Diffusion of vitamin B12 in gellan gum-carbon nanotube hydrogels

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Publication Details  
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Keywords
hydrogels, b12, gellan, gum, carbon, diffusion, nanotube, vitamin

Disciplines
Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

This conference paper is available at Research Online: http://ro.uow.edu.au/scipapers/4250
Diffusion of vitamin B$_{12}$ in Gellan Gum-Carbon Nanotube Hydrogels

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Abstract — We report on the diffusion of vitamin B$_{12}$ through gellan gum-carbon nanotube hydrogels. The addition of carbon nanotubes to gellan gum reduced the vitamin B12 diffusion coefficient from $1.70 \times 10^{-6}$ cm$^2$/s to $0.70 \times 10^{-6}$ cm$^2$/s.

Keywords – gellan gum; hydrogel; diffusion; conductivity

I. INTRODUCTION

Nanobionics: the merging of biology and electronics using the most recent advances in nanotechnology will play a significant role in the future development of medical implants and prosthetic devices. The realisation of novel material platforms that facilitate nerve cell regeneration, can control muscle cell regrowth, or that act as efficient platforms for endothelial cell growth is envisaged. In addition, the development of artificial muscle fibres for use in wearable prosthetics will benefit from advances in nanotechnology and the development of bio-composite materials.

The field of Bionics has to date been dominated by the use of traditional conductors such as metals and by traditional electromechanical devices such as motors. The advent of organic conductors (such as carbon nanotubes) with the emergence of nanotechnology may revolutionize the field of Bionics, enabling us to more effectively bridge the gap that currently separates the interface between biology and electronics. However, carbon nanotubes (CNTs) alone will not provide the blend of biological, mechanical and electronic properties required for the next generation of Bionics.

CNTs possess unique electronic and mechanical properties. A key challenge in exploiting these phenomenal properties is to overcome practical difficulties in processing them from their as-produced state. These difficulties arise as a result of the hydrophobic nature of CNTs and associated strong attractive interactions between adjacent CNTs. Considerable effort is currently being directed towards combining polymers and carbon nanotubes into composites with unprecedented mechanical and electrical properties.

A range of biomaterials have proven suitable hosts for CNTs, and potentially provide the basis of highly effective material platforms for Bionics. Chitosan is used in single-polymer scaffolds, as it is structurally similar to the extracellular matrix component glycosaminoglycans and is biodegradable in humans. Gellan gum is US FDA and European Union (E418) approved for food and medical usage, and has found wide application as a multifunctional gelling, stabilising and suspending agent as well as an emerging material for tissue engineering applications.

These biopolymers can be processed into hydrogels (a class of highly hydrated polymer materials) which are emerging as a viable scaffold material for tissue engineering applications due to their bio-degradability, process-ability, and similarities to the natural extra-cellular matrix. Physical cross-linking of a biopolymer solution via addition of cross-linking agents such as Ca$^{2+}$ is a well established fabrication method for hydrogels. Previously we applied this technique to gellan gum solutions and gellan gum-CNT dispersions.

We report the formation of hydrogels based on the addition of conducting carbon nanotube fillers to the biopolymers and investigate the diffusion of vitamin B12 in gellan gum and gellan gum-CNT hydrogels.

II. EXPERIMENTAL METHODS

A. Fabrication of gellan gum hydrogels

Solutions of endotoxin-free gellan gum (Gelzan CM, CP Kelco, Lot #7K1383A) in Milli-Q water (mH$_2$O) (18 MΩ cm$^{-1}$) were prepared at concentration of 1.0% w/v by adding the appropriate mass of gellan gum powder to mH$_2$O and stirring for ~2hrs at a temperature of ~80ºC. To the resultant hot gellan gum solution, a volume of hot 1M CaCl$_2$ solution (prepared from CaCl$_2$.2H$_2$O salt, UNIVAR Lot #712009) was added with continued stirring, so that the final Ca$^{2+}$ concentration in solution was 5 mM. The Ca$^{2+}$ acted as a cross-linking agent, allowing the formation of a hydrogel. The volume of CaCl$_2$ solution added was small, and consequently did not significantly affect the concentration of gellan in solution.

Solutions were then injected into a range of moulds, depending on the purpose of the particular gel, and cooled at room temperature to form a hydrogel.

B. Fabrication of gellan gum-carbon nanotube hydrogels

Single-walled carbon nanotubes (SWNT) (UNIDYM, Lot #P0348) or thin multi-walled carbon nanotubes (MWNT) (Nanocyl SA, Lot #NFL65) were added to hot gellan solutions prior to the addition of Ca$^{2+}$ ions, and consequently did not significantly affect the concentration of gellan in solution. Solutions were then injected into a range of moulds, depending on the purpose of the particular gel, and cooled at room temperature to form a hydrogel.
were kept in a hot water bath (~80°C) throughout the sonication process to prevent the onset of gelation. CNTs were initially dispersed at a concentration twice that required in the final gel, followed by the addition of an equivalent volume of an un-sonicated gellan gum solution to the gellan gum-CNT dispersion. This enabled the formation of a stable hydrogel, and did not appear to adversely affect dispersion quality. Final CNT concentrations ranged from 0.25 ~ 0.50% w/w. Hydrogels were cross-linked as described previously by the addition of hot CaCl₂ solution to a hot, stirred gellan-CNT dispersion.

C. Electrical characterisation

For the electrical characterisation gellan gum-CNT hydrogels were set on a substrate consisting of two highly porous reticulated vitreous carbon (RVC) electrodes fixed to a glass side. The RVC electrodes were connected to electrical characterisation equipment consisting of an arbitrary waveform generator (Agilent 33220A) coupled to a digital multimeter (Agilent 34410A). Current–voltage characteristics were measured by applying a triangular DC voltage waveform (0.5 V amplitude, 10 mHz frequency) across hydrogel samples, while current data were recorded at a rate of 2 s⁻¹.

D. Diffusion Studies

Diffusion through gellan and gellan-CNT hydrogels was characterised utilising a method similar to that employed by Li et al to measure diffusion coefficients thorough alginate and agarose hydrogels. The rate of solute uptake by hydrogel cylinders from a stirred solution of limited volume was determined by measuring the decrease in solute concentration in the surrounding solution over time, allowing the diffusion coefficient to be calculated using an unsteady-state transport model. The solute chosen was for this investigation was Vitamin B₁₂ (Sigma-Aldrich, approx. 99%, lot#117K1520). Vitamin B₁₂ was chosen as it is a physiologically relevant molecule that is readily dissolved in water, and its concentration in solution may be monitored easily by absorption at 550nm.

Hydrogel cylinders (length~8cm, diameter = 0.5cm) of various compositions were set in latex tubing. For each hydrogel composition, three identical cylinders were placed in separate 15mL vials containing 13.5mL of a 0.25mg/mL solution of Vitamin B₁₂ in mH₂O. This Vitamin B₁₂ concentration had previously been determined to be the most constant temperature of 37ºC. Over the course of three days, the time previously determined necessary for solute concentrations to reach equilibrium, 200µL aliquots were removed from the surrounding solutions at regular intervals (8 measurements) and placed in 96 well plates. At the conclusion of this period, Vitamin B₁₂ concentration was determined by reading absorption in the wells at 550nm. This was achieved utilising a micro-plate reader (SPECTRAMax Plus 384, Molecular Devices) interfaced to SOFTmax PRO software (Molecular Devices). Concentration of Vitamin B₁₂ at each measurement time was subsequently calculated from a calibration curve.

III. RESULTS AND DISCUSSION

Stable, self-supporting hydrogels (Figure 1) could be easily formed by combining equivalent volumes of an un-sonicated gellan gum solution with a sonicated gellan gum-CNT dispersion prior to addition of the Ca²⁺ cross-linker. The electrical conductivity of the resulting hydrogels was ~10⁻³ S/m in good agreement with our previously reported values.

The effect of CNTs on the diffusion of solutes through gellan gum hydrogels was carried out by investigating the transport of Vitamin B₁₂, a physiologically relevant molecule important in cell metabolism through the hydrogels at 37 ºC. Diffusion coefficients of Vitamin B₁₂ through cylindrical hydrogels were analysed using the method described by Carmen and Haul and Crank, and recently employed by Li et al. to characterise diffusion through agarose and alginate hydrogels.

Equation 1 describes the diffusion of a solute from a stirred solution of limited volume into a solid gel cylinder of length L and radius r, where L>>r, and the diffusion coefficient D is assumed constant.

\[
\frac{C_t - C_\infty}{C_0 - C_\infty} = \sum_{n=0}^{\infty} \frac{4\alpha(1+\alpha)}{4 + 4\alpha + \alpha^2 q_n^2} \exp \left( -\frac{Dtq_n^2}{r^2} \right).
\]

where \( C_t, C_0 \) and \( C_\infty \) are the concentrations of the solute in the batch at time t, equilibrium and time = 0, \( \alpha = K \times (\text{bath solution volume})/(\text{gel solution volume}) \), K is the equilibrium partition coefficient, D is the diffusion coefficient of the solute, t is time, r is the radius of the gel cylinder and \( q_n \) are the positive, nonzero root of the equation \( \alpha q_n J_0(q_n) + 2J_1(q_n) = 0 \), \( J_n \) are Bessel functions of \( n \)th order.

Carmen and Haul have tabulated values of \( q_n \) as a function of \( \alpha \), and by interpolation one can obtain \( q \) for a given value of \( \alpha \) characterising a particular sample. By measuring the concentration of the solute in the solution surrounding the hydrogel cylinder over time, the diffusion coefficient can be determined. This is achieved by plotting the following terms:

\[
\frac{C_t - C_\infty}{C_0 - C_\infty} \text{ versus } \left[ -\frac{Dt}{r^2} \right]
\]
and calculating the unknown parameter $D$ through non-linear regression\(^9\) of Equation 2 until the root-mean-square error between the experimental data and theoretical curve is minimised.

After conducting this analysis for a range of hydrogels, the data listed in Table 1 was utilised to obtain a ratio of the diffusion coefficients of Vitamin B\(_{12}\) in each hydrogel ($D_{\text{gel}}$) compared to that in water ($D_{\text{water}}$) at 37\(^\circ\)C, which are compared for hydrogels of differing CNT content in Figure 2.

**Table 1. Molecular Weight, Radius and Diffusion Coefficient in Water ($D_{\text{water}}$) for Vitamin B\(_{12}\).**

<table>
<thead>
<tr>
<th>Molecular Weight (Daltons)</th>
<th>1,300</th>
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<tbody>
<tr>
<td>Stokes radius (Å)</td>
<td>~7.7</td>
</tr>
<tr>
<td>$D_{\text{water}}$ (cm(^2)/s at 37(^\circ)C)</td>
<td>5.00 x 10(^{-6})</td>
</tr>
</tbody>
</table>

Even with no added CNTs, the rate of vitamin B\(_{12}\) diffusion through a hydrogel gel is approximately 0.34 times the rate of diffusion in water. This rate of diffusion is similar to that reported for vitamin B\(_{12}\) through alginate hydrogels\(^9\).

Figure 2 shows that addition of CNTs to the hydrogel significantly decreases the rate of vitamin B\(_{12}\) diffusion. Incorporating 0.25\% w/v SWNT or 0.25\% w/v MWNT into a gellan gum hydrogel reduces the diffusion coefficient of vitamin B\(_{12}\) in the hydrogel ($D_{\text{gel}}$) from 1.70 x 10\(^{-6}\) cm\(^2\)/s to 0.70 x 10\(^{-6}\) cm\(^2\)/s.

These results demonstrate that the addition of CNTs to a hydrogel comes at a cost of restricted solute diffusion through the hydrogel bulk. It is suggested that the greater proportion of dry mass (CNTs) in a gellan gum-CNT hydrogel compared to a gellan gum hydrogel may be responsible for the observed reduction in $D_{\text{gel}}$.

**IV. Conclusion**

CNTs were incorporated into gellan gum hydrogels in a step towards the development of conducting hydrogels that could potentially entrap and electrically stimulate cells, inducing functional tissue formation. Dispersion of CNTs was successfully achieved using sonication, and stable, self-supporting hydrogels could be formed if un-sonicated gellan was mixed with the sonicated CNT-containing solution prior to gelation. Quantitative measurements undertaken using vitamin B\(_{12}\) revealed that the diffusion coefficient through gellan gum hydrogels was ~0.34 that in water, and addition of CNTs further reduced the rate of diffusion to ~0.14 that in water.

This work contributes towards the development of conducting hydrogel materials for applications in tissue engineering and nanobionics.

**Acknowledgment**

This work was supported by the University of Wollongong and an ARC Future Fellowship (M. in het Panhuis). CP Kelco is thanked for provision of materials.

**References**