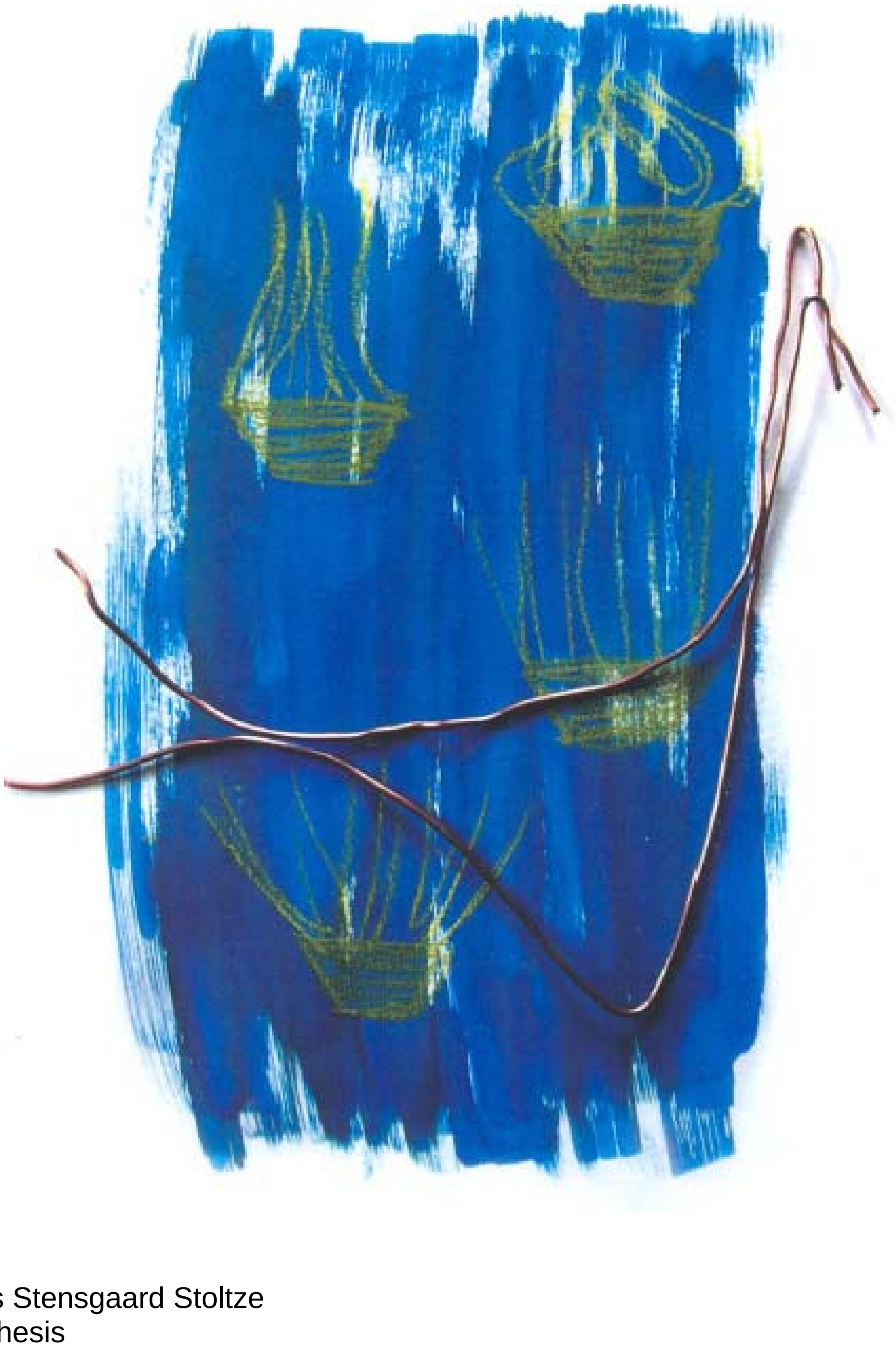
Investigation of Water Binding in Aqueous Solutions of Poly(ethylene glycol), Poly(propylene glycol) and Symmetric Triblock Copolymers of Poly(ethylene glycol) and Poly(propylene glycol)



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Preface

This M.Sc. thesis is based on the work carried out at the 9th and 10th semester at the Section of Chemistry, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University.

I would like to thank Associate Professor Søren Hvidt, Department of Science, Systems and Models, Roskilde University, for providing Pluronic copolymers to my experimental work.

The *Harvard System* (Author Date Method) is used for references throughout the thesis.

Table of Contents

Abstract

The aim of this thesis was to investigate the water binding in aqueous solutions of poly(ethylene glycol) (PEG), poly(propylene glycol) (PPG) and amphiphilic symmetric triblock copolymers of PEG and PPG by water activity measurements as function of temperature and concentration. Furthermore, Differential Scanning Calorimetry (DSC) was used to determine the critical micelle temperature (cmt) of the triblock copolymers. Eight different PEG polymers, one PPG polymer and three different triblock copolymers were applied; however the PPG polymer was omitted as it could not be dissolved properly.

The water activity measurements showed that the water activity increases with increasing temperature and decreasing concentration, i.e. water becomes a poorer solvent with increasing temperature because the polymer-water interactions are reduced. Excess enthalpies for the water binding were derived from the water activity measurements, but these are too large due to insufficient equilibrium time in the system.

The DSC measurements showed that at a given concentration the cmt increases with increasing hydrophilicity of the copolymer.

Resume (Danish Abstract)

Formålet med dette afgangsprojekt var at undersøge vandbindingen i vandige opløsninger af polyethylenglykol (PEG), polypropylenglykol (PPG) og amfifile symmetriske treblok copolymerer af PEG og PPG ved måling af vandaktivitet som funktion af temperatur og koncentration. Endvidere blev skanning kalorimetri (DSC) brugt til at bestemme den kritiske micelle temperatur (cmt) for treblok copolymererne. Otte forskellige PEG polymerer, en PPG polymer og tre forskellige treblok copolymerer blev anvendt; dog blev PPG polymeren undladt da den ikke kunne opløses ordentligt.

Vandaktivitetsmålingerne viste at vandaktiviteten stiger med stigende temperatur og aftagende koncentration, dvs. at vand bliver et dårligere solvent med stigende temperatur fordi polymer-vand interaktionerne reduceres. Overskudsentalpier for vandbindingen blev udregnet fra vandaktivitetsmålingerne, men disse er for store pga. utilstrækkelig ligevægtstid i systemet. DSC målingerne viste at ved en given koncentration stiger cmt med stigende hydrofil karakter af copolymeren.

1 Introduction

Protein behavior, such as enzyme activity, is a function of the thermodynamic activity and hydration of the macromolecule [Ng *et al.* 1990]. Thus, it is important to account for this non-ideal behavior when modeling such systems [Ng *et al.* 1990]. Many studies of the kinetics and equilibria of biochemical reactions have been done in dilute solutions, but as physiological media normally have a high macromolecular content, these studies are not applicable in describing the same reactions in the crowded cellular environment [Ng *et al.* 1990]. It has been suggested that the polymer poly(ethylene glycol) (PEG) is useful in such model studies of the interaction mechanisms of water with hydrophilic surfaces and macromolecules due to its unusual good solubility at high concentrations [Branca *et al.* 2002]. It is assumed that amphiphilic symmetric triblock copolymers of PEG and poly(propylene glycol) (PPG) are also useful in studies of the interaction mechanisms of water with macromolecules because of their ability to assemble in microstructures that resemble micelles [Alexandridis and Hatton 1995]. As PPG is a constituent of these copolymers, it is also useful to study this polymer.

With regard to macromolecules/polymers the solution is expected to be non-ideal due to both the large volume occupied by the polymer and specific water binding to the polymer. The non-ideality of aqueous solutions can be investigated by measuring the water activity, a_w, which is given by [Atkins and de Paula 2006; Clement *et al.* 2004]

$$a_w = \gamma_w x_w = \frac{p_w(T)}{p_w^*(T)} = RH \qquad (1)$$

where γ_w is the activity coefficient of water, x_w is the mole fraction of water, $p_w(T)$ is the partial vapor pressure of water at the temperature T, $p^*_w(T)$ is the saturated vapor pressure of water at the temperature T, and RH is the relative humidity above the solution. If the thermodynamic properties of the solution are ideal, the activity of water equals the mole fraction, i.e. $\gamma_w = 1$, and the measured effects are solely colligative [Blandamer *et al.* 2005].

It is seen from Equation 1 that at equilibrium the water activity of a solution is equal to the relative humidity above the solution. As it is fairly easy to measure the relative humidity of solutions, this enables one to conduct comprehensive studies of the water binding of polymer solutions.

The aim of this thesis is to investigate the water binding in aqueous solutions of PEG, PPG and amphiphilic symmetric triblock copolymers of PEG and PPG by water activity measurements as function of temperature and concentration.

To fulfill the aim stated above it is necessary to gain knowledge about the properties of the polymers in aqueous solution and how the water activity is expected to correlate with solution temperature and polymer concentration. Furthermore, a theoretical study of the interpretation of the water activity measurements is convenient.

2 Theory

First, a description of the polymers and their properties will be given, followed by the theoretical aspects of the interpretation of the water activity measurements.

2.1 Poly(ethylene glycol)

PEG, also known as poly(ethylene oxide) (PEO) or poly(oxyethylene) (POE), is a synthetic polyether of ethylene glycol. The polymers are identified by their average molecular weight, e.g. *PEG 200* for a polymer with an average molecular weight of 200 g/mol.
The PEG polymer has the general structure as shown in Figure 1, where the subscript *n* denotes the number of repeating units within the PEG molecule.

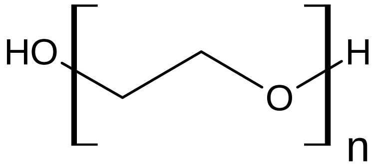

Figure 1: General structure of a PEG molecule. The subscript *n* denotes the number of repeating units in the PEG molecule.

The PEG-water system is well-studied due to the remarkable solubility of the polymer in water; actually it is soluble in all proportions at temperatures below the boiling point of water [Branca *et al.* 2002]. One of the most pronounced characteristics is the clouding behavior, i.e. the tendency for the polymer-water interactions to become less favorable with increasing temperature [Almgren *et al.* 1995]. Numerous explanations have been given to account for this behavior, and three will be discussed here [Almgren *et al.* 1995]. The first explanation is based on the properties of water; it is suggested that the PEG molecule fits well into the water lattice at room temperature. As the temperature is increased the water structure diminishes, and the compatibility between PEG and water is reduced. This could explain why PPG is not near as water soluble as PEG. But as the clouding behavior of PEG is observed in other polar solvents as well, this theory does not seem valid. A second explanation states that hydrogen bonding between the ether oxygen atoms of PEG and water stabilizes the PEG molecule. As the temperature is increased, the hydrogen bonds are broken and the polymer-polymer attractions become more pronounced. But clouding of PEG has been observed in a very weakly hydrogen bonding solvent (*tert*-butyl-acetate), which does not support this explanation. Furthermore, if this hypothesis is correct, poly(methylene glycol) (PMG) should be water soluble and exhibit clouding – which is not the case. A third model is based on the conformational properties of the -OCCO- sequence in the polymer chain. It is suggested that the majority of the possible conformations from rotation around the C-C bond are relatively non-polar, whereas only two are strongly polar. These two conformations have the lowest energy and interact most favorably with water. As the temperature is increased, the non-polar conformations are favored and will start to dominate the properties of the chain, which will make interactions with water less favorable. ^{13}C NMR studies have confirmed these non-polar conformations to be favored at high temperatures. The theory has the advantage that it can explain why PPG is water soluble (although to a lesser extent than PEG) and PMG is not; PMG does not contain the -OCCO- sequence in the polymer chain.

The fact that the polymer-water interactions become less favorable with increasing temperature will also have an impact on the water activity of a solution of PEG. It has been shown by Christiansen *et al.* [2007] that the water activity increases with increasing temperature and decreasing concentration.

Another issue that has caught a lot of attention is the determination of the number of water molecules bound to each monomeric unit of PEG. Studies by Makogon and Bondarenko [1985], Ng *et al.* [1990] and Branca *et al.* [2002] show that approx 2 water molecules are bound to every repeating unit at room temperature. Drying experiments of PEG performed by Christiansen *et al.* [2007] indicates that the number of water molecules bound per monomeric unit decreases with increasing temperature. This is in good agreement with the fact that the polymer-water interactions become less favorable with increasing temperature, and that the water activity of a PEG solution increases with increasing temperature.

2.2 Poly(propylene glycol)

PPG, also known as poly(propylene oxide) (PPO), is a synthetic polyether of propylene glycol. As with PEG the PPG polymers are identified by their average molecular weight, e.g. *PPG 1000* for a polymer with an average molecular weight of 1000 g/mol.

The PPG polymer has the general structure as shown in Figure 2, where the subscript *n* denotes the number of repeating units within the PPG molecule.

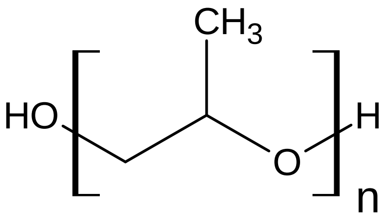

Figure 2: General structure of a PPG molecule. The subscript *n* denotes the number of repeating units in the PPG molecule.

PPG has a remarkable lower solubility in water than PEG. This is explained by the fact that the lower critical solution temperature is below the freezing point of water for high molecular weight polymers, whereas it is above room temperature for low molecular weight polymers [Almgren *et al.* 1995]. Figure 3 shows the temperature-concentration phase diagrams of PPG 1000 and PPG 2000 in water (data adapted from Weilby [2008] and Hvidt *et al.* [1994], respectively). Below the solid lines the system is a one-phase system whereas a two-phase system exists above the solid lines.

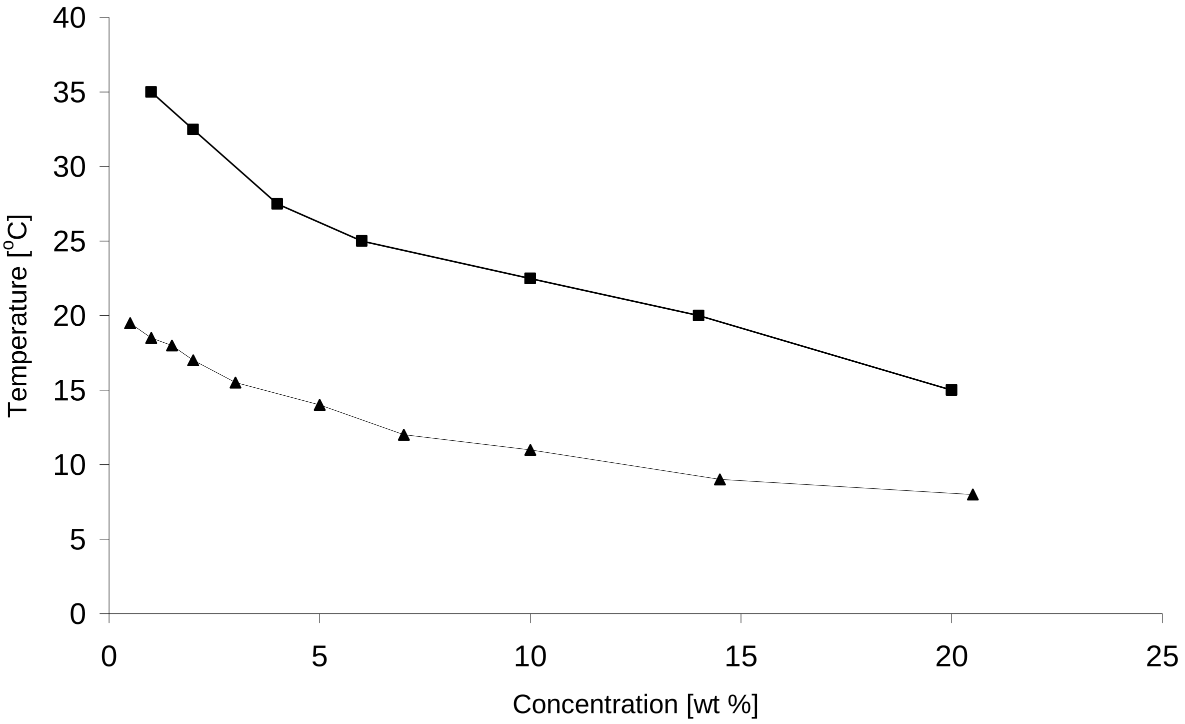

Figure 3: Temperature-concentration phase diagrams of PPG 1000 (-■-) and PPG 2000 (-▲-) in water. Below the solid lines the system is a one-phase system whereas a two-phase system exists above the solid lines. Adapted from Weilby [2008] (PPG 1000) and Hvidt *et al.* [1994] (PPG 2000).

As expected it is seen from Figure 3 that the higher molecular weight polymer PPG 2000 phase separates first as the temperature is increased. It has been shown that ΔH is positive and ΔC_p is negative for the phase separation of PPG and water [Hvidt *et al.* 2002]. As the phase separation is spontaneous, it must be accompanied by a gain in entropy. This can be explained by the breakdown of the ordered water lattice around the polymer, i.e. the PPG polymers are dehydrated during the phase separation.

In analogy to PEG the water activity of a PPG solution is expected to increase with increasing temperature and decreasing concentration, and above the cloud point it should be near unity as the polymer-water interactions are reduced significantly.

2.3 PEO-PPO-PEO Copolymers

Water-soluble symmetric triblock copolymers of PEO and PPO, often denoted $(EO)_x(PO)_y(EO)_x$, are non-ionic macromolecular surface active agents that are available in a range of molecular weights and PPO/PEO composition ratios. The surfactants are sold under the commercial names Poloxamers (manufactured by ICI) and Pluronics (manufactured by BASF) [Alexandridis and Hatton 1995]. In the Pluronic nomenclature the code for a polymer is given by a letter and two or three numbers, e.g. Pluronic P85 or Pluronic L101. The letter states the physical form of the copolymer at room temperature; L (liquid), P (paste) of F (flakes). The fist number (or the first two numbers in a three digit code) multiplied by 300 indicates the approximate molecular weight of the PPO block, and the last number multiplied by 10 gives the percentage weight fraction of PEO in the copolymer [Alexandridis and Hatton 1995].

The PEO-PPO-PEO triblock copolymers have the general structure as shown in Figure 4, where the subscripts *x* and *y* denote the number of ethylene oxide and propylene oxide units, respectively.

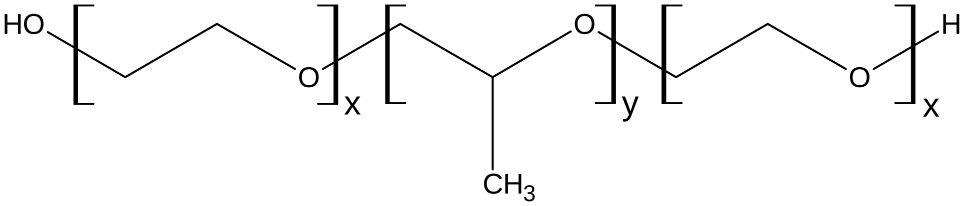

Figure 4: General structure of a PEO-PPO-PEO triblock copolymer. The subscripts *x* and *y* denote the number of ethylene oxide and propylene oxide units, respectively.

The most striking difference between the PEO-PPO-PEO triblock copolymers and the PEG and PPG polymers is the ability of the copolymers to form micelles and gels. This gives a more complex phase diagram as illustrated in Figure 5 where the temperature-concentration phase diagram of Pluronic P85 is shown [Glatter *et al.* 1994].

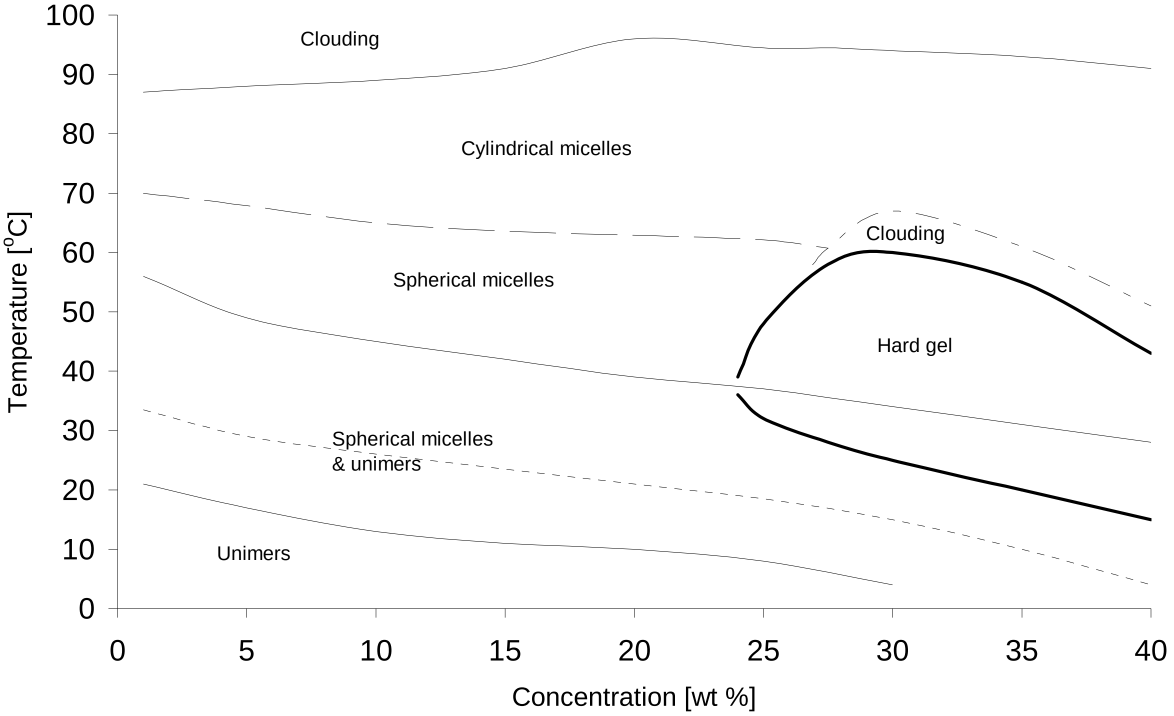

Figure 5: Temperature-concentration phase diagram of Pluronic P85. Adapted from Glatter *et al.* [1994].

At low temperature and concentration the copolymers are dissolved as unimers, but as the temperature is increased micelles start to form as indicated by the lower solid line in Figure 5. It is seen that the micellization occurs over a broad temperature interval between the lowest two solid lines. The dotted line in between indicates the maximum in micelle formation rate. Around 70 °C the shape of the micelles are changed as illustrated by the dashed line, and eventually the copolymer will phase separate at high temperatures as depicted by the upper solid line. At concentrations above 25 wt % the copolymer solution will form a hard gel when heated. This hard gel will ‘dissolve’ again above 60 °C as indicated by the bold solid line.

The micellization of symmetric triblock copolymers of PEO and PPO is believed to be a simple equilibrium process of the form [Hvidt 1995]

$$\text{n unimers} \leftrightarrows \text{micelle} \qquad (2)$$

where *n* denotes the number of unimers per micelle. The micellization process is endothermic (positive ΔH) and has a negative ΔC_p [Hvidt *et al.* 2002]. Since the micelles are stable the process must be characterized by a positive entropy change [Hvidt 1995]. From the equilibrium in Equation 2 it would be intuitively to predict a negative entropy change since micelles are more ordered structures than free unimers. Simultaneous Differential Scanning Calorimetry (DSC) studies of PEG 6000, PPG 2000 and Pluronic P94 have shown that PPG 2000 and Pluronic P94 undergo endothermic transitions (phase separation and micellization, respectively) with very equal onset temperatures, while PEG 6000 does not show any transition [Hvidt *et al.* 1994]. This gives the following explanation for the micellization process [Hvidt *et al.* 1994; Hvidt 1995; Hvidt *et al.* 2002]: At low temperature and concentration the copolymers are dissolved as unimers, and both the PEO and PPO part is hydrated. As the temperature is increased the PPO block becomes insoluble and undergoes a transition like it is seen in the phase separation of a PPG solution. The PEO blocks do not undergo any transitions but prevent the copolymer from phase separating by keeping the PPO block in solution as micelles, i.e. the micelles consist of a core of PPO and a hydrated mantle of PEO. The positive enthalpy and entropy change along with the negative change in the heat capacity is explained as a breakdown of the structured water around the PPO block and/or removal of hydrated water from the PPO block.

As for both PEG and PPG the water activity of a solution of these triblock copolymers is expected to increase with increasing temperature and decreasing concentration. Especially at the point of micellization, where the PPO core is dehydrated, it is expected that the water activity will show a sharp increase. The magnitude of this increase is believed to correlate with the enthalpy of micellization.

2.4 Interpretation of Water Activity Measurements

Two approaches will be used in interpreting the water activity measurements; the van't Hoff equation and the BET isotherm.

2.4.1 The van't Hoff Equation

To interpret the water activity measurements it is necessary to study the equilibrium for water in the system. Regardless of the type of polymer in solution there must be the following general equilibrium considering the adsorption of water to the polymer

$$H_2O_{(g)} \leftrightarrows H_2O_{(ads)} \qquad (3)$$

where $H_2O_{(g)}$ represents water vapor and $H_2O_{(ads)}$ water adsorbed to the polymer. The equilibrium constant, K, is given by

$$K = \frac{1}{p} \qquad (4)$$

where p is the partial pressure of water. The van't Hoff equation is then applied [Atkins and de Paula 2006]

$$\frac{d \ln K}{d(1/T)} = -\frac{\Delta_r H^\circ}{R} \qquad (5)$$

where T is the temperature, R is the gas constant, and $\Delta_r H^0$ is the standard reaction enthalpy. By inserting Equation 4 in Equation 5 one obtain

$$\begin{gathered} \frac{d \ln(1/p)}{d(1/T)} = -\frac{\Delta H_{ads}}{R} \\ \Updownarrow \\ \frac{d \ln p}{d(1/T)} = \frac{\Delta H_{ads}}{R} \end{gathered} \qquad (6)$$

where ΔH_{ads} is the reaction enthalpy of the reaction in Equation 3. From Equation 1 it is seen that $p = a_w \cdot p^*$, and by inserting this in Equation 6 one obtain

$$\begin{gathered}\frac{d \ln\left(a_w \cdot p^*\right)}{d(1/T)} = \frac{\Delta H_{ads}}{R} \\ \Updownarrow \\ \frac{d \ln a_w}{d(1/T)} + \frac{d \ln p^*}{d(1/T)} = \frac{\Delta H_{ads}}{R}\end{gathered} \tag{7}$$

The term $\frac{d \ln p^*}{d(1/T)}$ in Equation 7 refers to the enthalpy of condensation of pure water, and hence $\frac{\Delta H_{ads}}{R}$ can be expanded as follows

$$\frac{d \ln a_w}{d(1/T)} + \frac{d \ln p^*}{d(1/T)} = \frac{\Delta H_{excess}}{R} + \frac{\Delta H_{con}}{R} \tag{8}$$

where ΔH_{excess} is the excess enthalpy, i.e. the extra energy associated with water binding, and ΔH_{con} is the enthalpy of condensation of pure water (it has the same numerical value as the enthalpy of vaporization but opposite sign). Hence, by plotting $ln\ a_w$ as function of $1/T$ it is possible to find $\frac{\Delta H_{excess}}{R}$ as the slope of a linear fit (assuming that ΔH_{excess} is temperature independent). The value of ΔH_{excess} will be negative as energy is released during adsorption.
If the solution is ideal Equation 7 should be written as

$$\frac{d \ln x_w}{d(1/T)} + \frac{d \ln p^*}{d(1/T)} = \frac{\Delta H_{ads}}{R} \tag{9}$$

As the mole fraction of water does not depend on temperature the term $\frac{d \ln x_w}{d(1/T)}$ equals zero, and there is no excess enthalpy regarding the condensation/vaporization of water.

2.4.2 The BET Isotherm

It is believed that the water activity measurements can be interpreted using a BET-like isotherm. The BET isotherm accounts for multilayer adsorption to a surface and is given by [Atkins and de Paula 2006]

$$\frac{V}{V_{mon}} = \frac{cz}{(1-z)\{1-(1-c)z\}} \qquad \text{with} \quad z = \frac{p}{p^*} \qquad (10)$$

where V is the total volume of adsorbed material, V_{mon} is the volume corresponding to monolayer coverage, p is the vapor pressure, p^* is the vapor pressure above a layer of adsorbate that is more than one molecule thick and which resembles a pure bulk liquid, and c is a constant which is large when the enthalpy of desorption from a monolayer, $\Delta_{des}H^0$, is large compared with the enthalpy of vaporization, $\Delta_{vap}H^0$, of the liquid adsorbate

$$c = e^{\left(\Delta_{des}H^o - \Delta_{vap}H^o\right)/RT} \qquad (11)$$

Although the BET isotherm fits experimental data moderately well, it has the disadvantage that it underestimates the extent of adsorption at low pressures and overestimates it at high pressures [Atkins and de Paula 2006].
It is convenient to rewrite Equation 11 as proposed by Keiding and Pedersen [Unpublished note]

$$\frac{Y}{Y_{mono}} = \frac{k \cdot RH}{(1-RH)\cdot(1+(k-1)\cdot RH)} \qquad \text{where} \quad RH = \frac{p}{p^*} \qquad (12)$$

where Y is the water content [kg water/kg solid], Y_{mono} is the water content of the monolayer [kg water/kg solid], k is a constant reflecting the same properties as c in Equation 10, and RH is the relative humidity of the solution. If the function is inverted it gives the relative humidity of a solution as function of the water content, hence as function of the concentration of polymer. By fitting the isotherm to experimental data it is possible to determine Y_{mono} and k. On the basis of Y_{mono} the number of water molecules per monomeric unit, $\left(\frac{n_{\text{water}}}{n_{\text{PEG monomer}}}\right)$, can be calculated, and from the value of k the difference $\Delta_{des}H^0 - \Delta_{vap}H^0$ can be estimated as seen in Equation 11. This difference should be a measure of the extra energy needed to vaporize a water molecule from the monolayer compared to a water molecule in the bulk liquid.

3 Materials and Methods

Eight different PEG polymers with a broad molecular weight range and one PPG polymer (M_w = 2000) was used. The Pluronic copolymers P85, F87 and F88 were selected because PPG 2000 resembles the central PPO block (M_w ~ 2400 g/mol) and PEG 1000, 3000 and 4000 resembles the PEO blocks. Furthermore, these specific copolymers are well described in the literature.

The dry matter content of all stock reagents was determined, but besides this the polymers were used as received.

Differential Scanning Calorimetry (DSC) was used to determine the critical micelle temperature (cmt) and the enthalpy of micellization of the Pluronic copolymers at different concentrations to be able to identify the expected sharp increase in the water activity.

The relevant information of the applied polymers is listed in Table 1.

Table 1: Basic information about the applied polymers, including average composition, average molecular weight and supplier.

	Calculated Average Composition	Average Molecular Weight, M_w [g/mol]	Supplier
PEG 200	$HO\text{-}[CH_2CH_2O]_4\text{-}H$	200	Sigma-Aldrich
PEG 400	$HO\text{-}[CH_2CH_2O]_9\text{-}H$	400	Sigma-Aldrich
PEG 600	$HO\text{-}[CH_2CH_2O]_{13}\text{-}H$	600	Sigma-Aldrich
PEG 1000	$HO\text{-}[CH_2CH_2O]_{22}\text{-}H$	1000 (950-1050)[a]	Sigma-Aldrich
PEG 2000	$HO\text{-}[CH_2CH_2O]_{45}\text{-}H$	2000	Sigma-Aldrich
PEG 3000	$HO\text{-}[CH_2CH_2O]_{68}\text{-}H$	3000 (2700-3300)[a]	Sigma-Aldrich
PEG 4000	$HO\text{-}[CH_2CH_2O]_{90}\text{-}H$	4000 (3500-4500)[a]	Sigma-Aldrich
PEG 8000	$HO\text{-}[CH_2CH_2O]_{181}\text{-}H$	8000	Sigma-Aldrich
PPG 2000	$HO\text{-}[CH_2CHCH_3O]_{34}\text{-}H$	2000	Sigma-Aldrich
Pluronic P85	$EO_{25}PO_{41}EO_{25}$	4600	BASF
Pluronic F87	$EO_{60}PO_{41}EO_{60}$	7700	BASF
Pluronic F88	$EO_{103}PO_{41}EO_{103}$	11400	BASF

a) Molecular Weight Distribution, M_n [g/mol]

3.1 Determination of Dry Matter Content in Stock Reagents

To account for any water in the stock reagents the dry matter content was determined (duplicate determination) [Dansk Standardiseringsråd 1980]. Aluminum weighing vessels were dried in a furnace at 105 °C for two hours. Afterwards they were placed in a desiccator and weighed, and an appropriate amount of stock reagent was added. The weighing vessels were then dried in a furnace at 105 °C for at least 20 hours and finally weighed to determine the water loss.

3.2 Preparation of Polymer Solutions

Solutions of PEG, PPG and Pluronic copolymers were prepared gravimetrically using distilled water ($\rho = 18.2\ \mathrm{M\Omega{\cdot}cm}$), and the polymers were allowed to dissolve and mix at 5 °C with gentle shaking for at least 15 hours. After the preparation they were kept at 5 °C. An overview of the prepared polymer solutions is given in Table 2.

Table 2: Overview of the prepared polymer solutions. The nominal and corrected concentration refers to the fact that some solutions were prepared before the determination of dry matter content, i.e. the corrected concentration is the actual concentration of the solution. NB: The table continues on the next page.

No.	Sample ID	Reagent	Nominal Concentration [wt %]	Corrected Concentration [wt %]
1	PEG 200-40	PEG 200	N/A	40
2	PEG 200-60	PEG 200	N/A	60
3	PEG 400-40	PEG 400	N/A	40
4	PEG 600-40	PEG 600	N/A	40
5	PEG 1000-40	PEG 1000	N/A	40
6	PEG 2000-40	PEG 2000	N/A	40
7	PEG 2000-60	PEG 2000	N/A	60
8	PEG 3000-40	PEG 3000	N/A	40
9	PEG 4000-40	PEG 4000	N/A	40
10	PEG 8000-40	PEG 8000	N/A	40

11	PPG 2000-0.1	PPG 2000	0.1	0.1
12	PPG 2000-1	PPG 2000	1	1
13	PPG 2000-5	PPG 2000	5	5
14	PPG 2000-10	PPG 2000	10	10
15	PPG 2000-15	PPG 2000	15	15
16	PPG 2000-20	PPG 2000	20	20
17	P85-1	Pluronic P85	1	0.45
18	P85-5	Pluronic P85	5	2.23
19	P85-15	Pluronic P85	15	6.69
20	P85-30	Pluronic P85	30	13.38
21	F87-1	Pluronic F87	1	0.58
22	F87-5	Pluronic F87	5	2.88
23	F87-15	Pluronic F87	15	8.63
24	F87-30	Pluronic F87	30	17.25
25	F88-1	Pluronic F88	1	0.76
26	F88-5	Pluronic F88	5	3.82
27	F88-15	Pluronic F88	15	11.46
28	F88-30	Pluronic F88	30	22.92

Solutions no. 12-16 contained undissolved PPG after preparation, thus no experiments were made with PPG 2000.

3.3 Water Activity Measurements

The water activity was measured with an accuracy of ± 0.003 using a water activity meter (AquaLab 4TE, Decagon Devices, USA) which utilizes a cooled-mirror dew point sensor to determine the water activity of the sample.

This method is based on dew point depression, i.e. the difference between the temperature of the air and the temperature at which the air is saturated with water vapor (the dew point temperature) [Clement *et al.* 2004]. The sample is placed in the measurement chamber where it equilibrates with the surrounding air at the selected temperature [Decagon Devices 2008]. At equilibrium the relative humidity of the air inside the chamber equals the water activity of the sample. When measuring the air is cooled by a thermoelectric (Peltier) cooler, and the appearance of water on the mirror is detected by a photoelectric cell [Decagon Devices 2008]. The temperature at which condensation occurs (the dew point temperature) is recorded by a thermocouple attached to the mirror [Decagon Devices 2008]. The sample temperature and the dew point temperature are related to the relative humidity of the air, and hence the water activity of the sample, through the Clausius-Clapeyron equation [Atkins and de Paula 2006]

$$\frac{p}{p^*} = e^{-\frac{\Delta H_{vap}}{R}\left(\frac{1}{T}-\frac{1}{T^*}\right)} \qquad (13)$$

where p is the partial pressure at the temperature T, p^* is the partial pressure at the temperature T^*, ΔH_{vap} is the enthalpy of vaporization (assumed temperature-independent) and R is the gas constant. As $\frac{p}{p^*} = a_w$, the water activity of the sample is obtained directly from the dew point depression $\left(\frac{1}{T}-\frac{1}{T^*}\right)$, hence T is the sample temperature and T^* is the dew point temperature.

3.3.1 Water Activity as Function of Temperature

The water activity meter was placed in a climate room at 8 to10 °C as it had difficulties in cooling the samples below ambient temperature when placed in the laboratory. 5 mL of sample was transferred to a sample cup and placed in the measurement chamber of the device. The water activity was measured at 15 to 40 °C with 5 degrees interval. For sample no. 1, 3-6 and 8-10 three successive measurements were taken, and the mean value was used, while for sample no. 17, 20, 21, 24, 25 and 28 the water activity was measured until a stable value was obtained; defined as a change in ‘direction’ of the water activity over time. It was noticed that for sample no. 1, 3-6 and 8-10 there was a general tendency for the water activity to increase during the three successive measurements. All samples were weighed before and after the experiment to account for vapor loss (~ 1 %). Furthermore it was observed that sample no. 20, 24 and 28 had formed a gel at the end of the experiment.

3.3.2 Water Activity as Function of Water Content

The water activity meter was placed in the laboratory at ambient temperature. 3 mL of sample was transferred to a sample cup and placed in the measurement chamber of the device. The water activity of solutions no. 1, 2, 6, 7, 10 and the PEG 2000 stock reagent was measured continuously at 50 °C until a constant value was obtained; defined as three successive measurements that fluctuated no more than ± 0.0005 a_w. During the measurements a general tendency for the water activity to decrease towards this constant value was observed. Then 0.5 mL distilled water (ρ = 18.2 MΩ·cm) was added, and the procedure was repeated until maximum 3.5 mL water had been added. Before each addition of water the sample was weighed to account for vapor loss.

The *Solver Function* in Microsoft Excel was used to fit the measured data with the inverse function of Equation 12 by changing the values of k and Y_{mono}.

3.4 Differential Scanning Calorimetry

DSC is the measurement of the change of the difference in the heat flow rate between a sample and a reference sample while they are subjected to a controlled temperature program [Höhne *et al.* 2003]. The basic principle of this method is to heat/cool the sample and the reference at a desired rate. If the sample undergoes a phase transition, a differential temperature signal is generated which is proportional to the difference between the heat flow rates to the sample and reference. The differential temperature signal is then used to calculate the output heat flow rate [Höhne *et al.* 2003].

It is necessary to calibrate the instrument with regard to onset temperature of the transition and peak area which is done by the following procedure [Höhne *et al.* 2003]: At least two calibration standards, covering the temperature interval of interest, are used. The standard is measured at three different scanning rates, β, and the extrapolated peak onset temperature, T_e, is determined (defined as the intersection between the auxiliary line and the baseline). Then T_e is plotted as function of β and extrapolated to zero heating rate. This temperature, $T_e(\beta \rightarrow 0)$, is compared with the literature value of the transition temperature, and a temperature correction, $\Delta T_{corr}\ (\beta = 0)$, is established. This procedure is repeated for each standard, and $\Delta T_{corr}\ (\beta = 0)$ is plotted as function of $T_e(\beta \rightarrow 0)$. This curve will give the corrections to be applied to the measured values $T_e(\beta \to 0)$ of the samples. The peak area is calibrated by measuring on a standard and comparing the measured area with the literature value of the heat of transition.

3.4.1 Determination of CMT and Enthalpy of Micellization

A Perkin Elmer DSC 7 scanning calorimeter connected to a Perkin Elmer Thermal Analysis Controller, TAC 7/DX was used to study the heat capacity of solutions no. 17-28. Approx 30 mg of solution was placed in an aluminum pan which was sealed. Thermograms were obtained in heating scans at different rates (10 to 80 °C/min) at temperatures between -10 and 100 °C with an empty aluminum pan as reference. A water bath (TopTech FP50-ME, Julabo Labortechnik GmbH, Germany) set at -40 °C was used to cool the furnace, and the furnace was purged with nitrogen gas. The melting point of water and gallium was used for temperature calibration while water was used for peak area calibration. The enthalpies of transitions were calculated as the area of the peaks relative to the baseline, which was drawn as a straight line between the baselines prior to and after the transition, using the Pyris Software from Perkin Elmer.

4 Results

In the first section the water activity measurements of PEG will be presented followed by a section where the calorimetric data and water activity measurements of the PEO-PPO-PEO triblock copolymers are presented. The data from the determination of dry matter content can be found in Appendix A – Dry Matter Content of Stock Reagents. Furthermore, all data can be found on the enclosed CD-ROM.

4.1 Poly(ethylene glycol)

4.1.1 Water Activity as Function of Temperature

Figure 6 shows the water activity as function of temperature for all 40 wt % PEG solutions along with literature values for 40 wt % PEG 400 and 40 wt % PEG 8000 from Stanley and Strey [2003].

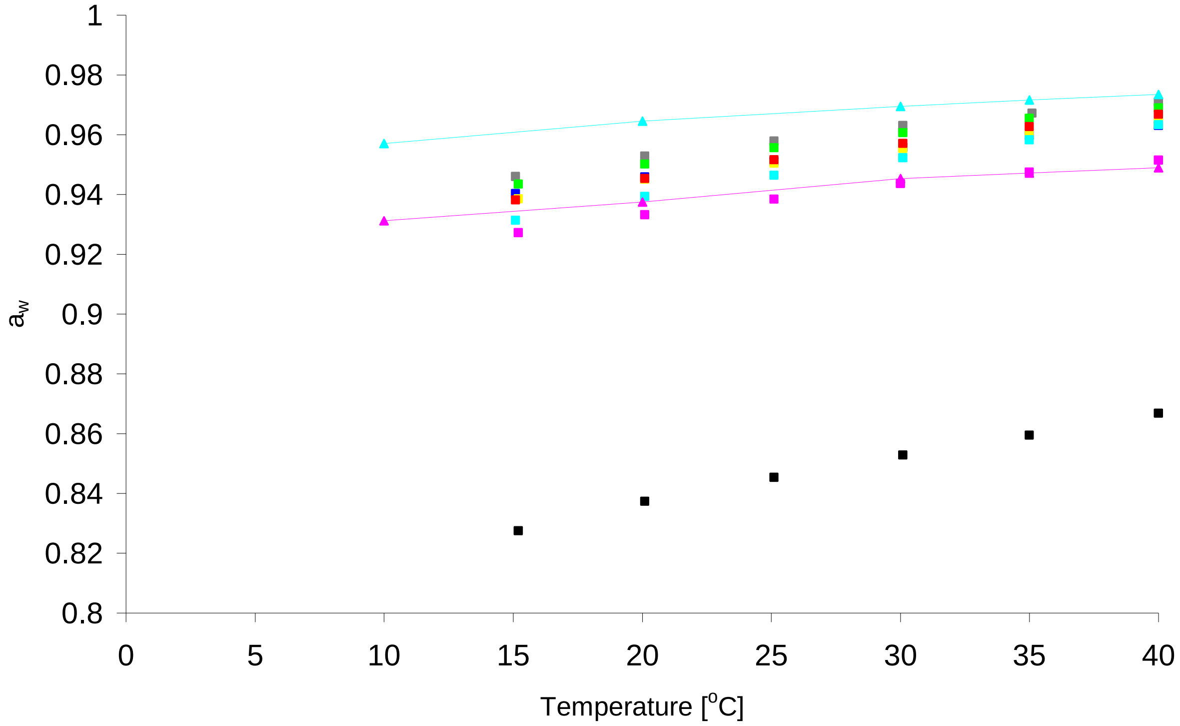

Figure 6: Water activity as function of temperature for PEG 200-40 (■), PEG 400-40 (■), PEG 600-40 (■), PEG 1000-40 (■), PEG 2000-40 (■), PEG 3000-40 (■), PEG 4000-40 (■), PEG 8000-40 (■). Literature values from Stanley and Strey [2003] are given for 40 wt % PEG 400 (-▲-) and 40 wt % PEG 8000 (-▲-).

A general trend in Figure 6 is that the water activity increases with increasing temperature for all 40 wt % PEG solutions. Additionally, it is seen that the water activity measurements of PEG 200-40 lies significantly lower than the measurements of the remaining PEG solutions. The water activity data of PEG 400-40 are in reasonable consistence with the values found in the literature, while this is not the case for PEG 8000-40.

The ΔH_{excess} values are presented in Table 3 along with the standard deviation and literature values from Stanley and Strey [2003] given in parentheses.

Table 3: Calculated values of ΔH_{excess} for the 40 wt % PEG solutions along with the standard deviation. Literature values from Stanley and Strey [2003] are given in parentheses.

	ΔH_{excess} [J/mol]
PEG 200-40	-1380 ± 24
PEG 400-40	-779 ± 15 (-481 ± 30)
PEG 600-40	-719 ± 16
PEG 1000-40	-805 ± 13
PEG 2000-40	-774 ± 18
PEG 3000-40	-809 ± 18
PEG 4000-40	-911 ± 17
PEG 8000-40	-1016 ± 18 (-417 ± 27)

It is seen from Table 3 that PEG 200-40 has the largest value of ΔH_{excess}. Otherwise, there is no correlation between the molecular weight of the PEG polymers and the ΔH_{excess} values.

In order to evaluate the data in Figure 6, other than from the literature values already given, the water activity measurements for all 40 wt % PEG solutions at 20 °C are converted to osmotic pressure, Π[1]. The osmotic pressure is then plotted against the molecular weight of the PEG polymers as seen in Figure 7. Literature data from Money [1989] for solutions of 100, 200 and 300 g/L, i.e. approx 10, 20 and 30 wt %, respectively, are given for comparison.

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[1] $\Pi = -\frac{RT}{V_m}\ln a_w$, where V_m is the molar volume of water [Atkins and de Paula 2006].

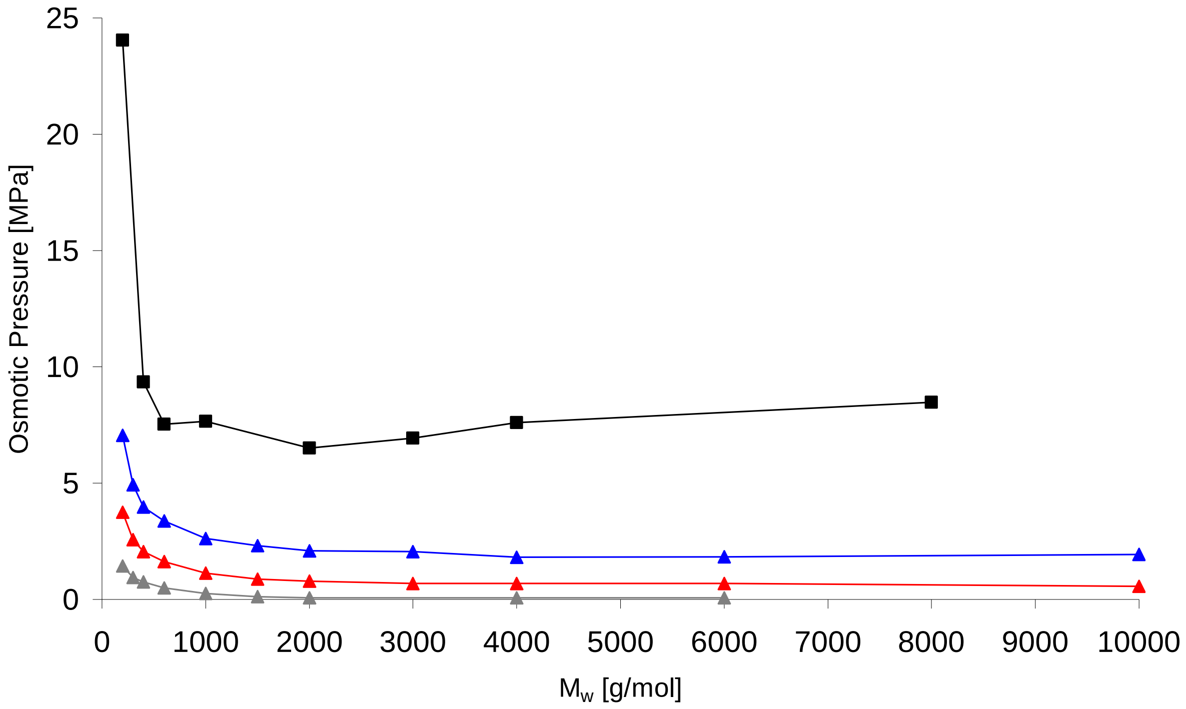

Figure 7: Osmotic pressure as function of molecular weight for the 40 wt % PEG solutions at 20 °C (-■-). Literature data from Money [1989] for solutions of 100 (-▲-), 200 (-▲-) and 300 g/L (-▲-), i.e. approx 10, 20 and 30 wt %, respectively, are given for comparison.

As indicated in Figure 7 the osmotic pressure of the 40 wt % solutions is higher than the osmotic pressure data from the literature at all molecular weights. Below 2000 g/mol the osmotic pressure has a tendency to increase with decreasing molecular weight, and the osmotic pressure increases with increasing concentration at a specific molecular weight.

4.1.2 Water Activity as Function of Water Content

Figure 8 shows the water activity as function of water content at 50 °C for PEG 200 (PEG 200-40 and PEG 200-60) along with the fitted BET-isotherm.

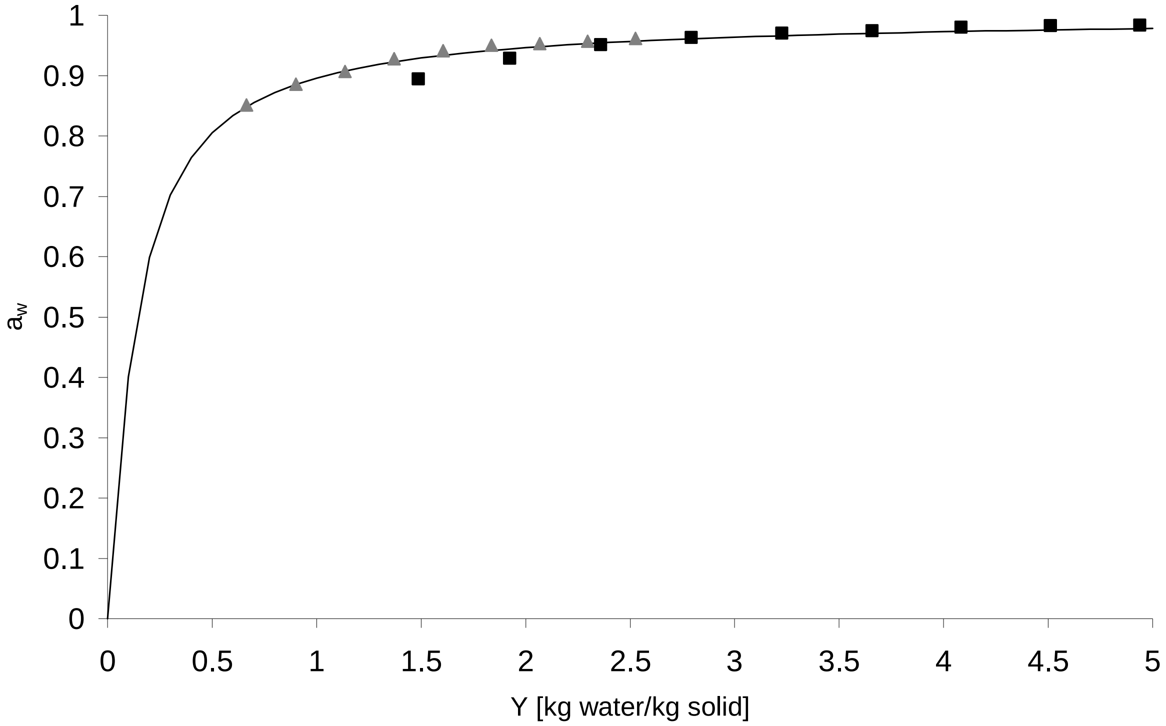

Figure 8: Water activity as function of water content at 50 °C for PEG 200 (PEG 200-40 (■) and PEG 200-60 (▲)) along with the fitted BET-isotherm (–).

Figure 9 shows the water activity as function of water content at 50 °C for PEG 2000 (PEG 2000-40, PEG 2000-60 and the stock reagent of PEG 2000) along with the fitted BET-isotherm.

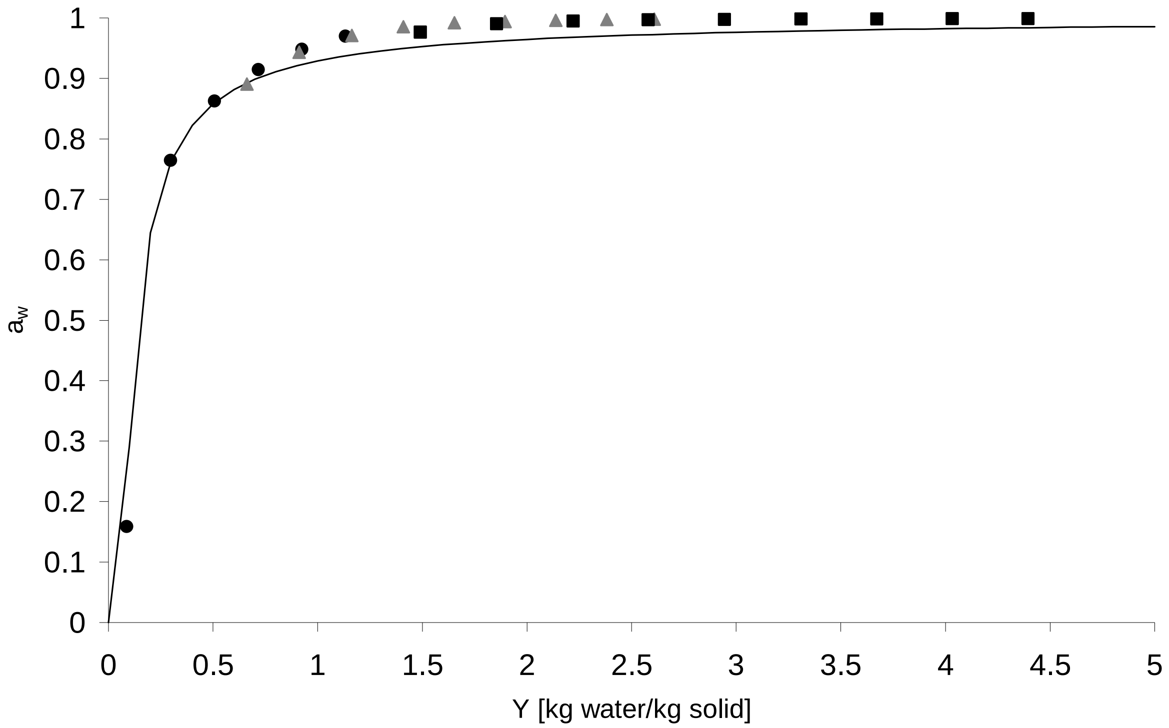

Figure 9: Water activity as function of water content at 50 °C for PEG 2000 (PEG 2000-40 (■), PEG 2000-60 (▲) and the PEG 2000 stock reagent (●)) along with the fitted BET-isotherm (–).

From Figure 8 and Figure 9 it is seen that the water activity decreases with decreasing water content, i.e. increasing polymer concentration. The values of *k* and Y_{mono} obtained from the modeling of the BET isotherm in Figure 8 and Figure 9 are given in Table 4 along with the calculated values of $\Delta_{des}H^0 - \Delta_{vap}H^0$ and $\left(\frac{n_{\text{water}}}{n_{\text{PEG monomer}}}\right)$.

Table 4: BET fitting parameters along with the derived values of $\Delta_{des}H^0 - \Delta_{vap}H^0$ and the number of water molecules per PEG monomer.

	k	Y_{mono} [kg water/kg solid]	$\Delta_{des}H^0 - \Delta_{vap}H^0$ [J/mol]	$\frac{n_{\text{water}}}{n_{\text{PEG monomer}}}$
PEG 200	1.75	0.11	1505	0.3
PEG 2000	2291	0.07	20776	0.18

It is seen from Table 4 that there is a large difference between the two *k* values, and hence a large difference between the values of $\Delta_{des}H^0 - \Delta_{vap}H^0$. The difference in Y_{mono}, and hence $\frac{n_{\text{water}}}{n_{\text{PEG monomer}}}$ is less pronounced.

Figure 10 shows the water activity as function of water content at 50 °C for PEG 8000-40 along with two data sets from Christiansen *et al.* [2007] obtained by another method where a sample of PEG 8000 is dried continuously at 50 °C. The data for PEG 8000-40 is not fitted with the BET isotherm.

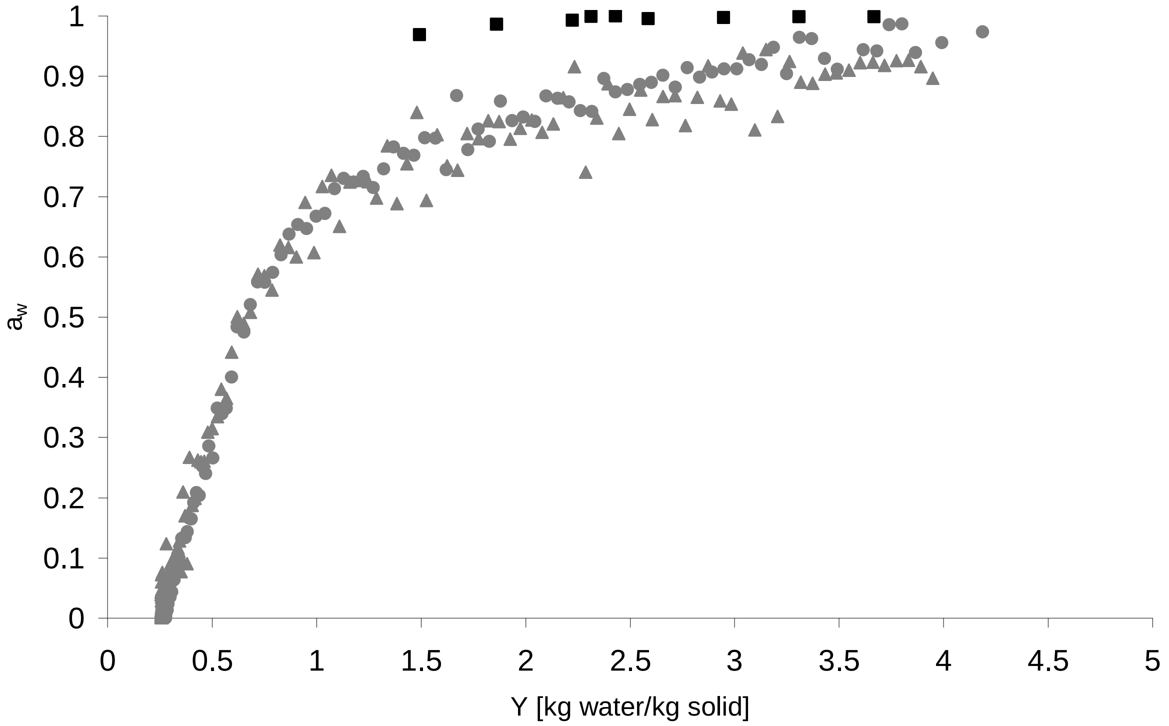

Figure 10: Water activity as function of water content at 50 °C for PEG 8000-40 (■) along with two data set (▲ and ●) from Christiansen *et al.* [2007] obtained by another method where a sample of PEG 8000 is dried continuously at 50 °C.

It is clearly seen from Figure 10 that the data for PEG 8000-40 lies significantly higher than the data from Christiansen *et al.* [2007]. The water activity of the literature data clearly decreases with decreasing water content, i.e. increasing polymer concentration.

4.2 PEO-PPO-PEO Copolymers

4.2.1 Determination of CMT and Enthalpy of Micellization

The extrapolated onset temperature of micellization as function of corrected concentration for Pluronic P85, F87 and F88 is shown in Figure 11 along with literature values for Pluronic P85 and F88 from Alexandridis *et al.* [1994].

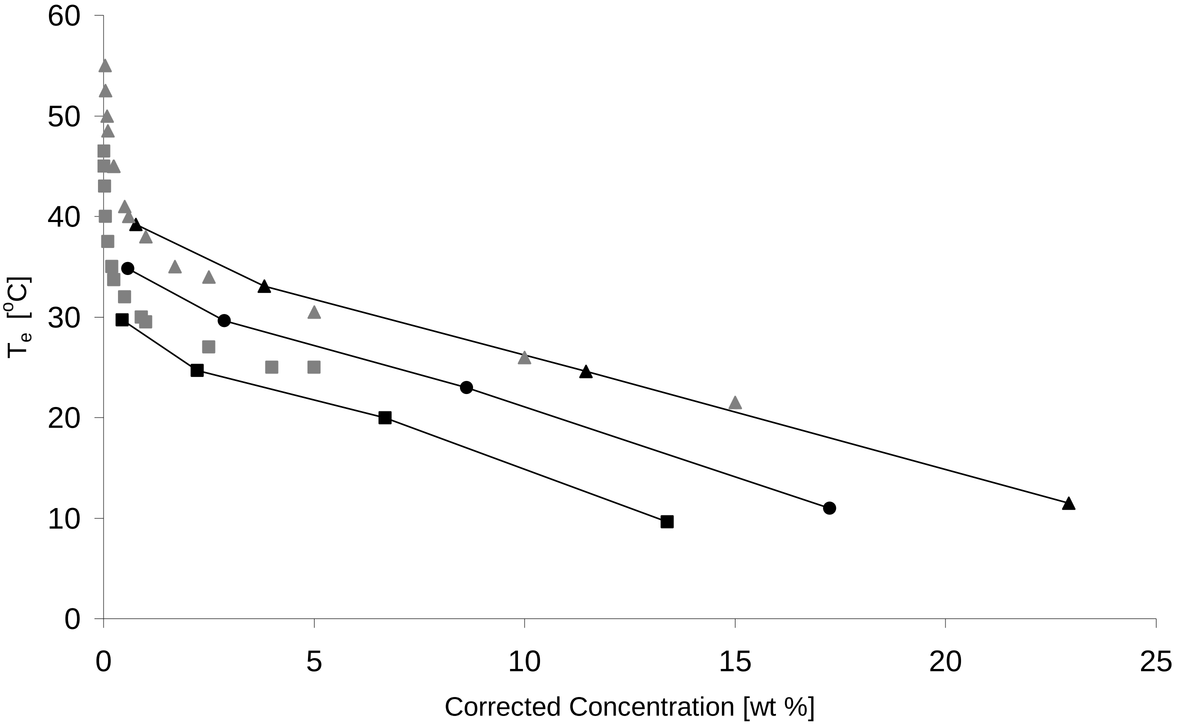

Figure 11: Extrapolated onset temperature of micellization as function of corrected concentration for Pluronic P85 (-■-), F87 (-●-) and F88 (-▲-) along with literature values from Alexandridis *et al.* [1994] for P85 (■) and F88 (▲).

It is seen from Figure 11 that both the critical micelle concentration (cmc) and the critical micelle temperature (cmt) increase in the order P85 < F87 < F88. The measured values for P85 lie slightly below the corresponding literature values while the values for F88 fit quite well.

The enthalpy of micellization as function of corrected concentration for Pluronic P85, F87 and F88 is presented in Figure 12.

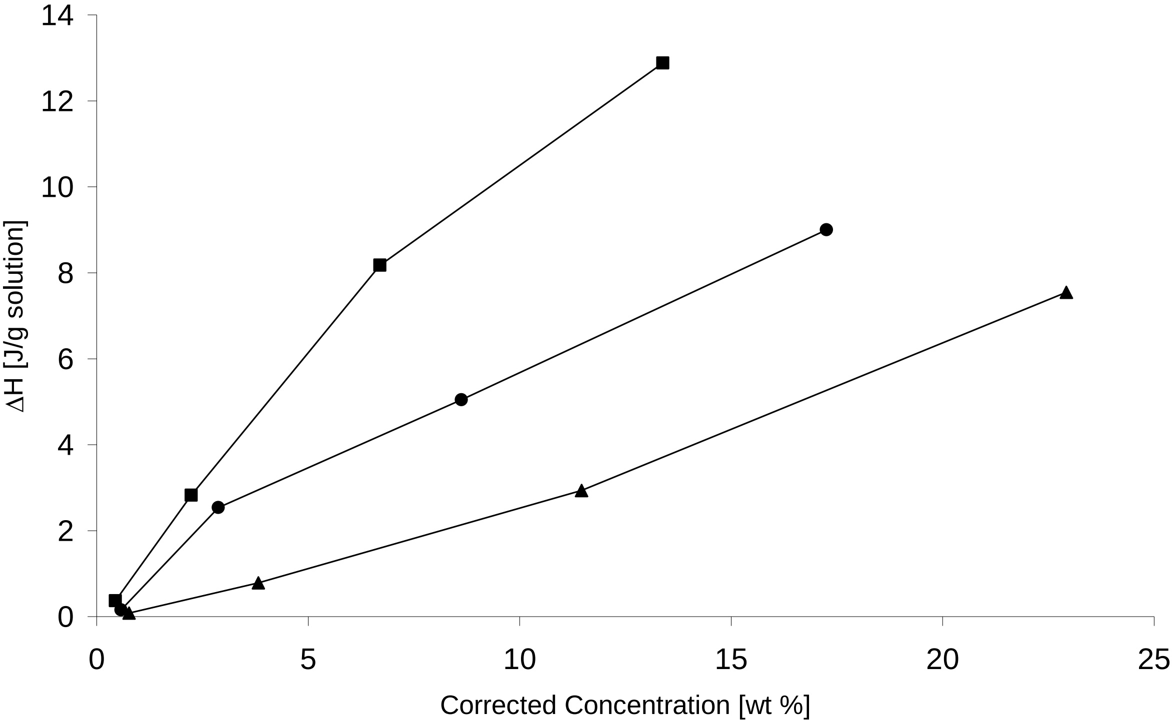

Figure 12: Enthalpy of micellization as function of corrected concentration for Pluronic P85 (■), F87 (●) and F88 (▲).

Figure 12 clearly indicates that at a given concentration the enthalpy of micellization increases in the order F88 < F87 < P85.

4.2.2 Water Activity as Function of Temperature

Figure 13 depicts the water activity as function of temperature for P85-1 and P85-30. The whiskers indicate the uncertainty of the water activity meter (± 0.003).

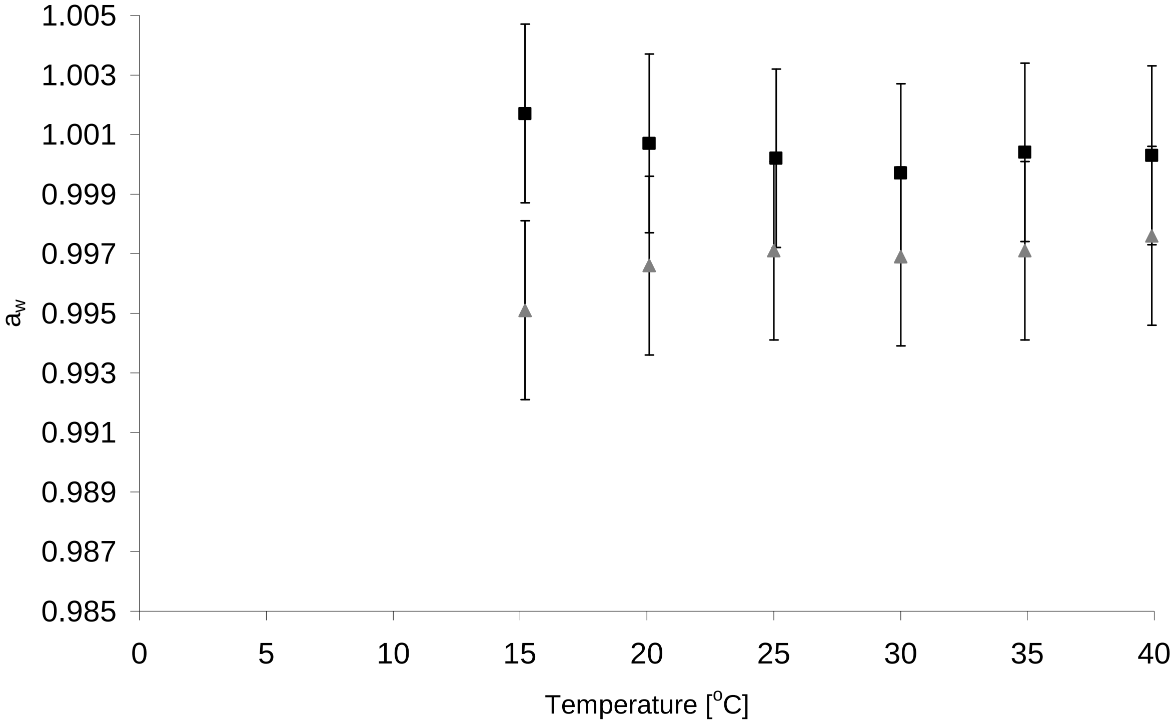

Figure 13: Water activity as function of temperature for P85-1 (■) and P85-30 (▲). The whiskers indicate the uncertainty of the water activity meter (± 0.003).

It is difficult to make any quantitative observations in Figure 13 as all measurements are above a water activity of 0.995 with an uncertainty of ± 0.003, thus it is neither possible to identify any sharp increase in the water activity at the cmt nor to determine ΔH_{excess}. However, there is a tendency for the measurements of P85-1 to have a higher water activity than P85-30. The data for F87-1, F87-30, F88-1 and F88-30 are very similar to those of P85-1 and P85-30 and are given in Appendix B – a_w Data for Pluronic F87 and F88.

5 Discussion

In this chapter the results are discussed and compared with the theory, and the methods are evaluated. The discussion is divided into sections according to the applied methods.

5.1 Water Activity Measurements

5.1.1 Water Activity as Function of Temperature

The comprehensive study of the water activity as function of temperature of 40 wt % solutions of eight different PEG's clearly demonstrates that the water activity increases with increasing temperature (Figure 6). This agrees well with the fact that the polymer-water interactions become less favorable, i.e. that water becomes a poorer solvent for PEG, with increasing temperature. However, it is seen from Figure 6 that the water activity measurements of PEG 8000-40 are lower than the literature values. An explanation to this could be that the reaction given in Equation 3 has had insufficient time to come to equilibrium. The samples were stored at 5 °C and the initial measurements in the water activity meter were done at 15 °C. This temperature change definitely causes the water activity of the sample to increase, but for the 40 wt. % PEG samples only three successive measurements were taken before the temperature was raised. Hence, the timescale for the sample to reach equilibrium is only 10 to 15 minutes at each temperature. This hypothesis is supported by the fact that the water activity measurements have a tendency to increase during these three successive measurements at each temperature, and by the fact that the measured values of both PEG 400-40 and PEG 8000-40 approach the literature values as the temperature is increased (see Figure 6). The former indicates that the water activity is increasing towards a constant value that is not reached, and the latter suggest that the equilibrium time decreases with increasing temperature. For PEG 8000-40 in particular it was observed that the sample was very viscous which could explain a long equilibrium time. To evaluate the measurements of the 40 wt % PEG solutions further the water activity data at 20 °C are converted to osmotic pressure and compared with literature values (see Figure 7). As expected the 40 wt % solutions have a higher osmotic pressure, i.e. a lower water activity, than the solutions of 10, 20 and 30 wt %. If the 40 wt % solutions should exhibit the same interrelationship as the solutions of 10, 20 and 30 wt %, it seems that the level of the measurements of the 40 wt % solutions is too high, especially for PEG 200 and

PEG 8000. Hence, the water activity measurements for the 40 wt % PEG solutions as function of temperature (Figure 6) are too low; in particular the measurements of PEG 200-40 and PEG 8000-40. The fact that the water activity measurements for the 40 wt % PEG solutions are too low due to insufficient equilibrium time, especially at low temperatures, has an impact on the derived ΔH_{excess} values (Table 3). As seen from the two reference values given for 40 wt % PEG 400 and 40 wt % PEG 8000, the calculated values are too high.

The water activity measurements as function of temperature for the Pluronic copolymers are too close to 1 to distinguish them from one another (see Figure 13 and Appendix B – a_w Data for Pluronic F87 and F88). Hence, it is not possible to identify any increase in water activity as a consequence of the micellization process. The reason that the water activity measurements are so close to 1 must be that the concentration of the copolymer solutions is too low. For P85-30, F87-30 and F88-30 it is also possible that the water activity is close to 1 because micellization already occurs below 15 °C as indicated in Figure 11. At high water activities it will be more convenient to measure the osmotic pressure of the solution as the accuracy is improved significantly, e.g. the osmotic pressure (at 25 °C) corresponding to a water activity of 0.995 is 6.81 atm while it is 1.36 atm for a water activity of 0.999.

It was observed that the samples P85-30, F87-30 and F88-30 had formed a gel at the end of the water activity experiments (45 °C). Considering the corrected concentration of P85-30 (13.4 wt %) this does not correspond to the phase diagram given in Figure 5 which states that a gel is not formed at a concentration below 25 wt %. However, Brown *et al.* [1991] have performed oscillatory shear measurements on Pluronic P85 that shows gelation in the concentration range 3 - 40 wt %. At 13.4 wt % the gelation temperature is reported to be approx 40-45 °C.

5.1.2 Water Activity as Function of Water Content

Water activity measurements as function of water content were done at 50 °C on PEG 200, PEG 2000 and PEG 8000, see Figure 8, Figure 9 and Figure 10, respectively. As expected the water activity decreases with decreasing water content. For PEG 200 it is seen that the two data points with the lowest water content for the PEG 200-40 series differ from the data points of the PEG 200-60 series. This is believed to be due to insufficient equilibrium time, although the equilibrium time in these experiments were significantly longer (hours) compared to the experiments where the water activity was measured as function of temperature (10-15 minutes). The lack of data points at low water contents has a great impact on the shape of the BET isotherm, and hence the values of k and Y_{mono}. Thus it is difficult to conclude anything about the $\Delta_{des}H^0 - \Delta_{vap}H^0$ values (Table 4) for PEG 200 and PEG 2000, although it seems implausible that the desorption energy is 21 kJ higher than the energy of vaporization of pure water. The number of water molecules per PEG monomer for both PEG 200 and PEG 2000, 0.3 and 0.18, respectively, is below 2 which is the expected value at room temperature. This agrees well with the fact that the water-polymer interactions become less pronounced with increasing temperature, i.e. the polymer is less hydrated at higher temperatures. Christiansen *et al.* [2007] found that the number of water molecules per PEG monomer is 0.7 for PEG 8000 at 50 °C which is higher than the values found for PEG 200 and PEG 2000.

The data for PEG 8000-40 (Figure 10) is not fitted with the BET isotherm due to lack of data points. Instead the data is compared with data from Christiansen *et al.* [2007] who has conducted drying experiments on PEG 8000. The literature data clearly shows a decrease in water activity with decreasing water content as expected. However, it is seen that the data for PEG 8000-40 does not fit the literature data. This is believed to be due to the differences between the two methods used to obtain the data. The method of Christiansen *et al.* [2007] is based on continuous drying of a PEG solution at constant temperature, while the method used in this thesis is based on stepwise addition of water to a PEG solution. The former method has the disadvantage that the equilibrium between water in the sample and in the air is disrupted constantly due to the continuous drying. Hence, the relative humidity of the air above the sample is too low. The method used in this thesis has the disadvantage that the system is disturbed every time water is added, but if the system is given sufficient time to equilibrate reliable values should be achieved.

5.2 Differential Scanning Calorimetry

As expected the data shows that the cmc and cmt increases in the order P85 < F87 < F88 (Figure 11), i.e. the values are shifted towards higher concentration/temperature the more hydrophilic the copolymer is. At a given concentration the enthalpy of micellization increases in the order F88 < F87 < P85 (Figure 12) as the number of copolymers, hence the number of PPO blocks, in solution increases with decreasing molecular weight of the copolymer.

6 Conclusion

Solutions of PEG and symmetric triblock copolymers of PEG and PPG were prepared at different concentrations and studied by water activity measurements as function of temperature and concentration. Additionally, the symmetric triblock copolymers of PEG and PPG were studied by DSC measurements.

The water activity measurements as function of temperature for the PEG solutions show that the water activity increases with increasing temperature, i.e. water becomes a poorer solvent with increasing temperature, giving excess enthalpies in the range -719 to -1380 J/mol. These excess enthalpies are too large due to insufficient equilibrium time in the system. The water activity measurements as function of concentration for the PEG solutions show that the water activity increases with decreasing concentration, but fitting with the BET isotherm is problematical due to lack of data points, especially at low water contents. The water activity measurements as function of temperature for the symmetric triblock copolymers of PEG and PPG are all above 0.995 and impossible to distinguish due to the uncertainty of the water activity meter. The DSC measurements of the symmetric triblock copolymers of PEG and PPG show that both the cmt and cmc increase in the order P85 < F87 < F88 and that the enthalpy of micellization at a given concentration increases in the order F88 < F87 < P85.

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8 Appendix A – Dry Matter Content of Stock Reagents

The results from the dry matter determination of the stock reagents are given in Table 5 along with the standard deviation.

Table 5: Average dry matter content of the stock reagents along with the standard deviation.

	Average Dry Matter Content [wt %]
PEG 200	71.7 ± 1.5
PEG 400	92.0 ± 1.5
PEG 600	94.9 ± 0.5
PEG 1000	91.1 ± 1.0
PEG 2000	91.5 ± 0.1
PEG 3000	89.9 ± 0.6
PEG 4000	87.3 ± 0.1
PEG 8000	83.5 ± 0.6
PPG 2000	99.8 ± 0.1
Pluronic P85	44.6 ± 18.2
Pluronic F87	57.5 ± 3.8
Pluronic F88	76.4 ± 0.7

It is noticed that the standard deviation for Pluronic P85 is relatively high.

9 Appendix B – a_w Data for Pluronic F87 and F88

Figure 14 shows the water activity as function of temperature for F87-1 and F87-30. The whiskers indicate the uncertainty of the water activity meter (± 0.003).

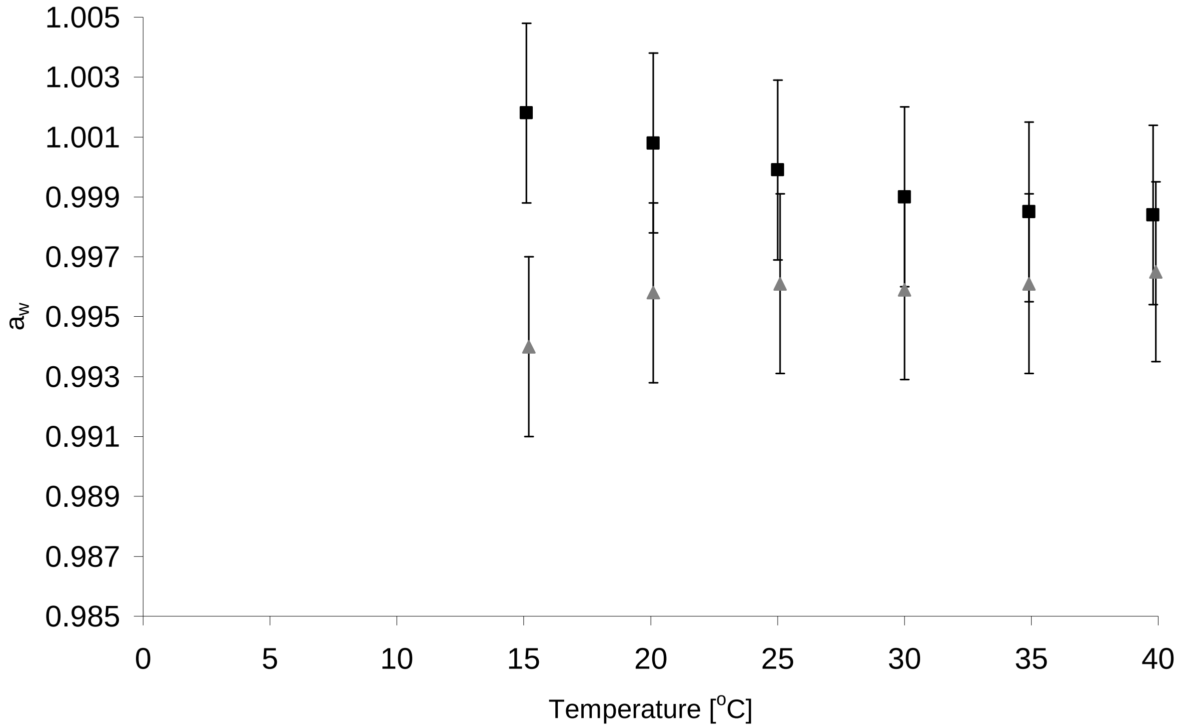

Figure 14: Water activity as function of temperature for F87-1 (■) and F87-30 (▲). The whiskers indicate the uncertainty of the water activity meter (± 0.003).

Figure 15 shows the water activity as function of temperature for F88-1 and F88-30. The whiskers indicate the uncertainty of the water activity meter (± 0.003).

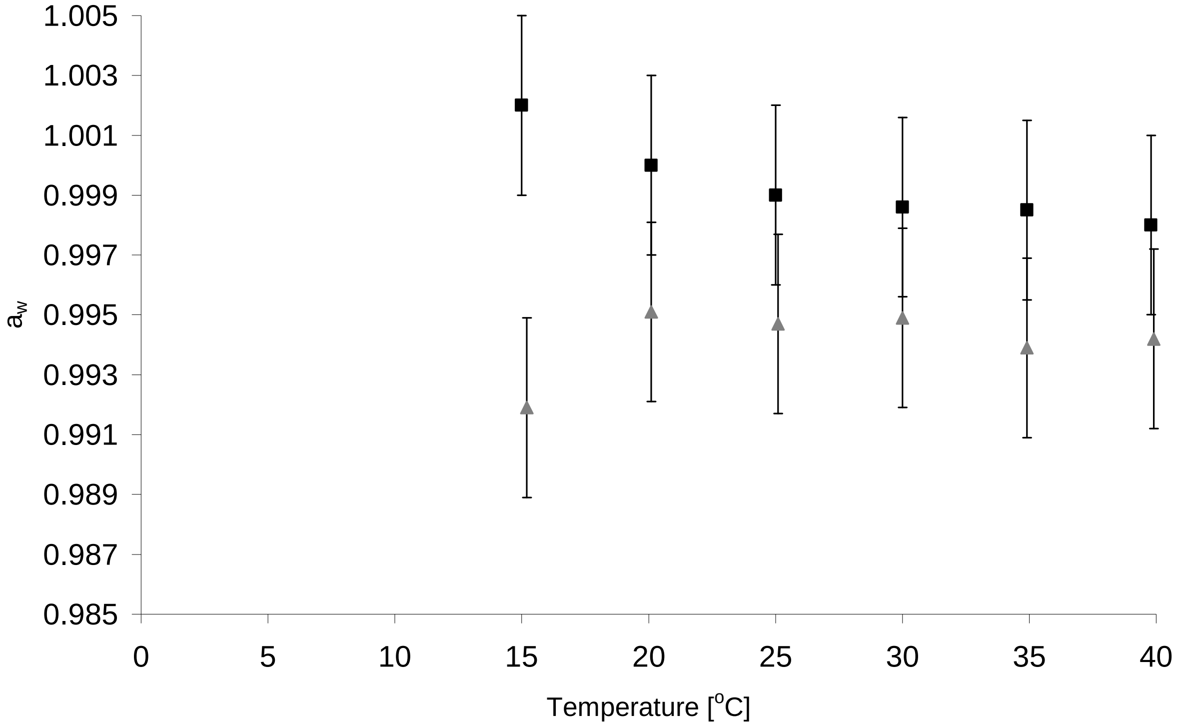

Figure 15: Water activity as function of temperature for F88-1 (■) and F88-30 (▲). The whiskers indicate the uncertainty of the water activity meter (± 0.003).