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Studies on the use of needle-free injection device on proteins

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This paper is dedicated to the memory of Professor Csaba Horváth, the "father of HPLC", a friend, a mentor, an exceptional scientist and a true renaissance man.

Abstract

In the following communication we report the evaluation of 18 proteins that were processed by a specific needle free injection device. The processed protein samples were analyzed by two HPLC techniques, reversed-phase liquid chromatography (RPLC) and size-exclusion chromatography (SEC). These techniques are two of the most widely used analytical techniques in the biopharmaceutical industry for the characterization, integrity assessment and stability study of peptide and protein products. The results indicate that needle free injection, using the specific device of this study, is not damaging to the studied proteins and does not generate aggregates. We found no evidence of the predicted possible effects of needle free injections, and concluded that needle free delivery is in general not different than any other delivery system and that its use should be evaluated on a case by case basis. It has to be noted that there are various needle free device designs and our work was performed using an Iject® from Bioject. Our conclusions therefore should be limited to the Iject® design we used in this study. In the reported experiments we used commercially available (economical) model proteins, which facilitate the use of the results for future comparison and reference. The work reported here can serve as a reference to illustrate the benign nature of our needle free injection device. It also highlights an interesting analogy between a set of phobias that were seen to have plagued the early stages of biochemistry and HPLC, on the one hand, and some attitudes that appear to hinder the widespread acceptance of needle free injection at present time, on the other. These phobias were identified and named by Professor Csaba Horváth, the father of HPLC, as barophobia, siderophobia and lithophobia. Today a wealth of evidence is available to indicate that those phobias are ungrounded and that the negative observations can be explained in most cases by adsorption and prevented by proper formulations and solvent conditions.

Keywords: Needle-free injection; Protein stability; Protein HPLC; Pressure injection; Biopharmaceuticals; Drug delivery

1. Introduction

Needle-free injection technology has been used in clinical practice for many decades and has been shown to be safe and effective for the administration of many different medications for a variety of applications, including immunization and mass inoculation of large populations [1–9].

Needle free injection based delivery systems for the delivery of peptide and protein based biopharmaceuticals are becoming increasingly popular for a number of reasons. One of them is that the method eliminates the fear of injection, which can involve *diatrypophobia*, the fear of piercing, or *belonephobia*, the fear of needles generally. Patients such as diabetics who need to inject themselves on a regular basis are very much in favor of this comfortable delivery method. However, one of the major roadblocks for the more general use of needle free injection is that only limited information is available in the public domain on the effects of needle free injection on the integrity of peptides and proteins. The reason for this is due partially to the novelty of this delivery system, as well as partially to misinformation about possible negative effects on biopharmaceuticals. It is telling that previously used names like jet injection or pressurized injection

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have been eliminated to minimize the negative perceptions those names created. Current knowledge of the benefits of this drug delivery system is limited to a select few scientists within the pharmaceutical industry, and the details of the experimental results they generated have been shielded by confidentiality agreements.

The essence of needle free injection is that the pharmaceutical drug product is injected through the skin by pressure instead of by a traditional needle and syringe method [10,11]. The benefits of this unique delivery system are numerous. It is convenient, physiologically accepted, fast, no sterilization of the device is required and most importantly it is ideal for the delivery of peptide and protein pharmaceuticals [12,13]. One of the great benefits of needle free delivery is that biopharmaceuticals delivered by needle free injection do not have to pass the gastrointestinal system and its degradation industry [14–18]. The opportunity to have biopharmaceuticals delivered more efficiently can thus prove extremely beneficial or in some cases may be the only viable option.

However, the widespread implementation of any new device, technology or even industry often invites rejection based on our previous experience, which is too often influenced by prejudices or phobias.

Most researchers would agree that there exist three major fears (phobias) based on long standing scientific reflexes that come into play with any type of protein handling.

(1) The proteins' primary structure could be affected due to degradations (primary structure).
(2) Irreversible conformational changes could occur (secondary and tertiares structures).
(3) Protein aggregation also could occur (quaternary structure).

In the case of needle free injection one of the most often used arguments is that the injection can damage a protein. The potential effects can depend on the combination of applied pressure, flow path design and the material characteristics of the nozzle.

It is interesting that similar fears surfaced in the early days of protein HPLC and initially hindered the wide acceptance of HPLC in the field of peptide and protein separation. Biochemists were concerned about the effects of pressure, flow through small pores and the materials used in HPLC on the integrity of peptides and proteins.

Because of these apparent similarities we feel justifying to reminding ourselves of those concerns and the way they were handled. Some of those fears were named by Professor Csaba Horváth as *barophobia*, or the fear of pressure, *lithophobia*, or the fear of cuts, which could occur when protein solution is forced through the pores of the porous silica beads, and *siderophobia*, or the fear of any iron containing transport line which could cause damage to proteins. These fears were ultimately resolved by thoroughly executed experimental research and over a few years it became obvious that in general the fears were unfounded. When in some individual cases problems did surface, they were shown to be more characteristic to the individual proteins than to the technique itself. Detailed studies helped to clarify the problems and in most cases these were not related to the defined phobias, but were rather the results of the presence of surfaces, and they were overcome by appropriate formulations and solvent conditions.

The methods selected for this study on the effect of needle free injection on proteins are aimed at following changes in the primary and quaternary structure of proteins. The fundamental concern is about degradation and aggregation, changes that are most devastating if they occur during any delivery method. Other changes should be studied separately and are beyond the objectives of this study.

It is ironic that in this work we use an analytical technique, HPLC, to resolve some of the phobias related to needle free injection, which itself had to overcome the same phobias in the 1980s [19–21].

2. Materials and methods

2.1. Chemicals

All proteins and phosphate buffered saline (PBS) were from Sigma (St. Louis, MO, USA). The chromatographic solvents, 1-propanol and trifluoroacetic acid (TFA) were purchased from Fisher (Pittsburgh, PA, USA).

2.2. Chromatographic conditions

The RPLC column was an SB-300 C8 from Agilent (Wilmington, DE, USA). Column dimensions are 150 mm × 4.6 mm and it is packed with 5 μm StableBond silica particles with 300 Å pore size. We employed gradient elution using 0.1% TFA in water as A solvent and 50% 1-propanol and water containing 0.1% TFA as B solvent. The linear gradient ascended from 5 to 50% B solvent. The flow rate was 0.5 mL/min. The elution of proteins was detected at 215 and/or 280 nm.

The SEC column, TSK G2000SWXL was from Tosoh Biosciences (Montgomeryville, PA, USA). We used 2× PBS as mobile phase, 0.5 mL/min flow rate and the elution was detected at 215 or 280 nm.

2.3. Needle free injection device

The Iject was from Bioject (Portland, OR, USA) is a prefilled single-use disposable injection device configured to administer 0.5–1.00 ml subcutaneous or intramuscular injections. The device is distributed "ready to use". Thus, it requires no additional parts or modifications for function. The device is activated by rotating the trigger sleeve 180°, and an injection is administered by advancing the trigger sleeve while the nozzle is held against the injection site. The Iject needle-free injection system is an investigational device, subject to the US Food and Drug Administration clearance for commercial distribution.