Postfabrication Encapsulation of Model Protein Drugs in a Negatively Thermosensitive Hydrogel

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ABSTRACT: A postfabrication encapsulation technique was developed for loading model protein drugs into an intelligent and biodegradable hydrogel film, which exhibits negative thermosensitivity with a desirable phase transition temperature between refrigerator temperature and body temperature. The hydrogel comprises mainly poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer, and oligo(lactide). The model proteins Hemoglobin and Bovine Serum Albumin were loaded into the hydrogel films by soaking the gels at 4°C, at which the hydrogel film was swollen. The loaded drug was released gradually in PBS at 37°C, where the hydrogel film was shrunken. Because the hydrogel is biodegradable, the loaded drug could be released completely. It is confirmed that proteins can, in their native structures, be included in the hydrogel via the present technique, as characterized by FTIR, Raman spectrum, UV/VIS spectrum, and circular dichroism spectrum. The highlight of our approach is avoidance of high temperatures and organic solvents in encapsulation, making it ideal for protein drug delivery systems. © 2005 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 94:1676–1684, 2005

Keywords: biodegradable polymers; proteins; encapsulation; hydrogels; controlled release; intelligent

INTRODUCTION

Rapid progress in genetic engineering has resulted in the availability of a wide variety of protein drugs. Several hundred protein drugs are currently undergoing clinical trials.[1] Associated protein drug delivery systems have been a challenge in the fields of drug controlled release and biomaterials. Proteins are usually very sensitive to high temperatures (higher than body temperature) and organic solvents, which are, however, hard to be avoided in most of cases of classic small molecular drug loading, and in current protein drug encapsulation techniques.

Hydrogels have been of great interest as protein drug carriers due to their excellent biocompatibility, hydrophilicity, and their flexibility in tailoring physiochemical properties.[2–7] One attraction is the possibility that drug loading and release might be controlled by changing the network structures of so-called intelligent hydrogels with environmental stimuli.[8–13] Reports, however, of potentially applicable hydrogels as protein drug delivery vehicles with both intelligence and biodegradability as well as other necessary characteristics of delivery systems are still rather limited. Kim et al.[14] and Zentner et al.[15] developed injectable drug delivery systems using the thermosensitivity of the copolymers. The copolymers exhibit sol-gel transition due to

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temperature-dependent self-assembly. Bioactive substances in the copolymer aqueous solution were entrapped in the physical hydrogels on cooling[14] or warming.[15] Organic solvents are avoided in these physically crosslinked gels.

Chemical crosslinking is a highly versatile method to create hydrogels with good mechanical stability.[16,17] Unfortunately, the traditional *in situ* encapsulation technique of forming hydrogels in the presence of drug has some limitations. So, if protein drugs can be encapsulated into a hydrogel AFTER the latter's preparation, many of the classic problems in protein drug loading can be resolved. A reasonable postfabrication encapsulation technique is to soak the preformed and purified hydrogel in a drug-containing solution. But one falls into a new dilemma. Either it is really hard for a macromolecular drug to penetrate into the hydrogel after gellation, or the burst release is very serious if the postfabrication loading is relatively easy. As summarized in literature,[1,19] size-exclusion from the hydrogel network makes drug loading very low: on a dry basis, loading levels are often less than 0.1%.[18] Gehrke et al. greatly improved the loading of ovalbumin and α-amylase into dextran hydrogels using the principles of aqueous two-phase extraction,[19] but the drugs were completely released from the dextran hydrogel before degradation occurred.

Degradation is very important for many biomaterials.[20–22] In the work presented in this article, an intelligent and biodegradable hydrogel was prepared. The "intelligence" of the hydrogel comes from the macromer having a negatively thermosensitive central block with a phase transition between 4°C (refrigerator temperature) and 37°C (human body temperature). The hydrogel swells and includes proteins at 4°C, which enhances drug loading. At the human body temperature, the hydrogel shrinks, which avoids a striking burst release. The hydrogel and associated postfabrication encapsulation is schematically presented in Figure 1.

The central block in the macromer is extended with oligomers of L-Lactide and terminated with acrylate groups. The hydrogel is thus biodegradable with an adjustable degradation rate. Drug release can be regulated by the biodegradation rate of the hydrogel. To our knowledge, there is no literature yet reporting on postfabrication encapsulation of proteins by this kind of thermosensitive and biodegradable hydrogel. Our work provides a unique way for controlling the loading and release of protein drugs.

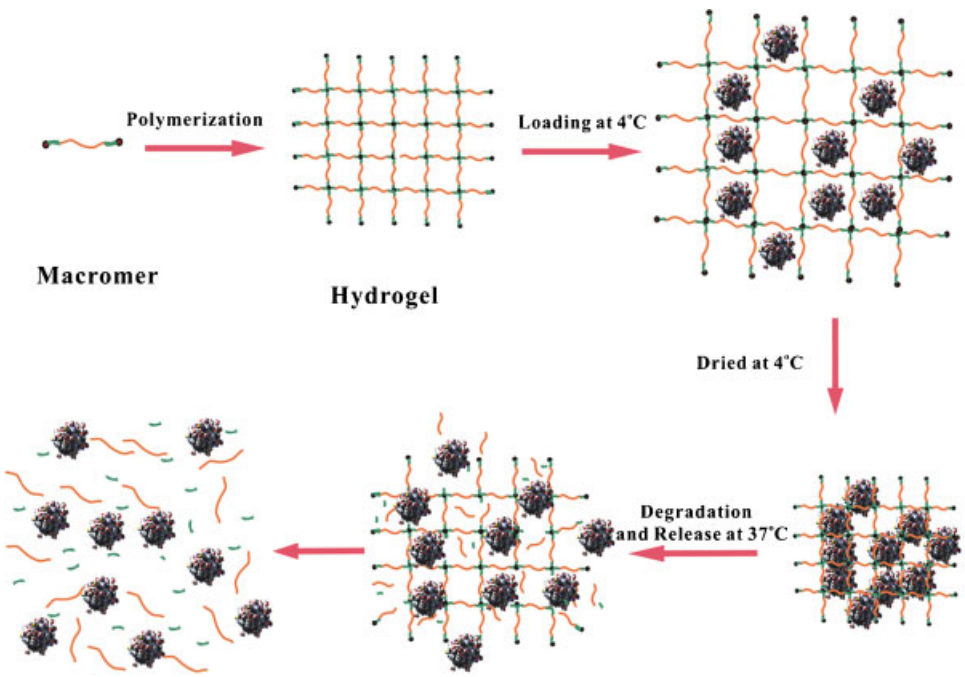

Figure 1. Schematic presentation of the intelligent and biodegradable hydrogel obtained by polymerization of macromers and employed as postfabrication encapsulation of protein drugs. [Color figure can be seen in the online version of this article, available on the website, www.interscience.wiley.com.]