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Comparison of two-dimensional gel electrophoresis patterns and MALDI-TOF MS analysis of therapeutic recombinant monoclonal antibodies trastuzumab and rituximab

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ABSTRACT

The principal objective of this study was the evaluation of two-dimensional gel electrophoresis (2-DE) in combination with MALDI-TOF MS, after tryptic digest with regard to suitability for qualitative characterization and identification of therapeutic recombinant monoclonal antibodies trastuzumab and rituximab. Moreover, the impact of post-translational modifications of these glycoproteins on the electrophoresis behavior has been evaluated. 1-D SDS-PAGE, in reducing and non-reducing conditions, and 2-DE were used for the assessment of M_r and the monitorization of deglycosylation efficiency. In addition, 2-DE was used for the determination of *pIs*. 2-DE gels revealed characteristic glycoprotein migration behavior, highly complex spot pattern, typical for recombinant monoclonal antibodies. N-linked oligosaccharides were released with PNGase F; enzymatic desialination was studied with sialidase and carboxypeptidase B was used for the study of lysine truncation. Peptide spots resolved in 2-DE gels were *in gel* tryptically digested, resulting peptides were subjected to MALDI-TOF MS analysis and peptide mass fingerprinting (PMF) has been used for the identity confirmation of both monoclonal antibodies.

1. Introduction

Trastuzumab, [FDA, USAN, INN], Herceptin® [TN], Syn: *huMab 4D5-8*, is a humanized recombinant monoclonal antibody (*rmAb*), produced in Chinese Hamster Ovary (CHO) cells. It selectively targets the extracellular domain of the human epidermal growth factor receptor 2 protein (HER-2). The antibody is an IgG1 that contains human framework regions with the complementarity-determining regions (CDR) of a murine anti-p185^{HER2} antibody that binds to HER-2 [1,2]; see Fig. 1.

Rituximab (FDA, USAN, INN), Rituxan®, MabThera® (TNs) is a genetically engineered chimeric rmAb, produced in CHO cells. This IgG1-κ antibody contains murine light and heavy chain variable regions, and human gamma 1 heavy chain and kappa light chain constant regions; see Fig. 2

Both *rmAbs* are composed of four polypeptide chains linked via disulfide bonds: two light and two heavy chains.

Trastuzumab contains 1328 amino acids, its theoretical molecular weight (M_r), calculated from the amino acid sequence of the reduced non-glycosylated form is 145.45 kDa, however, the apparent M_r is higher due to the presence of N-linked oligosaccharides [7]. Each heavy chain contains 450 amino acids and each light chain 214 amino acids, with their respective theoretical M_r, 49.284 kDa and 23.443 kDa and respective theoretical *pI* values, 8.49 and 7.76, calculated using *PeptideMass* program [8]. Trastuzumab contains only one N-glycosylation site at the conserved heavy chain asparagine 300 [3].

Rituximab contains 1328 amino acids, the theoretical M_r of reduced non-glycosylated form is 144.544 kDa and the apparent M_r is higher due to the heavy chain glycosylation at asparagine N-301 [9]. Each heavy chain contains 451 amino acids, and each light chain contains 213 amino acids, with their respective theoretical M_r 49.214 kDa and 23.057 kDa and respective theoretical *pI* values 8.67 and 8.26. Glycosylation of *rmAbs* has no effect on antigen binding. However, it is required for the *Fc* mediated effector functions such as antibody-dependent cell-mediated cytotoxicity and complement dependent cytotoxicity (CDC) [10]. Moreover, rapid elimination of IgG-antigen complexes from circulation is strongly influenced by IgG glycosylation [11].

Trastuzumab and rituximab exhibit glycane profiles basically consisting of asialo, neutral, fucosylated, complex biantennary oligosaccharide type, as determined by CE-LIF of PNGase released glycans, after derivatization with 3-aminobenzoic acid,

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Light chain: DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC; and heavy chain: EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR IYPTNGYTRY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCSRWG GDGFYAMDYW GQGTLVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQY**N** STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

*Complementarity determining regions highlighted and the heavy chain N_{300} glycosylation site indicated in bold type.

Fig. 1. Amino acid sequence and CDRs of trastuzumab [3,4].

2-aminobenzoic acid, and 1-aminopyrene-3,6,8-trisulfonate, respectively. With these techniques, only minor amounts of terminal sialinated oligosaccharides (1–5% of total glycan amount) have been detected [12–15]. Furthermore, CE-ESI-MS and LC-ESI-MS methods were used for the glycosylation studies of these *rmAbs* [9,16,17].

Charged variants of *rmAbs* can be studied using different methods, such as high-performance ion exchange chromatography (HPIEC), isoelectric focusing (IEF), and capillary zone electrophoresis (CZE) [18]. Charged variants which can occur at the four N-termini and the two heavy chain C-termini of rituximab were investigated with HPIEC, after papain digestion [19]. For the

Light chain: QIVLSQSPAI LSASPGEKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT SNLASGVPVR FSGSGSGTSY SLTISRVEAE DAATYYCQQW TSNPPTFGGG TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC; and heavy chain: QVQLQQPGAE LVKPGASVKM SCKASGYTFT *SYNMH*WVKQT PGRGLEWIG*A* *IYPGNGDTSY* *NQKFKG*KATL TADKSSSTAY MQLSSLTSED SAVYYCAR*ST* *YYGGDWYFNV* WGAGTTVTVS AASTKGPSVF PLAPSSKSTS GGTAALGCLV KDYFPEPVTV SWNSGALTSG VHTFPA VLQS SGLYSLSSVV TVPSSSLGTQ TYICNVNHKP SNTKVDKKAE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY **N**STYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFF L YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K

*Murine variable domains highlighted, complementarity determining regions underlined and italicized and the heavy chain N_{301} glycosylation site indicated in bold type

Fig. 2. Amino acid sequences of rituximab [5,6].

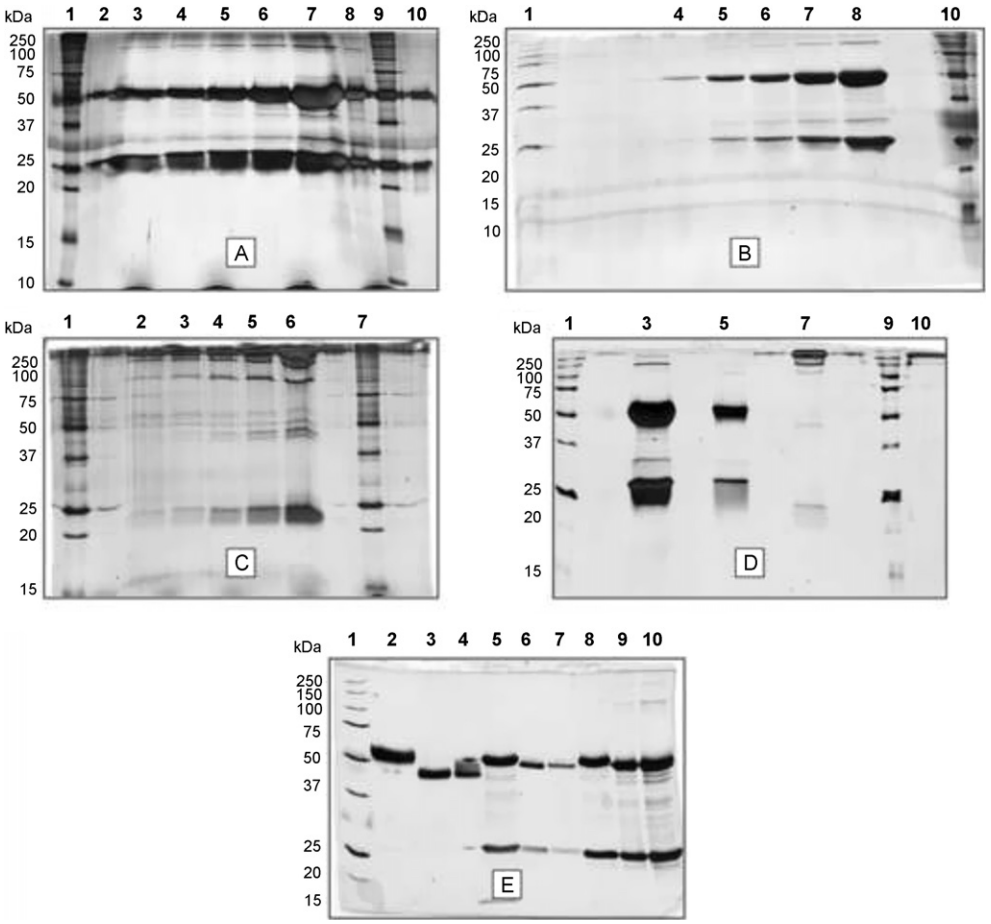

Fig. 3. 1-D SDS-PAGE analysis of trastuzumab and rituximab. (A) trastuzumab reducing conditions. Lanes 2–6, trastuzumab (1.3–20.5 μg), lanes 1, 7 *MW* standards; (B) rituximab, reducing conditions. Lanes 4–8, rituximab (0.1–5 μg), lanes 1, 10 *MW* standards; (C) trastuzumab, non-reducing conditions. Lanes 2–6 trastuzumab (1.3–20.5 μg), lanes 1, 7 *MW* standards; (D) rituximab: lanes 1, 9 *MW* standards; lanes 3, 5, reducing conditions (15 and 1.5 μg, respectively), lanes 7, 10, non-reducing conditions (15 and 1.5 μg, respectively); (E) deglycosylation pattern: abatacept (lanes 2, 3 and 4), trastuzumab (lanes 5, 6 and 7) and rituximab (lanes 8, 9 and 10), 2 μg each. Lane 1, *MW* standards, lanes 5, 8, glycosylated rituximab and trastuzumab, lanes 6, 7, 9 and 10, deglysolylated trastuzumab and rituximab (slightly different conditions); silver stained acrylamide gels: 12.5% T; 2.6% C.

determination of trastuzumab and rituximab serum levels, flow cytometry and ELISA have been described [20–22]. CDC assay was used for the determination of the rituximab potency [23], whereas potency of trastuzumab was determined by antiproliferation activity test [24].

High-performance liquid chromatography (HPLC) coupled with ESI-MS was used for the quantitation of intact trastuzumab [25] and for quantitative analysis of trastuzumab capillary isoelectric focusing (cIEF) and SDS-capillary gel electrophoresis (SDS-cGE) have been evaluated, demonstrating adequate sensitivity, precision and linearity for the use in a quality control environment [26]. Cation-exchange chromatography, a validated method used for the release of trastuzumab production lots, was used for the study of variants arising from the deamidation of asparagine and aspartate isomerization [3] and terminal lysine processing was studied using RP HPLC. In the latter case, cyanogen bromide cleavage following methionin 431, released C-terminal peptides that were studied with RP-HPLC [27]. Methionin oxidation in *rmAb* trastuzumab was studied by papain digestion of *Fab* and *Fc* fragments and by the detection of oxidized *Fc* fragments using hydrophobic interaction chromatography [28]. Peptide mapping was used to assess genetic heterogeneity of recombinant cell lines [29].

Furthermore, it has been widely recognized that 2-DE is a powerful method for the analysis of complex protein mixtures, able to be complemented with downstream MALDI-TOF MS analysis for identification purposes [30]. Additionally, the approval of 2-DE for the routine quality assurance of recombinant drugs has been proposed [31]. In this study we have examined the suitability of 2-DE and subsequent PMF analysis for the study of identity, purity and post-translational modifications of therapeutically relevant *rmAbs* trastuzumab and rituximab.

2. Materials and methods

Isoelectric focusing. 1 ml aliquot rehydration solution (urea 8 M, thiourea 2 M, CHAPS 4%, Triton-X100 0.5%, bromophenol blue 0.005%), 5 mg/ml DTT and 5 μl/ml IPG buffer of selected pH interval (GE Healthcare Bio-Sciences AB Uppsala, Sweden) were mixed prior to use. In 340 μl of this solution, 10 μl (1–5 μg/μl) of sample solution were mixed and shortly vortexed. The mixture was allowed to stay 1 h at room temperature followed by centrifugation (15,000 g, 5 min). Strip Holders were put onto the cooling plate/electrode contact area of the IPGphor strip holder platform (IPGPhor™ IEF system, GE Healthcare, Biosciences AB, Uppsala, Sweden). Prepared samples were applied onto commercially available Immobiline DryStrip gels (6–9 *L* and 3–11 *NL*, length 18 cm ±2 mm, GE Healthcare Bio-Sciences AB Uppsala, Sweden) by *in-gel* rehydration [32]. The temperature was set to 20 °C and the current limited to 50 μA/IPG strip. Focusing was run to a total 72 kVh.

Second dimension, SDS-PAGE. The second-dimension, SDS-PAGE, was run on a vertical system (PROTEAN® II Xi Cell for vertical

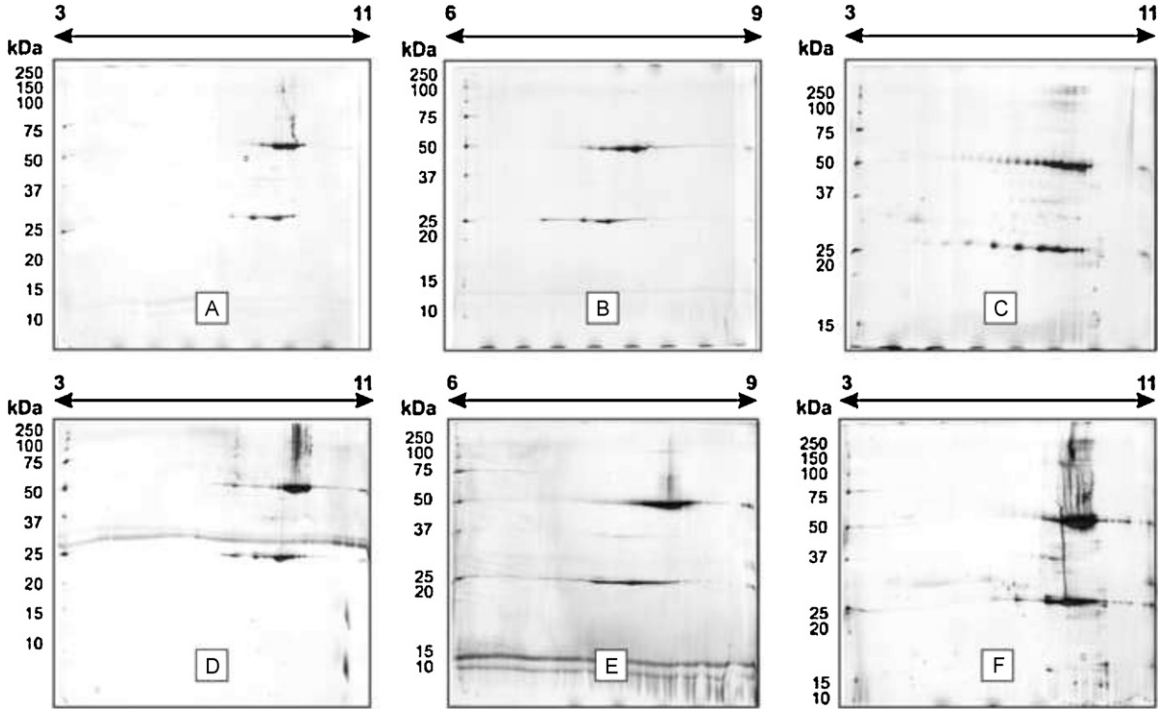

Fig. 4. 2-DE analysis of trastuzumab and rituximab. (A) IPG 3–11 *NL*, non-deglycosylated, 10 μg trastuzumab; (B) IPG 6–9 *L*, non-deglycosylated, 10 μg trastuzumab; (C) IPG 3–11 *NL*, deglycosylated, 50 μg trastuzumab; (D) IPG 3–11 *NL*, non-deglycosylated, 10 μg rituximab; (E) IPG 6–9 *L*, non-deglycosylated, 10 μg rituximab (F), IPG 3–11 *NL*, deglycosylated, 50 μg rituximab. Silver stained acrylamide gels: 12.5% T; 2.6% C.

electrophoresis, 20 cm × 20 cm, Bio-Rad Laboratories, USA). Focused IPG strips were equilibrated twice, each time for 15 min, by gently shaking. The first equilibration step was performed in 5 ml equilibration buffer (Tris 0.05 M, urea 6 M, glycerol 30%, SDS 2%, bromphenol blue 0.005%) containing 1% DTT, followed by the second equilibration in 5 ml equilibration buffer containing 2.5% iodoacetamide [33].

Equilibrated IPG gel strips were placed on top of the vertical SDS gel. Molecular weight standards (Precision Plus Unstained Protein™ Standards, Bio-Rad Laboratories, USA) were placed at

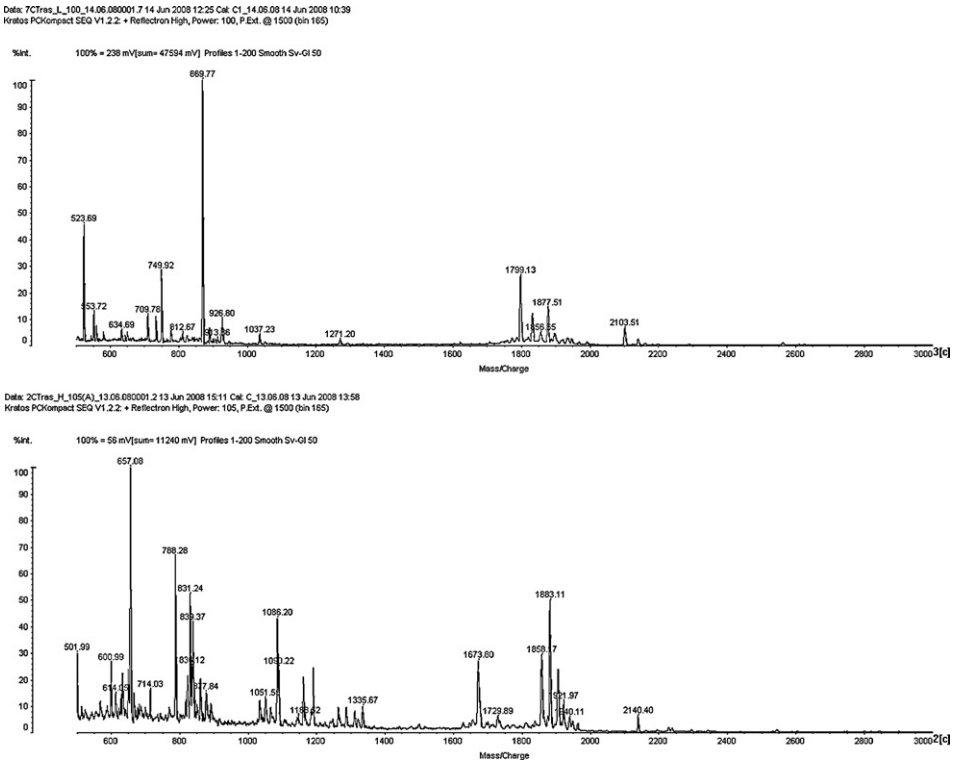

Fig. 5. MALDI-TOF MS spectra (peptide mass fingerprints) of peptides generated from deglycosylated, 2-DE separated and *in situ* tryptically digested trastuzumab light (up) and heavy chain (down).

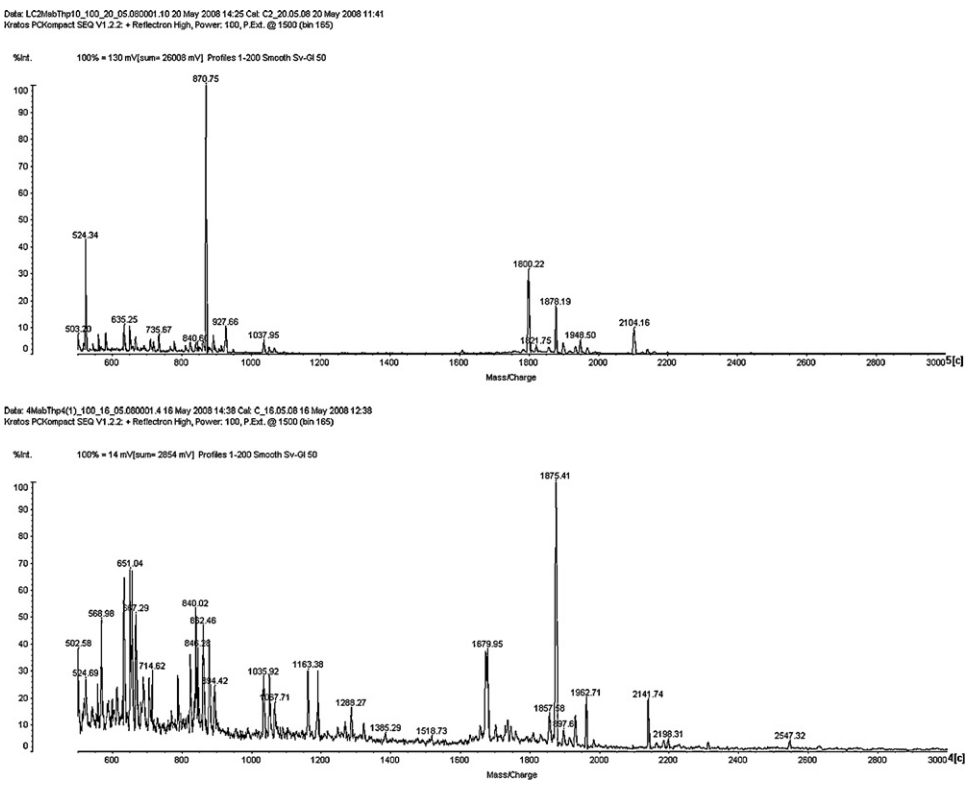

Fig. 6. MALDI-TOF MS spectra (peptide mass fingerprints) of peptides generated from deglycosylated, 2-DE separated and *in situ* tryptically digested rituximab light (up) and heavy chain (down).

the anodic end of the SDS-PAGE gel. Second dimension electrophoresis run (lab made gels 12.5% T, homogenous, 2.6% C; 200 mm × 200 mm × 1 mm) was performed under constant current of 45 mA/gel.

One-dimensional, SDS-PAGE was performed according to Laemmli [34], on the mini-gel system Mini-PROTEAN® 3 Cell, 8 cm × 7.3 cm, (Bio-Rad Laboratories, USA).

Silver staining was performed according to Blum et al. [35]. Coomassie staining (Coomassie Brilliant Blue G 250, Serva Electrophoresis GmbH, Heidelberg Germany) was performed according to the manufacturer's instructions. All chemicals used were reagent and HPLC grade. Gels were scanned (Biorad Densitometer GS 710) and analyzed with PDQuest 6.2.1. (Bio-Rad Laboratories, USA).

In-gel digestion. For silver stained gels, *in situ* reduction, alkylation and tryptic digestion of proteins resolved from 2-DE gels, with prior destaining, was performed according to methods described elsewhere [36,37].

Sample redissolution and MALDI-TOF sample presentation. Dry pellets of digestion extracts were resuspended in 5 μl, 0.1% TFA. Peptide mixtures were analyzed using a saturated matrix solution of α-cyano-4-hydroxycinnamic acid (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) in 70% ACN: 30% of 0.1% TFA (*v*/*v*). All mass spectra were acquired on Compaq SEQ (Kratos Analytical, Manchester, UK) MS instrument operated in positive reflectron mode, peptides were observed as $[M+H]^+$ ions. For external calibration, bradykinin fragment 1–7, angiotensin II, ACTH fragment 18–39 and insulin oxidized beta chain, were used.

Peptide mass fingerprinting. PeptideMass program was used to *in silico* cleave entered respective trastuzumab and rituximab light and heavy chain sequences with trypsin and to compute generated peptide masses with their specified modifications [8]. During database searching taxonomy parameter were not specified, selected enzyme was trypsin and only peptide masses higher than 500 Da have been displayed. Other search parameters were set to allow up to 1 miss cleavage, methionines in oxidized form (variable modification), cysteines in carbamidomethylated form (fixed modification), average mass, peptide ions in protonated $[M+H]^+$ form, mass tolerance (MT) parameter was set from ±1 to ±2 Da. Protein molecular mass was not specified.

Enzymatic deglycosylation. Deglycosylation of *rmAbs* was performed according to the procedure described in Kamoda et al. [12]. Solutions of the pharmaceutical preparations, trastuzumab (Herceptin®) 21 mg/ml and rituximab (MabThera®) 10 mg/ml, collected from the vials immediately after clinical use in Vienna Hospital Rudolfstiftung, were dialyzed against MilliQ-water (3 days, 4 °C) using cellulose dialysis tube (cut off 12,000–14,000 Da, Japan Medical Science, Tokyo, Japan). After dialysis, solutions were freeze dried and lyophilized and samples of pharmaceutical preparations, 0.5 mg each, were dissolved in 49 μl phosphate buffer (pH 7.5) to obtain a final concentration of 500 μg/50 μl. PNGase F of *Flavobacterium meningosepticum*, recombinant, from *E. coli*, 100 U/100 μl, (Roche Diagnostics GmbH, Mannheim, Germany) was used for the cleavage of N-linked oligosaccharides, (15 U/150 μg *rmAb*). Neuraminidase (sialidase) from *Arthrobacter ureafaciens* 1 U/100 μl, 30 mU/150 μl *rmAb* (Roche Diagnostics GmbH, Mannheim, Germany) was used for the cleavage of sialic acids and carboxypeptidase B, 750 U/ml, from pig pancreas (Roche Diagnostics GmbH, Mannheim, Germany) was used for the study of lysine truncation (0.75 U/150 μg *rmAb*). After digestion (overnight at 37 °C) samples were stored at −80 °C.

3. Results and discussion

3.1. 1D-SDS-PAGE analysis of trastuzumab and rituximab

In order to examine relevant quality parameters, including detection, and purity assessment, 1-D SDS-PAGE experiments,

Table 1
Summary of MALDI-MS data for the identification of trastuzumab light and heavy chains.

Rank of result	Database	Protein name/*accession number*	Additional comments	Mass tolerance	Sequ. cov.	Score	Matched peptides
Trastuzumab light chain							
1	NCBInr	Crystal structure of extracellular domain of human Her2 complexed with Herceptin Fab, chain A; *gi28948772/1N8Z_A*		±1 Da	65%	219	18
1	MSDB	Fab fragment of humanized antibody 4d5, version 4, chain A – human (*1FVDA*)		±2 Da	73%	194	17
2	MSDB	Mixture of		±2 Da		166	18
		(a) *peptide CH68665/CQ890830*	protein sequence is identical with the constant domain of trastuzumab light chain (residues 108–214)	±2 Da	97%	106	10
		(b) *1 FVCC*(Fv fragment of humanized antibody, 4d5, version 8, chain C – human)	variable fragment of trastuzumab light chain (residues 1–108)	±2 Da	65%	74	8
1	SwissProt	Ig kappa chain C region, *IGKC_HUMAN*	Sequence is identical with trastuzumab light chain residues 109–214				
Trastuzumab heavy chain							
1	NCBInr	Mixture of		±1.2 Da		226	28
		(a) Enzyme deglycosylated human Igg1 Fc fragment, chain A; *gi254574798/3DNK_A*	Amino acid sequence is identical with the Fc fragment of herceptin (residues 238–450); D-62 (aspartic acid) of this protein corresponds to trastuzumab asparagine residue, N-300 (non-deglycosylated form)	±1.2 Da	71%	136	16
		(b) *Fab* fragment of trastuzumab, *gi442924/1FVE_B*			55%	85	12
1	MSDB	Mixture of		±1.2 Da		222	26
		(a) Human *CAC21795/CAC21795*	Protein sequence is identical to trastuzumab residues 218–450 (except C5S mutation) including CH2 and CH3 regions	±1.2 Da	65%	135	16
		(b) Fab fragment of trastuzumab, *1FVEB*		±1.2 Da	55%	85	12

under reducing and non-reducing conditions, were performed. Under reducing conditions both *rmAbs* were resolved in two distinct M_r species which migrated in two bands (upper band at ~50 kDa and lower band ~23 kDa) confirming the migration behavior typical for IgG antibodies which are composed of two identical subunits each composed by two polypeptide chains: two heavy and two light chains, linked via disulfide bonds (Fig. 3, gels A and B).

Under non-reducing conditions, single bands of *rmAbs* at M_r ~150 kDa have been observed (sum of two heavy and light chains) and in contrast to reducing conditions the band sharpness was reduced (Fig. 3, gels C and D). Sensitivity and linearity were assessed, as well. After N-deglycosylation, only a small decrease in M_r of *rmAbs* heavy chains was observed indicating the presence of N-linked glycans whereas M_r of light chains was not affected. For comparison purpose, deglycosylation experiments were performed for recombinant fusion protein abatacept (Fig. 3, gel E).

3.2. 2-DE analysis of trastuzumab and rituximab

Recombinant *mAbs* are biological products derived from rDNA technology, produced in mammalian CHO cells. Therefore, it is expected to have a certain degree of heterogeneity and isoforms. As the heterogeneity of these products defines their quality, the characterization of the degree and profile of the heterogeneity constitutes an essential part of regulatory requirements [38].

The applied 2-DE method was optimized for the *pI* gradient (IPG strips 3–11 *NL*, 6–9 *L*), gel concentration and visualization procedure. 2-DE gels of glycosylated antibodies revealed the characteristic glycoprotein migration behavior resulting in highly complex, poorly resolved spot patterns with small differences in M_r, but different *pI*s (Fig. 4). 2-DE analysis revealed that heavy chain spots of *rmAbs* migrated at M_r ~50 kDa whereas their light chains migrated at M_r ~23 kDa. Respective heavy chain *pI* ranges for trastuzumab and rituximab were 6.5–8.7 and 6.3–9.0; and respective light chain *pI* ranges were 5.7–8.1 and 6–8.5. Higher sensitivity of silver versus Coomassie staining and improved spot resolution at lower sample amounts was observed, as well (data not shown). No improvement in spot resolution could be documented applying IPG 6–9 *L*.

No significant differences were observed in 2-DE spot patterns between N-deglycosylated, desialinated and carboxypeptidase B digested *rmAbs* and their glycosylated forms. Investigated r*mAbs* harbour only one glycosylation site, containing predominantly neutral N-linked glycans which contribute to only 2% in their M_r [39], in addition sialic acid content is only 1–5% of total glycan amount. Expected *pI* and M_r differences after N-linked glycan release are therefore too small to be unambiguously detected with 2-DE. Calculated *pI* differences between glycosylated and N-deglycosylated, and 450-lysine and truncated lysine variants is only ~0.13 [8], which is also too small to be detected on 2-DE gels considering a certain variance regarding to gel-to-gel reproducibility. Respective

Table 2
Summary of MALDI-MS data for the identification of rituximab light and heavy chains.

Rank of result	Database	Protein name/*accession number*	Additional comments	Mass tolerance	Sequ. cov.	Score	Matched peptides
Rituximab light chain							
1	NCBInr	Fab of rituximab (crystal structure of Rituximab Fab in complex with an epitope peptide, chain L) *gi 146387638/2OSL_L*	Human constant region (residues 107–213)	±1.1 ±1.5 ±1.7	35%42%45%	97141157	9 12 13
Rituximab heavy chain							
1	NCBInr	Fc fragment of rituximab (bound to a minimized version of the B-Domain from protein A called Z34c, chain A); *gi20664303/1L6X_A*		±1.5 ±2.0	42% 57%	66 103	8 11

2-DE gels of N-deglycosylated trastuzumab and rituximab are presented in Fig. 4 (gels C and F, 2-DE gels of desialinated and carboxypeptidase B treated antibody samples are not shown).

3.3. MALDI-TOF MS and peptide mass fingerprinting analysis of *rmAbs*

For identification purpose, tryptic *in-gel* digestion of selected silver stained 2-DE gel spots, corresponding to heavy and light chains of trastuzumab (Fig. 4, gel C) and rituximab (Fig. 4, gel F), respectively, was performed. Peptide masses determined by MALDI-TOF MS analysis were compared to *in silico* (theoretical) peptide masses by applying the enzyme cleavage rules, from three amino acid sequence databases: MSDB, NCBInr and SwissProt using MASCOT [40] and PROWL (ProFound) [41] search engines. Antibody concentration, amount of trypsin, digestion time, extraction of generated peptides and other factors related to the MALDI-TOF sample preparation have been studied and optimized. In Figs. 5 and 6, respective MALDI-TOF spectra of generated peptides from deglycosylated light and heavy chains of trastuzumab and rituximab are presented.

3.3.1. MALDI-TOF MS and peptide mass fingerprinting analysis of *trastuzumab*

For the identification of trastuzumab light chain, a list of 18 peptide masses determined by MALDI-TOF MS from corresponding protein spots were compared to data from sequence databases NCBInr, SwissProt and MSDB using the search engines MASCOT and PROWL [40,41] (Fig. 4C). The generated top score results for trastuzumab light and heavy chain including important MS parameters are shown in Table 1.

For the identification of trastuzumab heavy chain, a list of 29 peptide masses determined by MALDI-TOF MS was compared to the same databases using MASCOT and PROWL/*ProFound* [40,41] search engines (Table 1).

3.3.2. MALDI-TOF MS and peptide mass fingerprinting analysis of *rituximab*

For amino acid sequence confirmation of rituximab light chain, a list of 13 peptide masses experimentally determined by MALDI-TOF MS were compared to sequence databases as described above. Results are summarized in Table 2.

The databases MSDB and SwissProt, respectively, generated the highest score results for IgG kappa light chain and fragment of monoclonal antibodies suggesting that in this databases rituximab light chain sequence information is not mentioned.

For amino acid sequence confirmation of rituximab heavy chain, a list of 14 peptide masses experimentally determined by MALDI-TOF MS was compared to the same databases (Table 2). SwissProt and MSDB generated similar results.

Some MALDI spectra are available as supporting information.

4. Concluding remarks

Experimental results show that 1-D SDS-PAGE analysis can be used as a simple, fast and reliable method for the quality assessment of *rmAbs*, trastuzumab and rituximab. This technique allows the estimation of antibody M_r and purity. In contrast to 1-D SDS-PAGE, 2-DE analysis delivered more comprehensive information regarding identity and structural integrity of *rmAbs*, and apart from the fast estimation of M_r and *pI*, 2-DE proved to be a very suitable technique for the illustration of charge heterogeneity, as well. Experimental data revealed that MALDI-TOF peptide mass fingerprinting could be used as rapid method for the identification of *rmAb* characteristics, provided that the sequence information is present in respective databases. Apart from its application during the production process of *rmAbs*, 2-DE in combination with subsequent MALDI-TOF MS analysis in our opinion might be used as a routine technique in quality control laboratories, suited in the regulatory/pharmacopoeia environment, as well.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.07.006.

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