Controlled Release of Vitamin B-12 Using Hydrogels Synthesized by Free Radical and RAFT Copolymerization in $scCO_2$

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Summary: Research in polymer network synthesis is mostly focused on materials with absorbent or controlled-release properties. They are important in healthcare applications. Free radical copolymerization is the main route to obtain gels, using monomers and crosslinking agents, but the materials produced by this route are highly heterogeneous. These heterogeneities reduce the efficiency of chemical compounds used in controlled-release applications. Our research is focused on creating new routes of polymer synthesis to reduce these heterogeneities. In this article, we compare gels synthesized by conventional free radical and by reversible addition-fragmentation chain transfer (RAFT) copolymerization of acrylic monomers in supercritical carbon dioxide. These gels are evaluated for controlled drug delivery applications with vitamin B12. The materials were characterized by scanning electron microscopy (SEM), nitrogen adsorption (BET), differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). These characterization techniques allowed us to determine the morphology and texture of each hydrogel and to explain how vitamin release performance is affected by these characteristics of the hydrogel.

Keywords: controlled release; hydrogels; RAFT polymerization; supercritical carbon dioxide; vitamin B12

Introduction

Reversible deactivation radical polymerization (RDRP) has taken relevance since the 1990's.[1–4] One of the techniques of RDRP is reversible addition-fragmentation chain transfer (RAFT) polymerization. This technique uses particular chemical compounds known as RAFT agents, based on thiocarbonylthio compounds, which control the length of the produced polymer molecules, reducing their molar mass dispersity ($Đ$). Although the research on RAFT polymerization was initially focused on synthesis of linear and branched polymer molecules, RAFT agents have also been used to promote homogeneity of crosslink density distribution in the synthesis of polymer networks.[5,6] Polymer networks

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synthesized by conventional vinyl/divinyl copolymerization are known to be intrinsically heterogeneous.[7] Polymer networks are used as absorbents or to release chemical compounds, such as pharmaceutical drugs and fragrances, among others.[8,9]

Divinyl monomer units in crosslinked polymers are not well distributed along the polymer network structure, thus promoting heterogeneities. These heterogeneities are caused by two main factors. The first one is that at the beginning of polymerization process, polymer chains are short and move freely, reacting freely with other polymer chains, either linear or branched. The second factor is that at the beginning of the process, high amounts of crosslinker are present in the reaction medium, thus promoting the production of polymer networks with very tight crosslink points. However, as time elapses, the concentration of crosslinker decreases, promoting the formation of polymer networks with wider or more separate crosslink points.[5,10–14]

Heterogeneities in polymer networks are explained by the fact that crosslink points are separated from each other by different numbers of monomer units along the main chain, thus reducing the amount of accessible volume for active substances, such as fragrances or drugs, to be stored in the polymer network structure.[10,11,15–18]

The reduction of these heterogeneities inside polymer networks is a complicated task, with few alternatives. One alternative is to modify the polymerization process. Instead of operating in batch mode, semicontinuous or continuous processes can be used. Different operation policies in semicontinuous operation, aimed at reducing heterogeneities in polymer networks, have been proposed.[19] Other solutions involve micro or nanotechnology approaches.[20]

Another approach is to use RDRP techniques in polymer network production.[21–24] At first glance, the idea of using RDRP in polymer network formation seems to be an accessible technology. In the case of linear or even branched polymers, it is not difficult to follow their growth by measuring their molecular weight distribution using gel permeation chromatography (GPC), or studying their thermal or rheological behavior by differential scanning calorimetry (DSC), dynamical mechanical (DMA) or rheometry analysis of solid or molten samples.[18]

However, the characterization techniques mentioned before do not provide accurate information on the structural heterogeneity of polymer networks.[25] The reason is that polymer networks are highly crosslinked materials grouped into particles with low or almost negligible molecular mobility, so that they can not be dissolved by the solvents used in GPC analyses; they are unable to be molten for rheometry analyses, and it is very difficult to compress them into solid samples for DMA.[10,26] Spectroscopic analytical techniques provide limited information on the structure and heterogeneity of polymer networks. For instance, infrared spectroscopy (FTIR) can only detect the presence of certain functional groups in concentrations above 4 wt-%. Proton or carbon nuclear magnetic resonance (^{1}H-NMR or ^{13}C-NMR) techniques require to dissolve the polymer in a specific solvent; if a probe for solid samples is used, resolution in small details or shifts is lost.[10,26] Further options to determine the differences between polymer networks obtained by free radical (FRP) or RDRP polymerization techniques are limited.[10,27,28] Another approach is to address the study of polymer networks by using analytical techniques used in the analysis of porous materials, such as nitrogen or solvent adsorption, where adsorption isotherms are generated and analyzed.[27] The drawback of these techniques is the selection of an adequate solvent or gas.

Microscopy techniques are also useful to characterize the morphology of polymer networks. Scanning electron microscopy (SEM) is used to determine the shape of polymer network aggregates, their size distribution and, under certain conditions, the distribution and quantification of their constituent elements.[28]

In some way, these characterization techniques require polymer networks of the same composition, but generated by different synthetic routes or procedures. Yet another approach to compare the structural characteristics of polymer networks synthesized by different routes is to evaluate their performance for a specific application, such as their capacity for absorption or release of chemical substances.[8]

It is clear from the above discussion that the measurement and understanding of crosslink density distribution of polymer networks, in order to determine their degree of structural homogeneity (or heterogeneity), requires the use of as many characterization techniques as possible. This is particularly important when trying to confirm or reject the apparently accepted claim that polymer networks synthesized by RDRP are structurally more homogeneous than the corresponding materials synthesized by FRC.

The complexities found in the characterization of polymer networks are not the only challenge when studying such materials. The synthesis itself has its own challenges. For instance, solvents are needed in the synthesis for viscosity control or as heat removal medium; however, the solvent needs to be removed when purifying the final product. This energy demanding stage is more complicated for polymer networks, compared to linear or branched materials, because of the high swelling characteristics of the former. These complexities can be overcome, or at least attenuated, by using supercritical fluids as solvents in the synthesis of polymer networks.[2,5,21–24] At supercritical conditions, the density of the fluid is low, in the order of magnitude of a gas. One advantage of using supercritical fluids as solvents is that once the synthesis of the polymer network has been completed, the solvent can be easily removed by only releasing pressure in the reactor. This technique is promising for applications that require FDA approval of the produced materials.

In this contribution, the characteristics and performance in control release of vitamin B12, of two polymer networks (hydrogels), synthesized by conventional free radical (FRP) and RAFT copolymerization of 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA), using supercritical carbon dioxide ($scCO_2$) as solvent, are compared. Given the complexity of the hydrogels produced, and in order to understand the differences between these two polymer networks, and how the polymerization process affects the final product, a combination of several characterization techniques (SEM, DSC and DMA) was required.

Experimental Section

Synthesis of Polymer Networks

Polymer networks were synthesized using 25 mmol of 2-hydroxyethyl methacrylate (HEMA) monomer (97%, Aldrich) and 1.25 mmol of ethylene glycol dimethacrylate (EGDMA) (98%, Aldrich) as comonomer and crosslinker. 0.1 mmol of Azobisisobutyronitrile (AIBN) was used as initiator. For the sample synthesized by RAFT copolymerization, 0.05 mmol of 4-cyano-4-(dodecyl sulfanyl thiocarbonyl) sulfanyl pentanoic acid (CTA) (97%, synthesized in our laboratory at ITT-Mexico) were used. All substances were introduced into a 38 cm^3 high pressure vessel. Carbon dioxide (99.99%, Praxair) was pumped at 103 bar using an ISCO syringe pump. The pressurized cell was introduced into a water bath at 70 °C. Pressure was then adjusted to 172 bar. The polymerization was allowed to proceed for 16 hours. The sample synthesized by conventional free radical copolymerization was identified as G322 whereas the sample synthesized by RAFT copolymerization was identified as G325.

Characterization of Polymer Networks

Polymer gels were characterized by different techniques. Scanning electron microscopy (SEM) analyses were performed in a

JEOL 5900-LV microscopy, 20 KV and at 3500× of magnification to analyze the morphology of the particles. Differential scanning calorimetry (DSC) analyses, aimed at obtaining the glass transition temperature (Tg) of the gels, were performed in a TA Instruments DSC, model 2920. Samples were heated, cooled and heated again from room temperature to 200 °C, at 10 °C/min. Dynamic mechanical analyses (DMA) were carried out by compacting 0.5 g of gel at 550 bar and then placing the sample in a compression device. The analysis conditions used were the following: heating from 80 °C to 200 °C at 10 °C/min, 1 Hz frequency, 0.8 microns amplitude, and 0.4 mN force. Nitrogen adsorption analyses, aimed at determining porosity, were performed in a Quantachrome equipment.

Controlled Release Tests for Vitamin B12

100 mg of each gel were swelled in 4 ml of ethanol under vigorous agitation for 30 minutes. Meanwhile, 20 mg of Vitamin B12 were dispersed in 2 ml of ethanol. Both alcoholic samples were blended and stirred vigorously for a few minutes and then left to sit for 48 hours. Afterwards, the dispersion was centrifuged at 4000 rpm to separate the swollen gel into sol and gel fractions. Ethanol was analyzed by UV-spectroscopy to determine the amount of remaining vitamin B12. 15 mg of vitamin loaded hydrogel were immersed into a 10 ml phosphate buffer solution at 0.1 M and pH = 7.4. This dispersion was contained within a dialysis permeable bag (Spectra/Pro® Dialysis Membrane MWCO:12-14,000). The dialysis bag containing the gel loaded with vitamin B12 was hung inside a jacketed vessel containing 100 ml of buffer solution at pH = 7.4, at 36.5 °C, which corresponds to a human body temperature. Vessel contents were stirred slowly to guarantee uniformity of the dispersion outside the bag. Samples of the buffer solution were taken to determine the amount of released vitamin. Vitamin content in solution was determined using UV-visible spectroscopy in an Agilent equipment at a wavelength of 361 nm.

Results and Discussion

As observed in Figure 1, there were significant differences in glass transition temperature (Tg), between the two analyzed samples, despite the fact that both

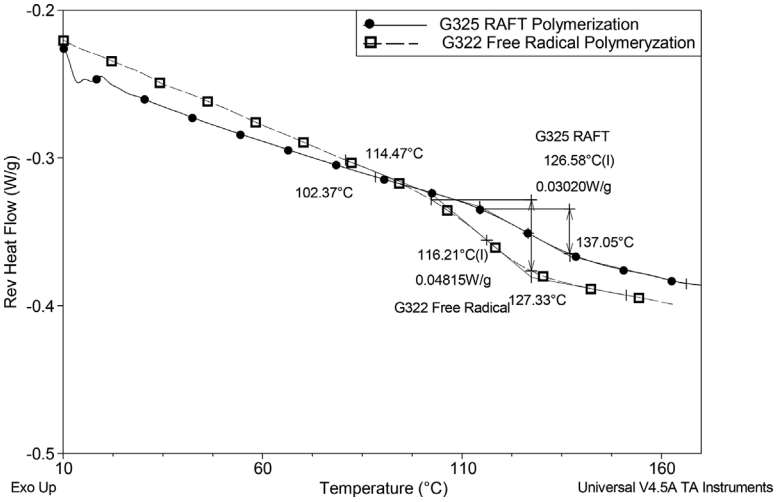

Figure 1.
DSC charts of reversible heat flow vs temperature for the second heating cycle.

samples were synthesized at the same conditions, except for RAFT agent content. Tg for sample G322, obtained by DSC, was 116 °C, 10 °C lower than the corresponding value for sample G325 (Tg = 126.6 °C). When carrying out the DSC measurement, we observed that sample G325 required less power (energy) (0.03020 watts/g) than sample G322, which required 0.04815 watts/g for this transition. This change in energy per sample mass during the glass transition process is related to the amount of heat transformed into kinetic energy within the polymer network. This energy allows the polymer network segments to experience some mobility. The sample that requires more energy has either higher molecular weight (if the material was linear or branched), or it is highly crosslinked.[10,30]

On the other hand, the Tg measured by DMA was almost the same (141 °C for G322 and 146 °C for G325) for both samples. However, it is observed in Figure 2 that the tan(δ) vs. temperature profiles were significantly different. In sample G322, synthesized by conventional free radical copolymerization, the base of the peak shown in the plot is almost 80 °C wide, whereas in sample G325 the corresponding amplitude is 42 °C. In the case of linear polymers, these differences are explained by the molecular mass distribution of each sample. Polymers with high dispersities of molar mass (high Đ values) have wider bases of the transition peak. Likewise, narrow bases of the transition peak are associated to polymers with narrow Đs. Following a similar argument, in the case of polymer networks we can consider that the amplitude of the base of a peak in a tan(δ) vs temperature profile can be related to the heterogeneity (variance) of the crosslink density distribution. Namely, wide amplitudes of the base of a peak in a tan(δ) vs temperature profile can be related to broad crosslink density distributions. This would then indicate that the sample produced in the presence of a RAFT agent (sample G325) is less heterogeneous than the one synthesized in its absence (sample G322).

Similar differences were also observed in the nitrogen adsorption and SEM analyses, as shown in Figures 3 and 4. The nitrogen adsorption isotherms shown in Figure 3a indicate that sample G322 is a non-porous material or a material having large spaces among its particles (type II isotherm under IUPAC classification; see graphical representation in Figure 4c). On the other hand, sample G325 corresponds to a type IV isotherm (Figure 3b), with bottle neck pores, or pores with narrow entrances, present in agglomerates of particles (see graphical representation in Figure 4d).

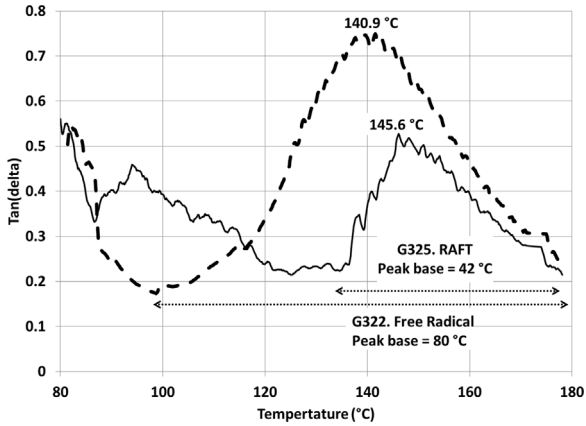

Figure 2.
DMA results: tan(δ) vs temperature.

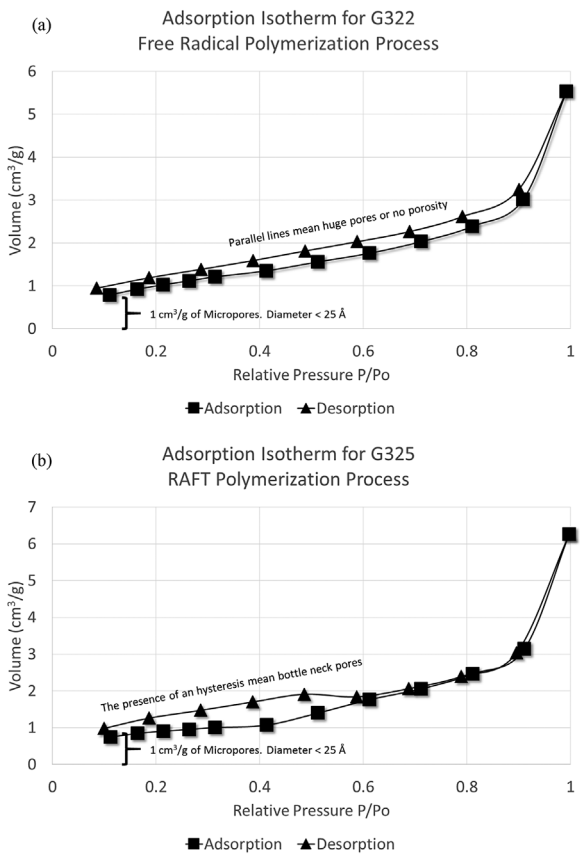

Figure 3.
Nitrogen adsorption profiles for polymer networks synthesized by (a) free radical copolymerization (G322); (b) RAFT copolymerization (G325).

The morphologies inferred from the nitrogen adsorption analyses were corroborated by SEM, as shown in Figure 4. Particle sizes in sample G322, synthesized by conventional free radical copolymerization, were large (above 50 microns). On the other hand, small spherical particles gathered as bunches like a raspberry, and these also grouped in larger bunches like grapes, were observed in sample G325, synthesized by RAFT copolymerization. This morphology is typical of structured materials with high porosities, such as Amberlyst-15, a sulfonic styrene-divinyl benzene ion exchange resin from Dow Chemical, which is described in Sherrington and Hodge.[31] Also shown in Figure 4 (4c and 4d) are the shapes of each morphology, graphically illustrating the differences when nitrogen is adsorbed.[27,29,31] These suggested shapes are based on IUPAQ's classification of sorption isotherms and hysteresis shapes.[29,31]

As shown in Figure 5, performance differences between the two samples were evident not only in characterization results, but also in the vitamin B12 loading tests. Hydrogel G325 absorbed large amounts of vitamin B12, almost four times the amount of vitamin absorbed by sample G322. This behavior is explained by the structured porous morphology of sample G325, as evidenced from the SEM and nitrogen adsorption characterization analyses. On the contrary, sample G322 had a non-porous morphology (microporous structure).

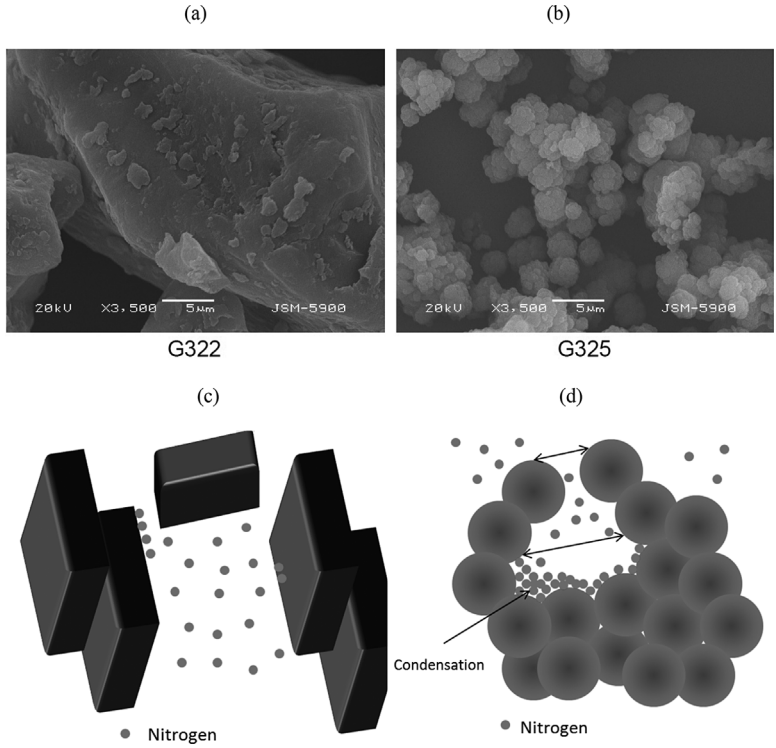

Figure 4.
(a) SEM image of a polymer network synthesized by free radical copolymerization (sample G322); (b) SEM image of a polymer network synthesized by RAFT copolymerization (sample G325); (c) schematic representation of idealized shapes of particles found in SEM pictures for sample G322; (d) schematic representation of idealized shapes of particles found in SEM pictures for sample G325.

Both samples had similar releasing times but very different loading capacities. At the beginning of the test, sample G322 desorbed an insignificant amount vitamin B12. This went on for about one hour (see white squares in Figure 5). According to the characterization results, and due to its non-porous structure above 25 Å in sample G322, vitamin B12 might have been strongly retained in the microporous

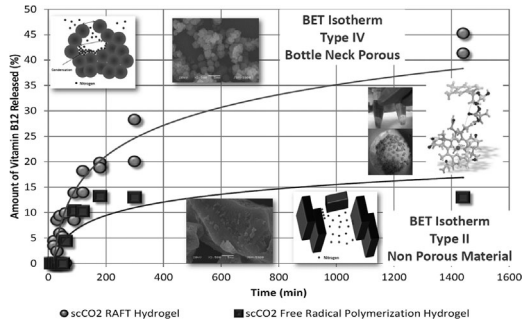

Figure 5.
Release profiles for vitamin B12.

cavities of the polymer network structure (vitamin B12 is about 26.5 Å length and 12 Å width). The trapped vitamin molecule could not be released until the solvent had swollen the polymer network after a given amount of time, 60 minutes in our case. It is only then that vitamin B12 was able to be released in sample G322.

Sample G325, synthesized by RAFT copolymerization, behaved differently. The dark dots of Figure 5 represent vitamin B12 releasing profile for sample G325. An asymptotic curve with two releasing regions is observed. The first region corresponded to a fast vitamin B12 releasing rate (an almost vertical slope for short times is observed). The second region had a very slow releasing rate (an almost horizontal slope). This behavior can be explained by the shape of the polymer network aggregates, where the large pores allow fairly easy removal of vitamin B12 molecules. However, as elapses, vitamin B12 starts being released through the smaller pores, preserving the rate of desorption without a change. Due to the different pore size distributions and the apparent higher volume of sample G325, compared to sample G322, the amount of loaded vitamin B12 is higher in sample G325.

Conclusion

The hydrogel samples synthesized by conventional and RAFT copolymerization of vinyl/divinyl monomers, in $scCO_2$, for this study were characterized by DSC, DMA, adsorption and SEM techniques and presented important structural and morphological differences. The hydrogel particles synthesized by RAFT copolymerization were much more porous than the non-controlled ones, and they were able to retain and release higher amounts of vitamin B12 within and through their structures. These results strongly suggest that the controlled particles are significantly less heterogeneous (nodes more evenly distributed within the polymer network structure) than the particles synthesized in the absence of RAFT controller. The research results reported herein derived in a patent application.[24]

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