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# Characterization of microcapsules: recommended methods based on round-robin testing

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Alginate beads, as well as microcapsules based on alginate, cellulose sulphate and polymethylene-co-guanidine, were produced at diameters of 0.4, 1.0 and 1.5 mm. These standard materials were tested, by independent laboratories, in regards to water activity, bead or capsule size, mechanical resistance and transport behaviour. The water activity and mechanical resistance were observed to increase with bead and capsule size. Transport properties (ingress) were assessed using a variety of low molar mass and macromolecular probes. It was observed that the penetration of Vitamin B12 increased with bead diameter, as did dextran penetration. However, for the membrane-containing microcapsules, larger membrane thickness, observed for the larger capsules, retarded ingress. The authors, who are part of a European working group, recommend that permeability be assessed either using a large range of probes or a broad molar mass standard, with measurements at one or two molar masses insufficient to simulate the behaviour in application. Mechanical compression is seen as a good means to estimate elasticity and rupture of beads and capsules, with the sensitivity of the force transducer, which can vary from μN to tens of N, required to be tuned to the anticipated bead or capsule strength. Overall, with the exception of the mechanical properties, the precision in the inter-laboratory testing was good. Furthermore, the various methods of assessing transport properties agreed, in ranking, for the beads and capsules characterized, with gels having smaller radii being less permeable. For microcapsules, the permeation across the membrane dominates the ingress, and thicker membranes have lower permeability.

*Keywords*: Microcapsules, alginate gel beads, encapsulation, characterization, properties, mass transport, permeability, mechanical strength, water activity, size distribution.

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## Introduction

Microencapsulation is applied in a variety of industries, including those that embrace high value-added products, such as therapeutics, as well as commodity-based seeds used in agriculture. Intermediate applications in probiotic development for animal and human consumption complement this broad spectrum. Specific examples of microencapsulated 'bioactives' include drug delivery, where controlled release profiles are desired, biocatalysts for fine chemical manufacturing, and bioartificial organs, the latter of which involves primary or genetically-modified mammalian cells.

The optimization of the microcapsule's properties in the aforementioned applications is a function of its release characteristics, generally represented by the permeability, as well as the mechanical stability and durability, including elasticity and compressive modulus, and water content. The latter is critical in pore formation and in the preservation of foods. Macromolecular parameters, such as the chemical composition and copolymer sequence length distribution, are also critically related to biocompatibility. This publication reviews the recent advances, and remaining challenges, in the characterization of sub-millimetre polymeric membrane-containing capsules, in regards to their diffusive, mechanical and hygroscopic characteristics.



### *Capsule size and distribution*

Over the past two decades, there has been an increasing interest in colloidal and microsphere formulations in a wide range of industries and in the biomedical sciences in particular (Washington 1992, Barber and Healthcare 1993, USP 2000). This gradual unfolding has been parallelled by a rapid development in techniques to study these systems, in particular using light scattering methods such as laser diffraction and photon correlation. The study of microbead and microcapsule dimension is of central importance as well as properties such as permeability, mechanical and chemical stability or drug solubility, all of which correlate with particle size.

Particle size distribution estimation by analytical sieving is most suitable where the majority of the particles is larger than $\sim$100 μm. Among the limitations of the sieving method is the difficulty in separating (cohesive) particles that tend to clog the sieve openings. An alternative method for characterizing the size of particles is by optical microscopy. The major advantages that microscopic techniques possess over most other methods are that the particle profile itself is measured, instead of parameters which are dependent on particle size, and that the operator can select the individual particles to be studied. However, the measurement requires the manual manipulation of particles, which is time-consuming, particularly if a sufficient large number of particles $(n > 30)$ are counted to obtain good precision. Computer analysis of microscope images enables the user to rapidly handle a large amount of information in a typical picture. A suitable colour image software is one which works independently on red, green and blue components that can be combined to quantify an image.

For accurate spherical particles, size is defined by the diameter. However, as microcapsules are often irregularly shaped, the type of the equivalent diameter measured must be defined. The most frequently used descriptors are Feret's

diameter (distance between pairs of parallel tangents to a randomly oriented projected area of the particle and perpendicular to the ocular scale) and Martin's diameter (dimension, parallel to the ocular scale, that divides a randomly oriented particle into two equal projected areas).

*Transport properties*

Permeation is a function of both transport and thermodynamic properties, with some indicators combining both considerations into a single parameter. For example, permeability, is represented by the product of the diffusion $(D)$ and partition $(K)$ coefficients (Gehrke *et al*. 1997). In contrast, the molar mass cutoff (MMCO) is thermodynamic in nature, while other measurements, such as the diffused mass, are purely kinetic. Studies have been reported in regards to permeability measurements based on thermodynamic, kinetic or combined considerations. The former is size dependent, while the latter is influenced by hydrophobic and electrostatic interactions between macromolecules comprising the membrane. As the primary transport mechanism is via water-filled pores (Schuldt and Hunkeler 2000), the water activity, which will be discussed in detail below, can also be critical in defining a microcapsule's release or immunoprotective properties. Methods to estimate the permeability of microcapsules include both ingress- and egress-based techniques, as is summarized in a recent review (Remunan-Lopez and Bodmeia 1997). Typical solutes include biomacromolecules such as proteins (Nurdin *et al*. 2000), haemoglobin (Chandy *et al*. 1999) and cytokines (Tai *et al*. 1995) for the ingress experiments, and dextran (Whatley *et al*. 1991) or synthetic, narrowly distributed, water-soluble polymers for measurements based on exodiffusion.

The commonly employed molar mass cutoff concept (MMCO) often misrepresents membrane properties, as the diffusion across the gel is generally controlled by size, with the molecular size cutoff itself differing by a factor of two if biomacromolecules or synthetic polymers are employed. Therefore, the method requires standardization, which is one of the goals of the present work. For the typical alginate-calcium bead, the MMCO is on the order of 230 kDa (Brissova *et al*. 1996), with a poly-$^{L}$-lysine coating reducing this to ~150 kDa (Awrey *et al*. 1996). It has been reported that multiple coatings, which are generally alternating polyanion and polycations (the so-named 'onion' capsules), control the release profile of loaded drugs (Arica and Hasirci 1993). Furthermore, permeability generally decreases with higher calcium concentrations for the alginate beads, due to an increased density of the outer film. The porosity is also enhanced with the alginate G-block length (Thu *et al*. 1996).

Microbead and microcapsule transport properties can also be assessed via kinetic measurements. For example, the 'mass diffused' expresses, in percentage terms relative to the initial mass, the total mass ingress. Concentrations are monitored as a function of time in such measurements. Speciality capsules include those with pH-sensitive permeabilities achieved, for example, by grafting polymers with dissociating side chains onto thermoplastics (Okahata, Haguchi and Seki, personal communication, 2001). The slowing of the release profile of polyelectrolyte complex-based microcapsules can be achieved by complexing oppositely-charged macromolecules at the electrical equivalent pH (Daniels and Mittermaier 1995).

*Mechanical stability*

Microcapsule mechanical stability has been evaluated by micromanipulation (Zhang *et al.* 1999), the percentage of broken capsules (Leblond *et al.* 1996), and other techniques which will not be summarized here due to the need for brevity. In terms of the former, precisions of ±10% have been obtained with a 10 μm probe coupled to a force transducer. Mechanical properties for polyelectrolyte-based microcapsules are a function of the polymer molar mass distribution (Dautzenberg *et al.* 1996) and can be as high as 7 N per microcapsule. In contrast, a capsule's bursting forces can be sensed as low as 20 μN.

For the common alginate gel, mechanical properties, as assessed under compression, increase with calcium chloride levels (Leroux *et al.* 1999). The compressive modulus also increases with alginate concentration and the length of the guluronic acid blocks, with the highest reported modulus at a G-length of 10–14. A capsule's resistance to mechanical deformation is influenced by coating (Kung *et al.* 1995) and often varies in a contrary manner, in regards to material characteristics, with permeability.

*Water activity*

The stability of freeze-dried live lactic acid bacteria in different dry food products, such as infant formulae and cereals, is mainly dependent on the water activity of the bacteria preparation. It should be kept below 0.2 to obtain good storage stability at ambient temperature (Ishibashi *et al.* 1985). Higher water activity must be compensated for by storing the product at lower temperatures, which, in most cases, is not possible for the aforementioned products—particularly during transportation, where the cold chain is generally not respected.

The water activity of a formulation is a measure of the energy status of the water. It shows how tightly-bound, chemically or structurally, the water is. It equals the relative humidity of air, at equilibrium with a sample, in a sealed measurement chamber. The moisture, in contrast, is the total amount of water present in the sample (Gomez-Diaz 1992). The water activity of a sample that has been dried is affected by the combination of the drying process used and the composition of the sample. In this test, the observed water activity is a relative measure of the possibility to dry beads of different size and composition.

Water activity is important in microbeads, which are fully gelled microcapsules, since the main mechanism of hydrogel degradation is via osmotic swelling. In the case of alginates, this is caused by Donnan equilibrium of the carboxy groups. Assessing the influence of buffer type and storage conditions on the microcapsule thermodynamics is, therefore, important for beads, polyelectrolyte complex-based systems, and microcapsules containing phase inversion membranes. When scale-up becomes important, the cell density per mL of polymer, and, hence, water activity, can change (de Vos *et al.* 1997), indicating a role for thermodynamic considerations in microtechnology, even with reaction times as low as 1 s.

In phase inversion membranes, the water activity can increase permeability two-fold to test solutes such as glucose, insulin and albumin, due to macroporosity (Crooks *et al.* 1990). For polyelectrolyte-based microcapsules, the water activity is often higher for systems with unreacted charges, indicating a role for water activity in capsule diagnostics and quality control (Kono *et al.* 1996). As the primary

transport mechanism is diffusion through water-filled pores, the water activity also, clearly, influences microcapsule performance in its ultimate application.

## Experimental

### *Materials*

Keltone HV-sodium alginate (Alg) (Lot 54650A) was purchased from Kelco/Nutra Sweet (San Diego, CA). Cellulose sulphate (CS) was supplied by Acros Organics (Geel, Belgium) as cellulose sulphate sodium salt (Lot A006986201). Polymethylene-co-guanidine Hydrochloride (PMCG) was purchased from Scientific Polymer Products (Ontario, NY, USA).



### *Microcapsule preparation*

Poly(methylene-co-guanidine) hydrochloride/calcium chloride/sodium alginate/sodium cellulose sulphate (ACPMG) microcapsules, with diameters between 400–1500 μm, were produced at the Swiss Federal Institute of Technology, Lausanne, Switzerland, in the Laboratory of Polyelectrolytes and BioMacromolecules (LPBM). Their production involved a two-stage procedure, which comprised the formation of calcium/polyanion beads followed by a membrane forming stage where the beads are suspended in a solution of polycation, in this case PMCG. The following concentrations were employed: 0.6% Alg and 0.6% CS in 0.9% NaCl as polyanion mixture, 1% $CaCl_2$ in 0.9% NaCl as gelation bath. The ACPMG capsules were sheered off a syringe via air-stripping. Following a washing step, in 0.9% NaCl, in order to remove the excess of polycation, the beads were transferred into the 'outer' polymer solution, a receiving bath containing 1.2% PMCG in 0.9% NaCl. The final step was washing in three consecutive baths of 0.9% NaCl, during 1 min. The dropping time of polyanion was 3 min, the gellation time was 1 min and the membrane formation period was 15 s, 1 min and 3 min for a diameter of 400, 1000 and 1500 μm, respectively.

### *Microbead preparation*

Alginate solutions were prepared by mixing 2 g of SG 150 alginate (SKW, France) with 100 mL of distilled water. The solutions stood overnight at room temperature (Poncelet *et al.* 2001) and then were dropped into 100 mL of a 1% calcium chloride solution using an electrostatic droplet generator (Poncelet *et al.* 1999a).

### *Capsule and bead size and distribution*

Capsule size was visually examined under a standard inverted-light microscope (Axiovert 100, Carl Zeiss Jena GmbH, Jena, Germany) at EPFL-LPBM, a Laser difractometer at Vienna, an inverted microscope (MicroInstruments Ltd., Oxon, UK) in Birmingham and an optical microscope (crystallographic type) in Warsaw.

Figure 1 provides the distribution of capsule sizes obtained for ACPMG capsules measured in different laboratories. In figure 2, the correspondence between the values of alginate bead diameters, measured in different laboratories, is presented. One can observe that the agreement between the measurements is satisfactory.

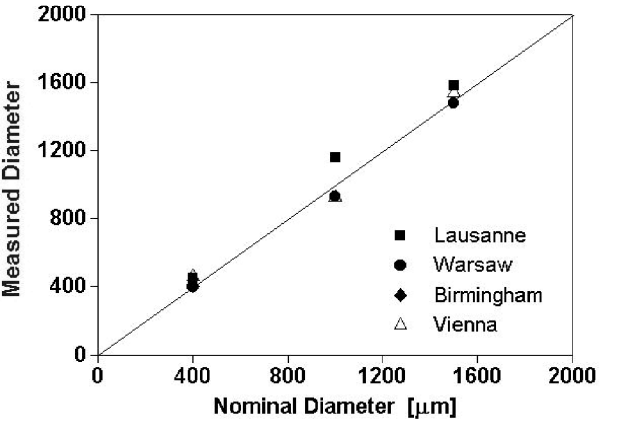



Figure 1. Mean capsule size for Alg/CS/$CaCl_2$/PMCG microcapsules, measured in various laboratories, as a function of the nominal diameter (◆): Birmingham, (●): Warsaw, (■): Lausanne-LPBM, (△): Vienna.

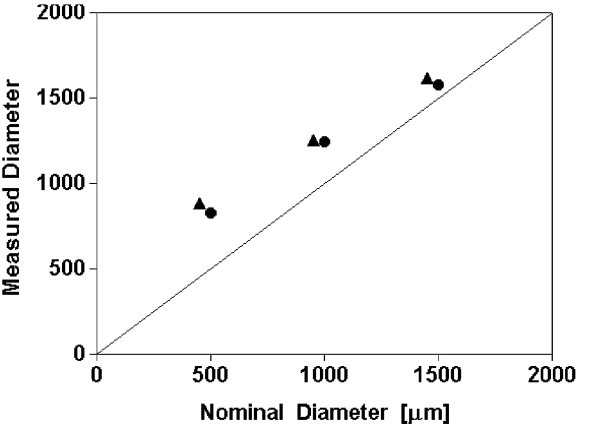



Figure 2. Mean bead size for Alg/$CaCl_2$ microbeads, measured in various laboratories, as a function of the nominal diameter (●): Warsaw, (▲): Lausanne-LPBM.

For particle size analysis, a stereo-microscope with a moving table and micrometric drive or a micromanipulation apparatus equipped with a microscope (Zhang *et al.* 1999) was used. The mean particle size and the standard deviation of a normal distribution were calculated. For individual beads or capsules, the arithmetic average between the largest and smallest diameter on the image was taken as the diameter. Figure 3 is a schematic illustrating the calculation of average diameters for non-spherical capsules.

*Water activity*

The alginate beads were dried in a laboratory freeze-drier FD8, equipped with shelves, supplied by Heto (Denmark). The beads, together with the storing buffer, were poured onto aluminium trays with a diameter of 80 mm and frozen to –37°C in the freeze drier. The filling height was ~5 mm. The beads were dried for 3 days. The water activity was measured with an AquaLab CX-3 instrument from

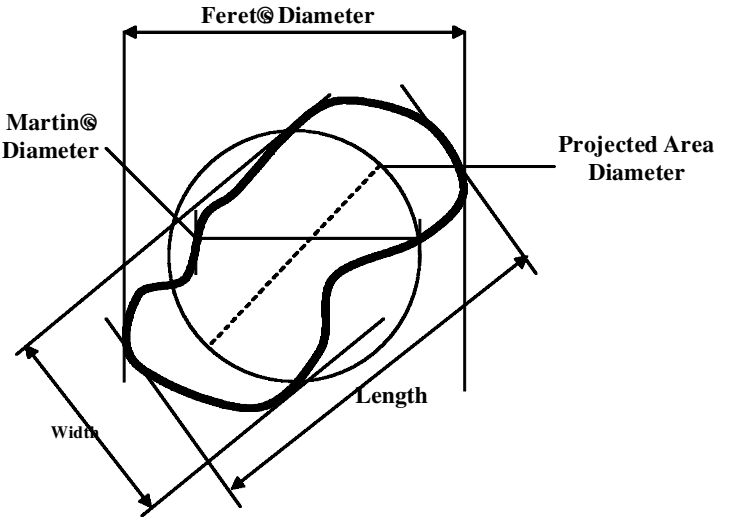



Figure 3. Schematic illustrating the calculation of Martin's diameter and Feret's diameter for non-spherical capsules.



Decagon Devices (USA). The CX-3 uses the chilled-mirror dew point technique to measure the water activity.

*Permeability*

*Vitamin $B_{12}$.* Diffusion of vitamin $B_{12}$ (Sigma-Aldrich ,V2876), from its solution in 0.9% NaCl, into microcapsules was investigated by shaking of equal volumes of solution and microcapsules (2 mL) at 37°C for 90 min. The decrease of vitamin $B_{12}$ concentration as a function of time was measured spectrophotometrically after collecting samples of 50 µl volume at time 0 and subsequently after 2, 5, 10, 20, 30, 60 and 90 min. Vitamin $B_{12}$ concentration was determined by measuring absorbance at wavelength 360 nm on a Shimadzu UV-160 spectrophotometer. From the investigations, the determination of equilibrium concentration had an error of ±5%. Results were collected as the kinetic curves of relative concentrations of investigated solute as a function of time.

*Dextran standards.* One millilitre of 0.2% solution of dextran standards of different molecular masses (10, 40, 70, 110 and 220 kDa), in 0.9% NaCl containing 0.01% sodium azide, was added to 1 mL of microcapsules placed in 10 mL vial, mixed continuously. Samples of dextran solution were collected and analysed by size exclusion chromatography using a universal calibration based on dextran standards. Aliquots were withdrawn at time zero and after 4 h, and injected into a liquid chromatograph equipped with Shodex SB-G and SB-803 HQ columns. The eluent was 0.9% NaCl with 0.01% sodium azide at the flow rate 0.5 mL/min.

*Pullulan standards.* One millilitre of a 0.1% solution of pullulan standards (Shodex, Japan) of different molecular masses (47.3, 112, 212, 404 and 788 kDa), in 0.9% NaCl was added to 1 mL of microcapsules placed in a 10 mL vial with gentle agitation. Samples of the supernatants were withdrawn immedi-

ately, and after 2 h of contact, and the quantity of standard analysed by size exclusion chromatography on a column (OHpak SB-804 HQ, Shodex, Japan) connected to a RI detector (Nerma, ERC-7515A, Ercatech AG, Bern, Switzerland). The eluent was degassed 0.9% NaCl containing 0.01% sodium azide at a flow rate of 0.5 mL/min. Bead permeability was obtained from the difference in peak height of the two samples. Beads were considered permeable to molecules of molecular weight comparable to the standard when the relative difference of the peak heights was less than 95%.



*Mechanical properties*

The mechanical stability measurements, carried out at the Swiss Federal Institute of Technology's Laboratory of Polyelectrolytes and BioMacromolecules (LPBM) and Laboratory of Chemical and Biological Engineering (LGCP), were performed on a Texture Analyser (TA-2xi, Stable Micro Systems, Godalming, UK). The mobile probe was driven at a constant speed of $0.5\,mm\,s^{-1}$ during the microcapsule compression. The experiment was carried out on a series of 25 microcapsules, the average and SD being calculated (Grigorescu *et al.* 2002).

The mechanical tests, carried out at the Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland, were accomplished on a manually operated device. The bursting force was obtained using a mobile piston, pressing on a single capsule, placed on the plate of a weight balance. The experiments were performed on a series of 12 microcapsules, the average and SD being calculated.

At ENITIAA (Nantes, France), the mechanical resistance was correlated to the membrane characteristics by applying a negative pressure to the membrane with the help of a microcapillary (Poncelet *et al.* 1999b). The deformation of the membrane in function of the negative pressure provided information on the membrane elasticity and resistance itself. Membrane mechanical properties were determined by placing a microcapsule under an inverted microscope (Leica Model DMR, Germany). A capillary was placed close to the microcapsule with the help of a micromanipulator. The capillary was connected to a syringe and a mercury manometer. By moving the syringe, a negative pressure is applied in the capillary. The deformation of the microcapsule membrane is recorded as a function of the negative pressure.

Another method used for mechanical stability determination was developed at the School of Chemical Engineering, University of Birmingham, with the details provided in a prior publication (Rehor *et al.* 2001). This micro-manipulation technique allows the mechanical properties of single microcapsules as small as 1 μm in diameter to be characterized, along with the observation of the bursting event, under the microscope.

In Warsaw, mechanical characterization was, as in the other cases, via compression with a piston. The force value was read when the piston stopped. When the force transducer read 0, a further compression step ensued. This 'discrete' method differs from the aforementioned continuous procedures, and will lead to differences in the observed mechanical properties, as will be further discussed herein.

*Membrane tension*

Membrane mechanical tension was determined by placing a microcapsule under a microscope (LEICA, DMR-Germany). A capillary was moved close to the microcapsule with the help of a micromanipulator. The capillary is connected to a syringe and a mercury manometer (figure 4). By moving the syringe, a negative pressure is applied in the capillary $(\Delta P)$. The deformation of the microcapsule membrane $(x)$ (figure 5) is recorded in function of the negative pressure. While a semi-permeable membrane separates two fluids at different pressures, it become convex toward the lower pressure side. Assuming a circular symmetry deformation, the Laplace's law states that:

$$\Delta P = \frac{2T}{r} \tag{1}$$

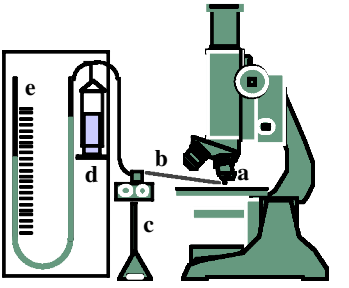
where $\Delta P$ is the pressure difference, $r$ is the curvature radius and $T$ the membrane tension or stiffness. Equation (1) could be applied for both the microcapsule itself and its deformation in the capillary (figure 5), providing:

$$P_m - P_e = \frac{2T}{r_m} \tag{2}$$

and

$$P_m - P_c = \frac{2T}{r_d} \tag{3}$$

where $P_m$, $P_e$ and $P_c$ are the pressure inside and outside of the microcapsules, and in the capillary, and $r_m$ and $r_d$ the microcapsule radius and the curvature radius of the deformation. The pressure difference between the external media and the capillary can be found by combining equations (2) and (3):




**a** microcapsule under microscope **b** capillary
**c** micromanipulator **d** syringe **e** manometer

Figure 4. Schematic of the evaluation system of membrane mechanical properties.

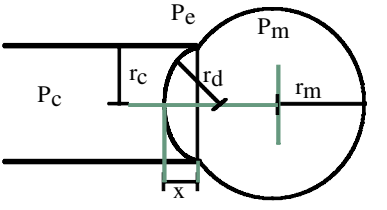



Figure 5. Schematic of the deformation of the microcapsule under negative pressure.

Table 1. Water activity of alginate beads of various diameters.

| Nominal bead diameter (mm) | Water activity (dimensionless) |
|---|---|
| 0.5 | 0.22 |
| 1.0 | 0.24 |
| 1.5 | 0.26 |

$$\Delta P = P_e - P_c = 2T\left(\frac{1}{r_d} - \frac{1}{r_m}\right) \tag{4}$$

From geometric consideration (figure 5), one could observe that:

$$\frac{1}{r_d} = \frac{2x}{x^2 + r_c^2} \tag{5}$$

Equations (4) and (5) allow determination of the membrane tension, $T$.

## Results

### *Water activity*

Table 1 reveals that it was not possible to dry any of the microbeads below a water activity of 0.2. However, a clear tendency between bead diameter and water activity is observed. The smaller surface area per unit volume likely results in greater difficulties in drying the larger alginate beads. The long drying time employed herein indicates that it will not be possible to obtain a lower water activity with the formulation that was used, suggesting that a simple electrolyte-alginate gel will not be applicable for food-grade applications. Alginate-based hydrogels may, though, find applications in biocatalysts and bioartificial organs, where their mechanical properties and permeability dominate the utilization criterion. Therefore, table 1 serves as a means to correlate changes in mechanical properties, where water acts as a plasticizer, and in permeability properties, where water pores actuate diffusion across the membrane. This will be the topic of the following sections of this paper.

### *Capsule size*

Samples from three different charges of ACPMG capsules were distributed to four laboratories for size distribution measurement. The results presented in table 2 show clearly that values for mean diameters of capsules of one batch are corresponding very well, the relative SD are in a range between 7–11%. Additionally, the small deviations of measured diameters, and their 95% confidence intervals, from the nominal values validate the preparation techniques (figures 1 and 2).

### *Mechanical characterization*

Capsule strength depends on capsule size, shape, membrane density and thickness, with the bursting force a complex parameter describing the strength

Table 2. Size distribution of ACPMG capsules.

| Laboratory | Particle diameter (SD), μm | | | Number of capsules tested |
|---|---|---|---|---|
| A | 450 (310) | 1160 (230) | 1580 (260) | 20/20/20 |
| B | 400 (50) | 930 (50) | 1480 (130) | 30/30/30 |
| C | 405 (16) | — | — | 37/–/– |
| D | 457 (27) | 923 (52) | 1535 (76) | 30/30/30 |
| Overall average with 95% confidence intervals | 428 ± 26 | 1004 ± 181 | 1532 ± 100 | 112/80/80 |

Average diameters and standard deviations of ACPMG capsules; size distribution measurements were carried out by microscopy in four different laboratories (A: Lausanne; B: Warsaw; C: Birmingham and D: Vienna).



of the capsule membrane as well as the core strength (Rehor *et al*. 2001). Therefore, standard methods to evaluate microcapsule strength do not exist, motivating the present study. Specifically, the aim of this round-robin test was to compare the mechanical stability results obtained in different laboratories, as well as to evaluate the influence of storage and transport conditions on capsules properties.

Figure 6 presents the bursting force for ACPMG capsules as it was measured in different laboratories. As expected, the bursting force increases with the diameter. One can observe a good agreement for all the measurements performed on the capsules with the size 400 μm. However, when increasing the capsule size, i.e. increasing the reaction time, the differences between the measurements become more significant. This can be due to the differences between the membrane thickness, which influences the mechanical strength. In a previous paper (Rehor *et al*. 2001) the influence of membrane thickness on microcapsule strength has been discussed. For capsules of identical membrane thickness, the bursting force increases with increasing capsule size when the membrane thickness is less than 20% of the capsule radius. However, when the membrane thickness is superior

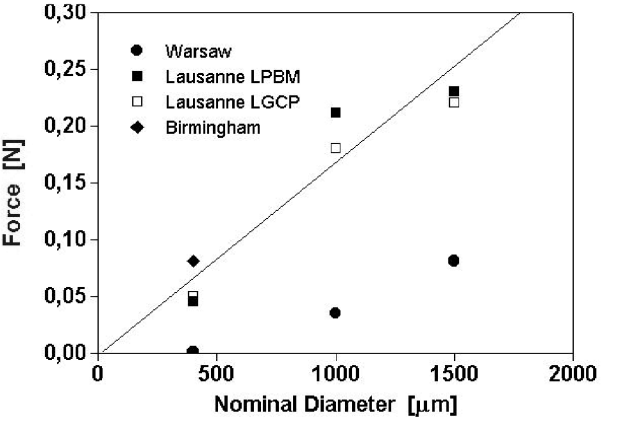



Figure 6. Mechanical strength for Alg/CS/$CaCl_2$/PMCG microcapsules, measured in various laboratories, as a function of the nominal diameter (◆): Birmingham, (●): Warsaw, (■): Lausanne-LPBM, (□): Lausanne-LGCP.

Table 3. Membrane tensions determined by the micropipette aspiration method for the quaternary capsules (ACPMG).

| Nominal capsule diameter (m) | Membrane tension $T$ (Pa.m) |
|---|---|
| 400 | — |
| 1000 | $16.5 \pm 0.7$ |
| 1500 | $13.5 \pm 0.8$ |

Table 4. Mean and variance of the microcapsule and microbead bursting strengths.

| Nominal diameter (μm) | Average bursting force (N) |
|---|---|
| ACPMG capsules | |
| 400 | $0.045 \pm 74.3\%$ |
| 1000 | $0.148 \pm 67.07\%$ |
| 1500 | $0.28 \pm 26.72\%$ |
| Alginate beads | |
| 500 | $0.035 \pm 60.60\%$ |
| 1000 | $1.232 \pm 150.77\%$ |
| 1500 | $1.529 \pm 140.25\%$ |



to 20% of the capsule radius, lower bursting forces are obtained for a given capsule size, since the membranes are sufficiently thick to actually touch during deformation. The micropipette aspiration method shows that the membrane resistance for the capsules with diameter 1000 μm is higher than the value obtained for the capsules with diameter 1500 μm, while with the compression method the capsules with 1500 μm are the most resistant (table 3).

The mechanical properties obtained by the various methods for ACPMG capsules and alginate beads, with the means and variances are summarized in table 4. As would be expected, the composite alginate beads, with the inorganic salt (which acts to reinforce the matrix), have significantly higher mechanical properties than the ACPMG microcapsules. The latter have a liquified core and their mechanical properties are derived from a thin outer membrane. As the size of the beads and capsules increases, the difference between the modulus due to the inorganic support and the membrane (alginate beads and ACPMG capsules, respectively) increases, as is shown in table 4.

*Transport properties: microbead and microcapsule penetrability*

Both kinds of investigated microcapsules, alginate gel beads (Alg) and alginate-cellulose capsules with poly(methylene-co-guanidine) based outer layer (ACPMG), showed similar patterns of vitamin $B_{12}$ decrease in time during ingress experiments. This is exemplified in figure 7 for ACPMG capsules with nominal diameter 1000 μm. Following a rapid decrease of concentration during the first minutes of the experiment, an equilibrium concentration was attained, amounting to ~50% of the initial concentration. The equilibrium relative concentration values

Table 5. Percentage ingress of vitamin $B_{12}$ or dextran standards.

| Capsule type and nominal size (mm) | Percentage ingress vitamin $B_{12}$ 1355 [Da] | Percentage ingress of dextran standards | | | | |
|---|---|---|---|---|---|---|
| | | DS 10 [kDa] | DS 40 [kDa] | DS 70 [kDa] | DS 110 [kDa] | DS 220 [kDa] |
| ACPMG 400 | — | — | — | — | 19.44 | 15.63 |
| ACPMG 1000 | 45.30 | — | — | — | 13.53 | 9.87 |
| ACPMG 1500 | 57.00 | — | — | — | 10.62 | 4.90 |
| ALG 500 | 57.00 | 29.63 | — | 11.77 | — | 0 |
| ALG 1000 | 58.30 | 30.19 | — | 3.23 | — | 0 |
| ALG 1500 | 75.70 | 29.63 | — | 6.25 | — | 0 |

were then calculated as an average value for the three last experimental points (concentrations after 30, 60 and 90 min). The results of the determination of percentage of mass diffused for vitamin $B_{12}$ and dextran standards, for all investigated samples, are shown in table 5. Vitamin $B_{12}$, the neutral marker with small molecular mass of 1355 Da, penetrates easily all investigated microcapsules and between 50–60% of its initial mass is distributed inside the investigated gels. The single exception is the Alg 1500 sample, where an unexpectedly large mass is removed from the solution. The permeability slightly increases with increasing diameter of the microcapsule.

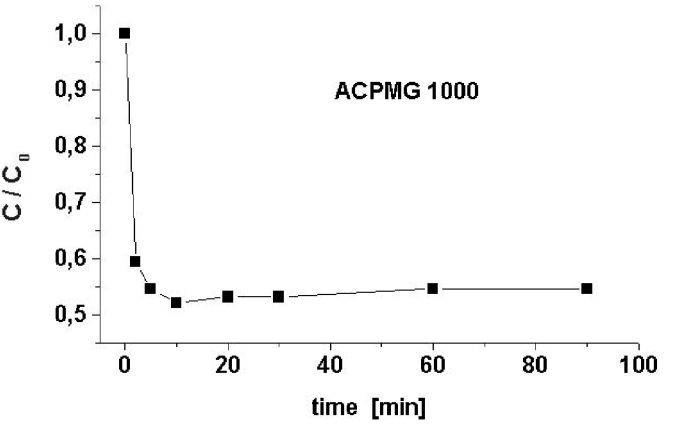
To evaluate a possible influence of different surface, along with changing sample, one can determine the amount of the selected marker diffused per unit surface of capsules, i.e. vitamin $B_{12}$ and dextran penetration in $mg/cm^2$. The total surface of different microcapsule samples was estimated from the measurements of the size distribution and average diameter in each sample. The results of this standardization, with respect to the total surface of microcapsules in the sample, in the case of vitamin $B_{12}$, is presented in figure 8.

As expected, the microcapsule penetration decreases with increasing molecular mass of dextran standard. As can be seen in figure 9, the penetration in all groups of capsules increases with increasing average diameter of beads. This phenomenon could be related to differences in total outer surfaces of investigated microcapsules caused by different bead diameters. When pullulan standards were employed in ingress experiments, the molar mass cut-off was found to decrease with capsule size due to the presence of a thicker membrane (table 6).

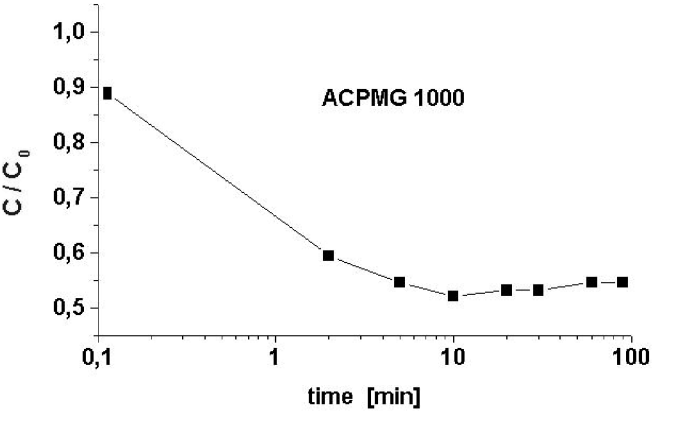
For the alginate beads, as expected, the permeability decreases with dextran molar mass and capsules size. The molar mass cut-off was not precisely determined, although it is estimated to be ~70 kDa. In the case of the membrane-containing ACPMG capsules, a lower permeability was also observed with increasing dextran molar mass and capsule size. For this chemistry, the molar mass cut-off was determined to be ~245 kDa for 400 μm capsules, ~196 kDa for 1000 μm capsules and ~156 kDa for 1500 μm capsules, respectively.

## Discussion

Concerning the amount of mass diffused into capsules, one would expect to obtain values of ~50% of initial mass with the assumption of free diffusion and




(*a*)




(*b*)

Figure 7. Experimental kinetic curve for vitamin $B_{12}$ diffusion into ACPMG-1000 microcapsules.

Table 6. Molar mass cutoff for ACPMG capsules as assessed with Pullulan standards.

| Nominal diameter (mm) | MMCO (kDa) |
|---|---|
| 0.4 | 245 |
| 1.0 | 196 |
| 1.5 | 156 |

total accessibility of gel interior for the marker molecules. In the case of a low molecular mass marker, such as vitamin $B_{12}$, the majority of results for all samples lies between 50–60%, with one exceptional alginate sample (Alg 1500, 76%) where diffusion is likely accompanied by a considerable adsorption of the marker for unknown reasons.

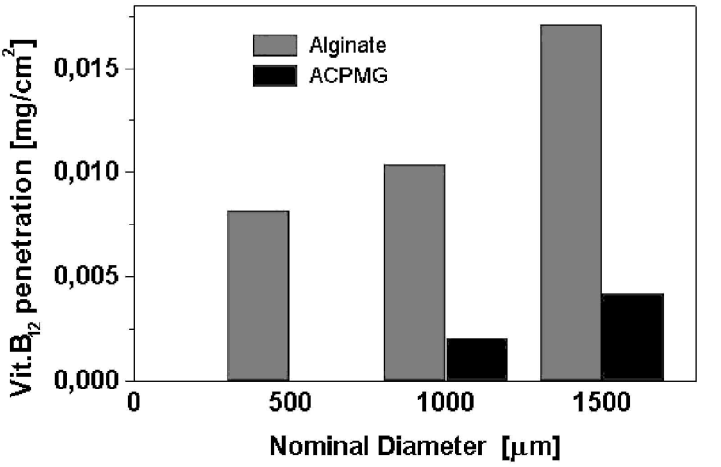



Figure 8. Vitamin $B_{12}$ penetration related to a unit surface of microcapsules for all samples.

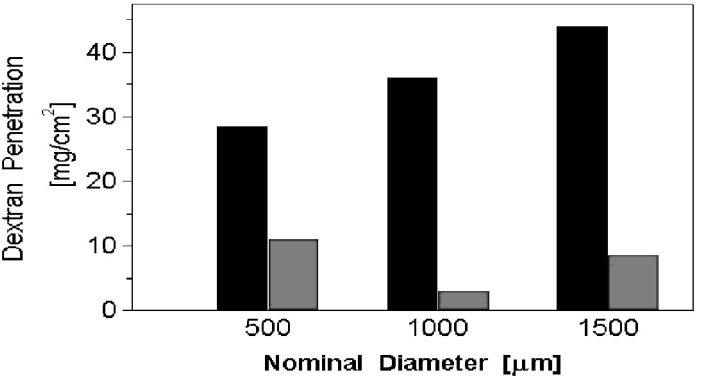



Figure 9. Dextran penetration related to a unit surface of microcapsules for alginate beads. Dextran 10 kDa: black bars, dextran 70 kDa: grey bars.

There are several conclusions which can be drawn from the investigations of permeability of microcapsules for dextran standards. First, it can be seen (table 5, figures 9–11) that dextran standards of larger molar mass generally penetrate microcapsules to a smaller extent than do the small molecular mass standards (e.g. a maximum of 30% ingress for dextrans versus over 50% for vitamin $B_{12}$). This observation is valid for both types of investigated materials. Secondly, there is surprisingly small penetration of alginate beads by more massive dextrans (DS of 70 kDa for all alginate beads). This observation should be compared with the other experimental data, e.g. regarding the extent of penetration of immunoglobulin G (IgG, 160 kDa) into alginate beads, manufactured for the purpose of islets xenotransplantation, where ~40% of the initial marker mass disappears from solution after 8 h (Lanza *et al.* 1995). Most probably, this inconsistency can be explained on the grounds of differences in structure of dextrans and globular proteins in the solution. Finally, a reasonable comparison of results of both methods of transport investigations (transfer of vitamin $B_{12}$ and dextran penetra-

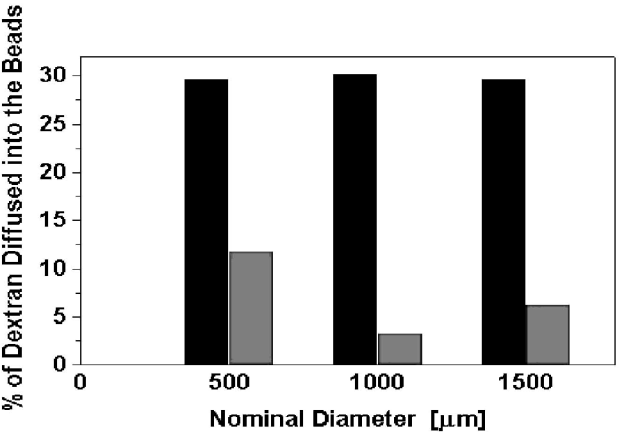



Figure 10. Permeability of alginate beads for dextran with molar mass of 10 kDa (black bars) and 70 kDa (grey bars), respectively. Permeability is assessed as the percentage of dextran ingress.

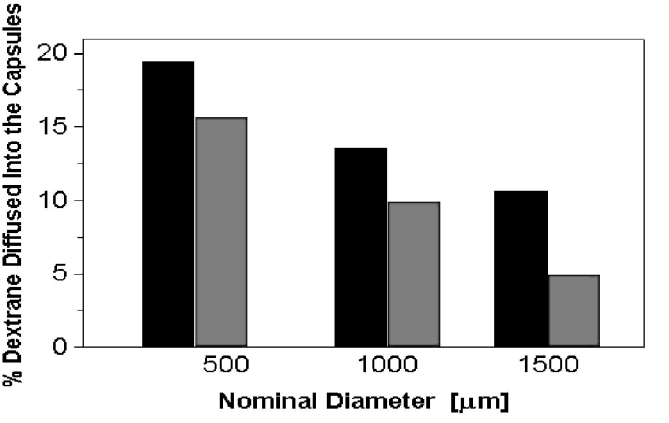



Figure 11. Permeability of Alg/CS/$CaCl_2$/PMCG microcapsules for dextran with molar mass of 110 kDa (black bars) and 220 kDa (grey bars), respectively. Permeability is assessed as the percentage of dextran ingress.

tion) requires elements of standardization of results with respect to microcapsule dimensions. Therefore, standardization of dextran penetration results to unit surface of microcapsules was proposed (penetration mg/$cm^2$, figures 8 and 9).

The observed phenomenon of increasing penetration with increasing microcapsule diameter demonstrates that it is easier for the applied marker to penetrate larger capsules than smaller. There is no reason for such an effect if capsules of different diameters have similar internal structures and are homogeneous. However, this is not the case with the investigated capsules. ACPMG capsules clearly have an outer layer, or skin, with different properties than their interior. On the other hand, alginate beads are produced in an inhomogeneous gellation process and, thus, have a denser outside gel layer and a less dense fluid core. At least in the case of alginate beads, the thickness of the denser layer decreases with increasing diameter. If the mass transfer resistance of this layer is crucial in mass transfer

from an outside solution into the microcapsules, this could explain the observed relationship between mass diffused and diameter. The observed tendency of increase of dextran penetration with increasing average diameter of microcapsules could be interpreted using the same arguments as with mass of vitamin $B_{12}$ diffused inside, i.e. concerning microcapsule inhomogeneity. Nevertheless, other reasons are also not excluded.

The increased permeability of capsules to small molecule standards (table 5) is likely due to the higher surface available per unit volume of the capsule. The membrane-containing ACPMG capsules also had thicker membranes than similar capsules of smaller diameters. Therefore, it is to be expected that the capsules with thicker membranes have lower molar mass cutoffs, as is observed in table 6.



## Conclusions

In general, the following conclusions can be drawn from the round-robin studies:

- A high precision was observed, in inter-laboratory testing, regarding the measurement of capsule diameters.
- Microbead water activity increased moderately with diameter, although in all cases the activity was above 0.2.
- Both methods of transport investigations seem to be complimentary concerning ingress of substance into microcapsule. The first method, with vitamin $B_{12}$ ingress, is well suited for screening of samples of different materials. It enables one to find differences between various materials (see figure 8), is simple, fast and cost-effective. It is also suitable as the first screening step to choose the proper dextran standard markers for cut-off determination.
- The ranking of the beads according to the ease of transport through the hydrogel was the same for small molecule and macromolecular probes, although higher molar masses provide reduced sensitivities. The repeatability in ingress measurements is good, with data within $\pm$10%.
- Larger capsules had more rapid ingress to small molecules, due to the larger surface area, although a lower molar mass cut-off, due to the thicker membranes.
- The bursting force was observed to increase with bead and capsule size.
- There is a distribution of bursting forces, within a batch of microcapsules with the same nominal diameter with a standard error of $\sim\pm$20%.
- Bursting force varies with the compression speed.
- If water egress is observed during capsule compression, than the associated force measured is an artefact.

## Recommendations

This study concludes with the following recommendations:

- Ingress should be estimated using a range of probes varying from oligomeric (several hundred or thousand daltons) to macromolecules as high as 250 kDa.

Systems where one or two standards are used, of selected molar mass, provide limited information and are not recommended. Alternatively, a broad molar mass standard can be employed.

- Mechanical properties can be estimated via compression, with the rupture at break, ultimate force and elasticity assessable. Force transducers are available which sense upwards from the μN range, and these need to be matched to the anticipated properties of the hydrogel or microcapsule in question.
- Mechanical compression should be measured at a deformation rate sufficiently large to prevent water egress during compression.



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