

ANALYTICAL INSTRUMENTS FOR BIOPHARMACEUTICAL CHARACTERIZATION

Darinka Gjorgieva Ackova¹, Katarina Smilkov¹, Emilija Janevik-Ivanovska¹, Trajče Stafilov², Zorica Arsova-Sarafinovska^{1,3}, Petre Makreski²

¹Faculty of Medical Sciences, Goce Delčev University, 2000 Štip, R. Macedonia

²Institute of Chemistry, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, 1000 Skopje, R. Macedonia

³Institute of Public Health of the Republic of Macedonia, Centre of Reference Laboratories, 1000 Skopje, R. Macedonia

Introduction

Drugs with biological origin or biopharmaceuticals are products where the active substance is composed of or derived from living organisms and often represent the crossing of the boundaries of medical science and different fields of research. They can be composed of carbohydrates, nucleic acids, proteins, or complex combinations of these substances, or may be composed from live cells or tissues. Unlike chemically synthesized small molecules that have a well-defined structure and are thoroughly characterized, the pharmaceutical products with biological origin are complex in structure and therefore their full characterization is great achievement.

Experimental

Here, we used a multiple set of analytical techniques (HPLC, SDS-PAGE, ICP-MS, MALDI-TOF, TLC, UV/VIS, FTIR and Raman Spectroscopy) for extensive characterization of immunocomplexes of antibody rituximab intended for use as biopharmaceutical.

Results

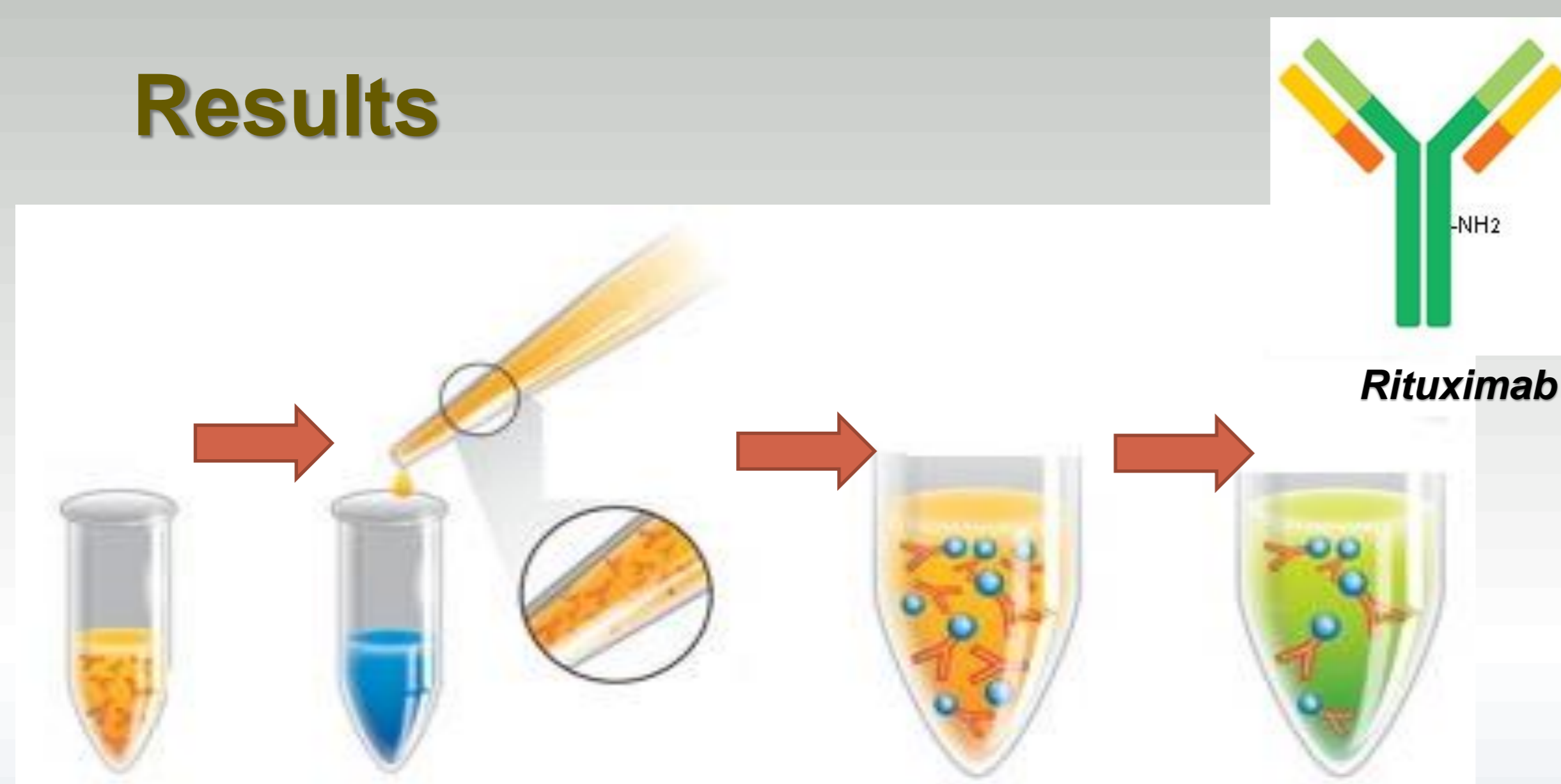


Fig. 1. Preparation of lyophilized formulations of immunocomplexes of antibody rituximab

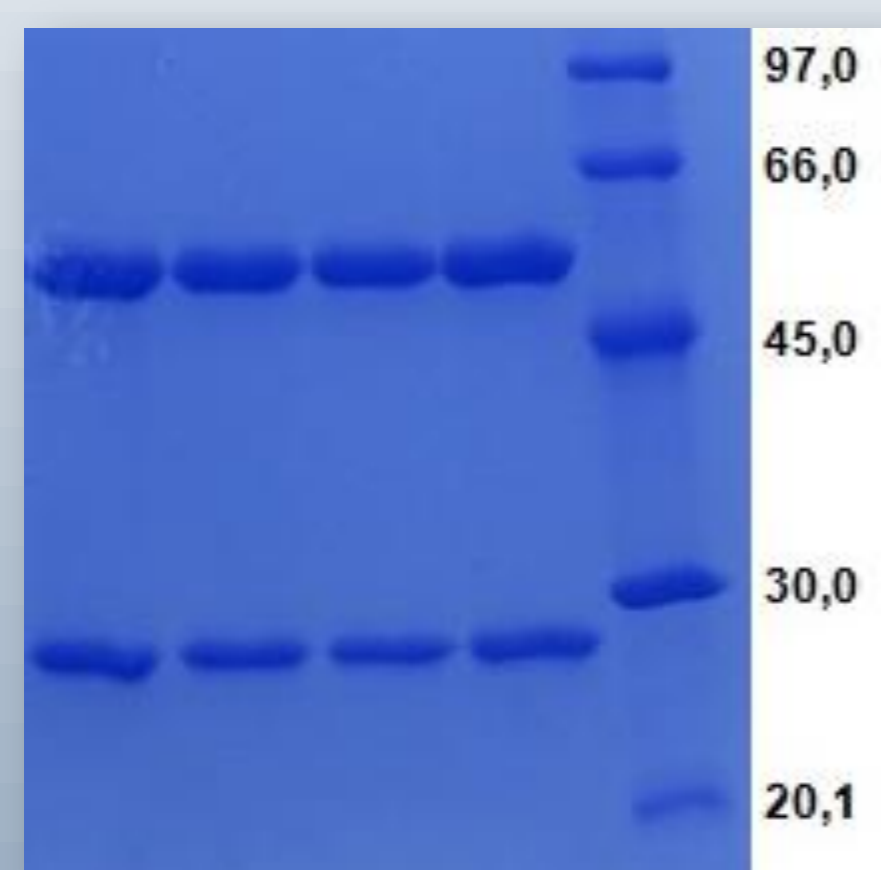


Fig. 2: Reducing SDS-PAGE lane patterns of rituximab (1), *p*-SCN-Bn-DOTA-rituximab (2), *p*-SCN-Bn-DTPA-rituximab (3), 1B4M-DTPA-rituximab (4).

Fig.1: MALDI-TOF results for three complexes of rituximab with:
1: *p*-SCN-Bn-DOTA; (binding of 6.1 groups)
2: *p*-SCN-Bn-DTPA (binding of 8.8 groups) and
3: 1B4M-DTPA (binding of 8.3 groups)

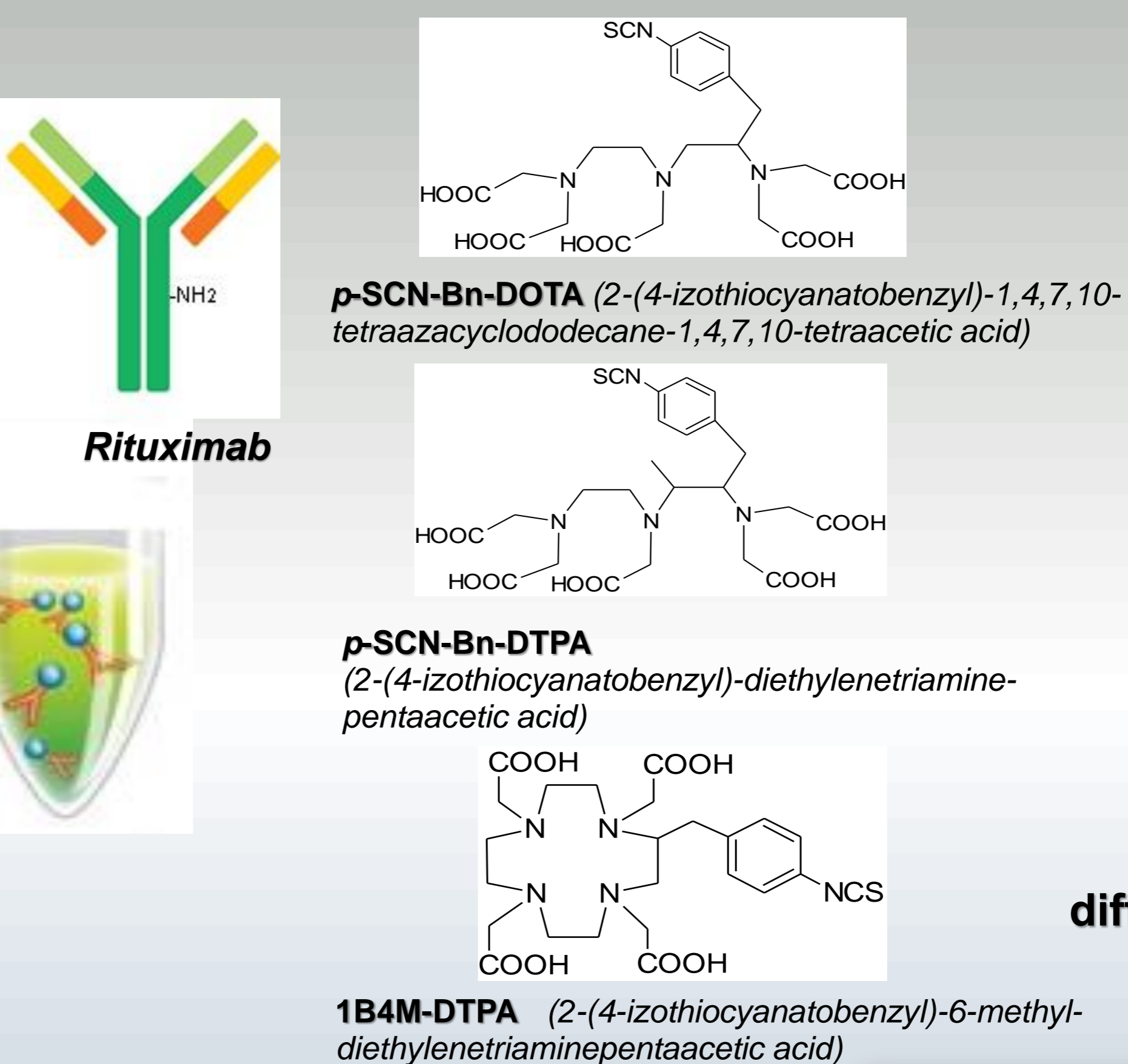


Fig.2. Immunocomplexes with different BFCA's in lyophilized state.

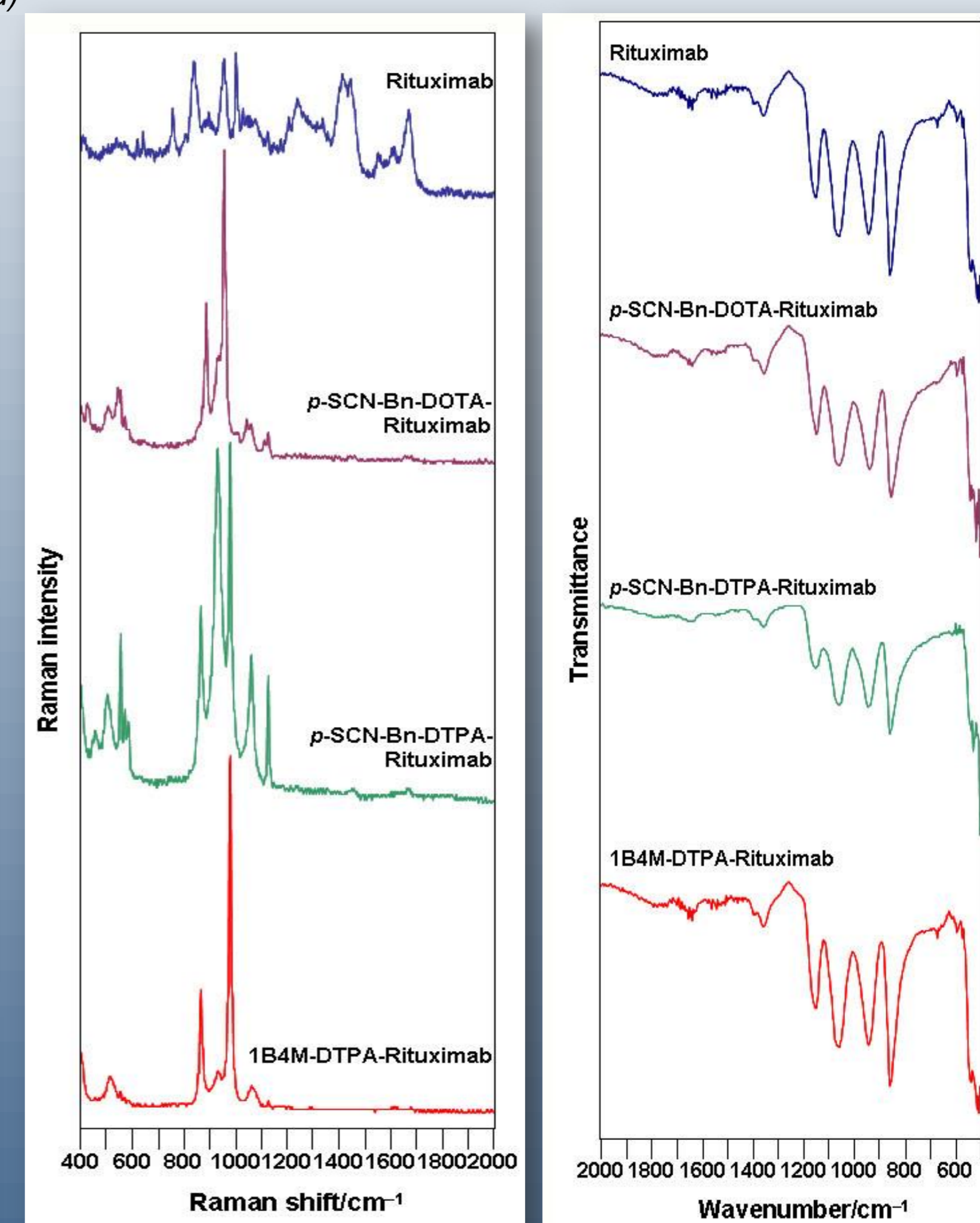
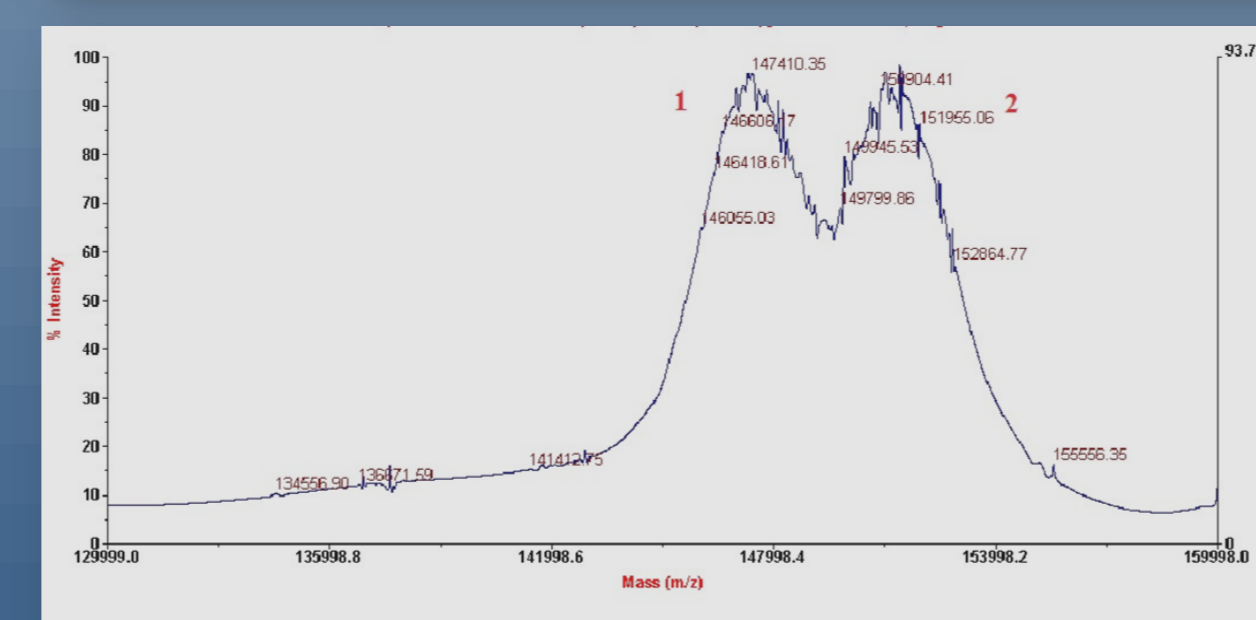
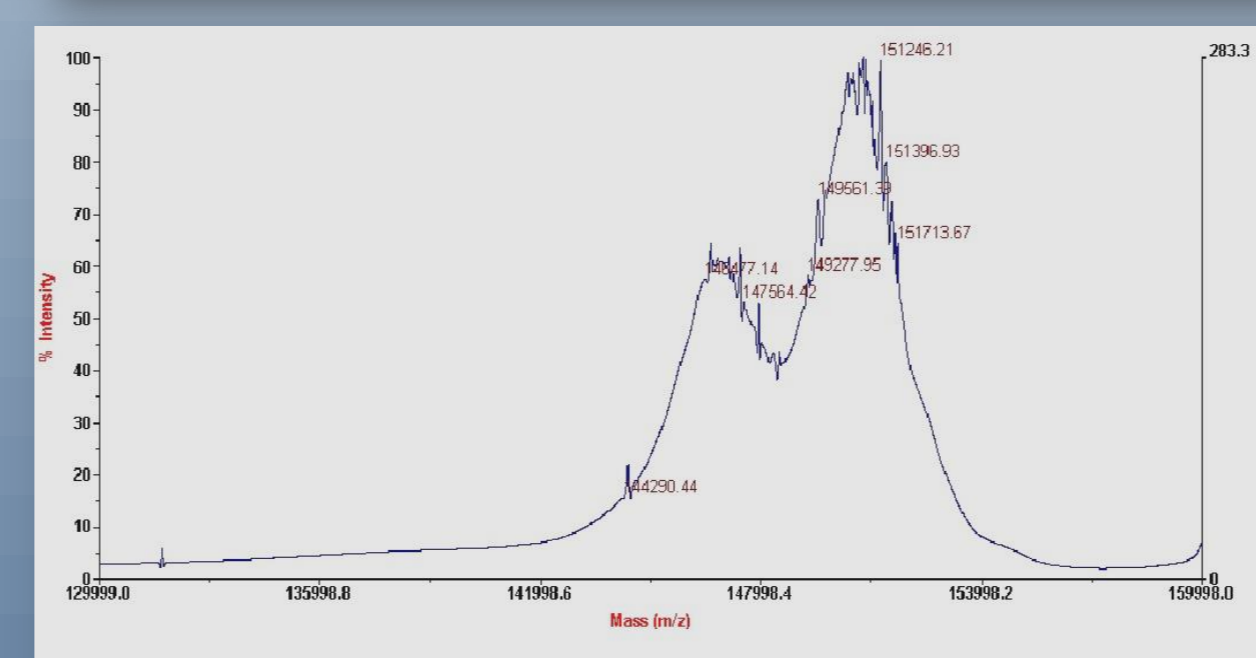
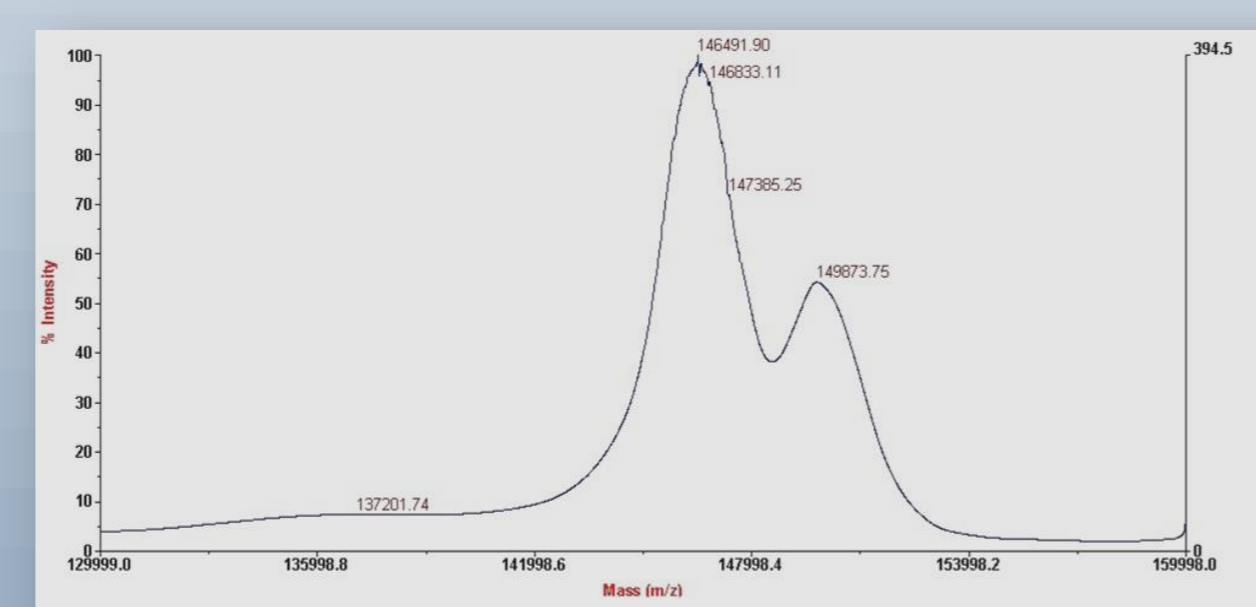


Fig. 3: Raman and ATR-IR spectra of rituximab and three types of experimental complexes

Conclusions

Analytical methodology that is currently in use enables a comprehensive characterization and identification of biopharmaceuticals (determination of molecular weight and particle size, physico-chemical properties, identity, purity and homogeneity, biological activity, immunochemical properties, purity/impurities (if present), present isoforms and structures of higher order, and stability of the product) and covers implementation of variety of physico-chemical, immunological and biological methods.