

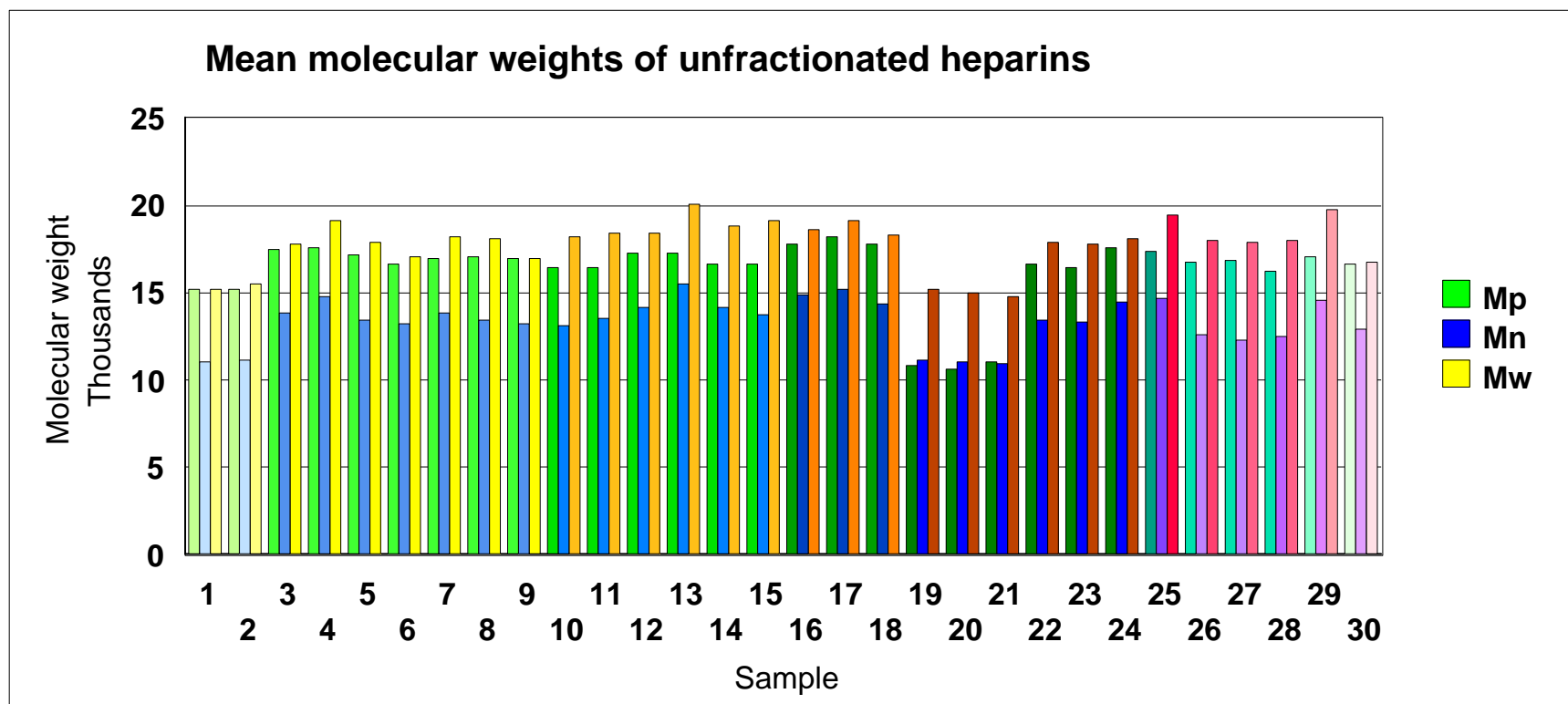
# Molecular weight distributions for UFH: a potential reference material.

Barbara Mulloy



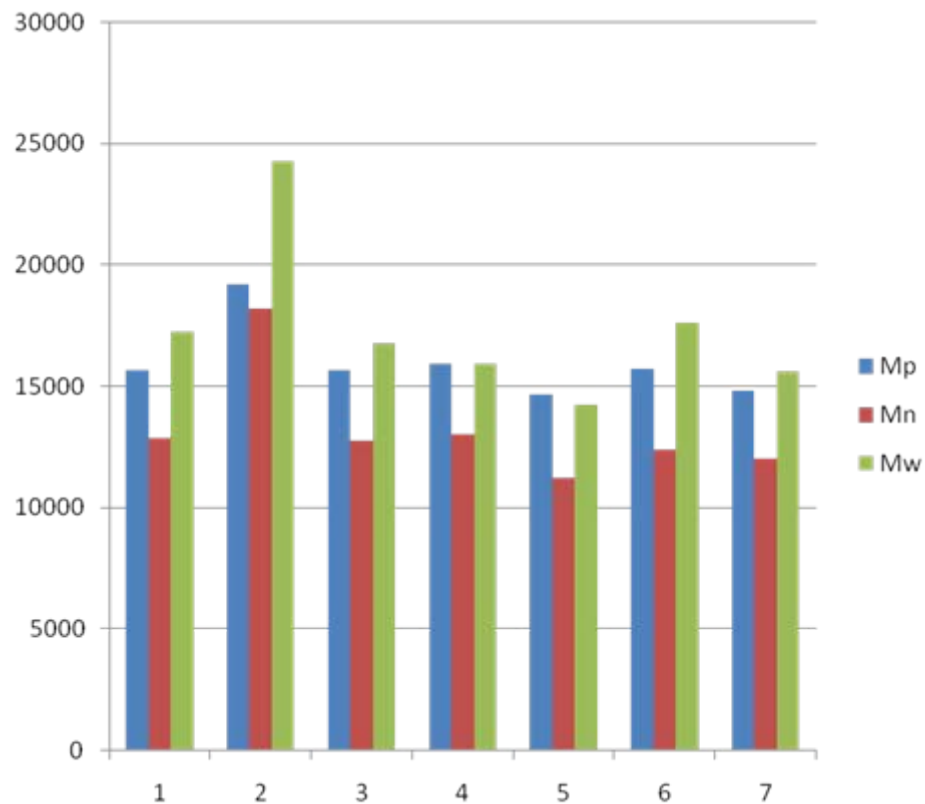
- Molecular weight is not a defining characteristic for unfractionated heparin.
- Pharmacopoeial monographs do not make reference to molecular weight for UFH.
- No compendial method exists, and there is no easily available calibrant.

# Molecular weights of UFH samples: 1998

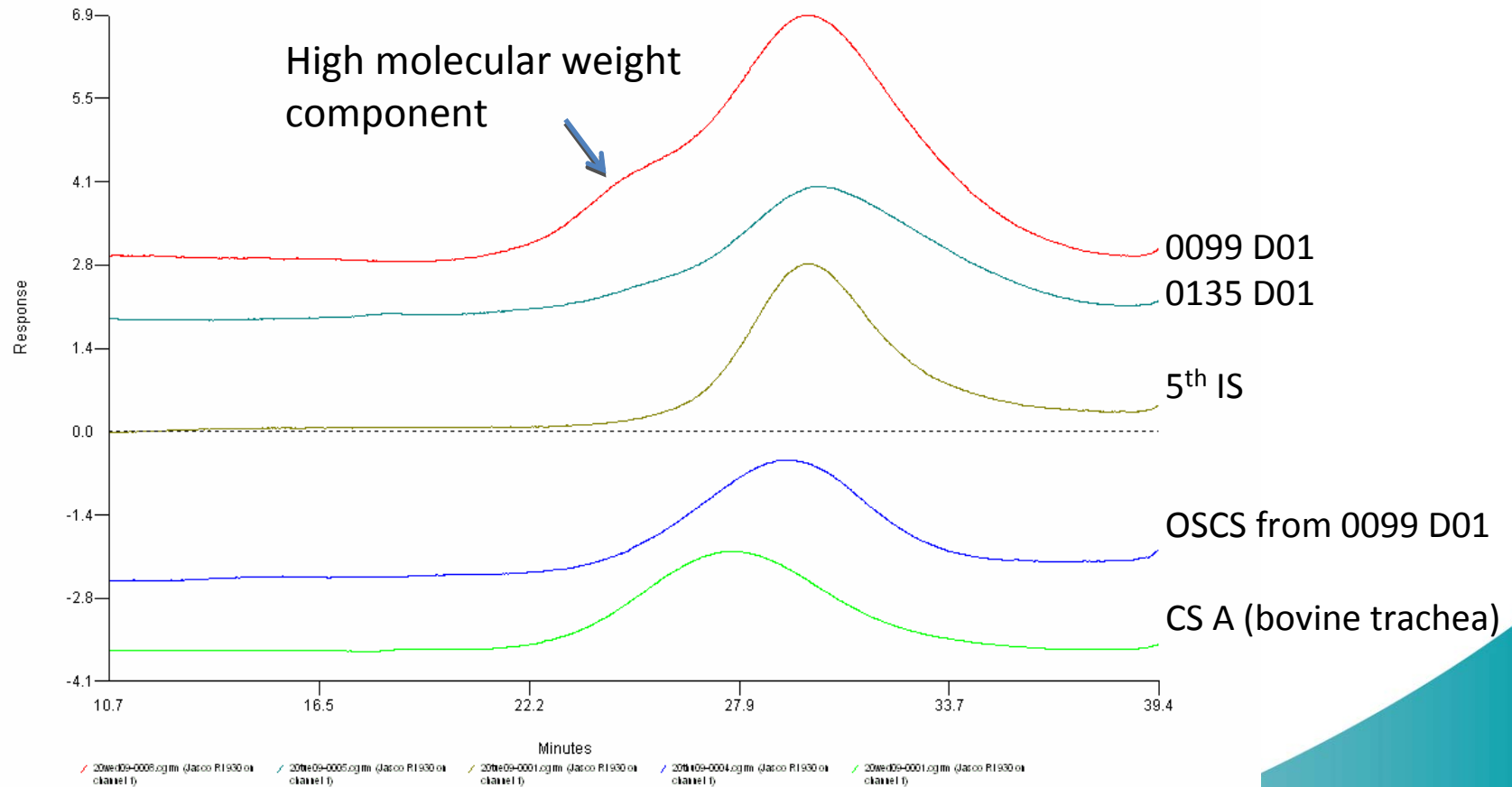


B. Mulloy, E. Gray and T. W. Barrowcliffe, "Characterisation of Unfractionated Heparin Samples: Comparison of samples from the last 50 years" *Thromb. Haemostas.* **84** (2000) 1052-1056.

# Molecular weights of UFH samples 2008



# Effect of OSCS contamination on MW of UFH



# Calibration of GPC systems for MW measurement



- Individual laboratories have developed sets of narrow standards for their own use.
- A set of 19 standards from Sanofi has been used in several publications (including ours) but setting up such a system is too resource-hungry for use as an internationally available method.
- Light scattering is a possibility if a value for  $dn/dc$  can be agreed upon.

- Low angle laser light scattering (LALLs) and multiple angle laser light scattering (MALLS) can be coupled with GPC to give a thorough description of molecular weight distribution of a polydisperse sample.
- No calibrant is needed, but the relationship between RI and concentration ( $dn/dc$ ) must be known for the sample in question.
- For heparin, values for  $dn/dc$  vary between 0.11 and 0.18; most published values falling between 0.12 and 0.16.
- Light scattering needs two detectors and can be technically challenging.

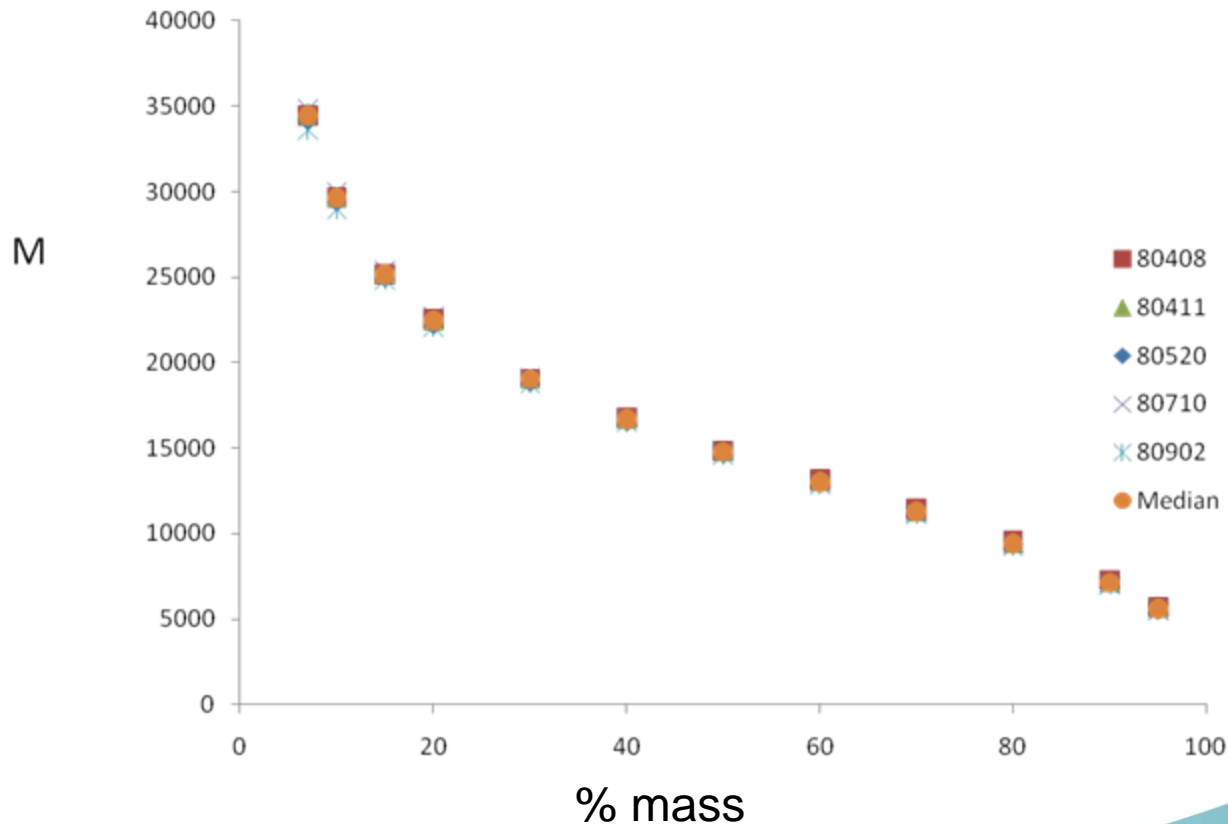
## Possible reference material: a Broad Standard?



- The 2<sup>nd</sup> International Standard LMW Heparin for MW Calibration (NIBSC 05/112) is supplied principally for use as a Broad Standard.
- In this calibration method, a polydisperse calibrant is used with a Table of molecular weights, with percent of the calibrant above and/or below that molecular weight.
- This provides a robust and simple calibration, very easy to use when specialist software is available, and not difficult even using a spreadsheet.

# Sample Z as a potential Broad Standard

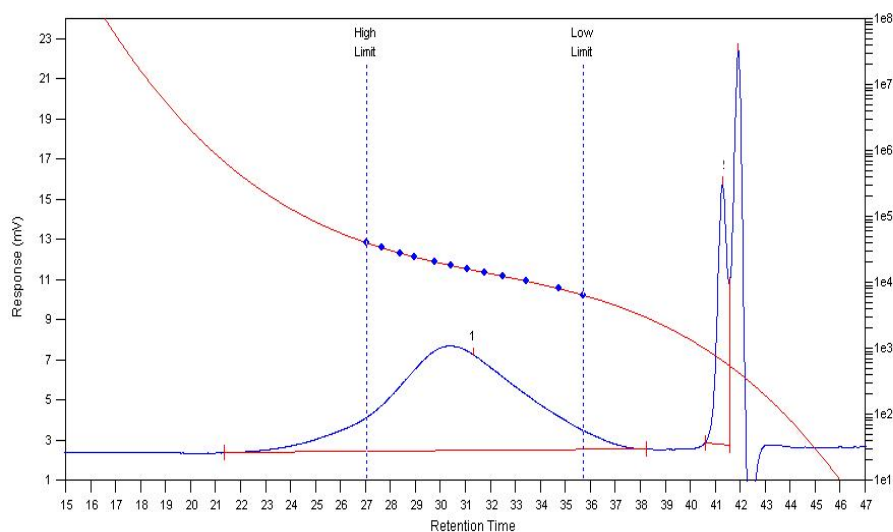
Molecular weight  $M$  measured using narrow standards plotted vs % mass above that value of  $M$ .



Five runs, same lab,  
same columns, over  
5 months.

# Sample Z as a Broad Standard

## Chromatogram of Z and calibration curve



Values from Narrow and Broad standard determinations, 3 UFH samples, 1 run.

	Mn NS	Mn BS	Mw NS	Mw BS
UFH1	12680	12645	16678	16595
UFH2	13026	12985	16101	16013
UFH3	11206	11180	14268	14201

This method efficiently transfers a MW calibration using a single reference material.

# Limitations of MW reference material



- The polydispersity of any UFH reference material is going to be only slightly larger than that of other UFH products, so that a small part of every UFH MW distribution will lie outside the calibrated range.
- If the trend to higher molecular weights for UFH continues, the proportion of material outside the high molecular weight end of the range will increase.

# Potential MW specifications



- Specifications for molecular weights of polydisperse polymers may be expressed in terms of 1) average molecular weights, or 2) proportion of the sample falling into particular molecular weight ranges.
- Bearing in mind the limitations in calibration using a UFH Broad Standard, the safe choice is to use option 2, specifying molecular weight ranges well within the calibrated range.
- For example, percent above 20,000 and below 10,000.

# Conclusions

- Characterisation of UFH molecular weight distribution by gel permeation chromatography needs either a reference material for calibration, or light scattering detection.
- A reference material which will calibrate well over the entire range of UFH molecular weights is difficult to make.
- A partial calibrant could be made using a UFH sample with high polydispersity; one such sample was a candidate for the 6<sup>th</sup> IS Heparin.
- In order to assure the acceptability of such a calibrant, a collaborative study will be needed

# Acknowledgements



- Donors of samples for 6<sup>th</sup> IS:
- Bioiberica
- Leo
- Opocrin
- Schering-Plough
- SPL
  
- John Hogwood, NIBSC