

# Sonochemically prepared BSA microspheres containing Gemcitabine, and their potential application in renal cancer therapeutics

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## Abstract

This report demonstrates the formation and characterization of sonochemically prepared bovine serum albumin (BSA)–Gemzar (Gemcitabine) microspheres and shows their increased anticancer activity compared to pristine Gemzar. The amount of loaded Gemzar was determined by light absorption measurements. The BSA–Gemzar composite was analyzed and characterized by optical microscopy and scanning electron microscopy. The release kinetics of Gemzar from the proteinaceous microspheres was tested. The BSA–Gemzar composite was examined for its anticancer activity (in vitro) in renal cancer cells (RCC, 786-O cells) using [<sup>3</sup>H]thymidine incorporation assays. It was found that the influence of the Gemzar-loaded microspheres on the cancer cells was significantly greater than that of an equimolar concentration of pristine Gemzar.

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## 1. Introduction

Macromolecular therapeutics are rapidly gaining interest in the area of nanomedicine for their ability to serve as alternatives to traditional drug regimens. Macromolecular conjugation offers improvements in the delivery of drugs by preventing their passive diffusion into highly circulated tissue systems throughout the body, resulting in lowered toxicity and volume of distribution. Drugs that would potentially benefit from such strategies are those exhibiting low bioavailability and limited therapeutic utility. The primary advantage in the use of macromolecules as drug-delivery vehicles is their mechanism of cellular internalization. The cell membrane is naturally impermeable to complexes larger than 1 kDa; however, cells possess

a variety of active internalization mechanisms to accommodate cellular entry of large molecular complexes. Here, the cell membrane will invaginate to engulf molecules and extracellular fluid in an intracellular membrane-bound vesicle, or endosome, that will subsequently travel through the cell, a process known as endocytosis. Molecules may reside near the membrane or directly interact with membrane proteins to enable their retention in these vesicles. The attachment of drug moieties to macromolecular carriers can help to target these complexes to specific cell populations in organ systems [1].

There is intense interest in biocompatible vesicles for drug delivery and biomedical contrast imaging [2–4]. Protein core–shell microspheres are highly biocompatible structures with an outer shell made from disulfide cross-linked protein roughly 50 nm thick, with a core containing air or nonaqueous liquid [5,6]. In 1990, Suslick and co-workers developed a method in which they used high-inten-

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sity ultrasound to make aqueous suspensions of proteinaceous microcapsules filled with water-insoluble liquids, and demonstrated the chemical mechanism of their formation [5–9]. This synthetic method can be used to produce liquid-filled proteinaceous microcapsules from various kinds of proteins such as bovine serum albumin (BSA) [5,6], human serum albumin (HAS) [6] and hemoglobin (Hb) [10]. Bovine serum is widely used for the preparation of proteinaceous microspheres due to its availability in a pure form and its biodegradability, nontoxicity and nonimmunogenicity [11]. In addition to its functionality for transporting different macromolecules in the bloodstream to target organs, it was also found that albumin accumulates in solid tumors [12,13], making it a potential macromolecular carrier for the site-directed delivery of antitumor drugs. A new process for the formation of microspheres was recently reported by Edirisinghe and co-workers [14,15]. They have used the novel electrohydrodynamic atomization technique and showed that the microspheres absorbed and encapsulated the liquid in which they were collected.

Cancer is one of the leading causes of morbidity and mortality in the United States, as well as in the rest of the world. From the latest statistics available from 2003 [16], cancer accounted for 556,902 out of the 2,448,288 deaths in the United States. Therefore, active research for the development of new treatment strategies for a number of cancers is very important. Our earlier study demonstrated the increased anticancer activity of Taxol encapsulated in BSA microspheres, as compared to pristine Taxol on a Mouse Multiple Myeloma cell line MPC-11. It was shown that a 3 min sonication forms a BSA microsphere and encapsulates over 90% of the Taxol drug in the microsphere [17]. The Taxol encapsulated in the BSA microspheres and organic solvent (mesitylene) was released from the microspheres after 24 h of incubation at the desired locality of the cancer cells. From our experiments was observed that Taxol continues to function in the same way (inhibits cell division at the G<sub>2</sub>-M interface) as pristine Taxol. In addition, it was found that the released mesitylene also caused the death of some of the cancer cells. In this context, was decided to use a more biocompatible organic solvent. Dodecane, a paraffin oil, is a suitable vehicle in which to suspend insoluble salts, such as calomel, mercury salicylate, etc., for hypodermic injection (under the skin) [18].

Dodecane was used for the encapsulation of another anticancer drug, Gemzar (Gemcitabine HCl), a nucleoside anticancer drug that has a broad spectrum of antitumor activity against various tumors [19]. Structurally, it is a deoxycytidine in which the deoxyribose moiety contains two fluorine atoms instead of hydrogens at the 2'-position (Fig. 1). Upon systemic exposure, Gemzar is metabolized by cytidine deaminase to the inactive metabolite 2',2'-difluoro-2'-deoxyuridine (dFdU), which represents the main catabolic pathway [20]. Gemcitabine penetrates cells via nucleoside transporters, and thereafter it is phosphory-



Fig. 1. The chemical structures of Gemzar (Gemcitabine HCl, dFdC).

lated to its mononucleotide by deoxycytidine kinase, and subsequently by nucleotide kinases to its active metabolites, Gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP). dFdCDP inhibits ribonucleotide reductase, which leads to a reduction in the concentrations of the deoxynucleotides required for DNA synthesis. dFdCTP is incorporated into DNA. After this addition, only one more nucleotide is added to the DNA strand before DNA polymerase activity is blocked. This last incorporation prevents the cellular repair mechanisms from removing the dFdC nucleotide. The cytotoxic activity of dFdC has been correlated with both the intracellular concentration of dFdCTP and with its incorporation into DNA. Furthermore, dFdCTP can be incorporated into RNA, leading to the inhibition of RNA synthesis [21].

According to a Mayo Clinic report, the American Cancer Society estimates that almost 51,000 people in the United States are diagnosed with kidney cancer each year [22]. Gemzar was chosen as an anticancer drug in this study as it is widely used in the treatment of various types of kidney cancer in humans. BSA microspheres are chosen as the delivery vehicle due to their small size (mean size  $\sim 1 \mu\text{m}$ ), longer circulatory time, and because they are easily synthesized and characterized. The purpose of the present study was to develop a delivery system for Gemcitabine based on immobilizing the drug with BSA microspheres, and to validate whether such a system inhibits the growth of renal cancer *in vitro*. This paper demonstrates the synthesis and characterization of sonchemically prepared BSA–Gemzar microspheres and shows their increased anticancer activity compared to pristine Gemzar. The formation of the microspheres modifies the Suslick recipe in two ways: the concentration of peptide (BSA) solution was reduced, and more biocompatible organic solvent was used for experiments.

The amount of loaded Gemzar was determined by absorption measurements. The BSA–Gemzar composite was analyzed and characterized by light microscopy and scanning electron microscopy (SEM) measurements. The release kinetics of Gemzar from the microspheres was tested. The BSA–Gemzar composite was examined for its anticancer activity (*in vitro*) in renal cancer cells (RCC, 786-O cells). The underlying problem regarding the present research work is whether anticancer drugs having a short half-life and extensive systemic toxicity can nevertheless be used by avoiding their exposure to cells via their encapsulation in a protein that is friendly to these cells.

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