Evaluation of Carbon Dioxide Sterilization of a Model Hydrogel

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Statement of Purpose: Rapid developments in surgical and implantable device technology challenge current sterilization methods. This is particularly true for temperature-sensitive biopolymers. The major sterilization methods used in hospitals include steam, ethylene oxide, gamma irradiation and gas plasma. Specifically for biopolymers, high temperatures, toxic or oxidative chemical agents, and radiation degrade performance and biocompatibility. The next generation of polymeric medical devices and heat sensitive biomaterials require new sterilization methods.
Carbon dioxide (CO_2)-based fluids have been tested for both inactivation and sterilization of organisms and compatibility with biomaterials. Compressed CO_2 kills many clinically relevant gram positive vegetative bacteria (e.g. *Staphylococcus aureus*) (Erkmen O. Food Sci. Technol. 1997;30:826-829) and gram negative vegetative bacteria (e.g. *Escherichia coli*) (Dillow AK *et al*. P Natl Acad Sci USA 1999;96:10344-10348). Also, polymers have been processed with compressed CO_2 without degrading chemical and mechanical properties (Sawan SP *et al*. Lowell, MA: Los Alamos National Laboratory, 1995:7-32). If this technology can be developed, then the entire field of implantable biopolymers, especially those being developed for cell-based tissue engineering, it could overcome a major barrier to commercialization. The purpose of the present work is to evaluate the CO_2 sterilization process in terms of both sterilization effectiveness and its influence on the physical properties of a model hydrogel.

Methods: Poly (acrylic acid-co-acrylamide) potassium salt powder was obtained from VWR. 30% hydrogen peroxide (H_2O_2) solution was obtained from Fisher Scientific. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 15597) were used as test microorganisms embedded in a polymeric matrix to investigate the bacteriocidal activity of pure supercritical (SC) CO_2 or SC $CO_2 + H_2O_2$.
An ISCO SFX 2-10 fluid extractor (Lincoln, NE) was used for treatment of as received and swollen gels at 40ºC and 27.6 MPa for 4 hours. The water content, equilibrium swelling ratio, thermal stability and surface morphology of the hydrogel were evaluated before and after treatments. CO_2 treated and untreated as received gel was hydrated (excess water removed with the aid of a Buchner funnel) and dehydrated (vacuum dried at 50ºC and 20 inHg) to evaluate its water content and swelling ratio. A PerkinElmer TGA 7 Thermo Gravimetric Analyzer was used for thermal analysis and the surface morphology of the hydrogel, before and after CO_2 processing, was examined by a JEOL 200CX Scanning Electron Microscope (SEM) at 2.5 kV.
To quantify bacterial kill, a standard plate counting technique was employed. The hydrogel was inoculated with a diluted bacterial suspension (either *S. aureus* or *E. coli*) and treated with CO_2 or $CO_2 + H_2O_2$. For control, an untreated sample was immersed in a temperature bath at 40ºC for 4 hours and inactivation quantified.

Results/Discussion: The drying curve of poly(acrylic acid-co-acrylamide) potassium salt after treatment with $CO_2 + H_2O_2$ is shown in Figure 1, which suggests that the water present in the sample was mostly bound water in a metastable state. After 17 hours of slow drying, a transition is reached, the hydrogel network collapses fairly rapidly, and drying proceeds to completion. Both curves overlap, indicating no change in hydrogel structure and therefore no change in properties after treatment. The average equilibrium swelling ratio for treated samples is 70.9 ± 3.5 and 71.7 ± 3.5 for untreated. Similar behavior was observed when samples were treated with pure CO_2. TGA thermograms of hydrated samples showed evidence of slight structural change due to CO_2 processing; this is under further investigation. SEM imaging showed no apparent structural changes due to treatments.

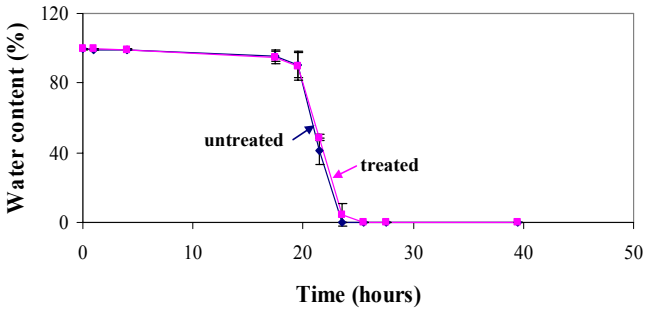

Figure 1. Drying curve of poly(acrylic acid-co-acrylamide) potassium salt after $CO_2 + H_2O_2$ treatment

S. aureus inactivation after pure CO_2 or $CO_2 + H_2O_2$ treatment was investigated. Complete bacteria kill was achieved, equivalent to an average of 7.72 log-reduction. Therefore, pure CO_2 treatment is sufficient to achieve a high level of deactivation. This sterilization process has been proven effective before in *S. aureus* suspended in a liquid solution but not when embedded in a polymeric matrix. The average log-reduction of the negative control was 1.09 ± 0.31 confirming that bacteria inactivation occurs due to CO_2 pressure and not because of thermal deactivation. A 7.93 log-reduction of *E. coli* was achieved (after CO_2 and $CO_2 + H_2O_2$ treatments) at the same experimental conditions.

Conclusions: A novel CO_2 process completely sterilizes both E. coli and S. aureus on a model hydrogel, poly(acrylic acid co-acrylamide). The physical properties were largely unaffected by the CO_2 process which suggest promise to employ this process for environment-sensitive gels. More extensive testing of a variety of materials is needed.