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# Mechanical characterisation of agarose-based chromatography resins for biopharmaceutical manufacture

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ABSTRACT

Mechanical characterisation of agarose-based resins is an important factor in ensuring robust chromatographic performance in the manufacture of biopharmaceuticals. Pressure-flow profiles are most commonly used to characterise these properties. There are a number of drawbacks with this method, including the potential need for several re-packs to achieve the desired packing quality, the impact of wall effects on experimental set up and the quantities of chromatography media and buffers required. To address these issues, we have developed a dynamic mechanical analysis (DMA) technique that characterises the mechanical properties of resins based on the viscoelasticity of a 1 ml sample of slurry. This technique was conducted on seven resins with varying degrees of mechanical robustness and the results were compared to pressure-flow test results on the same resins. Results show a strong correlation between the two techniques. The most mechanically robust resin (Capto Q) had a critical velocity 3.3 times higher than the weakest (Sepharose CL-4B), whilst the DMA technique showed Capto Q to have a slurry deformation rate 8.3 times lower than Sepharose CL-4B. To ascertain whether polymer structure is indicative of mechanical strength, scanning electron microscopy images were also used to study the structural properties of each resin. Results indicate that DMA can be used as a small volume, complementary technique for the mechanical characterisation of chromatography media.



## 1. Introduction

Manufacturers must ensure that chromatography media meet a broad range of requirements before use for the separation/purification of biological products. These requirements include a number of safety considerations (leachables, toxicology), performance (capacity, specificity, throughput), cost (capital investment, longevity) and stability, among others [1]. Stability can be split broadly into two categories – chemical and mechanical. The chemical resistance of chromatography media is dependent on the coupling chemistry as well as the choice of spacer and ligand chemistry and stability. Whereas, the mechanical stability is dependent largely on the choice and composition of the base material, particle size distribution, particle porosity, and to a lesser extent, ligand and ligand deployment [2,3].

The base material is chosen based on a number of factors such as cost, the properties of the material to be processed and surface area and mass transfer characteristics, giving rise to parameters such as dynamic binding capacity (DBC) maximum flow rates, maximum number of cycles etc. Based on this, different manufacturers use different composite materials for their chromatographic media [4]. Agarose is a commonly used base matrix material in biopharmaceutical purification as it relatively straightforward to manufacture and customise certain properties such as porosity and specific binding properties. This paper focuses particularly on MabSelect™, Sepharose ™ and Capto™ media (GE Healthcare, Uppsala, Sweden).

Agarose is one of two main constituents of agar and is generally extracted from seaweed. It is composed of a polysaccharide polymer material formed of repeating units of 1–3-linked β-D galactose and 1,4-linked 3,6-anhydro-α-L-galactose [5]. Once the agar has been processed, the agarose is in the form of a dry powder. It is then dissolved in an aqueous solution >85° C, causing the chains to degrade [3,6]. When the solution reaches a certain viscosity, it is cooled and poured, whilst simultaneously being stirred into a non-polar organic solvent which contains an emulsifier. These conditions induce the formation of spherical beads (emulsification). The stirring and cooling rates are a key parameters in determining certain structural characteristics such as porosity, pore size distri-

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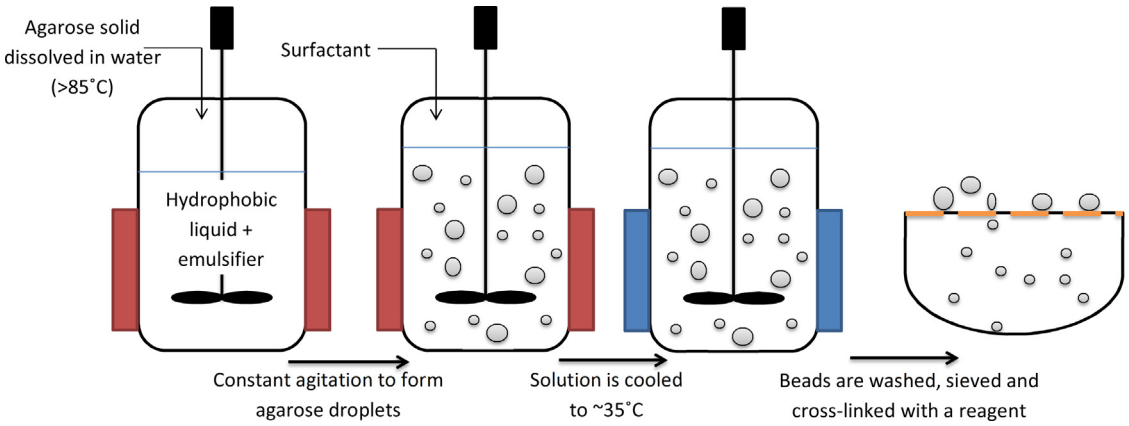


**Fig. 1.** General method for making porous agarose beads. The agarose solid is dissolved in water heated to about 90° C. This is then added to a stirred vessel containing a hydrophobic solution (eg. Toluene or mineral oil) together with an emulsifier. The solutions are immiscible meaning that constant agitation causes the formation of agarose droplets. A surfactant is added to prevent droplet coalescing. The solution is then cooled to below the gelation point of agarose (~35° C) and the beads are then washed, sieved to narrow the size distribution and cross-linked with a reagent.

bution and particle size distribution, which tends to range from 20 to 300 μm [4] (Fig. 1).

Upon formation, the beads are insoluble and sediment into the higher density water phase, as opposed to the organic solvent phase. The beads are subsequently cross-linked with a reagent such as epichlorohydrin. The extent to which this is done is one of the critical factors that determine the rigidity of the matrix. However, caution must be taken at this step as over-cross-linking may reduce porosity, ligand deployment and compressibility characteristics [7,8]. When the process is completed, the resin can be used in various applications such as size exclusion and desalting. It may alternatively go on to be functionalised with different ligand chemistries, after which it can be used in a number of modes for various biopharmaceutical applications [1,3,9–11].

To ensure consistency in the structural and mechanical properties of chromatography media, the media has to be well characterised. Structural integrity testing involves looking at pore size and particle size distributions and porosity, which can generally be ascertained indirectly by observing titration curves or static capacity. There have also been reports on developing lab-based procedures that involve the use of micromanipulation [9,12]. A better idea of mechanical and column performance is usually determined by pressure-flow characterisation. This technique involves gradually increasing the flow rate and observing a rise in the pressure profile in the column. At a certain flow velocity, the pressure in the column will continue to rise without further increase to the flow rate. It is at this point that the critical velocity has been reached and the column has 'failed' [13].

The advantages of this method include the ability to determine the behaviour of chromatography media in a packed bed and how mechanical properties vary with media viscosity, pH, ionic strength etc. However, a drawback of this method is that it requires that the operator adheres to stringent packing criteria to obtain meaningful data. When packing columns, several re-packs may be required to achieve the desired asymmetry and each resin, depending on its chemical and mechanical properties, has its own specific packing criteria. Furthermore, it is necessary to use a column of a suitable diameter, such that wall effects that support the resin in narrow columns do not dominate [14]. The bed height also needs to be representative, as pressure drop directly correlates to the height of the bed, meaning heights of 15 cm or greater are typically used [15]. For these reasons, the pressure-flow technique consumes large quantities of chromatographic media and buffers, which is costly [16].

To address these drawbacks, we have developed the use of dynamic mechanical analysis (DMA) (Fig. 2). This technique

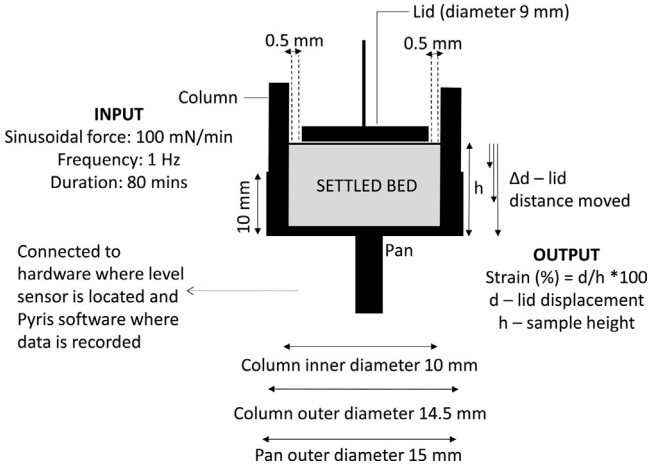


**Fig. 2.** Schematic of DMA set up and dimensions (not drawn to scale). The lid has a diameter of 10 mm and the column that sits inside the pan has an inner diameter of 11 mm and an outer diameter of 14 mm. A sinusoidal force of 100mN is applied at a frequency of 1 Hz over a period of 80 min. The output is strain v time, where strain is the displacement of the lid relative to the sample height.

involves applying a small deformation to a sample in a cyclic manner and allows for the sample material to respond to changes in stress, temperature, strain, frequency, force as well as other parameters. It is used widely in the bioengineering sector and the field of biosciences to characterise the viscoelastic properties of various biological tissue and other biomaterials. Traditionally, the stress and strain parameters are used to calculate Young's modulus to give an indication of changes in elastic properties. Moroni et al., 2006 [17] used the technique to investigate the use of scaffolds to mimic human tissue. They found that the technique was particularly sensitive to pore size changes in scaffolds. With increasing porosity in the scaffolds, there was a decrease in elastic properties, which corresponded to an increase in strain. It has also been used to look at the mechanical properties of materials similar to agarose gels, such as hydrogels. Meyvis & Stubbe 2002 [18] used DMA as a comparative technique to shear rheometry to investigate mechanical properties of pharmaceutical hydrogels. They found a strong correlation between the two techniques but observed that DMA can be used to investigate many more mechanical parameters than solely viscoelasticity.

We have applied the use of DMA to investigate the viscoelastic properties of small quantities of seven agarose-based

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