A. Lüdke[1)], U. Flenker[1)], S. Lüdke[1)], W. Schänzer[1)]

Influence of storage on the mobility values of human erythropoietin

[1)]Institute of Biochemistry, German Sport University, Cologne, Germany

Recombinant erythropoietin (rhEPO) can be distinguished from human endogenous erythropoietin (hEPO) by isoelectric focusing (IEF) (Lasne *et al*., 2002) and SDS-PAGE (Kohler *et al*., 2007; Reichel *et al*., 2009). While the separation by IEF is based on differences in the isoform profiles of the EPO-forms, the SDS-method divides endogenous and recombinant EPO according to their molecular weight and migration velocity in the gel (see Figure 1). In the SDS-method the relative mobility (rMob) of the different EPO-forms (B) is given as a ratio of two distances: **rMob = (C-A) / (C-B)**, with the two internal standards Darbepoetin α (A) and rat erythropoietin (C) (see Figure 2). At a relative mobility value of rMob ≥ 0,578 a sample is regarded suspicious for rhEPO and has to be confirmed by IEF (Kohler *et al*., 2007). In this study the influence of storage on the mobility of hEPO in urine was evaluated. Urine was stored at different temperatures for several weeks to determine possible variations in the relative mobility values.

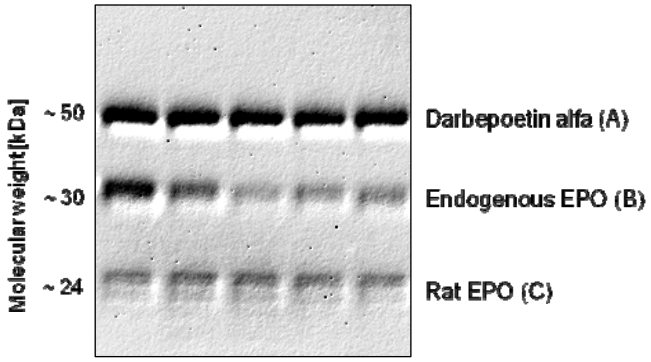

Figure 1: Separation of EPO by SDS-PAGE
SDS-PAGE of urine samples collected for the stability study to detect endogenous EPO (B). The internal standards are Darbepoetin α (A) and rat EPO (C). The latter is produced in insect cells

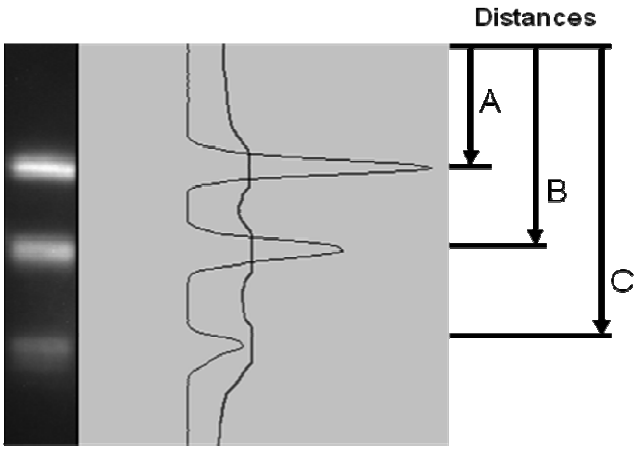

Figure 2: Calculation of the peak distances from the top of the membrane
Upper peak: Darbepoetin α; Middle peak: hEPO; Lower peak: Rat EPO

Method

We studied the influence of storage on the relative mobility of hEPO in urine. The urine of eight subjects (four male, four female) was collected. The storage temperature for untreated and concentrated (Lasne *et al*., 2002) urine samples were 4°C and -20°C, respectively. The samples were analysed by SDS-PAGE at the beginning of the study, then weekly for five to six weeks. The relative mobility value rMob was calculated for each sample.

Results

Change of the relative mobility over time

The relative mobility values decreased significantly over time (-0.002 per week, $p < 0.001$) (see Figure 3). This could derive from a degradation of glycans and thus a decrease of the molecular size. We found considerable variations of the decrease between the subjects (± 0.0013 per week) (see Figure 3 and 4). Consequently, the trends can be regarded as largely individual.

Influence of storage temperature and of the sample preparation before storage

No effect of the storage temperature could be detected as was concluded from likelihood statistics (see Figure 5). No improvement of the model fit was achieved by incorporation of this factor ($p = 0.71$). We obtained clues, that hEPO, which started at a higher rMob, decreased stronger than hEPO that started at a lower value. The change of the relative mobility values over time did not depend on the concentration state of the sample. The preparation of the samples before storage (untreated at 4°C, concentrated at -20°C) had no effects on the relative mobility.

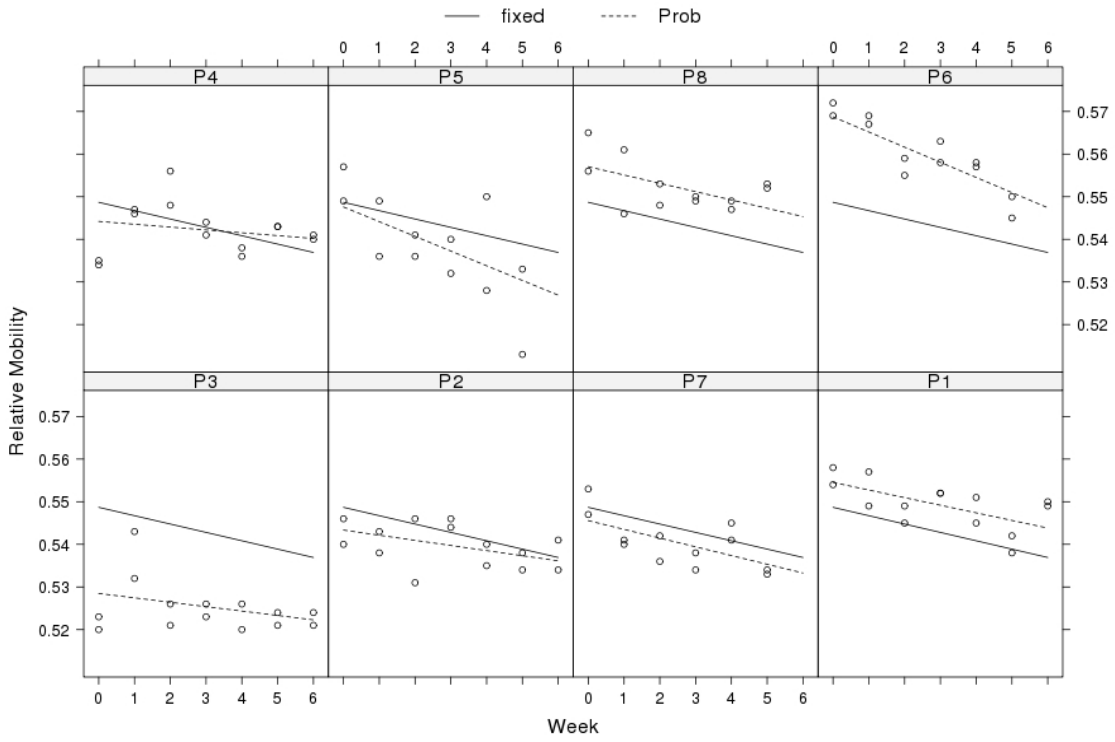

Figure 3: Relative mobility values of subject P1 to P8 depending on time
The fixed line (solid line) reflects the mean value of the eight subjects. It shows a significant decrease of the relative mobility values over time. The individual values (dotted line) show largely individual trends.

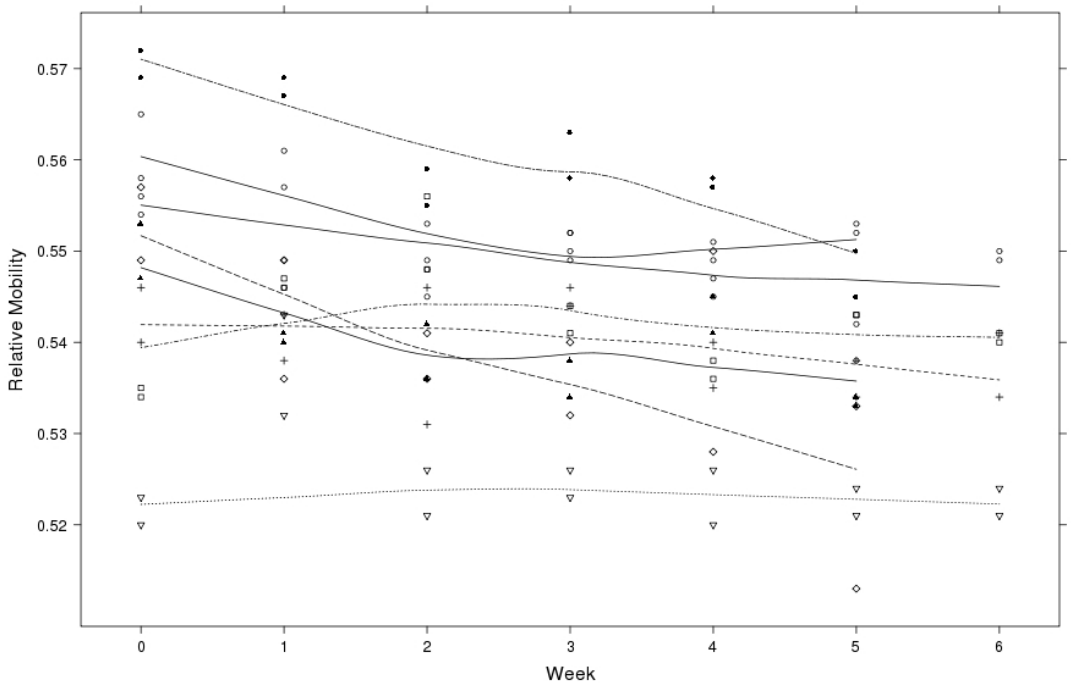

Figure 4: Variance between the subjects
The variance between the subjects is larger than the variance within a subject. Erythropoietin which starts at a higher rMob decreases stronger than EPO that starts at a lower value.

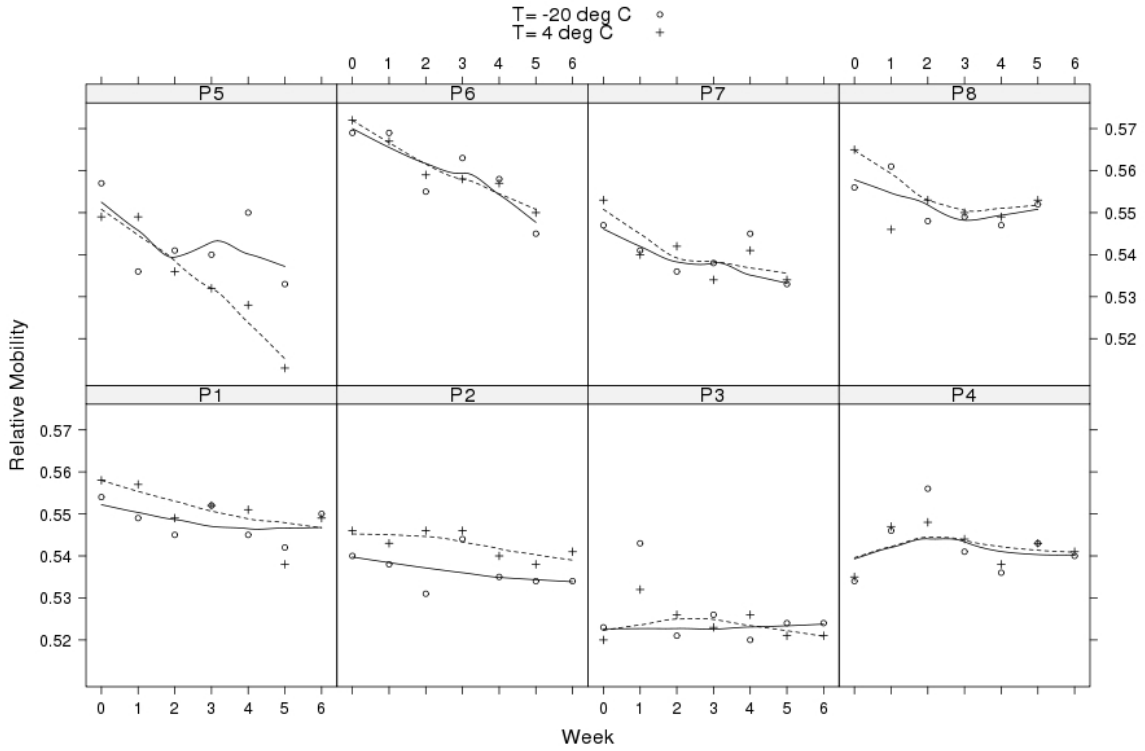

Figure 5: Influence of the storage temperature
The storage temperature of 4°C (dashed line) and -20°C (solid line) had no significant effect on the relative mobility values.

References

Kohler M, Ayotte C, Desharnais P, Flenker U, Lüdke S, Thevis M, Völker-Schänzer E, Schänzer W (2008) Discrimination of Recombinant and Endogenous Urinary Erythropoietin by Calculating Relative Mobility Values from SDS Gels. *Int J Sports Med.* **29,** 1-6.

Lasne F, Martin L, Crepin N, De Ceaurriz J (2002) Detection of isoelectric profiles of erythropoietin in urine: differentiation of natural and administered recombinant hormones. *Anal Biochem.* **311**, 119-126.

Reichel C, Kulovics R, Jordan V, Watzinger M, Geisendorfer T (2009) SDS-PAGE of recombinant and endogenous erythropoietins: benefits and limitations of the method for application in doping control. *Drug Test Analysis* **1**, 43-50.