# DIRECT MEASUREMENT OF OSMOTIC PRESSURE OF GLYCOSAMINOGLYCAN SOLUTIONS BY MEMBRANE OSMOMETRY

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## INTRODUCTION

Articular cartilage is a resilient bearing material capable of withstanding loads reaching several times body weight. The tissue's ability to swell creates a prestress in the cartilage matrix, and enhances its ability to bear load. This swelling pressure has been reported to vary from 0.02 to 0.2 MPa [1-3]. Other studies have also reported the osmotic pressure of chondroitin sulfate solutions, the primary glycosamnioglycan (GAG) found in cartilage proteoglycans [2,4]. Despite several studies in the literature [1-4], the available data on osmotic pressure has been obtained through indirect chemical equilibration measurement, where the osmotic contribution of cartilage or GAGs is measured relative to the osmotic pressure of uncharged macromolecules such as polyethylene glycol, PEG. Furthermore, this indirect technique has been further complicated by the nonlinear behavior of PEG at different temperatures (i.e its osmotic pressure at 4°C and 25°C are not linearly related, as dictated by Raoult's Law [4]). Therefore, the goal of this study was to design an apparatus for directly measuring the osmotic pressure of chondroitin sulfate solutions, equipped with a sensor measuring the pressure in a 1ml chamber. This dialysis chamber is separated from the bathing solution by a porous membrane, permeable to water and free ions only, therefore trapping the large macromolecules inside the chamber and creating a concentration gradient for driving flow. The osmotic pressure of PEG has also been measured in this study to validate this design and technique against prior measurements in the literature.

## MATERIALS AND METHODS

**Direct Membrane Osmometer (DMO)** A custom designed stainless steel cylindrical device (Ø 2.5 cm × 4.5 cm long) was used to measure the osmotic pressure of polymer solutions (Fig. 1). The device consists of a centrally located fluid chamber (Ø 0.8 cm × 2.5 cm deep) with a Ø 0.85 mm port at the bottom. A piezoresistive microchip pressure transducer (NPC 1210-100G; Lucas Novasensor, Fremont, CA) with a range of 0-0.69MPa is bonded to the bottom of the chamber, with the ports of the pressure transducer and chamber aligned. The voltage output of the transducer was recorded with a data acquisition card and Labview software (National Instruments, Austin, TX), and stored for later analysis.

**Experimental Setup** In each trial, 1 ml of a polymer solution was injected into the fluid chamber using a syringe and needle. A magnetic micro stir bar (Fisher Scientific, USA) was placed in the fluid chamber with its long axis parallel to the bottom of the chamber. A moist dialysis membrane (molecular weight cutoff: 1KDa, Ø 2.2cm; Spectrum Laboratories, Rancho Dominguez, CA) was laid down on the solution meniscus, while ensuring that air bubbles did not get trapped in the fluid chamber. A stainless steel wire mesh (McMaster-Carr, Type 316 SS woven wire cloth, no. 9319T575) was laid on top of the membrane to prevent it from bulging when pressurized, and the lid of the device was tightened against an O-ring seal. The DMO was then inverted and placed in a NaCl buffer (0.15M, 25 °C, 200 ml volume, pH=7), leaving it standing on its 3 pegs. This setup allows the NaCl to penetrate into the chamber via the buffer portal, flowing perpendicular to the membrane/mesh. Another magnetic stir bar was placed in the buffer bath and the entire setup was placed on a magnetic stir plate. The resulting pressure was measured as a function of time until equilibrium was reached.

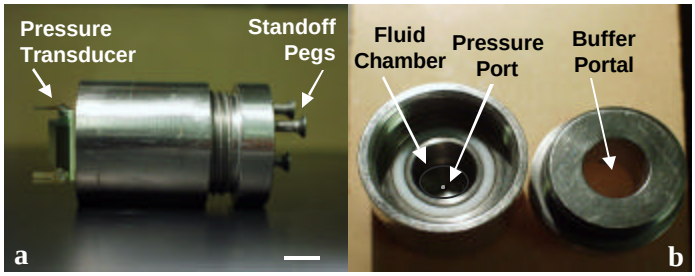


**Figure 1: Direct Membrane Osmometer (a) assembled device (b) fluid compartment, port for pressure transducer and access for buffer solution (scale bar=0.5 cm)**

**Polymer Solutions** The osmotic pressure of polyethylene glycol (PEG, 20 KDa; Sigma, St. Louis, MO) and chondroitin-6-sulfate (Sigma) solutions was measured over a range of concentrations (50 – 200 mg/ml). Each polymer was dissolved in 0.15M NaCl, and vortexed for 1 hour to ensure maximal solubility. At the end of each trial, the chondroitin sulfate (CS) solution was collected and analyzed for GAG content using the 1,9 dimethylmethylene blue assay of Farndale et al. [5] with chondroitin-6-sulphate (Sigma) as the standard. The fixed charge density ($c^F$) was calculated as $c^F = z_{CS} c_{GAG} / M_{CS}$, where $c_{GAG}$ is GAG content per ml of water, and $M_{CS}$ and $z_{CS}$ are the molecular weight and number of charges per CS disaccharide, respectively. ($z_{CS} = 2$ charges and $M_{CS} = 513$ g per repeating unit) [6].

## RESULTS

Four tests at each concentration were performed for both PEG and CS, totaling 16 trials for each. Upon tightening of the device lid, the pressure rapidly increased to ~ 0.2MPa in less than 10 seconds. Once placed in the buffer bath, the pressure increased or decreased nonlinearly (depending on concentration), reaching equilibrium within

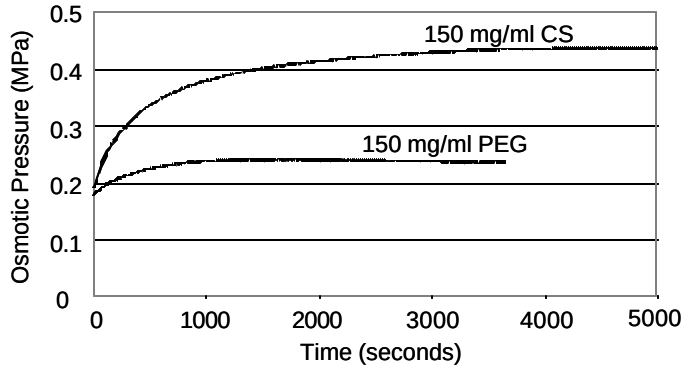


**Figure 2: Sample pressure vs. time curves**

1 to 3 hours (Fig. 2). The osmotic pressure response of polyethylene glycol (PEG) as a function of concentration is shown in Fig. 3. The repeated measure of pressure within each concentration group showed minimal variability. The pressure increased nonlinearly with increasing concentration and was well characterized with a quadratic polynomial, whose coefficients represent the first and second virial coefficients of osmotic pressure (Fig. 3). The osmotic pressure of CS solutions also increased nonlinearly with increasing fixed charge density (FCD) and was described well by the ideal Donnan Law ($R^2$=0.98) (Fig. 4). The maximum pressure attained was ~ 0.6 MPa and for physiological FCD, i.e. ~ 0.25 mEq/ml, the osmotic pressure measured was ~ 0.2 MPa.

## DISCUSSION

The osmotic pressure was accurately measured with good repeatability for a range of concentrations for both PEG and CS solutions. A comparison with the data available in the literature reveals excellent agreement ($R^2$=0.97) between the direct measure of PEG pressure with the calibration of Wachtel and Maroudas [7], also performed at 25 °C (Fig. 3). The PEG calibration of Ehrlich *et al.* [4], performed at 4 °C, slightly overestimates the pressure measured at room temperature. Direct measurement of CS osmotic pressure at 25°C also shows good agreement with the results of Basser *et al.* [3], for pressure of proteoglycans extracted from human femoral head cartilage, measured at 4 °C. More importantly, a comparison to ideal Donnan law (Fig 4.), with the osmotic and activity coefficients taken to be unity, reveals good correlation with the results from the current study as well as those from Basser *et al.* [3]. However, the results of Ehrlich *et al.* [4], measured with the chemical equilibration technique [2,4], are 30-50% below the current results (Fig. 4) and were justified by the authors through the limitations of Donnan ideal Law [4].

In this study, we have developed a new membrane osmometry device for the direct measurement of osmotic pressure of chondroitin sulfate solutions. Future studies will focus on osmotic pressure measurements over a wide range of ionic strengths of NaCl buffers, as well as investigate both the electrostatic and non-electrostatic (i.e. excluded volume) contributions of osmotic pressure.

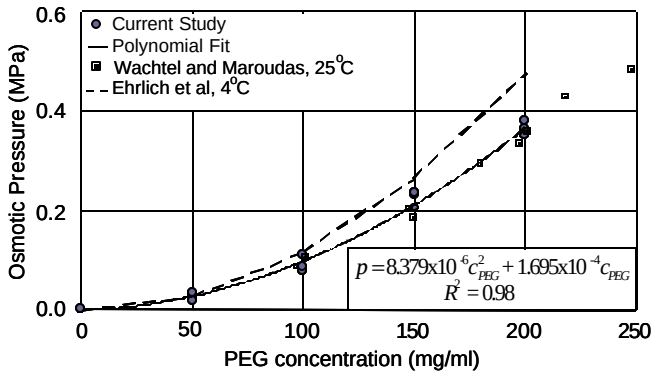


**Figure 3: PEG calibration of osmotic pressure meter.**

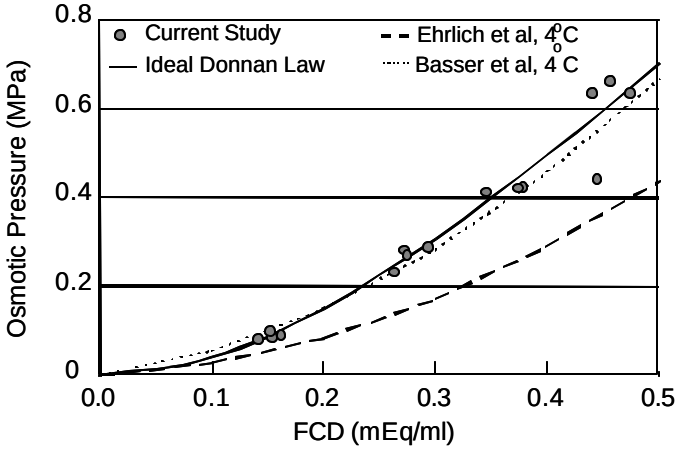


**Figure 4: Osmotic pressure of CS solutions.**

**Ideal Donnan Law:** $p = RT[\sqrt{c^{F^2} + 4c^{*2}} - 2c^*]$; $c^* = 0.15M$

## REFERENCES


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