

UNIQUE ORAL DRUG DELIVERY SYSTEM

Raphael M. Ottenbrite and ZHAO Ruifeng

(Chemistry Department, Virginia Commonwealth University, Richmond, VA 23284-2006, USA)

Sam Milstein

(Emisphere Technologies Inc., 15 Skyline Drive Hawthorne, NY 10595, USA)

ABSTRACT

An oral drug delivery system using proteinoid microspheres is discussed with respect to its unique dependence on pH. It has been found that certain drugs such as insulin and heparin can be encapsulated in proteinoid spheres at stomach pH's (1–3). These spheres also disassemble at intestinal pH's (6–7) releasing the drug for absorption. Using this technique low molecular weight heparin and human growth hormone have been orally delivered successfully to several animal species. Future work has been proposed to study the interaction and binding of the specific drugs with synthesized oligopeptides.

INTRODUCTION

Among the many drug delivery systems available, oral drug delivery has many obvious advantages. The oral route is the most convenient and preferred route for administering most medications. Using this technique, patients do not need to take drugs using inconvenient methods such as injections. This is particularly important for chronic situations which require medication on a daily basis such as insulin in the case of diabetics.

The objective of any oral drug delivery is to deliver the agent safely and efficiently to the target area without permanently altering its structure or function while in the gastrointestinal (GI) system. Therefore, drugs and drug carriers are required to withstand the harsh environment of the gastrointestinal (GI) tract, which includes very acidic conditions, pH 1–2, in the stomach and

a pH range of 6–7 in the intestine, as well as exposure to digestive enzymes throughout most of the GI tract^[1]. Many small molecules are chemically and structurally stable and remain unchanged by oral delivery. However, macromolecular drugs such as heparin, insulin and human growth hormone, whose activity is directly dependent on specific molecular structures, are very sensitive to the components in the GI tract. Specific digestive processes can efficiently transform these agents into molecular fragments that are pharmacologically ineffective^[1]. Currently these drugs and new biotechnology developed drugs have to be administrated via injection.

To effect oral delivery, essentially two barriers need to be overcome. First, the cargo must be transported from the mouth to the anatomical site where absorption occurs. Second, transit from the lumen of the GI tract to the blood must occur. Successful oral delivery requires that the cargo-carrier be resistant to the impact of enzymes and pH gradients that can span 7 units. Assuming that sufficient amounts of drug arrive at the appropriate anatomical site in the GI tract, several hundred lipid molecules must move for every drug molecule that crosses the intestinal epithelium. In addition, oral administration of protein and polar macromolecular drugs usually result in extremely low to no bioavailability due to their extensive hydrolysis, by acids and enzymes, and poor membrane permeability in the gastrointestinal tract^[2-3]. In the last decade, the use of microspheres or nanoparticles to protect those drugs from degradation has been of considerable interest^[4-5].

PROTEINOID MICROSPHERES

During the mid 1980's, a new proteinoid microsphere oral drug delivery system was developed^[1]. The core technology is aimed at delivering therapeutics such as polysaccharides and proteins which are being presently administrated via injection. Small amino acid-based compounds called proteinoids were used. These were made simply by thermal condensation of specific amino acids. The solid material obtained, after the condensation process, was finely ground and extracted with sodium bicarbonate solutions. The extracts were lyophilized to yield a slightly colored product. This proteinoid material was composed of small peptide molecules ranging in size from 300 to 800 daltons, mostly consisting of

tri- and tetrapeptides. The constructs are diastereomeric mixtures of linear, cyclic or branched chained peptides. The hydrophilic and hydrophobic characteristics of these proteinoids can be modified by using specific aliphatic amino acids and aromatic amino acids^[1].

Under specific pH conditions (< 3), these proteinoids spontaneously form stable microspheres. At higher pH conditions (> 5), the microspheres disassemble, releasing the encapsulated material^[1]. To encapsulate a drug, the proteinoid material is dissolved in water at pH 7. This solution is added at 40°C to a therapeutically desirable drug dissolved in a low pH solution (typically 1.7 N citric acid) with vigorous shaking. Under these acidic conditions with agitation, the proteinoids form microspheres with encapsulated drugs ranging in size from 5 to 10 microns (Fig. 1)^[6]. The size is usually dependent on the proteinoid used and the agitation, i. e., smaller spheres are formed by ultrasonic sound agitation (0.5 microns) compared to hand agitation (10 – 15 microns). The unique characteristic of these microspheres is that they remain intact in the acidic environment of the stomach (pH ~ 1). However, when they enter the upper GI tract, the higher (pH $\sim 6-7$) causes the microspheres to disassemble, allowing the drug to be released for absorption^[1]. In this manner, the proteinoid microspheres

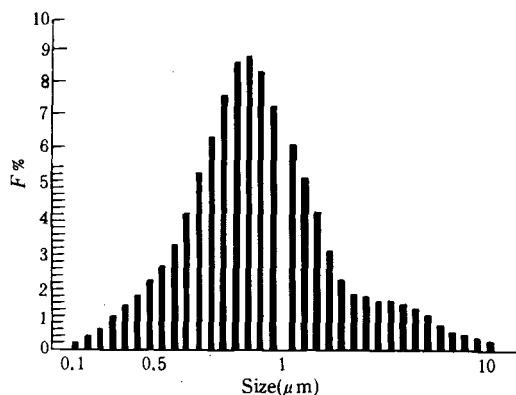


Fig. 1 Particle size distribution histogram of proteinoid microspheres, reproduced from ref. [6]

are able to protect a therapeutic agent from gastric acids and enzymes in the stomach and then release it into the intestinal absorption area.

The ability of these proteinoid materials to inhibit the activity of proteases in the intestine has been examined by the in vitro assays. The results indicate that some proteinoids permit a competitive inhibition process of both trypsin and chymotrypsin at concentrations in the millimolar range. The K_i

values have been seen for these proteinoids in the 10–400 mM range. Interestingly, most of the carriers studied show inhibition of both trypsin and chymotrypsin within this range even though the structures of the proteinoids would suggest greater specificity for one enzyme over another.

ORAL DRUG DELIVERY

Standard heparin and low molecular weight heparin (LMWH) microspheres were chosen as the model system. Heparin is a well-known anticoagulant and widely used for the prevention and treatment of thrombotic diseases. Low molecular weight heparin has been shown to possess improved pharmacological properties over standard heparin. Currently, however, both standard heparin and LMWH have to be administered parenterally to ensure clinical efficacy. In vivo data on six different animal species, which include mice, rats, guinea pigs, dogs, and primates, as well as a completed phase 1 human trial, with LMWH encapsulated in proteinoid microspheres demonstrated successful oral delivery with this system (Fig. 2)^[1].

In vivo evidence indicates that the LMWH delivered by this technique was rapidly absorbed. It was found that heparin orally administered in proteinoid spheres resulted in a significant increase in clotting time, which was detectable 30 minutes after dosing. This same phenomenon has been observed in all of the species that have been tested^[1].

Recently Ma et al.^[7] were able to freeze-dry heparin-loaded proteinoid microspheres without dramatically changing the integrity of the drug activity when sugars were used as cryoprotectants in the formulations. The cryoprotective effect of sugars on heparin-loaded microspheres was: trehalose > lactose > glucose > sucrose > galactose - maltose; while the order of cryoprotective strength for empty proteinoid microspheres was: lactose > sucrose > galactose - maltose - glucose > trehalose. The optimum formulation for heparin-loaded proteinoid microspheres was: 5% (w/v) trehalose added after microsphere formation. The optimum freezing temperature was found to be < -70%, and the optimum resuspending temperature was 40°C. Under these conditions the heparin-loaded proteinoid microspheres can be isolated, formulated into tablets or capsules and safely stored.

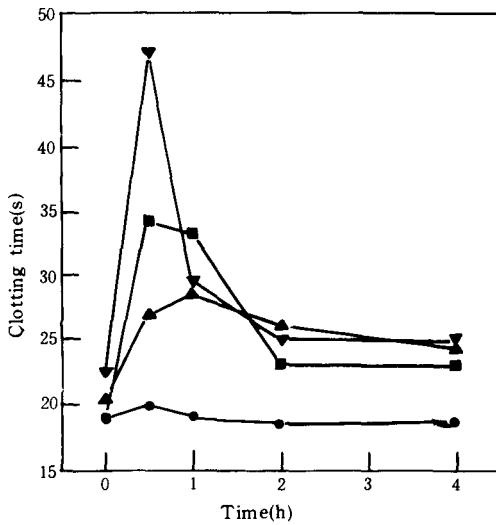


Fig. 2 Oral dosing of heparin to rats, reproduced from ref. [1].

—■— Hep+E266 —●— Baselines
 —▼— Hep+E261 —▲— Subcut. inj.
 Oral drug dose = 25 mg/kg
 Subcut dose = 2.5 mg/kg
 Volume dose = 3 ml/kg

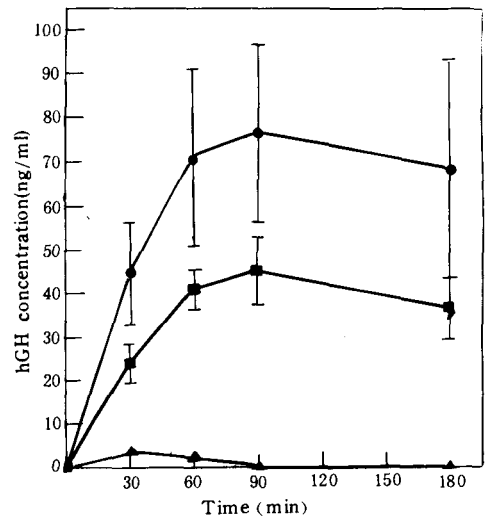


Fig. 3 Oral dosing of human growth hormone to rats, reproduced from ref. [1].

—●— hGH 1 mg/kg + E-198 p.o.
 —■— hGH subcut. 0.125 mg/kg
 —▲— hGH oral 1 mg/kg

Other in vivo evaluated drugs are heparinoids, calcitonin, human growth hormone, vaccines, monoclonal antibody 1gG2a, and polar compounds such as sodium cromolyn and desferoxamine. The size of these agents vary from the small neurotransmitter dopamine (150 daltons), to insulin (6,000 daltons) and vaccines (2.5 million daltons). Studies have documented both delivery into the blood stream and therapeutic activity. For example, it had been demonstrated that in rats a single dose of an influenza vaccine encapsulated in proteinoids and administered orally produced an early immune response. In contrast, control animals that received an unencapsulated vaccine did not produce a detectable immuneresponse^[1]. Human growth hormone (hGH) has also been successfully delivered using proteinoid material (Fig. 3)^[1]. While the drug taken orally alone is ineffective, a hGH solution dose (1 mg/kg) encapsulated in proteinoid spheres, delivered orally provided (75 ng/ml) of growth hormone in 90 minutes in the blood compared with a hGH solution (0.125 mg/kg) delivered subcuta-

neously which provided 40 ng/ml in the same time period.

CURRENT AND FUTURE RESEARCH

In essence, proteinoids have a dual function: in the microsphere phase proteinoid have the ability to protect drug cargoes from acids and enzymes, and as soluble materials, the proteinoids facilitate the absorption of a therapeutic agent from the intestine into the blood stream^[1]. Further evidence to determine the mechanism is being obtained by radio isotope experiments such as ¹⁴C labeled proteinoids and ¹²⁵I, ³H labeled drugs.

More recent studies have been concerned with proteinoid-drug binding. One such study showed that proteinoid spheres could bind a model dye compound (sulforhodamine 101) with sufficient strength to survive centrifugation^[1]. The possibility of studying these binding processes by thermal and spectrometric methods which have been used to evaluate interactions and complex formation between natural polysaccharides and proteins^[8-10], is being investigated. The thermal methods include differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC). The spectrometric methods include circular dichroism and nuclear magnetic resonance spectroscopy^[8-10].

Based on the preliminary studies by differential scanning calorimetry, heparin and human growth hormone have the thermally-induced transition peaks at 82°C and 78°C, respectively. Upon addition of oligopeptide, these transition peaks were not observed by DSC due to the "denaturation" of heparin or human growth hormone by oligopeptides. The indication from this data is that these oligopeptides interact with both heparin and human growth hormone, which causes a perturbation of the thermodynamic transitions^[11].

Our work is presently focusing on the synthesis of several oligopeptides and evaluation of their interaction with the polysaccharide drugs such as heparin, and protein drugs such as human growth hormone and interferon^[11-12]. One major purpose of this work is to synthesize oligopeptides with specific structure and specific amino acid sequences and to evaluate their ability to form microspheres with and without the drug^[11]. Another objective of our work is to study the complexation or binding of these oligopeptides with specific drugs such as heparin, human growth hormone and interferon^[11]. Based on the information

obtained with respect to the specific oligopeptides ability to form complexes with the specific drugs, in vivo experiments will be designed to evaluate the effectiveness of these oligopeptides to orally deliver these drugs to the host.

Keywords:

Oral drug delivery system; Proteinoid microspheres; Oligopeptides

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