant |
| c | arbitrary constant |
| d_p | particle diameter (μm) |
| E | Young's modulus of the sphere (MPa) |
| F | force (mN) |
| h | deformation by compression (μm) |
| K_0 | empirical constant |
| l | actual length (deformed particle) (μm) |

| | |
|------------|--|
| l_0 | initial length (particle diameter) (μm) |
| l_p | pore length (μm) |
| L | bed height (cm) |
| Δp | pressure difference in a chromatographic bed (MPa) |
| ΔP | pressure difference in the pore (MPa) |
| r | mean pore radius (nm) |
| R_p | particle radius (μm) |
| t | time (s) |
| u | linear flow rate (cm/min) |
| \dot{V} | volumetric flow (cm^3/min) |

Greek letters

| | |
|-----------------|--|
| ε | strain |
| ε_B | interstitial bed porosity |
| η | fluid viscosity [kPa s] |
| μ | appropriate choice for the Poissons's ratio was 0.5 for all calculations |
| σ | stress (Pseudostress), pressure (MPa) |

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