# *In Vitro* and *In Vivo* Evaluation of a Once-weekly Formulation of an Antidiabetic Peptide Drug Exenatide in an Injectable Thermogel

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**ABSTRACT:** An injectable thermogel composed of poly(lactic acid-*co*-glycolic acid)–poly(ethylene glycol)–poly(lactic acid-*co*-glycolic acid) (PLGA–PEG–PLGA) triblock copolymers was evaluated as the matrix of a long-acting drug delivery system of exenatide (EXT), an antidiabetic peptide. The optimal gel formulation containing 2 mg/mL EXT and three pharmaceutical excipients (1.25 wt % zinc acetate, 5 wt % PEG200, and 5 wt % sucrose) was injected subcutaneously, and its pharmacokinetics was investigated. Both *in vitro* and *in vivo* release profiles exhibited a sustained release of EXT over 1 week. After a subcutaneous injection of the EXT formulation into db/db mice, the blood glucose level was maintained in a normal range up to 7 days and meanwhile the growth of body weight was suppressed. The *in vivo* results were consistent with the *in vitro* EXT-release profile. Moreover, twice injections of the gel formulation resulted in the higher blood insulin level and lower plasma concentration of glycosylated hemoglobin compared with twice-daily injections of an EXT solution for 18 days. Histological observations manifested the protection of islet due to administration of the gel formulation. Therefore, the PLGA–PEG–PLGA thermogel provided an excellent candidate for a once-weekly delivery system of EXT, and the optimal EXT formulation not only afforded therapeutic efficacy but also improved patient compliance. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:4140–4149, 2013

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## INTRODUCTION

Glucagon-like peptide-1 (GLP-1), a hormone of 30 amino-acid residues, can stimulate insulin secretion in a glucose-dependent manner.[1] Because of its glucose-induced insulinotropic effect, GLP-1 controls hyperglycemia of type II diabetic patients without the risk of hypoglycemia. The bottleneck of its clinical application is the extremely short half-life in circulation (<2 min) owing to the rapid degradation by a ubiquitously expressed enzyme, dipeptidyl peptidase IV (DPP-IV).[1,2] This problem is fortunately resolved by the emergence of GLP-1 receptor agonists, which can resist the DPP-IV-induced degradation significantly.[2,3]

Exendin-4, a polypeptide of 39 amino-acid residues, is the most efficient and safe GLP-1 receptor agonist so far.[2,3] Although it shares approximately 53% sequence homology with GLP-1, the site of the proteolytic cleavage by DPP-4 is avoided.[2,3] Glucoregulatory actions of exendin-4 include enhancement of glucose-stimulated insulin secretion, inhibition of gastric emptying, and reduction of appetite. Moreover, exendin-4 can promote β-cell proliferation and consequently improve islet function.[2–5] The potency of the glycemic control of exendin-4 is 5000-fold of that of GLP-1.[3]

Exendin-4 was originally isolated from the salivary gland of the lizard Heloderma Suspectum (Glia monster).[6] Its synthetic product exenatide (EXT) and relevant solution injection (Byetta®) have been approved by both United States Food and Drug Administration (2005) and European Medicines Agency (2006) as a medication in treatment of patients with type II diabetes mellitus. Byetta® is administrated by twice-daily subcutaneous injection. A long-acting microsphere formulation of EXT (Bydureon®) was also developed and approved by both European Union (2011) and United States (2012) as a once-weekly injectable delivery system. Bydureon® shares many advantages such as reduced administration frequency, stable plasma drug level, and better patient compliance.[7,8] Nevertheless, a microsphere formulation often suffers from difficulties such as preparative complication, residual organic solvent, and high cost in sterilization. It is thus helpful for developing more formulation ways of EXT, for instance, a convenient implantation-type formulation.

The present paper concerns a subcutaneously injectable formulation in the form of hydrogels. Hydrogels have been investigated extensively for various biomedical applications.[9–17] Especially, injectable and biodegradable thermogels are promising as an implantation-type sustained-release carrier of bioactive proteins, peptides and other drugs.[18–24] Such a system is a low viscous solution at ambient temperature and undergoes a sol–gel transition upon heating. The drug is easily entrapped by mixing the aqueous polymer solution at room temperature without using any organic solvent. The polymer solution containing drugs is then readily injected using conventional syringe needles, and rapidly gelled at the physiological

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temperature, resulting in an automatic encapsulation of the drugs. Among all of these systems, the thermogelling triblock copolymer poly(lactic acid-*co*-glycolic acid)–poly(ethylene glycol)–poly(lactic acid-*co*-glycolic acid) (PLGA–PEG–PLGA) may be one of the most promising candidates for the clinical application due to its convenient synthesis, good profile of safety and adjustable properties.[25–30]

Recently, the first case of the thermogel formulation of EXT has been reported by us.[31] A "mixture hydrogel" composed of two PLGA–PEG–PLGA polymers yet with different block lengths was used as the delivery matrix. One polymer is too hydrophobic to dissolve in water, and the other is too hydrophilic to form a hydrogel at any concentration and temperature. Interestingly, the mixture with appropriate compositions could be soluble in water and form a physical hydrogel upon increase of temperature due to a subtle balance between hydrophilicity and hydrophobility when the polymer concentration is higher than the critical gel concentration (CGC).[32,33] By this way, the applicable window of the amphiphilic block copolymers has been broadened to a large extent, and the sol–gel transition temperature $T_{gel}$ could be easily adjusted by mix ratio to satisfy the medical requirements. Meanwhile, to suppress the burst effect of EXT and keep a uniform drug release from the gel, we introduced three excipients, zinc acetate, PEG, and sucrose into the formulation, and found surprisingly that the release profile of this water-soluble peptide could be significantly improved under appropriate conditions due to a synergetic effect.[31] Some key issues of this synergetic "mixture thermogel" still open, for example, more factors to modulate the *in vitro* release profiles, the *in vivo* release and its correlation with the *in vitro* one, the therapeutic efficacy in diabetic mammals. To answer these questions is vital for developing this promising antidiabetic formulation and very meaningful for shedding new insight of pharmaceutics concerning polypeptide drugs and/or physical hydrogels.

The present study is mainly emphasized on the pharmacokinetics (PK) and pharmacodynamics (PD) studies of the thermogel formulation of EXT, as schematically presented in Figure 1. The total copolymer concentration and drug amount will be examined as a regulator of the *in vitro* release profile of EXT in this study. The *in vivo* release will also be checked for the first time, and a comparison between the *in vivo* and *in vitro* profiles of the formulation with optimal condition will be made via transformation of the transient blood drug concentration into the cumulative drug release *in vivo*. db/db mice of type II diabetes will be employed to assess the therapeutic efficacy for 10 days (a bit longer than one week) upon a single subcutaneous injection of the optimal formulation. In addition, the plasma insulin level, concentration of glycosylated hemoglobin (HbA1c), and histological morphology of pancreatic islet will be examined after twice subcutaneous injections of the long-acting EXT thermogel formulation and compared with that of twice-daily subcutaneous injections of an EXT solution for 18 days.

## MATERIALS AND METHODS

### Materials

PEG [molecular weight (MW) 200, 1000, and 1500] and tin ethyl hexanoate (stannous octoate, 95%) were obtained from Sigma–Aldrich (USA) . D,L-lactide (LA) and glycolide (GA) were purchased from Purac (The Netherlands). Sucrose and zinc acetate were products of Sinopharm Chemical Reagent Company Ltd. (Shanghai, China). EXT was kindly provided via the Hangzhou Jiuyuan Gene Engineering Company, Ltd. (Hangzhou, China). All of chemicals were used without further purification. Two PLGA–PEG–PLGA triblock copolymers were synthesized as described previously.[31]

### Animals

Male Sprague–Dawley (SD) rats were provided from Shanghai Super-B&K Laboratory Animal Corporation Ltd. (Shanghai, China). db/db mice with body weight 50 ± 5 g were purchased from SLAC Laboratory Animal Corporation Ltd. (Shanghai, China). The animals were housed under a 12-h light/dark cycle with free access to food and water during the acclimatization period. All procedures concerning experimental animals obeyed the principles outlined in the Declaration of Helsinki and have been approved by the ethics review board of Novo Nordisk.

### Viscosity Measurements of Polymer Solutions and Hydrogels

Aqueous PLGA–PEG–PLGA solutions with 1/1 weight mix ratio of copolymer-1/copolymer-2 were prepared via dissolving polymer in deionized water. The viscosity measurements of aqueous polymer solutions as a function of temperature were

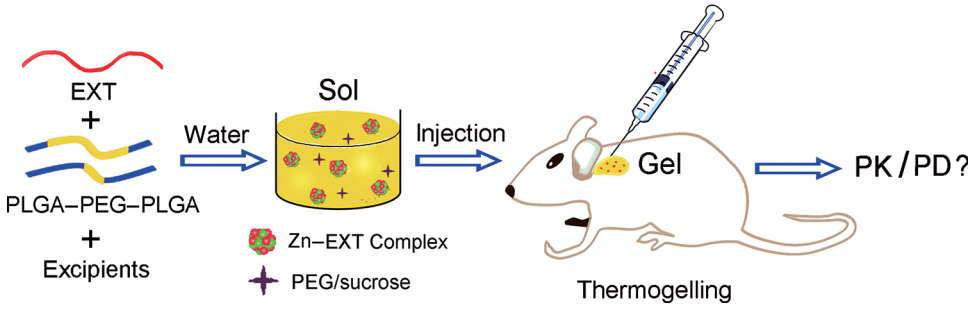


**Figure 1.** Schematic illustration of the present study of a hydrogel formulation of the peptide drug EXT. EXT with the amino acid sequence $NH_2$-HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-$CONH_2$ is soluble in water. Two amphiphilic copolymers composed of one hydrophilic block of PEG and two hydrophobic blocks of PLGA will be used with one copolymer dissolved in water and the other precipitated, yet their mixture is soluble in water and exhibits a sol–gel transition upon increase of temperature. The optimal EXT gel formulation contains three excipients (zinc acetate, PEG200 and sucrose). The formation of Zn-EXT complex is beneficial for reducing the initial burst release and the leaching of PEG200 and sucrose accelerates the late-stage release of EXT. Both PK and PD will be investigated after subcutaneous injections.