# Delivery of Antibodies to the Gastrointestinal Tract for Local Action

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## Introduction

There has been increasing interest in the oral administration of antibodies for localised treatment of infections or other conditions in the gastrointestinal (GI) tract. However, owing to the inherent instability of these molecules in this environment the dosed antibodies will quickly lose their activity, requiring a very large dose to be administered to elicit any desired pharmacological effect [1]. The main challenge in developing an oral antibody delivery system therefore is to release the antibody from the dosage form at the correct rate and location without loss of bioactivity.

The purpose of this study was to investigate the stability of immunoglobulin G (IgG) antibodies in simulated intestinal fluid (SIF) containing pancreatin at 37°C and, based on these stability data, to design and develop a suitable solid dosage form for oral delivery of antibodies to the intestine and colon for localised action.

## Methods

Normal human IgG, Gammagard S/D (Baxter), was used as a model drug in this study. Gammagard S/D is a highly purified freeze dried IgG derived from large pools of human plasma. The stability of IgG in SIF containing pancreatin was investigated by incubating the antibody in the SIF at 37°C. Stability samples were taken at predetermined time points and the remained antibody $F_{ab}$ activity was determined using in-house development human IgG $F_{ab}$ specific ELISA methodology.

In order to protect the unreleased IgG from degradation and meanwhile maintaining overall sustained release, a multi-tablet system was designed. In this system, three 7 mm sustained release tablets, each contained 115 mg Gammagard IgG with Eudragit RL, Ethyl cellulose of Eudragit RL / Ethyl cellulose 1:1 mixture as the matrix release controlling polymers, were prepared in order to achieve 2 – 3 hour drug release.

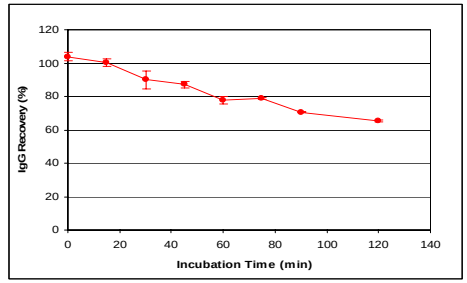
The optimum matrix type sustained release tablet was then coated with different combinations and thicknesses (as weight gain) of Eudragits L30D-55 and NE30D to achieve different delayed release times. The three tablets with each has different delayed release times were then incorporated in a size 00 hard gelatin capsule as a single formulation unit for use. In vitro release of IgG from individual and assembled system was characterised using USP Dissolution Test Apparatus II at a paddle speed 50 rpm. Dissolution testing was carried out in 0.1M HCl for two hours followed by adjusting the pH to 6.8 using sodium phosphate solution. Dissolution samples taken at pre-determined time points were filtered and analysed using a micro BCA method (BCA Protein Assay Kit, Pierce).

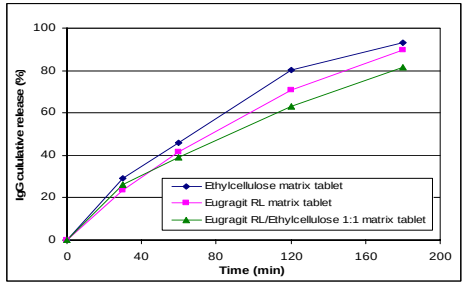
## Results and Discussion

Orally administered antibodies are not stable at low pH in the stomach and will be degraded by the proteolytic enzymes such as pepsin, trypsin and chymotryipsin in the GI tract. It has been reported that these enzymes initially degrade the antibodies to bioactive $F(ab')_2$ and $F_{ab}$ fragments and inactive $F_c$ [2]. The results from the IgG stability study confirmed that after 2 hours incubation at 37°C in SIF with pancreatin, more than 65% of the initial $F_{ab}$ specific IgG activity still remained, although some proteolytic enzyme degradation had occurred which could result in loss of the $F_c$ region (Figure 1). This suggests that the bioactivity of the antibody in the GI tract will depend on the conditions and duration of exposure. With an enteric coated stepwise delayed release system, it is possible to protect the loaded antibodies and release them at the correct rate directly into the intestine and colon without significant loss of its bioactivity for local action.

Gammagard IgG loaded core tablets containing Eudragit RL, Ethyl cellulose and Eudragit RL / Ethyl cellulose 1:1 mixture showed a sustained release of IgG for approximately 2 – 3 hours (Figure 2). The Eudragit RL based tablet has shown the better in vitro drug release profile (near zero order drug release with relative lower burst effect) and hence was selected for further development.

**Figure 1. Stability of IgG Antibody in SIF containing pancreatin at 37°C**

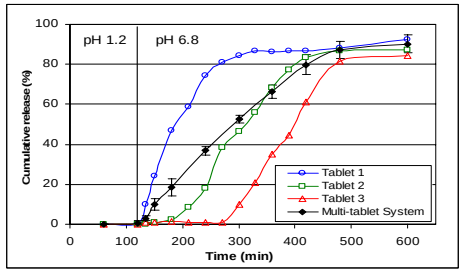



With ratios of Eudragits L30D-55 to NE30D between 1:0 to 7:3 and different coating thicknesses, delays in the sustained release of loaded IgG could be achieved from 0 to 150 min after 2 hours incubation in 0.1M HCl (Figure 3). The multi-tablet system containing three tablets with different coatings therefore showed no drug release at low pH for 2 hours, followed by an overall in vitro sustained release of IgG at pH 6.8 for up to 8 hours.

**Figure 3. Stepwise release of IgG antibody from a multi-tablet system**

**Coating conductions:**
**Tablet 1: Eudragit L30D-55 8% weight gain**
**Tablet 2: Eudragits L30D-55 / NE30D 7:3 12% weight gain**
**Tablet 3: Eudragits L30D-55 / NE30D 7:3 19% weight gain**




## Conclusions

The magnitude of the losses of the antibody bioactivity in the GI tract depends on the conditions and duration of exposure. A multi-tablet system has been developed with each of three tablets containing the same amount of antibody but with different coating layers, resulting in a stepwise sustained release of antibody. With such systems the loaded antibody can be effectively protected against degradation and then released in a controlled sustained fashion for up to 8 hours throughout the GI tract. Such systems should prove to be ideal delivery of antibodies or proteins to the intestine and colon for local action.

## References

1. B Tjellstrom et al (1997) Acta Paediatr 86: 221
2. RM Reilly et al (1997) Clin Pharmacokinet 32: 313

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