INTERACTIONS BETWEEN SURFACTANTS AND STARCH: FROM STARCH GRANULES TO AMYLOSE SOLUTIONS

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On the front cover: Scanning electron microscope image of wheat starch granules
To my mom
ABSTRACT

Starch is a mixture of two polysaccharides, amylose (AM) and amylopectin, which occurs naturally in the form of microscopic granules that are abundantly found in tubers, roots, cereal grains and fruits. In order to bring out their functional properties as thickeners and texture enhancers, starch granules are often disrupted by heating in excess water. This process, which is referred to as gelatinisation, causes the granules to swell and exude a fraction of the starch polysaccharides, resulting in a dramatic increase in the viscosity of the starch suspension. Surfactants are known to affect the different aspects of the gelatinisation process and, in particular, the swelling properties of starch. Surfactants are also known to form helical inclusion complexes with AM, the formation of which plays an important role in many of the instances in which starch and surfactants interact. This work was carried out in order to gain insight into how the surfactant structure (head group and chain length) influences the swelling properties of starch and the molecular mechanisms behind these effects. The investigations involved the study of the temperature-induced gelatinisation of starch in the presence of surfactants as well as studies on the association of surfactants to AM in solution and the solubility of the resulting AM-surfactant complexes.

Information on the extent of granule swelling upon heating was indirectly obtained by means of viscometry while insight on the molecular events taking place during gelatinisation was sought by means of differential scanning calorimetry (DSC) and confocal laser scanning microscopy (CLSM). Viscometric studies revealed that, with the exception of the cationic surfactants (alkyl trimethyl ammonium bromides), short-chain (C₁₀, C₁₂) surfactants induce an early swelling (swelling at lower temperatures than the control sample) in normal wheat starch granules, whereas their longer chain counterparts (C₁₄, C₁₆) have the opposite effect. Contrary to this finding, the effect of surfactants on the swelling of waxy wheat starch granules, an AM-free starch variety, is not influenced by the surfactant chain length but by the head group charge of the surfactant. The enhancing/restricting effect of surfactants on the swelling of waxy wheat starch granules, an AM-free starch variety, is not influenced by the surfactant chain length but by the head group charge of the surfactant. The enhancing/restricting effect of surfactants on the swelling of normal wheat starch is not correlated to their effect on the early aspects of gelatinisation (onset of the gelatinisation transition) but is, in most cases, associated with the dissociation temperature of AM-surfactant complexes formed simultaneously as the granules gelatinise. CLSM studies revealed that, compared to a longer-chain surfactant (C₁₆), a short-chain (C₁₂) surfactant has the ability to penetrate further into the granule matrix during gelatinisation, which may favour its availability for interacting with different starch granule components during gelatinisation.

Studies on the interactions between AM and surfactants with different chain length (C₁₂ vs. C₁₆) and head group (sodium sulphates vs. maltosides) revealed that the presence of a charged head group favours the water solubility of the resulting AM-surfactant complexes. However, this effect can be counteracted by the effect of the surfactant chain length: an increase in the chain length (C₁₂ vs. C₁₆) decreases the solubility of the complex.
SAMMANFATTING

Stärkelse är en blandning av två polysackarider; amylos och amylopektin. Stärkelse finns i naturen i mikroskopiska granuler som förekommer i rikliga mängder i rötter, rotknölär, spannmål och frukt. För att utnyttja stärkelsekornen som förtjockningsmedel eller konsistensgivare upphettas de i ett överskott av vatten. Genom denna process, som kallas gelatinisering, sväller granulerna samtidigt som vissa polysackaridfraktioner avyttras, vilket resulterar i en dramatisk viskositetsökning hos stärkelsesuspensionen. Tensider kan användas för att påverka olika aspekter av gelatiniseringsprocessen, och i synnerhet svällningsegenskaperna hos stärkelsen. Egenskaperna hos heliska inklusionskomplex med amylos spelar en viktig roll för denna process.

Detta arbete utfördes i syfte att klargöra effekten av tensidens huvudgruppskemi och kolkedjelängd på svällningsbeteendet hos stärkelsesuspensioner samt att utröna de bakomliggande molekylära mekanismerna. Undersökningarna inbegrep studier av gelatiniseringsprocessen, och lösligheten av de bildade tensid/amylos komplexen.


Studier av interaktionen mellan amylos och tensider med olika kolkedjelängd (C₁₂ och C₁₆) samt olika huvudgrupp (sulfat och maltosid) demonstrerade att en laddad huvudgrupp förbättrar vattenlösligheten hos amylos-tensidkomplexen. Detta kan dock motverkas genom en förlängning av kolkedjan som ger upphov till en sämre vattenlöslighet hos komplexen.
LIST OF PAPERS

This thesis is based on the following papers which will be referred to in the text by their roman numerals:

I. **Effect of surfactant structure on the pasting properties of wheat flour and starch suspensions.**
   Isabel Mira, Ann-Charlotte Eliasson and Karin Persson.

II. **On the effect of surface active agents and their structure on the temperature-induced changes of normal and waxy wheat starch in aqueous suspension. Part I. Pasting and calorimetric studies.**
    Isabel Mira, Karin Persson, V. Kurtis Villwock
    Carbohydrate Polymers, 2006, in press.

III. **On the effect of surface active agents and their structure on the temperature-induced changes of normal and waxy wheat starch in aqueous suspension. Part II. A confocal laser scanning microscopy study.**
    Isabel Mira, V. Kurtis Villwock, Karin Persson
    Carbohydrate Polymers, 2006, in press.

IV. **Binding of surfactants to amylose in aqueous solution: Part I. Dodecyl and hexadecyl maltosides**
    Isabel Mira, Karin Persson, Per M. Claesson
    Manuscript.

V. **Binding of surfactants to amylose in aqueous solution. Part II. Sodium dodecyl and hexadecyl sulphate.**
    Isabel Mira, Fredrik Hallberg, Karin Persson, István Furó, Per M. Claesson
    Manuscript.

I have conducted all the experimental work presented in these papers with the exception of the $^1$H NMR experiments in Paper V which were performed by my co-authors in Paper V, Fredrik Hallberg and István Furó.
Interactions between Surfactants and Starch: from Starch Granules to Amylose Solutions

Other papers by the author:


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Interactions between Surfactants and Starch: from Starch Granules to Amylose Solutions
1. INTRODUCTION

The term starch is used to refer to a mixture of two polysaccharides, i.e., polymers made up of sugar molecules, that are synthesized by plants as energy reserves. Starch is stored most abundantly in tubers (e.g., potato), roots (e.g., the sweet potato), the grains of cereals (e.g., wheat, corn, barley) and fruits. In all these, starch is found in the form of microscopic granules whose size, shape and characteristics are dependent on their botanical origin.

Starch is the cheapest priced and most abundant worldwide commodity. It is also one of the major nutrients in the human diet and finds its largest use in food-related applications. In this respect, starch is commonly used to enhance texture and viscosity in a wide variety of food products. However, starches have also found many applications as adhesives, paper and fabric coating agents, stabilizers and viscosity modifiers in paints and fluids used in the drilling of oil wells, just to mention a few.

For thickening food, during the baking process, for sizing and coating papers, sizing textiles, and use in drilling fluids or adhesive formulations as well as in a host of other applications, starch gelatinization in aqueous media is a first step. This process, which is commonly induced by heating, involves the disruption of the ordered structures within the starch granules and causes the extensive swelling of the granules and the leaching of the linear fraction of the starch polysaccharides (amylose). The increase in the volume fraction of the starch granules and the release of the starch polysaccharides into solution leads to an increase in the viscosity of the starch suspension, which is then referred to as a paste. Surfactants are well-known for their ability to affect different aspects of the starch gelatinisation process and, in particular, the swelling properties of starch and the characteristics of the resulting starch pastes.

Due to their relevance in many food-related applications, most investigations concerned with the effect of surfactants on the swelling and pasting properties of starch have involved the use of food-grade emulsifiers such a long-chain (C_{14}-C_{18}) monoglycerides and esters of sucrose. However, in an attempt to gain insight on the interaction mechanisms involved, non-food grade surfactants have also been used in model systems. Overall, despite the great deal of work that has been carried out in the area and the number of surfactants that have been studied in different investigations, no clear trends have emerged on the influence of the surfactant chemical structure on the effect of surfactants on the swelling properties of starches. This is partly due to the great difficulty to compare results from different studies which arises from i) the non-equilibrium nature of the swelling process, ii) the different susceptibility of starches of different botanical origins to the effect of surfactants and iii) the use of food-grade emulsifiers, which are commonly mixtures of different molecular species. Further, these complications have slowed down the increase in understanding of the interaction mechanisms involved.

This investigation has been carried out with the intention of gaining insight into the effect of surfactants on the swelling and pasting properties of starch and the interaction mechanisms involved in the swelling-enhancing effects of some surfactants and the swelling-restricting effects of others. In order to do this, the studies involved both the investigation of the interactions between surfactants and starch granules during gelatinisation and the study of interactions between surfactants and amylase (AM), the linear fraction of the starch polysaccharides, in solution. The latter was an important aspect of the investigation as
surfactants are well-known to form complexes with AM\textsuperscript{10-13} and the formation of these complexes plays a central role in many of the instances in which surfactants and starch interact.

In this work, the studies of starch gelatinisation involved the use of wheat starch, a common cereal starch largely used in breadmaking applications. Initial studies included a number of different food and non-food grade surfactants, but the number of these was reduced as the investigation proceeded and allowed for the identification of the most relevant surfactant structural features to be studied.
2. BACKGROUND

2.1. STARCH: FROM MOLECULES TO GRANULES

The term starch often applies to a mixture of two polymers: an essentially linear fraction, amylose (AM) and a branched counterpart, amylopectin (AMP). In both molecules glucose is the basic building block. These two polysaccharides are synthesised as energy reserves by many plant species. They occur naturally in a broad array of plant tissues in the form of water-insoluble, semi-crystalline granules, whose characteristics are dependent on the botanical origin. The nature and characteristics of the two starch polysaccharides will be briefly discussed before addressing those of the starch granules and some of the underlying processes that this work has been concerned with.

2.1.1. THE STARCH POLYSACCHARIDES: AMYLOSE AND AMYLOPECTIN

General features

Amylose (AM) is an essentially linear molecule consisting of ~ 99% (1-4) and ~1% (1-6) α-linked anhydroglucose units. On the other hand, Amylopectin (AMP) is a much larger, heavily branched molecule containing ~95% (1-4) and ~5% (1-6) α-linked anhydroglucose units (see Figure 1). Amylopectin is one of the largest biopolymers known with typical molecular weight (Mw) being in the range of $10^8$ g/mol. However, in spite of its large molecular weight, AMP is relatively compact due to its highly branched nature. Current models of the structure of amylopectin depict short linear chains, 10 to 20 units long, arranged in clusters on longer chains, with the longer chains spanning more than one cluster \(^{14}\) (see Figure 2). The molecular size, shape, structure and polydispersity of AM and AMP varies with botanical origin. The typical characteristics of these two polysaccharides are summarised in Table 1.

![Molecular Structure of Amylose and Amylopectin](image.png)

**Figure 1.** Molecular structure of amylose and amylopectin. Adapted from Tester et al.\(^{15}\)
Interactions between Surfactants and Starch: from Starch Granules to Amylose Solutions

Figure 2. The two-dimensional cluster model of amylopectin with short chains arranged in clusters on longer chains. A-chains, only linked to the rest of the molecule through its potential reducing end; B-chains, linked in the same way as A-chains but also carrying one or more A-chains; C-chains, carries the sole reducing group of the molecule. (Adapted from Parker et al.16)

Table 1. Typical characteristics of the two starch polysaccharides, amylose and amylopectin.

<table>
<thead>
<tr>
<th>Property</th>
<th>Amylose</th>
<th>Amylopectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular structure</td>
<td>Essentially linear</td>
<td>Highly branched</td>
</tr>
<tr>
<td>Molecular weight (Mw) (g/mol)</td>
<td>~1x10^5 - 1x10^6 a</td>
<td>~1x10^7 – 1x10^9 a</td>
</tr>
<tr>
<td>Degree of polymerisation (DP)</td>
<td>320 – 4900 a</td>
<td>9600 – 15900 a</td>
</tr>
<tr>
<td>No. of glucose units per chain b</td>
<td>n= ~1000 a</td>
<td>a = ~ 12-23 a</td>
</tr>
<tr>
<td>A-chains/ B-chain c</td>
<td>--</td>
<td>b = ~ 20-120 a</td>
</tr>
</tbody>
</table>

a Data from Tester et al. 15
b n, a and b defined as in Figure 1
c A and B-chains defined as in Figure 2
d Data from Parker et al. 16

Solution properties

In the starch polysaccharides the (1-4) α-linkages impart a gradual twist to the molecules which has important consequences for their solution behaviour. The solution conformation of AM has long been the subject of extensive research and is still a matter of certain controversy. The current view of AM in neutral aqueous solutions is that the average conformation resembles a highly disordered coil which involves many discernible sequences of short range helical structures that are irregular and labile.17

Two features of AM in solution have been recognized to be of particular interest to its functionality in starch-based products.18 The first one is its ability to form helical inclusion complexes when an appropriate ligand is present (the characteristics of such complexes will be discussed in more detail in the next sections of this thesis). One of the most well-known of those complexes is the one formed between AM and polyiodide ions in aqueous solution at room temperature.19 This association gives rise to a dark-blue complex which confers AM solutions a characteristic blue colour. The ability of AMP to form such complexes is much more restricted and association of this molecule with iodine in solution results in red-brown complexes. This difference is the basis for what has become one of the most widely used methods for identification of AM and AMP.
The second characteristic feature of AM in solution is related to its propensity to interchain associations mediated by local ordering of the polysaccharide chains. This makes AM solutions more unstable in comparison to AMP solutions. The occurrence of interchain associations in AM also results in the phenomenon termed retrogradation which takes place upon cooling of hot aqueous solutions of AM and which is believed to involve the subsequent crystallization of the AM molecule.

Beyond a certain critical concentration (∼1 w % for AM and ∼20 w % for AMP) the two starch polysaccharides have a tendency to form gels. Gelation occurs as a result of the formation of an interconnected network. Molecular association and/or crystallization that occur in gels results in hydrogen-bonded physical cross links. AM gels are stiff due to involvement of the molecule in more than one crystallite whereas AMP gels are soft due to fewer intermolecular interactions resulting from its cluster shape.

2.1.2. STARCH GRANULES

Composition

Starch granules can be found in a variety of sizes (∼1-100 μm in diameter), shapes (round, lenticular, polygonal) and size distributions (uni- or bi-modal) which are characteristic of the botanical source. A micrograph depicting the characteristic shape and size of wheat starch granules is shown in Figure 3.

![Figure 3](image)

Figure 3. Scanning electron micrograph of wheat starch granules. The granules exhibit the typical bimodal size distribution of wheat starches. The characteristic lenticular-shaped, big (>10 μm) A-granules and the spherical, small (<10 μm) B-granules can be clearly identified in the micrograph. Scale bar: 20 μm.

The two starch polysaccharides, amylose and amylopectin, constitute ∼98-99% of the dry weight of the starch granule. The ratio of the two polysaccharides varies according to the botanical origin of the starch. According to their overall AM content, starches can be classified as “waxy” (less than 15% AM), “normal” (20-35% AM) and “high” (amylo-) amylose starches (more than ∼40% AM).

The minor components of starch consist mainly of lipids and proteins. Despite being present in small amounts, both lipids and proteins have important effects on the functionality of starch granules. Lipids can be found mostly in cereal starches, where they are positively correlated with amylose content and may represent ∼1.5% of the granule. Starch lipids exist both in the form of surface and internal lipids. The surface lipids are mainly triglycerides,
free fatty acids, glycolipids and phospholipids whereas the internal lipids consist essentially of lysophospholipids and free fatty acids. The protein content in purified starches is typically < 0.6%. As with starch lipids, proteins occur both on the surface and within the matrix of the granules. Internal proteins have a higher molecular weight than surface proteins (~50-150 and ~15-30 kDa, respectively) and include residues of enzymes involved in starch synthesis, especially starch synthase. Starches also contain relatively small amounts (<0.4%) of minerals such as calcium, magnesium, phosphorous, potassium and sodium.

**Granule structure and organization**

The structure of native starch granules is currently thought to be hierarchically organised on four length scales: molecular (~Å); lamellar structure (~9 nm); growth rings (~100 nm) and the whole granule morphology (~μm). A schematic representation of a starch granule showing these different levels of organisation is shown in Figure 4. The crystalline regions in the starch granule consist of double helices of amylopectin, which are radially packed in either A-type (i.e. monoclinic) or B-type (i.e. hexagonal) arrays. The lamellae consist of alternating regions of amorphous and crystalline material whose total periodicity is ~9 nm in all species yet examined. Semi-crystalline zones made up of these lamellae, alternate, with a periodicity of ~ 100 nm, with amorphous zones in which amylopectin molecules are in a less organised state and in which much of the amyllose fraction is believed to exist. The repeating distance between these alternating zones of semi-crystalline and amorphous material represent what has been defined as growth rings (see Figure 4).

![Figure 4](image)

**Figure 4.** Schematic representation of the different structural levels of the starch granule and the involvement of amyllose and amylopectin. Reprinted from Buléon et al. with permission from Elsevier

As a consequence of the different levels of internal organization, native starch granules not only have a semi-crystalline character but also appear birefringent when viewed under
polarised light. The birefringence of the granules appears to be mostly due to the orientation of the molecular chains in the amorphous region and not to the crystallinity per se.\(^{20}\)

### 2.2. The Temperature-Induced Gelatinisation of Starch Granules

Starch is usually processed by heating in the presence of water. Such treatment induces a series of structural changes in the starch granules which are collectively referred to as gelatinisation. The gelatinisation process can be defined as the water-mediated disruption of the different levels of order within the starch granule. As a result of this loss of order, the granules swell irreversibly to many times their original size while at the same time AM, if present, is preferentially leached. Although the nature of the changes that starch granules undergo during gelatinisation is the same for all starches, the characteristics and extent of these changes, as well as the temperature interval over which they take place, are specific to their botanical origin. Figure 5 shows the typical temperature intervals over which the characteristic events associated with gelatinisation are observed in normal wheat starch.

Due to the large number of independent variables involved, such as botanical origin, growth conditions, extraction procedures, water content, heating rate and thermal history, the detailed mechanisms involved in the gelatinisation process proved to be surprisingly subtle and hard to elucidate even after exhaustive investigations with a number of different techniques.\(^{39-43}\) Results from recent investigations, in which the stages that occur during the gelatinisation of a range of starches in excess water have been followed by a combination of techniques (small and wide angle light scattering, calorimetry and small angle neutron scattering), have permitted a better insight.\(^{35}\) During the first stages of gelatinization, water enters the amorphous growth rings, and it is there where all the initial swelling forces are concentrated. The periodicity of the semicrystalline stack remains unchanged as long as the crystallites can still be identified. Then, as the temperature is raised further, the crystallites become destabilised and the crystallization index decreases progressively to zero. Concurrently with this loss of crystalline order, the molecular (i.e. double-helical) order within the granule is also lost.\(^{35, 40}\)
2.2.1. THE THERMAL TRANSITION ASSOCIATED WITH STARCH GELATINIZATION

Some of the changes taking place during the gelatinisation of starch involve thermal transitions. These thermal events are conveniently studied by means of differential scanning calorimetry (DSC), which is the most commonly used technique in studies of starch gelatinization. Thermal transitions associated with starch gelatinisation in the presence of excess water (i.e. > 70%) are well-known to occur as a single endothermic event in DSC thermograms.40 The DSC thermogram displays the heat flow as a function of temperature as starch is heated. An example of such thermogram is shown in Figure 6.

![Figure 6. DSC thermogram obtained during the gelatinization of wheat starch at a starch to water ratio of 1:3 and a scanning rate of 6°C/min.]

The DSC-endothermic transition takes place at temperatures similar to those at which structural changes are observed (see Figure 6). The enthalpy associated with this endothermic transition was for long considered to correspond quantitatively to the melting of the crystallites in the starch granules. However, experimental evidence from 13C NMR, X-ray diffraction and calorimetric studies has indicated that the enthalpy of gelatinization primarily reflects the loss of double-helical order (i.e. the unravelling of the double helices).40

2.2.2. GRANULE SWELLING AND LEACHING OF POLYSACCHARIDES

The swelling of the starch granule and the leaching of polysaccharides are often regarded as the final stages in the gelatinisation process, as they require the prior loss of at least some of the ordered structures within the starch granule. If present in the granules, AM will be preferentially leached over AMP during the process.26 The granule swelling and polysaccharide leaching processes have been shown to be highly correlated and the experimental evidence indicates that they cannot be altered independently.5, 7, 26, 44

The extent and rate of granule swelling, as well as the characteristic morphological changes that granules undergo as a result of the swelling, are specific to their botanical origin. Cereal starches, for example, are known to swell in two stages.5, 45 During the first stage (~60-75°C), granule swelling due to water uptake and the leaching of AM are rather limited. During the second stage (~75-95°C), extensive swelling and AM-leaching take place. Figure 7, shows a series of micrographs depicting the swelling pattern of wheat starch granules as they are heated in excess water. In these sequences of images it is possible to recognize the typical “saddle shape” that wheat starch granules develop as they swell.5, 46
Some insight into the swelling properties of starch can be gained by considering the granules as a network structure. However, the main limitation of this approach has been recognised to reside in the fact that, in contrast to the equilibrium swelling of polymer networks, the swelling of starch during gelatinisation is a profoundly non-equilibrium and irreversible process. This is illustrated by the observation that starches that have been subjected to different thermal histories, such as slow versus rapid heating, swell to different extent. As a consequence of this, if direct information on the granule-swelling and polysaccharide-leaching properties of starches at different temperatures is to be obtained, it is common practice to let the starches swell in excess water, in the absence of mechanical shear, at the desired temperature for at least 30 min. The time required for starches to reach their equilibrium swelling conditions at a given temperature will vary depending on the type of starch and, of course, the temperature. Wheat starch granules, for example, have been reported to exhibit a period of rapid (5-10 min) swelling, followed by further small increases in swelling that could span over a period of 5 hrs.

From the determination of the swelling properties of starches of different botanical origins, certain general trends have been identified regarding the relation between swelling power and starch granule composition. Starches with high AMP content, i.e. waxy varieties, swell much more rapidly and extensively than starches with normal or low AMP contents. The more restricted swelling of starches with normal or low AMP contents, has been found to be related not only to the presence of higher amounts of AM but also to a higher lipid and protein content. Removal of lipids and proteins present near or at the granule surface has indeed been found to result in an enhanced rate and extent of granule swelling, indicating that these components play an important role in restraining the swelling properties of starch granules.

2.2.3. PASTING OF STARCH SUSPENSIONS

As a consequence of the swelling of the starch granules and the leaching of polysaccharides, the flow behaviour of a granule slurry changes significantly as the suspension becomes a dispersion of swollen granules, partly disintegrated granules and molecularly dispersed granule contents. The latter is what is commonly referred to as a starch paste, although the term “particle gel” has also been used. The transition from a suspension of granules to a paste, i.e. the “pasting”, is accompanied by a large increase in apparent viscosity.

The starch pastes have been described as composite materials built up from a continuous polysaccharide phase with starch granule remnants as fillers. The properties of these pastes
have been found to depend not only on the characteristics of the dispersed and the continuous 
phase (i.e. deformability of the swollen granules and the polysaccharide concentration, 
respectively) but also on the interaction between the two phases.\textsuperscript{44, 45, 53} Upon cooling, the 
apparent viscosity of the paste increases due to the molecular association of leached or 
dispersed polysaccharides which results in a cross-linked network that increases the resistance 
of the paste to deformation. If the dispersion of the starch granule and its content has occurred 
to a great extent, these pastes can be regarded as macromolecular gels, where the mechanisms 
governing the solution behaviour of the polysaccharides will be of importance.\textsuperscript{18}

\textbf{Pasting curves}

Starch pastes are non-Newtonian fluids, whose rheological behaviour have been the subject of 
exhaustive investigation both by means of classical rheometers\textsuperscript{45, 48, 53, 54} and instruments that 
probe specific, relevant properties related to their end-use application. Some of the most 
widely-used of these instruments are the viscometers of the Brabender Viscoamylograph type, 
which record the torque required to balance the viscosity that develops when a starch slurry is 
subjected to a heating and cooling cycle under controlled shearing conditions. These 
viscometers are routinely used to characterise the pasting or “cooking” properties of starches 
as well as the properties of the hot paste and their behaviour upon cooling. The characteristic 
pasting curve that concentrated (> 6\% w/v) starch suspensions develop along the controlled 
temperature program is shown in Figure 8. This curve displays the apparent viscosity of the 
sample (i.e. the viscosity at a constant shearing rate) ($\eta_{\text{app}}$) and the temperature as a function 
of time.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8.png}
\caption{Typical pasting curve of a starch 
aqueous suspension showing some of the 
commonly measured parameters.}
\end{figure}

Early in the pasting test, the temperature is below that at which the gelatinisation of starch 
begins and the viscosity is low. As the temperature is raised, starch granules gelatinise and the 
viscosity of the suspension increases dramatically, essentially due to the mutual friction of 
highly swollen starch granules.\textsuperscript{44, 55} This point reflects the onset of the extensive swelling 
stage of the starch granules and denotes the beginning of the pasting process. After the onset 
of the pasting, the viscosity rises as the temperature is further increased, until it reaches a 
maximum or peak viscosity ($\eta_{\text{PEAK}}$). This peak in viscosity occurs at the point where the 
effects of further changes in granule swelling (which increases viscosity) and granule rupture 
and alignment of the leached amylose (AM) molecules in the direction of shear (which reduce
the viscosity) balance each other. Up to this point, the starch paste behaves very much like a suspension of solid particles, whose viscosity is mainly dominated by the particle volume fraction. Beyond the point of maximum viscosity, the viscosity of the starch paste decreases mainly due to the further disruption of the granules as they are subjected to constant high temperatures and mechanical shear. This process leads to a minimum in viscosity, also termed breakdown viscosity ($\eta_{\text{BREAK}}$). As the sample is subsequently cooled, re-association between leached starch polysaccharides (particularly AM) occurs, resulting in high sample viscosities at the end of the pasting program (final viscosity ($\eta_{\text{FINAL}}$)).

#### 2.3. SURFACTANTS AND THEIR INTERACTIONS WITH STARCH

#### 2.3.1. SURFACANTS AND THEIR BEHAVIOUR IN AQUEOUS SOLUTION

Surfactants are molecules that consist of two parts, one that is polar (or hydrophilic) and another one that is non-polar (or hydrophobic). Due to their dual nature, these molecules are also commonly referred to as amphiphiles, a word derived from the Greek words *amphi*, which means “both”, and *phile*, which means “friend”. The hydrophilic part of the surfactant is usually referred to as the “head group” and the hydrophobic part as the “tail” (see Figure 9). Surfactants are commonly classified on the basis of the charge of their polar head group. In consequence, surfactants can be anionic, cationic, non-ionic or zwitterionic, the latter class corresponding to surfactants that contain both an anionic and a cationic charge under normal conditions.

![Figure 9. Schematic illustration of a surfactant molecule](image)

As a consequence of their dual polar/non-polar nature, surfactant molecules cannot satisfy their dual affinity neither in a polar solvent, nor in non-polar one. This makes surfactant molecules prone to adsorb at surfaces and interfaces formed by two immiscible phases, where both parts of the molecule can locate themselves in the phase they have more affinity for. By doing so, surfactants lower their free energy and the one of the interface. The interfacial free energy per unit area is commonly referred to as interfacial tension and it can be taken to represent the amount of work required to expand the interface.

A second important consequence of the amphiphilic character of surfactants is their tendency to self-associate as their concentration increases in solution. In aqueous solution, the main driving force for this association is the hydrophobic effect. This effect is due to the fact that water molecules interact more strongly with each other than with hydrocarbons. Thus, the non-polar tails tend to associate creating structural aggregates termed micelles. The first formed micelles are often spherical and the concentration at which these start to form is referred to as the critical micelle concentration, cmc. The micellisation phenomenon occurs as a compromise between the effects that favour aggregation, i.e. the hydrophobic effect, and
those that oppose to it, namely the electrostatic and/or the steric repulsion between surfactant head groups.

2.3.2. INTERACTIONS BETWEEN SURFACTANTS AND STARCH POLYSACCHARIDES

Due to their central role in many food-related applications, most of the research on starch-surfactant interactions has involved the use of food-grade emulsifiers such as long chain (C\textsubscript{14}-C\textsubscript{18}) monoglycerides and esters of sucrose. Better-characterised, more water-soluble surfactants such as sodium dodecyl sulphate (SDS) or alkyl trimethyl ammonium bromides, have also been commonly used in model systems. In the starch literature the first group of surfactants is commonly referred to as “emulsifiers”, whereas the latter ones are sometimes referred to as “detergents”. Moreover, the term “polar lipid” is commonly used to refer to nature’s own surfactants and is thus applied to phospholipids and fatty acids that occur naturally in starch granules. No such distinctions will be made in this thesis, and the term “surfactant” will be used to refer to molecules with an amphiphilic character regardless of their actual nature or the application they are mostly used for.

The amylose-surfactant complex

The association between AM and surfactants is thought to involve complexed molecules located within single-helical conformations of the AM. There is strong evidence for this picture from X-ray diffraction studies of crystalline complexes in the solid state\textsuperscript{57-60} and some complementary support from structural studies in aqueous solution.\textsuperscript{61, 62} A molecular modelling representation of the AM-lipid complex is presented in Figure 10.

The ability of surfactants to form complexes with amylose is thought to reside in their capacity to satisfy the solvation requirements of the AM helical cavity (ca. 0.5 nm in diameter) which has a hydrophobic character. The helical cavity in AM has characteristics similar to those of cyclodextrins but it has higher flexibility in size and length of the guest binding sites. The complexed state represents an energetically favourable situation both for the AM and the surfactant molecule, which is reflected by the fact that the formed complexes are difficult to dissociate.
The complexes formed between AM and a number of amphiphilic molecules crystallise to give rise to a characteristic V-type X-ray diffraction pattern which arises from the crystallisation of AM in the form of single left-handed helices with a hydrophobic inner core and a hydrophilic outer surface. From studies of crystalline complexes of AM and a variety of organic molecules, it is known that each turn of the AM helix may consist of 6, 7 or 8 glucosyl residues, and that the number of residues depends on the size and shape of the guest molecules. Molecular modelling studies have indicated that in complexes of AM with amphiphilic molecules such as monoglycerides and fatty acids, there are on average two or three turns for each ligand molecule and that the polar head group is most likely not included within the helix due to both steric and/or electrostatic repulsions.

The thermal properties of the complexes of AM with a variety of amphiphilic molecules in the solid state have been extensively characterised by means of calorimetry. From these studies it is known that the thermal stability and the melting enthalpies of crystalline complexes are dependent on the chemical nature of the complexing ligand (chain length, unsaturation, nature of the polar head group), the degree of polymerisation of the amylose and the conditions (temperature, time and solvent) employed during complexation.

The amylopectin-surfactant complex

It was for long believed that surfactants interacted mainly with AM. However, it is now known that surfactants can also complex with amylopectin (AMP). This view is supported by strong evidence from binding studies of surfactants to AMP in solution as well as some indirect evidence from diverse calorimetric studies of waxy starch varieties in the presence of surfactants. Although the nature of this complex has not been completely resolved, the evidence indicates that the binding involves the formation of inclusion complexes with the AMP outer chains. Amylopectin has been reported to be able to bind similar amounts of surfactant as AM. However, in contrast to AM, the binding of surfactants to AM lacks cooperativity and it is extremely sensitive to small changes in the AMP molecular structure.

2.3.3. INTERACTIONS BETWEEN SURFACTANTS AND STARCH DURING GELATINISATION

Early stages of starch gelatinisation and related thermal events

From calorimetric studies it is known that surfactants affect the earlier aspects of the starch gelatinisation process. Numerous calorimetric studies of starches gelatinised (in excess water) in the presence of surfactants have shown that surfactants have the ability to alter the onset of the gelatinisation transition (TOGEL) as well as the enthalpy of the process (ΔH GEL). The effect of added surfactants on the onset of the gelatinisation transition usually involves changes in the order of 1-4°C, the actual magnitude of the effect depending on the type of starch, test conditions (heating rate) and type and concentration of surfactant. In this respect, most surfactants have been found to increase the TOGEL (i.e. to delay the onset of gelatinisation) of wheat and other cereal starches whereas the opposite effect has been found to be produced by sodium dodecyl sulphate (SDS).
As for the effect of surfactants on $\Delta H_{GEL}$, gelatinisation studies of wheat and other normal cereal starches in the presence of different surfactants have invariably found lower gelatinisation enthalpies than in the absence of these $^9, ^{72, 77, 80, 82}$. A similar effect has also been reported for normal tuber,$^8, ^{80}$ legume $^9$ and even waxy cereal starches.$^8, ^{81}$

In the DSC-trace of starches gelatinised in the presence of surfactants, a second endothermic transition is observed beyond the temperature range within which the gelatinisation transition occurs. This endotherm, which occurs near 100°C and is also observed in the DSC trace of lipid-containing starches, is ascribed to the order-disorder transition of AM-surfactant(lipid) complexes that are formed simultaneously as the granule gelatinises (See Figure 11). This endothermic event, which is usually attributed to the disordering of the complexes (V-helix$\rightarrow$coil transition) rather than to the melting of the crystallites,$^{68, 83, 84}$ is thought to involve the actual dissociation of the complex into free ligand and AM.$^{69}$ No such endothermic transition is observed in the DSC-trace of waxy (i.e. AM-free) starches gelatinised in the presence of surfactants. This has been attributed to the absence of cooperativity of the AMP-surfactant association (and dissociation) process.$^8$

In most cases, the enthalpy of dissociation of the AM-surfactant complex ($\Delta H_{cx}$) has been found to be comparable in magnitude to the reductions that added surfactants induce on the $\Delta H_{GEL}$ of starches.$^8, ^9, ^{77, 85}$ The decreasing effect of surfactants on $\Delta H_{GEL}$ has therefore been suggested to be the result of an exothermic effect associated with the formation of starch-surfactant complexes occurring simultaneously as the starch granule gelatinises.

The dissociation transition of the AM-surfactant complexes is reversible and can be observed, albeit with considerable hysteresis, both during heating and cooling. When this transition corresponds to complexes formed with lipids already present in starch granules the associated enthalpy remains, within experimental error, the same in all heating and cooling runs. On the contrary, when the peak is caused by added surfactants the enthalpy during first heating is only about 50 to 60% of the value observed on subsequent cooling and reheating. It has been suggested that this may be due to the fact that, immediately after gelatinisation, only a portion of the amylose is in a suitable state for complexing and that full complexing does not occur until the amylose has been leached from the granule.$^8, ^{85}$ An alternative view is that, on the first heating, a rather imperfect complex is formed perhaps as a result of steric constraints on the amylose molecules which may initially be non-covalently bonded to amylopectin chains.$^8$

**Later stages of starch gelatinisation: granule swelling and AM-leaching**

Surfactants are well-known to have the ability to alter the granule-swelling and AM-leaching processes that take place during the later stages of the starch gelatinisation process. The effect
produced by surfactants on these processes causes significant changes in the rheological behaviour of starch pastes. In this respect, the extent of the effect produced by surfactants on the swelling/AM-leaching properties of starches is known to be strongly dependent on the test conditions (heating and shearing), the type of starch and the type and concentration of surfactant.

The importance of the surfactant structure for the effect on the swelling properties of starches is indicated by the fact that, even for the same type of starch and under the same test conditions, some surfactants have the ability to restrict the swelling whereas others have the ability to enhance it. For instance, in the case of wheat and other cereal starches, surfactants such as long chain (C14-C18) saturated monoglycerides, esters of sucrose and lactic acid derivatives (sodium and calcium stearoyl lactylate) have been reported to restrict the swelling whereas others such as sodium dodecyl sulphate (SDS) and glycerol monocaprate have been found to have the opposite effect.

The restricted swelling of starch in the presence of surfactants has been proposed to be due to the presence of an insoluble surface layer of AM-surfactant complexes that would form and precipitate readily on the granule surface as AM leaches out during gelatinisation. It has also been suggested that surfactants may penetrate the granules and complex with AM thereby increasing the internal bonding and leading to a restricted swelling. The swelling-enhancing effect produced by a surfactant such as SDS has been proposed to be related to the increased solubility of the AM-surfactant complex, which would result from the high hydrophilicity of the charged surfactant head group and would favour water uptake by the granule. Although plausible, this theory cannot account for the swelling-enhancing effect produced by some non-ionic surfactants such as glycerol monocaprate or the restricted swelling observed in the presence of some anionic, food-grade surfactants such as sodium stearoyl lactylate, which is an indication that further work is required in order to elucidate the molecular mechanism involved.
3. MATERIALS AND METHODS

3.1 MATERIALS

**Flour, starches and starch polysaccharides**

In this work the interactions between surfactants and starch were studied within different contexts and in systems with different levels of complexity. Pasting studies of both wheat flour and wheat starch suspensions were performed. The sources and some characteristics of the flour and starches used throughout this work are summarised in Table 2.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Source</th>
<th>Composition/characteristics</th>
</tr>
</thead>
</table>
| Wheat flour                | Commercial flour milled from a Swedish winter wheat cultivar Tarso (Nord Mills, Sweden) | Protein (% w/w db): 11.5 b  
Ash (% w/w db): 0.6 b  
Starch (% w/w db): 69.5 e  
Moisture (% w/w): 15.0 d |
| Normal wheat starch        | CI Gel 2006, commercial sample (Cerestar, The Netherlands)             | AM (% w/w): ~30 e  
Moisture (% w/w): 11.6 d  
Granule size dist.: Bimodal  
Average granule size (μm): f  
A granules: 21.3 (10.7 % v)  
B granules: 3.0 (1.1 % v) |
| Waxy wheat starch          | Extracted from flour (Californian spring habit, hexaploid waxy wheat (USDA/ARS Western Wheat Quality Laboratory, USA)) by means of a gluten washing procedure. a | AM (% w/w): ~0-3 g  
Moisture (% w/w): 11.6 d  
Granule size dist.: Bimodal  
Average granule size (μm): f  
A granules: 21.3 (10.7 % v)  
B granules: 3.0 (1.1 % v) |

---

a See the “Materials and methods” section in Paper II for details.  
b Reported by the producer.  
c Determined by the Ewers’ method.  
d Taken as weight loss after heating at 120°C for 2 hr.  
e Average values taken from Soulaka & Morrison 87 and Buleon et.al. 14  
f Determined by means of a Malvern mastersizer light scattering instrument (Malvern instruments Inc., U.K.). See the “Materials and methods” section in Papers I-III for details.  
g Average values taken from Yoo & Jane 88 and Chakraborty et al. 89

Amylose (AM), type III from potato (Mw 3.5 ± 0.5 x 10^5 g/mol as determined by light scattering) was used for studies on the interactions between surfactants and starch polysaccharides in solution. In an attempt to minimize the presence of solvent impurities such as butanol, the AM was placed in an oven at 80°C for 2 hrs prior to use.
Surfactants

Table 3 summarises the surfactants used in this work. The molecular structure, type and abbreviations used throughout this thesis are also included in the table. All surfactants were analytical grade reagents and were used without further purification in most cases. Sodium dodecyl sulphate (C\textsubscript{12}Sulph) used for binding studies was purified by repeated recrystallization from water as described elsewhere.90

<table>
<thead>
<tr>
<th>Surfactant Group</th>
<th>Structure</th>
<th>Type</th>
<th>Abbreviation$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyl maltosides</td>
<td></td>
<td></td>
<td>C\textsubscript{n+1}Malt</td>
</tr>
<tr>
<td>Alkyl (mono)glycerides</td>
<td>C\textsubscript{n+2}Glyc</td>
<td>Nonionic</td>
<td>C\textsubscript{n+2}Glyc</td>
</tr>
<tr>
<td>Sucrose alkyl esters</td>
<td>C\textsubscript{n+2}Suc</td>
<td>Nonionic</td>
<td>C\textsubscript{n+2}Suc</td>
</tr>
<tr>
<td>Alkyl sodium sulphates</td>
<td>C\textsubscript{n+1}Sulph</td>
<td>Anionic</td>
<td>C\textsubscript{n+1}Sulph</td>
</tr>
<tr>
<td>Alkyl trimethyl ammonium bromides</td>
<td>C\textsubscript{n+1}TAB</td>
<td>Cationic</td>
<td>C\textsubscript{n+1}TAB</td>
</tr>
</tbody>
</table>

$^a$ n, number of CH\textsubscript{2} groups in the alkyl chain of the surfactant (see the schematic of the surfactant molecular structure in the table)

$^b$ The abbreviations SDS and SHS are used indiscriminately to refer to the surfactants C\textsubscript{12}Sulph and C\textsubscript{16}Sulph, respectively.

Two fluorescently-labelled, amphiphilic molecules were used as surfactant models in studies performed to gain insight on the possible influence of the alkyl chain length on the location and penetration patterns of amphiphilic molecules in starch granules. Both the molecular structure and the abbreviations used are presented in Table 4. The dyes were originally purchased in their neutral form and converted into their monoanionic salts by reacting them
with sodium hydroxide (see the “Materials and Methods” section in Paper III for further details).

**Table 4. Amphiphilic dyes used.**

<table>
<thead>
<tr>
<th>Amphiphilic dye</th>
<th>Structure</th>
<th>Abbreviation&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-alkanoyl 5-amino-fluorescein</td>
<td><img src="image" alt="" /></td>
<td>C&lt;sub&gt;n+2&lt;/sub&gt;-Fluor</td>
</tr>
</tbody>
</table>

<sup>a</sup> n, number of CH<sub>2</sub> groups in the alkyl chain of the dye (see the schematic of the dye molecular structure in the table).
3.2. METHODS

3.2.1. INTERACTIONS BETWEEN SURFACTANTS AND STARCH GRANULES

Viscometry

Viscometers have found, and still find, worldwide usage in the characterisation of starch pastes. Instruments like the Brabender Viscoamylograph, and the more recent Rapid Visco-Analyser (RVA), are viscometers especially configured for testing starch-based products. These instruments record the torque required to balance the viscosity that develops when a starch (or starch-based) slurry is subjected to a programmed heating and cooling cycle.52 Pasting studies performed in this work were carried out in an RVA (series 4, Newport Scientific, Warriewood NSW, Australia). In this instrument, the torque is measured by means of a paddle sensor which simultaneously ensures a homogeneous sample. Samples for testing are placed in disposable aluminium canisters, which are hydraulically clamped in a machined copper block. The temperature of the sample is controlled by means of the block, which in turn is heated by resistance elements mounted within it and cooled by running water through machined channels. This allows the RVA to achieve the accuracy of circulating water bath systems with the advantage of a much faster response.

![Viscosity trace developed by a starch aqueous suspension during the heating and constant-temperature steps of the RVA pasting test. The pasting temperature (PT) is indicated in the curve.](image)

During an RVA “pasting test”, concentrated starch aqueous suspensions (ca. 10% w/v) are sheared at a constant stirring speed as the temperature is typically raised from 50°C to 95°C, kept at this temperature for several minutes and then lowered again to 50°C. During the heating and cooling steps the temperature is varied at a constant rate which is usually 6°C/min in standard RVA pasting tests.

A characteristic pasting curve is developed along the controlled temperature program (See Figure 12). This curve displays the apparent viscosity of the sample (i.e. the viscosity at a constant shearing rate) ($\eta_{app}$) and the temperature as a function of time). A few parameters can be identified and used to characterise the sample from the recorded viscosity trace. Of special
interest in the present investigation is the pasting temperature (PT), which corresponds to the
temperature at which a steep increase in viscosity occurs (onset of pasting in Figure 12) due to
the extensive swelling of the majority of the starch granules. This temperature is defined as
that at which the rate of change in viscosity reaches a fixed value (4 cP/sec for a heating rate of
6°C/min).

The ability to accurately control and reproduce pasting conditions (temperatures and shearing
regimes) constitutes one of the main advantages of the RVA-type viscometers to indirectly
assess the swelling/AM leaching properties of starch granules. However, the instrument is
only meant for detecting large viscosity variations and thus not sensitive enough to register,
for example, the small viscosity changes occurring before the onset of the extensive
swelling/AM-leaching stage of cereal starch granules (i.e. changes below the PT). On the
other hand, typical pasting programs often involve the use of relatively fast heating rates. This
implies that the properties of the suspension during pasting are assessed under non-
equilibrium (thermal and physicochemical) conditions.

**Differential scanning calorimetry (DSC)**

Differential scanning calorimetry is a technique in which the heat-flow rate (power) to the
sample is monitored against time or temperature while the temperature of the sample, in a
specified atmosphere, is programmed. It is classified as a differential measuring technique
since the heat flow-rate to the sample is compared with that of a reference sample, and it is the
difference between these two that the instrument reports.\(^1\), \(^2\) A calorimeter of the heat-flux
type, with a disk-type measuring system (DSC 821\(\text{e}\), Mettler-Toledo, Switzerland), was used
in this work (see schematic diagram in Figure 13). In this type of instrument the measurement
signal is the temperature difference between sample and reference, a difference which is
proportional to the heat-flow rate.

The measuring principle of the DSC used in this study can be briefly described as follows.
When the furnace is heated (linearly with time), heat flows to the samples through a disc of
good thermal conductivity. When the arrangement is ideally symmetrical (samples of the
same kind), equally high heat-flow rates flow into the sample and the reference sample. The
differential temperature signal \(\Delta T\) is then zero. When this steady state equilibrium is disturbed
by a sample transition (e.g. phase transitions and chemical reactions), a differential signal is
generated which is proportional to the difference in the heat-flow rate to the sample (\(\Phi_{FS}\)) and
to the reference sample (\(\Phi_{FR}\)).

![Figure 13. Schematic of a DSC of the heat flux type used in this work. 1 disk, 2 furnace, 3 lid, 4 differential
thermocouple, 5 programe and controller, S crucible with sample substance, R crucible with reference
sample substance, \(\Phi_{FS}\) and \(\Phi_{FR}\) heat flow rate from furnace to sample crucible and to reference sample
crucible, respectively.](image-url)
From the resulting DSC trace (see Figure 14) it is possible to obtain information on characteristic temperatures ($T_i$, $T_0$, $T_{\text{PEAK}}$, $T_c$, $T_f$ in Figure 14) of transitions or reactions as well as the enthalpy change associated with these. In the latter case, this is done by subtracting the background curve (or baseline) from the transition curve and integrating the resulting curve to obtain an area that is proportional to the enthalpy.

![Figure 14. Schematic DSC trace showing an endothermic transition.](image)

DSCs allow heat-flow rates and their changes at characteristic temperatures to be measured quickly using small sample masses (milligram range) in wide temperature ranges and with an accuracy which is usually sufficiently high for the respective purpose. However, in order to obtain a direct indication of any transition with a DSC, not only must the transition be associated with a measurable enthalpy change but it also should occur over a fairly narrow (ca. 20-30°C) temperature range (otherwise the sample will behave as if it had a slightly higher or lower heat capacity). This, in turn, means that the process must have a significant degree of cooperativity, as is the case of phase transitions.8

**Confocal laser scanning microscopy (CLSM)**

Confocal laser scanning microscopy (CLSM) is a technique that allows imaging of fluorescent samples with high resolution.93, 94 Images are taken point-by-point and reconstructed with a computer, rather than projected through an eyepiece. The point-by-point principle implies that only one point of the sample is illuminated and only fluorescence from that point is detected at any given time. This is accomplished by focusing a laser into a small focal volume within a fluorescent or fluorescently-labelled specimen (see Figure 15). A mixture of emitted fluorescent light as well as reflected laser light from the illuminated spot is then recollected by the objective lens. A beam splitter separates the light mixture and allows only the fluorescent light into the detector. A pinhole aperture in front of the detector blocks all out of focus light so only the emitted fluorescence from the focused spot is used for image creation. High resolution images (ca. 150 nm in the x- and y-direction, ca. 500 nm in the z-direction) are then obtained by scanning the laser in the x-, y- and z-directions.

One of the key features of CLSM is its ability to perform direct, non-invasive serial optical sectioning of intact, thick living specimens with an absolute minimum of sample preparation. This in turn offers the possibility to perform repeated measurements on the same sample under different conditions or using different parameters.

As laser scanning confocal microscopy depends on fluorescence, a sample often needs to be treated with fluorescent dyes to make things visible. This in turn implies that when used for
the identification of certain components or processes in a given specimen, much of the reliability of the results depends on the choice of the right fluorescent probe.

**Figure 15.** Schematic diagram of the optical pathway and principal components in a laser scanning confocal microscope.

Imaging of starch granules in the presence of fluorescently labelled amphiphilic molecules was performed by means of a LSM 410 invert system (Zeiss, Germany) attached to an inverted microscope (Axiovert 135 M, Zeiss, Germany) fitted with an oil-immersion 40X / 1.3 NA lens.

### 3.2.2. INTERACTIONS BETWEEN SURFACTANTS AND AM IN SOLUTION

**Tensiometry**

In this work surface tension determinations were performed by means of a Krüss K12 tensiometer using the Wilhelmy plate method. This is a method based on the use of a plate of perfectly known geometry which is suspended from a precision balance.\(^{56, 95}\) To determine the surface tension the plate is immersed in the liquid of interest and the downward pull (i.e. the force) exerted by the liquid on the plate is measured (Figure 16).

**Figure 16.** Schematic picture of the measurement principle of the Wilhelmy plate method.
The force acting on the plate \( (F) \) will be the sum of a surface tension and a buoyancy contribution as given by the expression:

\[
F = 2(L_T + L_W)\gamma \cos \theta + L_T L_W \Delta \rho g h
\]  

(Eq. 1)

where \( \gamma \) is the surface tension of the liquid, \( \theta \) the contact angle at the three-phase line, \( L_T \) and \( L_W \) are the thickness and the width of the plate, respectively, \( \Delta \rho \) the density difference between the liquid and the vapour phase, \( g \) the gravitational constant and \( h \) the immersion depth of the plate in the liquid. A contact angle of zero degrees at the three-phase line is ensured by the use of a sand-blasted platinum plate. Thus, given that the immersion depth of the plate is zero (i.e. just before the detachment position of the plate), the surface tension of the liquid can be readily estimated by using the expression:

\[
\gamma = \frac{F}{2(L_T + L_W)}
\]  

(Eq. 2)

The Wilhelmy plate approach is one of the most commonly used techniques for the measurement of surface tension. However, the suitability of this method for the determination of the surface tension relies on the existence of a contact angle of zero degrees at the three phase line, i.e. the liquid must wet the plate completely. Thus, if adsorption of molecules from the liquid to the plate occurs, thereby altering the wettability of the plate, this condition will not be fulfilled and the method will not provide correct surface tension values. This is the case in, for example, aqueous solutions of cationic surfactants.

**Nuclear magnetic resonance (NMR) spectroscopy**

Nuclear magnetic resonance (NMR) spectroscopy is a technique which records transitions between energy levels of magnetic nuclei in an external magnetic field. This type of spectroscopy involves absorption of the energy of electromagnetic radiation in the radio-frequency region by a sample placed in an external magnetic field. The nuclei with non-zero spin (like \(^1\)H) behave as tiny magnets which align in external magnetic fields. These nuclei can then adsorb energy of specific radio frequencies (rf) that matches their transition from alignments with the field (lower energy state) to against the field (higher energy state). A plot of the absorption of rf energy versus the external magnetic field gives an NMR spectrum. The difference between the higher and lower energy state is influenced by the local environment of the nuclei, providing chemical shifts characteristics for different positions in the molecules. The absorbed rf frequency increases with the magnitude of the external field, which improves the separation between the peaks in the spectra.

In this investigation, \(^1\)H NMR spectroscopy was used to perform longitudinal relaxation measurements on the AM-SDS system as a means of getting information on the binding of SDS to AM in solution. Experiments were performed in a Bruker DMX 500 spectrometer with 500 MHz resonance frequency. The relaxation measurements are based on exciting the
molecules and observing how they relax into their equilibrium state. The half-time of the longitudinal relaxation process, commonly called spin-lattice relaxation time ($T_1$), provides information on the ease of tumbling, i.e. the mobility, of the molecule of interest.

**Transmittance**

Stability of AM-surfactant solutions against phase separation was assessed by means of transmittance determinations. The transmittance of the solution was quantified in terms of percentage of light transmitted through the sample by means of a Turbiscan Lab expert (Formulaction, France). In this instrument a light source (an electroluminescent diode in the near infrared ($\lambda = 880$nm)) is shone through the sample while the light transmitted through the sample is collected by synchronous optical sensors. Transmitted light flux values are reported as percentages relative to an internal standard (silicone oil). The instrument was set up to work in scanning mode. Under such mode, the optical reading head scans the length of the sample, thus providing curves of transmitted light flux as a function of the sample height.
4. SUMMARY OF KEY RESULTS AND DISCUSSION

4.1. GRANULE SWELLING AND PASTING OF STARCH SUSPENSIONS: SURFACANT STRUCTURE EFFECTS

Despite the great deal of work that has been carried out in the area, no clear-cut conclusions have emerged regarding the overall effect of the surfactant chemical structure on the swelling/leaching processes that lead to the pasting of starch suspensions. This has been partly due to the non-equilibrium nature of the swelling-leaching processes (which makes them very sensitive to test conditions), the different susceptibility of different starches to the effect of surfactants and the lack of studies involving a systematic variation of the surfactant structure (chain length and head group). Such a systematic study was performed in Paper I. In this study, information on the extent of granule swelling/AM-leaching was indirectly obtained by means of viscometry.

Figure 17 shows the pasting profile (apparent viscosity ($\eta_{app}$) vs. temperature and time) of a suspension of normal wheat starch (commercial-grade) depicting the dramatic increase in viscosity that characterises the onset of the pasting process and defines the pasting temperature (PT). The pasting profile of this starch in the presence of a long-chain (C16) alkyl maltoside ($\sim$1% w/w sb) (Heating rate 6°C/min). Note that PT occurs at $\sim$8 min (i.e. $\sim$86°C) in the control sample and at $\sim$9.5 min (i.e. $\sim$94°C) in the sample in the presence of the surfactant.

Figure 18 A summarises the effect of alkyl maltosides (alkyl-Malt), trimethyl ammonium bromides (alkyl-TAB) and sodium alkyl sulphates (alkyl-sulphates) of different chain length (C10 to C16) on the PT of normal wheat starch. The PT of the starch suspension in the absence of surfactants (control) is also indicated in the plot as a reference. As observed in Figure 18 A, the effect of the non-ionic and anionic surfactants on the PT (alkyl-Malt and alkyl-Sulph, respectively) was found to be strongly chain-length dependent. Despite differences in the extent of the effect, surfactants with 10 and 12 carbon atoms in their alkyl chain lowered the
PT (i.e. favoured granule swelling), whereas surfactants with 14 and 16 carbon atoms in their alkyl chain increased the PT (i.e. restricted the swelling). The same was not true for the cationic surfactants (alkyl-TAB), which were found to have a tendency to lower the PT regardless of the surfactant chain length.

Pasting studies of wheat flour (~70% w/w starch) in the presence of various surfactant groups, including also sucrose esters (alkyl-Suc) and monoglycerides (alkyl-Glyc), revealed similar trends regarding the chain length-dependence of the effect of surfactants on the PT (see Figure 18 B). Compared to starch, the changes induced by surfactants on the PT of the flour suspension were more moderate. This is taken to be an indication that interactions between surfactants and flour components other than starch (e.g. proteins) dampen the overall effect of surfactants on the swelling properties of starches, but do not alter the characteristic effect produced by the surfactant itself.

**Figure 18.** Pasting temperature (PT) of (A) wheat starch suspensions (10% w/v) and (B) wheat flour suspensions (14% w/v) in the presence of surfactants with different head groups and alkyl chain length (Surfactant concentration: 4.1x10^{-5} moles of surfactant/g starch or ~1% w/w sb; heating rate: 6°C/min). Lines are a guideline for the eye.

**Figure 19.** Pasting temperature (PT) of wheat flour suspensions (14% w/v) in the presence of (A) alkyl-Maltosides (C_{10}-C_{16}) and (B) C_{12} surfactants (sulphates, maltosides and trimethyl ammonium bromides) as a function of surfactant concentration (~0.5-15% w/w sb). Heating rate: 6°C/min. Arrows in Figure (B) indicate the cmc of the respective surfactant.
The effect of the surfactant concentration was explored for wheat flour suspensions in the concentration range $1.0 \times 10^{-5} - 3.1 \times 10^{-4}$ moles of surfactant/g starch (i.e. ∼0.5–15% w/w starch basis (sb)). These experiments revealed that an increase in surfactant concentration favoured the swelling-enhancing or swelling-restricting effect of the respective surfactants (see Figures 19 A and B). Increases in the surfactant concentration beyond $1 \times 10^{-4}$ moles of surfactant/g starch (i.e. ∼5% w/w sb) were found to have a marginal effect for all the surfactants tested except C12TAB.

Our findings confirmed previous reports on the swelling-enhancing effect of sodium dodecyl sulphate (C12Sulph) and the well-known restricted swelling induced by long-chain monoglycerides and sucrose esters. However, our results also showed that the swelling enhancing effect of some surfactants is not necessarily related to the presence of a charged head group and that, in most cases, the overall effect of the surfactant on the swelling properties of starch is strongly affected by the surfactant chain length. Moreover, our findings also showed that the overall solution properties of the surfactant, i.e. whether the surfactant is below or above the critical micelle concentration (cmc), do not play a major role in determining the type of effect on the PT. Evidence of this is found in the fact that surfactants with very different cmc’s, such as C16Sulph and C16Malt or C12TAB, C12Malt and C12Sulph (see Figure 19 B), had the same type of effect on the PT.

As was mentioned in the introductory section of this thesis, during an RVA pasting test the swelling properties of the starch are assessed under non-equilibrium conditions. For this reason, pasting studies were also carried out using a slower heating rate (1.5°C/min) with the intention of exploring the effect of surfactants on the swelling properties of starch under conditions closer to equilibrium. These experiments revealed that the type of effect induced by different surfactants on the PT is not affected by the heating rate (1.5°C vs. 6°C/min) used to induce the pasting (see Tables III-V in Paper III).

4.2. STARCH GELATINISATION IN THE PRESENCE OF SHORT- AND LONG-CHAIN SURFACTANTS:

4.2.1. PASTING VS. CALORIMETRIC STUDIES

Some insight on the molecular mechanisms behind the effect of short- and long-chain surfactants on the swelling properties of starch was sought by means of calorimetric studies. Calorimetric studies of starch gelatinised in the presence of surfactants yield information on the effect of surfactants on the earlier aspects of the gelatinisation process as well as on their ability to form inclusion complexes with AM as the granules gelatinise. A series of short-chain (C12) and long-chain (C16) surfactants were chosen for this investigation. Additional insight on the possible interaction mechanisms involved was also sought through the use of both normal and waxy wheat starch. Compared to normal wheat starches, waxy wheat starch has a much lower AM content (∼0-3% compared to ∼30% AM), thus being potentially very useful for disentangling effects due to interactions between surfactants and AM and/or AMP.

Surfactants were indeed found to have very different effects on the pasting properties of the two wheat starches. Figure 20 shows the pasting profiles of normal and waxy wheat starch in the presence of short- and long-chain sodium sulphates (Sulph), maltosides (Malt), trimethyl
ammonium bromides (TAB) and monoglycerides (Glyc). From the pasting profiles of the two starches in the absence of surfactants it is obvious that they have very different swelling properties. The lower PT and higher peak viscosity ($\eta_{\text{peak}}$) of the waxy starch evidence the more extensive swelling that this type of starches undergo as they gelatinise. This behaviour is usually attributed to the existence of a more loosely bound internal structure which is associated to the lack of AM as well as to the low contents of internal lipid.\textsuperscript{26, 97}

In contrast to normal wheat starch, short- and long-chain surfactants did not produce different effects on the PT of the waxy starch. In fact, only ionic surfactants were found to produce significant effects on the PT of waxy wheat starch (see Table III in Paper II for details). Regardless of their chain length, the ionic surfactants lowered the PT (i.e. had a swelling-enhancing effect) whereas the nonionic surfactants had either a very small or an insignificant effect on the PT. Furthermore, compared to normal wheat starch, the addition of surfactants was found to bring about rather minor changes in the pasting properties of waxy wheat starch. These findings suggested that AM-surfactant interactions play an important role in determining the overall effect that most short- and long-chain surfactants have on the PT of normal starch. As discussed below, further support to this view was provided by the results from calorimetric studies.

Figure 20. Pasting profile of normal and waxy wheat starch suspensions (10% w/v) in the presence of short-chain (C\textsubscript{12}) and long-chain (C\textsubscript{16}) surfactants (Surfactant concentration: 1x10^{-4} moles of surfactant/g starch or ~3-5% w/w dsb; heating rate: 6.5°C/min).
Effect of surfactants on the early stages of starch gelatinisation

Figure 21 shows the DSC traces of normal and waxy wheat starch in the presence of short-chain surfactants. These traces depict the characteristic features of the thermograms obtained in the presence of all the surfactants included in this study. In general, one, two or three distinctive endothermic transitions could be recognised in these traces. The single, well-defined transition associated to the gelatinisation of starch in excess water was seen in the DSC traces of the normal and waxy wheat starch in the temperature ranges 50-70°C and 50-80°C, respectively. The endotherm associated with the dissociation transition of the complex formed between AM and native polar lipids in starch was observed in the DSC trace of normal wheat starch in the absence of surfactants at ~90°C. A similar endothermic transition was also recognised in the DSC-trace of normal wheat starch gelatinised in the presence of all the surfactants included in this investigation, meaning that all these had the ability to form complexes with AM during the process. As expected, no evidence of such transition was seen in the thermograms of the waxy wheat starch in the presence of surfactants. The third transition, a sharper endotherm occurring at T<50°C in the thermograms of normal and waxy wheat starches in the presence of C12Glyc, is associated to the melting of surfactant crystals (i.e. the crossing of the Krafft boundary in the surfactant phase diagram). A similar transition was also observed in the DSC-trace of starches gelatinised in the presence C16Sulph and C16Glyc (see Figures 3 and 4 in Paper II). For the latter surfactant, this transition coincided with the initial part of the gelatinisation endotherm in both normal and waxy wheat starch, thus making it impossible to determine the actual onset temperature of the gelatinisation endotherm in both these cases.

The temperatures and enthalpies of the endothermic transitions of normal and waxy wheat starch in the presence of surfactants are presented in Table 5. A generalised summary of the effect of the different surfactants studied on the onset temperature of the DSC-gelatinisation transition \(T_{O GEL}\) is presented in Table 6. In this respect, it was found that contrary to their nonionic counterparts, short-chain ionic surfactants had a special ability to depress \(T_{O GEL}\) (i.e. had a granule destabilising effect) in both normal and waxy wheat starch, although the
reductions induced in waxy wheat starch were larger. On the other hand, the longer-chain surfactants had very different effects on the $T_{O\text{GEL}}$ of the two types of starches. These surfactants, regardless of their head group charge, were found to delay the onset of the gelatinisation endotherm in normal wheat starch while they produced the opposite effect on waxy starch. In general, these results are in good agreement with the previously reported effects of some short- and long-chain anionic surfactants on the $T_{O\text{GEL}}$ of normal cereal starches.8, 72

Table 5. Characteristic temperatures [onset ($T_{O\text{GEL}}$), peak ($T_{\text{PEAK GEL}}$)] and enthalpies ($\Delta H_{\text{GEL}}$) of the gelatinisation endotherm of normal and waxy wheat starch (starch to water ratio: 1:3) in the presence of surfactants. Surfactant concentration: $1 \times 10^{-4}$ moles of surfactant/g starch or $\sim 3-5\%$ w/w sb; heating rate: 6.5°C/min.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>$T_{O\text{GEL}}$ (°C)</th>
<th>$\Delta T_{O\text{GEL}}$</th>
<th>$T_{\text{PEAK GEL}}$ (°C)</th>
<th>$\Delta T_{\text{PEAK GEL}}$</th>
<th>$\Delta H_{\text{GEL}}$ (J/g)</th>
<th>$\Delta(\Delta H_{\text{GEL}})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal wheat starch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12Sulph</td>
<td>50.30 ± 0.25$^b$</td>
<td>-2.11</td>
<td>57.10 ± 0.17$^ab$</td>
<td>-0.25</td>
<td>7.05 ± 0.42$^b$</td>
<td>-3.00</td>
</tr>
<tr>
<td>C12TAB</td>
<td>50.10 ± 0.14$^{ac}$</td>
<td>-2.31</td>
<td>55.31 ± 0.11$^b$</td>
<td>-2.04</td>
<td>8.97 ± 0.30$^{eq}$</td>
<td>-1.08</td>
</tr>
<tr>
<td>C12Malt</td>
<td>52.72 ± 0.12$^{ad}$</td>
<td>0.30</td>
<td>57.76 ± 0.16$^{ab}$</td>
<td>0.41</td>
<td>7.82 ± 0.12$^{db}$</td>
<td>-2.23</td>
</tr>
<tr>
<td>C12Glyc</td>
<td>53.85 ± 0.07$^{def}$</td>
<td>1.44</td>
<td>59.30 ± 0.24$^{ac}$</td>
<td>1.95</td>
<td>8.59 ± 0.09$^{fg}$</td>
<td>-1.46</td>
</tr>
<tr>
<td>C16Sulph</td>
<td>53.54 ± 0.05$^{ef}$</td>
<td>1.12</td>
<td>57.38 ± 0.05$^{ab}$</td>
<td>0.23</td>
<td>5.45 ± 0.16$^f$</td>
<td>-4.60</td>
</tr>
<tr>
<td>C16TAB</td>
<td>53.62 ± 0.11$^{f}$</td>
<td>1.20</td>
<td>59.36 ± 0.36$^{ad}$</td>
<td>2.00</td>
<td>8.32 ± 0.37$^{gh}$</td>
<td>-1.73</td>
</tr>
<tr>
<td>C16Malt</td>
<td>52.93 ± 0.02$^{f}$</td>
<td>0.51</td>
<td>55.94 ± 0.26$^{bc}$</td>
<td>-1.41</td>
<td>7.67 ± 0.13$^h$</td>
<td>-2.38</td>
</tr>
<tr>
<td>C16Glyc</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

| Waxy wheat starch |
|-------------------|------------------------|--------------------------|--------------------------|-------------------------------|-------------------------------|
| C12Sulph   | 51.12 ± 0.21$^b$       | -5.48                    | 58.35 ± 0.25$^b$          | -4.84                        | 9.95 ± 0.70$^{gh}$            | -2.50                         |
| C12TAB     | 51.20 ± 0.01$^{bc}$    | -5.40                    | 59.65 ± 0.10$^d$          | -3.54                        | 10.77 ± 0.95$^{bc}$           | -1.68                         |
| C12Malt    | 55.92 ± 0.08$^{ad}$    | -0.68                    | 62.07 ± 0.01$^d$          | -1.12                        | 11.58 ± 0.84$^{ab}$           | -0.87                         |
| C12Glyc    | 56.67 ± 0.19$^{a}$     | 0.07                     | 62.63 ± 0.01$^{e}$        | -0.56                        | 9.64 ± 1.77$^{bc}$            | -2.81                         |
| C16Sulph   | 54.79 ± 0.32$^{ad}$    | -1.81                    | 60.66 ± 0.06$^{f}$        | -2.53                        | 8.85 ± 1.62$^{gh}$            | -3.60                         |
| C16TAB     | 54.28 ± 0.20$^{ef}$    | -2.32                    | 60.37 ± 0.09$^{f}$        | -2.82                        | 10.69 ± 0.08$^{bd}$           | -1.76                         |
| C16Malt    | 54.40 ± 0.52$^{f}$     | -2.20                    | 61.54 ± 0.16$^{h}$        | -1.65                        | 13.17 ± 0.42$^{ae}$           | 0.72                          |
| C16Glyc    | --                     | --                       | --                       | --                            | --                            | --                            |

$\Delta T_{O\text{GEL}}, \Delta T_{\text{PEAK GEL}}$ and $\Delta(\Delta H_{\text{GEL}})$ denote the difference between the respective calorimetric parameter in the presence and absence (control) of surfactant.

Reported values are means ± standard deviation of triplicates. Values within the same column followed by the same letter are not significantly different ($p \leq 0.05$).

Due to the nature of the processes that give rise to DSC-gelatinisation transition, the onset temperature of this endothermic event is expected to depend on a complex interplay of factors, among which water transport into the granules can be considered to play a very important role. Surfactant molecules may affect this process in many possible ways depending
on their relative affinity for the starch polysaccharides and other starch granule components. In consequence, it is perhaps not surprising to find that surfactants can have so different effects on $T_{O\text{GEL}}$. Even though it would be difficult to draw conclusions regarding the mechanisms behind these effects, the results found in this investigation clearly show that, for a given type of starch, the effect of surfactants on $T_{O\text{GEL}}$ is not entirely governed by the surfactant chain length or by the presence of a charged head group.

Table 6. Generalised summary of the effect of the different surfactants studied on the onset temperature of the gelatinisation transition ($T_{O\text{GEL}}$) as determined from DSC measurements. Surfactant concentration: $1 \times 10^{-4}$ moles of surfactant/g starch or $\sim 3\text{-}5\%$ w/w sb; heating rate: 6.5°C/min.

<table>
<thead>
<tr>
<th>Chain length type</th>
<th>Type of wheat starch</th>
<th>Surfactant</th>
<th>Normal</th>
<th>Waxy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C$_{12}$</td>
<td>C$_{16}$</td>
<td>C$_{12}$</td>
</tr>
<tr>
<td>Ionic</td>
<td></td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Nonionic</td>
<td></td>
<td>↓, ↑</td>
<td>↑</td>
<td>-</td>
</tr>
</tbody>
</table>

$\downarrow$, the surfactants lower $T_{O\text{GEL}}$; $\uparrow$, the surfactants increase $T_{O\text{GEL}}$; $\cdot\cdot\cdot$, the surfactants have an insignificant effect on $T_{O\text{GEL}}$.

In good agreement with previous findings on the effect of surfactants on the gelatinisation enthalpy ($\Delta H_{GEL}$) of normal wheat and other cereals starches, all the surfactants included in this investigation were found to produce significant reductions in the enthalpy of gelatinisation of normal wheat starch. These reductions were similar in magnitude to the transition enthalpy of the AM-surfactant complexes (see Table VIII in Paper II), thus supporting the view that the (exothermic) formation of these complexes during gelatinisation may partly account for the reductions that surfactants induce on $\Delta H_{GEL}$.

A comparison of the effect of surfactants on the early aspects of gelatinisation (onset of the gelatinisation transition as determined by DSC) and the later aspects of the process (onset of pasting due to granule swelling as determined by RVA) is presented in Figure 22. By comparing the results from the calorimetric and pasting studies in this way it is apparent that there is no correlation between the effect of surfactants on the swelling properties of the two wheat starches and their effect on the earlier aspects of gelatinisation. This is indicated by the fact that, compared to what is observed in the absence of surfactants, a lower (or higher) $T_{O\text{GEL}}$ does not coincide with a lower (respectively higher) $PT$.

The dissociation of the AM-surfactant complexes

The results presented in Figure 22 also show that, in contrast to waxy wheat, the extensive swelling in normal wheat starch granules (i.e. the PT) takes place at temperatures well above those at which the gelatinisation transition occurs. The presence of AM, native lipids and the interactions between these have been suggested to account for the restricted swelling of normal starches with respect to waxy varieties. In that respect, our results suggest that interactions between AM and added surfactants play a major role in determining the overall effect of the surfactant on the swelling properties of starch. Evidence of this is found in the
fact that the PT of normal wheat starch in the presence of all the studied surfactants, except the alkyl ammonium bromides, fell either within or very close to the temperature range within which the thermal transition associated to the dissociation of the AM-surfactant complex takes place (see Figure 22). In agreement with previous reports on the thermal stability of AM-surfactants complexes and the $\Delta H_{\text{CX}}$ the complexes formed between AM and the long-chain surfactants were found to have greater thermal stabilities (i.e. higher $T_{\text{O CX}}$) than the complexes formed by their shorter-chain counterparts. Our interpretation is that the conformational changes that the AM fraction undergoes upon formation of complexes inside and/or at the granule surface increase the internal bonding of the granules thereby restricting the swelling. Thus, once the AM-surfactant complexes begin to dissociate such constraints would disappear and the granule would swell unrestrained. Although this explains the chain-length dominated effect of some surfactants on the swelling properties of normal wheat starch, it cannot account for the swelling-enhancing effect of the cationic surfactants $C_{12}\text{TAB}$ and $C_{16}\text{TAB}$. Thus, other mechanisms may also be in effect.

Figure 22.- Temperature ranges for the gelatinisation and AM-surfactant complex transitions in the presence of surfactants in (A) normal and (B) waxy wheat starch. Triangles indicate the onset of the pasting (PT) at the same heating rate (Heating rate 6.5°C/min; surfactant concentration: $1x10^{-4}$ moles of surfactant/g dry starch). The continuous and dashed grey lines highlight the onset of the gelatinisation endotherm ($T_{\text{O GEL}}$ from DSC) and the onset of pasting (PT from RVA) for the control sample, respectively.

Surfactants like SDS (i.e. $C_{12}\text{Sulph}$, according to the notation used in this study) have been found to be very effective in removing starch granule surface proteins and lipids. Partial removal of surface protein and lipids (by means of SDS-treatments and other extraction methods) has in turn been found to result in an enhanced rate and extent of granule swelling. It is not known whether the ability to solubilise surface proteins and lipids is specific for SDS. Moreover, the available experimental evidence does not allow us to assess the extent to which a surfactant like SDS can remove proteins and lipids at the levels of surfactant concentration used in the present study or under the conditions imposed by RVA pasting experiments. In consequence, it cannot be ruled out that the ability to solubilise surface granule proteins and lipids contributes to the effects found to be produced by different surfactants on the swelling properties of normal wheat starch. Moreover, a special ability to interact and solubilise surface proteins and lipids may explain the distinctive capacity of $C_{12}\text{TAB}$ and $C_{16}\text{TAB}$ to enhance the swelling of normal wheat starch granules.
4.2.2. GRANULE ACCESSIBILITY

Much of the overall effect of surfactants on the gelatinisation of normal wheat starch has been explained in terms of their ability to form helical inclusion complexes with amylose (AM). Yet, very few studies have addressed the questions on where such complexation might take place and on which regions of the starch granules are accessible to surfactants. The study presented in Paper III was intended to address these questions by means of confocal laser scanning microscopy (CLSM) and the use of fluorescently-labelled amphiphilic molecules. Of particular interest was the investigation of the effect of the surfactant chain length on the location and penetration pattern of the surfactant into the starch granule. Differences of such nature had been previously reported to restrict the penetration of long-chain (C₁₄-C₁₆) amphiphilic molecules into the matrices of starch granules under certain conditions. Our previous investigations had also showed that most long- and short-chain surfactants had a distinctive effect on the swelling of normal wheat starch. It was therefore decided to explore whether short- and long-chain surfactants could access different regions of the granule during gelatinisation. This was done with the intention of exploring the extent to which differences of such nature could be behind the different effects produced by some short- and long-chain surfactants on the swelling of starch.

Short and long-chain (C₁₂ and C₁₆, respectively) amphiphilic, fluorescein-based dyes were chosen as surfactant models (see Table 7 for details on the molecular structure of the dyes). Although different with respect to common surfactants in terms of their hydrophilic-lipophilic balance, the structural differences of these two dyes with respect to each other was expected to reveal the existence of possible differences in granule accessibility relevant to typical short- and long-chain surfactants. Furthermore, the amylose complexability of these amphiphilic dyes under relevant conditions was demonstrated by means of DSC (see Paper III for details).

To make the results from this investigation relevant to actual practical situations, the experimental conditions were set up to be as similar as possible to the ones encountered when interactions between starch and surfactants are studied. Thus, the penetration pattern of the amphiphilic dyes into starch granules suspended in excess water was studied without any further preparation, dye removal or filtration steps. Normal and waxy wheat starch granules were used in this investigation.

**Dye location in ungelatinised starch granules**

The micrographs in Figure 23 show the location of the dyes in normal and waxy wheat starch granules after short (<1 hr) and long (24hr) contact times. The micrographs revealed that after short contact times the two dyes were confined to the outer regions of the starch granules. However, observations of the staining patterns in starch granules after longer times (24 hrs) clearly showed that the dyes had a tendency to penetrate the granules and that this tendency was influenced by the chain-length of the dye and the type of starch. In this respect, the penetration of the dyes through the matrix of the granules proved to be less restricted in waxy than in normal wheat starch. This is suggested to be associated with the more loosely bound internal structure of waxy starches, which in turn is though to be related to their high AMP contents as well as with their lower content of internal lipids and proteins.²⁶, ⁹⁷

For a given type of starch, the penetration of the longer chain dye (C₁₆Fluor) into the starch granule matrix was more constrained than that of the shorter-chain one (C₁₂Fluor).
Interactions of chemical nature between the dyes and some of the starch granule components (e.g. association with AM and AMP), are believed to be responsible for the differences in penetration of the two dyes.

<table>
<thead>
<tr>
<th></th>
<th>Short contact times (&lt;1hr)</th>
<th>Long contact times (24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal wheat starch</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₂Fluor</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>C₁₆Fluor</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Waxy wheat starch</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₂Fluor</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>C₁₆Fluor</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 23.** Staining patterns of C₁₂Fluor and C₁₆Fluor dyes in ungelatinised normal and waxy wheat starch granules after short and long contact times (dye concentration: 0.6x10⁻⁵ moles of surfactant/g starch or ~0.2% w/w sb; fluorescence signal intensity shown in a green colorscale; scale bar = 100 μm; ).

**Dye location during starch gelatinisation: Normal wheat starch**

Figure 24 shows a series of micrographs depicting the location pattern of the short- and the long-chain dye in normal wheat starch granules as they gelatinise. The micrographs show that as the starch gelatinisation proceeds and the granules swell, more and more of the amphiphilic dye diffuses into the matrix of the granules. Up to ~80°C the diffusion of both dyes was rather restricted. Yet, compared to its longer-chain counter part, the short-chain dye was found to diffuse in a less restrained fashion. The different ability of the two dyes to penetrate into the granule matrix became apparent at higher temperatures (>80°C) when the shorter-chain dye was observed to diffuse profusely through the granule matrix. Our interpretation is that as the granules gelatinise and the AM fraction gains more conformational freedom more and more of the dye will form complexes with AM. As mentioned before, calorimetric studies showed that the used dyes had the ability to form this type of complexes. In common with the complexes formed between AM and other short- and long-chain amphiphiles the complexes formed between AM and C₁₆Fluor exhibited higher thermal stability than the ones formed between AM and C₁₂Fluor (see Table II in Paper III). In the granule, complexation with AM could restrict the mobility of the dye, which in turn could only diffuse freely once it is no longer complexed to AM. Thus, upon heating, profuse diffusion of the fraction of complexed dyes through the starch granules could only take place above the temperature at
which the complexes start to dissociate, i.e. above <75°C for the short-chain dye and < 100°C for the long-chain one.

<table>
<thead>
<tr>
<th>C_{12}Fluor</th>
<th>C_{16}Fluor</th>
</tr>
</thead>
<tbody>
<tr>
<td>29°C</td>
<td></td>
</tr>
<tr>
<td>76°C</td>
<td></td>
</tr>
<tr>
<td>86°C</td>
<td></td>
</tr>
<tr>
<td>95°C</td>
<td></td>
</tr>
</tbody>
</table>

Figure 24. In situ gelatinisation of normal wheat starch in the presence of short- and long chain amphiphilic dyes (C_{12}Fluor and C_{16}Fluor, respectively) (dye concentration: 0.6x10^{-5} moles of surfactant/g starch or ~0.2% w/w sb; heating rate: 1.5°C/min). In the CLSM images (left) the fluorescence signal intensity is shown in a green colorscale (scale bar = 20 μm).

Overall, the ability of some amphiphilic molecules to penetrate further and more quickly into the granule matrix would result in higher local amphiphile/starch ratios, which may affect their overall availability to interact with the AM and/or AMP fraction in starch as well as with proteins and other starch granule constituents as starch granules gelatinise. Beyond their ability to form complexes with AM and/or AMP, the different penetration ability of short- and long-chain amphiphiles through starch granule matrices may contribute to the distinctive effect that some short- and long-chain surfactants have on the swelling properties of wheat starch.
4.3. INTERACTIONS BETWEEN SURFACTANTS AND AMYLOSE IN SOLUTION

The ability of a surfactant such as SDS to enhance granule swelling while long-chain (C_{16}-C_{18}) monoglycerides have the opposite effect, have previously been attributed to differences in solubility of a surface layer on the granules built of amylose-surfactant complexes formed as AM leached from the granule during gelatinisation. The high hydrophilicity of charged surfactant head groups was suggested to be responsible for the water solubility of the complex which in turn was believed to favour water uptake by the granule. In fact, poor water solubility of inclusion complexes of amylose and surfactants has been reported to be associated with the absence of electrostatic stabilisation, either because the surfactant is non-ionic, or because the concentration of non-complexed surfactant or added salt is sufficiently high to screen electrostatic effects. As it has already been discussed, the results from our previous investigations (Paper II and Paper III) indicated that the swelling-enhancing/restricting effect of different surfactants can not be entirely attributed to the water solubility of complexes formed between AM and ionic surfactants. Moreover, preliminary investigations on the solubility of complexes of AM and a series of short (C_{12}) and long-chain (C_{16}) surfactants indicated that, contrary to what had been expected, the solubility of the AM-surfactant complexes was not entirely governed by the presence of a charged surfactant head group. This finding indicated that a better understanding on the solution properties of these complexes was required.

This section summarises the results from the investigations presented in Papers IV and V on the solubility of complexes of AM and short- (C_{12}) and long-chain (C_{16}) non-ionic (maltosides) and anionic (sodium sulphates) surfactants. A prerequisite for the establishment of comparisons between different AM-surfactant complexes was the determination of the overall binding capacity of the AM molecule in solution. In this respect, studies of crystalline AM-fatty acids complexes have revealed that the stoichiometry of the complex varies with the chain length of the complexing agent. Similar results have also been found in studies on the binding capacity of AM in solution where it was reported that the amount of a fatty acid salt required to saturate AM decreased with increasing alkyl chain length of the fatty acid. Furthermore, results from various studies on the binding of different surfactants to AM in solution suggested that the characteristics of the surfactant head group could also affect the overall binding capacity of the AM molecules. By studying the association of surfactants to AM in solution, i.e. under conditions where the AM molecule has no conformational constraints, it is also possible to retrieve information on the characteristic features of the association process between different surfactants and this polysaccharide.

4.3.1. BINDING OF SURFACTANTS TO AM IN SOLUTION AS STUDIED BY MEANS OF SURFACE TENSION

The ability of a polymer to alter the surface tension of a surfactant solution, particularly when the polymer is not surface active in itself, is an indication of the occurrence of interactions (association) between the surfactant and the polymer in bulk. Under these conditions, it is usually assumed that surfactants bound to the polymer do not contribute to the surface tension. Thus, by comparing the surface tension of surfactant solutions with and without polymer, it is possible to estimate the amount of surfactant bound to the polymer at any given surfactant concentration. As AM is in itself a non-surface active polymer, the binding of surfactants to AM in solution can, in principle, be conveniently estimated by means of surface
SUMMARY OF KEY RESULTS AND DISCUSSION

tension determinations. Our studies revealed that, while this was true for the AM-alkyl maltosides systems, the information that could be extracted from the surface tension isotherms of AM-alkyl sulphate systems was rather limited.

Figure 25 A shows the surface tension isotherm (surface tension vs. surfactant concentration) of the nonionic surfactant dodecyl maltoside (C12Malt) in the presence and absence of AM (0.2% w/v) at 25°C. The surface tension isotherm of C12Malt in the presence of AM exhibits the familiar features of that of a surfactant in the presence of a non-surface active polymer and allows the identification of different association regimes. Between points 1 and 2 the higher surface tension of the system with respect to that of the surfactant alone indicates that only a fraction of the total amount of added surfactant remains free. Beyond point 2 and up to point 3, the surface tension of the system remains unchanged, thus indicating that within this concentration range essentially all the added surfactant binds to AM. Beyond point 3, the surface tension of the system drops progressively as the surfactant concentration is increased thus indicating the build up of the surfactant concentration in solution up to the point where surfactant micelles begin to form (point 4). The surface tension isotherms of the two alkyl maltosides (C12Malt and C16Malt) exhibited qualitatively the same features (see Figures 1 and 7 in Paper IV) and for these systems the binding isotherms (amount of surfactant bound vs. total amount of surfactant) and the binding capacity of AM, could be estimated.

Figure 25 B shows the surface tension isotherm of sodium dodecyl sulphate (SDS) in the presence and absence of AM at 25°C. The surface tension isotherm of SDS in the presence of AM is characterised by the occurrence of two clear break points: one occurring at high surface tension values and another occurring at low ones. Beyond the first break point (~0.3 mM), the surface tension falls below that of the pure surfactant, and the low-surface-tension break point takes place well below the cmc of the surfactant in the absence of AM. Thus, beyond the first break point, a synergistic adsorption of polymer-surfactant entities at the liquid-air interface takes place. As both the surfactant and the polymer contribute to the surface tension in this concentration region, the surface tension of the system cannot be used to provide an estimate of the free surfactant concentration in solution. However, a comparison of the surface tension isotherms of the SDS and AM-SDS systems in the presence and absence of electrolyte (100 mM NaCl) (see Figure 25), revealed that the first break point in the isotherm could be used as
an indication of the concentration up to which the surfactant would bind to AM through the formation of non-surface active complexes.

As observed in Figure 25 B, the surface tension isotherm of SDS in the presence of AM and electrolyte is characterised by the occurrence of one clear break point at high surface tensions and another one occurring at low surface tension values. Up to the surfactant concentration at which the first point takes place, hardly any change in surface tension is observed upon addition of surfactant. By contrast, in the absence of polymer, a significant lowering of the surface tension occurs in this SDS concentration range. Thus, this first break point in the isotherm can be unambiguously ascribed to the concentration up to which essentially all the added surfactant binds to the AM and form complexes with no surface activity. Results from binding studies of various surfactants to AM in aqueous solution 12, 62 have indicated that such a distinctive break point in the surface tension isotherm of the surfactant in the presence of AM, denotes the end of a highly cooperative association process of the surfactant to the polymer, which seems to involve the formation of helical inclusion complexes. As observed in Figure 25 B, the first break point in the surface tension isotherm of SDS in the presence of AM takes place at the same SDS concentration (ca. 0.3 mM) both in the presence and absence of electrolyte, demonstrating that the presence of electrolyte does not exert any significant influence on the interactions between SDS and AM up to this point. Thus, we propose that, by analogy, also in the absence of electrolyte all the added SDS binds to AM via formation of non-surface active helical inclusion complexes up to this concentration. Overall, as discussed below, the surface activity of the complexes formed between AM and the two sodium alkyl sulphates (SDS and SHS) within certain conditions and concentration ranges, limited the quantitative information that could be extracted from the surface tension isotherms. However, the information that surface active AM-SDS complexes form, whereas no surface active complexes are detected in the AM-maltoside systems, is intriguing and would be worth further studies.

**Binding of alkyl maltosides to AM: temperature and chain length effects**

The investigation of the effect of the surfactant chain length (C12 vs. C16) on the binding capacity of AM and the solubility of the AM-surfactant complexes was the main objective of our studies. However, the two long-chain surfactants used, i.e. hexadecyl maltoside (C16Malt) and sodium hexadecyl sulphate (SHS), have a high Krafft temperature (~ 45°C). Thus, binding studies of these surfactants to AM in solution could only be carried out above this temperature. To assess the extent to which the association of the surfactants to AM was affected by temperature, binding studies of the short-chain surfactants, C12Malt and SDS, were carried out both at room temperature and at 50°C.

Figure 26 shows plots of the amount of surfactant bound to AM (Cbound) as a function of the total surfactant concentration (Ctot), as determined from surface tension measurements. The curves obtained for the different systems studied, namely AM-C12Malt at 25°C and 50°C and AM-C16Malt at 50 °C, exhibited qualitatively the same features. These curves revealed that most of the binding of the surfactants to AM occurs as the result of a highly cooperative association process, which is evidenced by the steep increase in the amount of bound surfactant as the total surfactant concentration is increased. The total binding capacity of the AM molecule is given by the maximum amount of surfactant bound which, in these systems, seems to coincide with the end of the cooperative binding regime. The free energy gain of binding of the surfactant to AM compared to the formation of surfactant micelles (ΔG°),
which gives an indication of how favourable the binding process is, can be estimated by using the expression:

\[ \Delta G^o = kT \ln \left( \frac{\text{cac}}{\text{cmc}} \right) \]  

(Eq.3)

where \( k \) is the Boltzman constant, \( T \) is the temperature and \( \text{cac} \) is the critical association concentration of the surfactant to the polymer, which is determined by extrapolation of the linear portion of the binding isotherm, in a linear-linear scale, to zero binding. The higher the magnitude of \( \Delta G^o \) (absolute value), the higher the extra driving force for the association with AM compared to the association into surfactant micelles. The estimated cacs and calculated free energy gain (\( \Delta G^o \)) for the association of the alkyl maltosides to AM at 25°C and 50°C are listed in Table 7.

![Figure 26. Binding isotherms (C_bound vs. C_tot) of at (A) C12Malt to AM at 25°C and 50°C and (B) C12Malt and C16Malt to AM at 50°C as determined from surface tension measurements. C_bound, bound surfactant concentration reported as the number of surfactant molecules bound per 1000 glucose units (GU) in the AM molecule; C_tot, total surfactant concentration.](image)

**Table 7.** Characteristics of the association of alkyl-maltosides to AM.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>T (°C)</th>
<th>cmc (mM)</th>
<th>cac (mM)</th>
<th>( \Delta G^o ) (kT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12Malt</td>
<td>25°C</td>
<td>0.17</td>
<td>0.011</td>
<td>-2.7</td>
</tr>
<tr>
<td>C12Malt</td>
<td>50°C</td>
<td>0.21</td>
<td>0.083</td>
<td>-0.9</td>
</tr>
<tr>
<td>C16Malt</td>
<td>50°C</td>
<td>0.0035</td>
<td>≤ 0.001</td>
<td>≤ -1.3</td>
</tr>
</tbody>
</table>

A comparison of the binding isotherms and characteristics of the association of C12Malt to AM at 25°C and 50°C (see Figure 26 A and Table 7) revealed that an increase in temperature delays the onset of the association of the C12Malt to AM (i.e. the association begins at higher surfactant concentrations) and makes the binding process less favourable in terms of energy gain with respect to the formation of surfactant micelles (see the magnitude of \( \Delta G^o \) in Table 7). By contrast, an increase in the surfactant chain length (C16Malt vs. C12Malt) favoured the association of the surfactant to AM which is evidenced by the fact that the binding of C16Malt to AM begins at lower concentrations and involves a greater energy gain (Figure 26 B and Table 7).
The highly cooperative nature of the association of surfactants to AM has been attributed to the induction of a coil-helix transition in the AM molecule upon binding which would simplify the successive formation of inclusion complexes with AM. Support to this view is provided by conformational studies of AM in the presence of increasing amounts of fatty acid salts. The delayed onset of the association of C₁₂Malt to AM at higher temperatures and the less favourable nature of the association process with respect to the association at 25°C (given by the magnitude of ΔＧ°), is interpreted as being related to the effect of high temperatures on the solution properties of AM. Higher temperatures have been reported to favour a more disordered conformation of the AM molecule, which in turn, could counteract the formation of AM-surfactant inclusion complexes. The observed effect of an increase of the surfactant chain length could be interpreted as follows. The association between surfactants and AM is driven by interactions of hydrophobic nature between the surfactant alkyl chain and the inner core of the AM helix. An increase in the surfactant chain length will confer a more hydrophobic character to the surfactant molecule which, driven by a stronger tendency to minimise unfavourable water/alkyl-chain contacts, will then exhibit a stronger driving force for binding to the polymer.

Overall, the binding capacity of the AM molecule was not found to be strongly affected neither by an increase in temperature nor by an increase in the surfactant alkyl chain length. Under the conditions used in this study, the AM molecule was found to bind 25 and 30 C₁₂Malt molecules/1000 glucose units at 25°C and 50°C, respectively, and 27 C₁₆Malt molecules/1000 GU at 50°C.

**Binding of sodium alkyl sulphates to AM: temperature and chain length effects**

As discussed previously, the surface activity of the complexes formed between AM and sodium alkyl sulphates within certain concentration ranges, limited the information that could be extracted from the surface tension isotherms of these systems. In an attempt to gain further information on the binding of these surfactants to AM, these systems were explored by means of ¹H NMR spectroscopy. In contrast to surface tension measurements, NMR provides direct information on the interactions between the surfactant and AM in the bulk. However, two characteristic features of these systems severely limited the possibilities of analysis by means of ¹H NMR, namely i) the presence of a butanol residue in the AM sample that overlapped with the better-resolved surfactant peaks in the NMR spectrum and ii) the detrimental effect that the substitution of H₂O by D₂O proved to have on the stability of AM solutions (the use of deuterated water as a solvent is imposed by the choice of technique as described in the Experimental section in Paper V). Consequently, only the AM-SDS system at 50°C proved to be sufficiently stable for ¹H NMR measurements.
Figure 27 A shows the surface tension isotherm of SDS in the presence and absence of AM at 50°C. In contrast to what was found at 25°C, the surface tension of the AM-SDS system at 50°C does not remain unaltered as the surfactant concentration is increased up to the first break point in the isotherm. In fact, it is lower than the surface tension of the surfactant in the absence of AM. Thus, the higher temperature induces a change in the AM-SDS interactions within the concentration region where the surfactant is expected to bind to AM in a cooperative fashion to yield non-surface active helical inclusion complexes. As a consequence of this change, AM-SDS entities adsorb synergistically at the air-liquid interface. The investigation of this system by means of $^1$H NMR indicated that binding of SDS to AM decreases the hydrodynamic radius of AM, that is, compacts the AM molecule, and slows down the internal dynamics of the polymer.

As indicated by the surface tension isotherm of SHS in the presence of AM at 50°C (Figure 27 B), an increase in the surfactant chain length ($C_{12}$ vs. $C_{16}$) counteracts the molecular events that lead to a synergistic adsorption of AM-surfactant entities at the interface in the region where cooperative binding to AM is expected to occur. Moreover, the first break point in the surface tension isotherm of SHS in the presence of AM takes place at a concentration at which the surfactant would bring about important reductions of the surface tension in the absence of AM. In consequence, this point distinctly denotes the concentration up to which essentially all of the added SHS binds to AM. The lack of surface activity of the AM-SHS system up to this concentration allows for the estimation of the binding capacity of the AM helix which is found to be ca. 22 SHS molecules/1000 GU. This value is not very different to the SDS binding capacity of AM at 25°C, which was found to be ca. 26 SDS molec/1000 glucose units. It must be pointed out that these values are somewhat lower, yet not very different, than the $C_{12}$Mal and $C_{16}$Malt binding capacity of AM at the same temperatures (25 and 27 molecules/1000 GU, respectively).
4.3.2. SOLUBILITY OF AM-SURFACTANT COMPLEXES

AM molecules in solution have a strong propensity to form inter-chain associations. This tendency leads to the formation of molecular aggregates large enough to scatter light in the visible region. As a consequence, AM solutions become increasingly cloudy over time due to the progressive association into larger aggregates. As these aggregates increase in size they will start to settle. The series of molecular events responsible for the instability of dilute AM solutions are counteracted by an increase in temperature. In fact, at the AM concentrations used in this study (0.2% w/v), surfactant-free AM solutions at 25°C showed evidence of phase separation as a decrease in the transmittance of the bulk solution (i.e. an increase in the sample turbidity) already within the first 24 hrs after preparation and sedimentation of aggregates began to occur after ca. 11 days. On the contrary, surfactant-free AM solutions kept at 50°C remained clear and showed no signs of phase separation within the time scale of our observations (ca. 30 days).

In the studies presented in Papers IV and V the solubility (i.e. stability against phase separation) of different AM-surfactant complexes at different levels of surfactant binding was assessed by means of transmittance determinations. A high transmittance denotes clear solutions whereas low transmittance values evidence turbidity or cloudiness in the sample, which in turn indicates poor water solubility of the system.

The binding of the two alkyl maltosides, C_{12}Malt and C_{16}Malt, to AM resulted in insoluble complexes that phase-separated almost immediately and formed aggregates that settled shortly after (typically <30 min). The insolubility of the formed complexes was evidenced by the low transmittance (i.e. the cloudiness) of AM-surfactant solutions after the addition of the surfactant (see Figure 28). In the two AM-surfactant systems, higher surfactant concentrations resulted in more turbid solutions (lower %T). Moreover, the degree of cloudiness displayed a strong correlation with the extent of binding of the surfactant to the polymer as determined by surface tension measurements. However, a few noticeable differences between the effect of the short- and the long-chain alkyl maltoside could be identified. The addition of C_{12}Malt only had a destabilising effect on the AM solution (evidenced by low transmittance values) at concentrations beyond those at which considerable binding takes place (>5 molec/1000GU) (Figure 28). By contrast, even at low levels of binding (< 5 molec/1000GU), the C_{16}Malt was found to induce a substantial decrease in the transmittance of the solutions, in which a small amount of sediment was formed shortly after. Altogether, these findings indicated a more destabilising effect of the longer-chain maltoside on the AM with respect to that of the C_{12}Malt, which in turn pointed to the formation of complexes with different solution properties.

The stability of the AM solutions increased considerably in the presence of SDS. The extent of this effect was assessed by following the stability of AM solutions at 25°C in the presence of increasing amounts of SDS (Figure 29A). No signs of phase separation were observed within the time scale of our observations (ca. 14 days) in AM solutions in the presence of 0.55, 3 and 10 mM SDS. At lower SDS concentrations (0.03 and 0.2 mM), the AM solutions began to show signs of phase separation (i.e. a decrease in transmittance) after 7 days. Thus, at low concentrations the association of the surfactant to the polymer is not as efficient in preventing the molecular association and growth of aggregates that leads to phase separation in AM solutions.
SUMMARY OF KEY RESULTS AND DISCUSSION

Figure 28. Transmittance of (A) C_{12}Malt-AM and (B) C_{16}Malt-AM systems (0.2% w/v AM) 15 min after the addition of the surfactant at 50°C. The reported values are averages of the transmittance along the whole sample height. Transmittance data is contrasted with the amount of bound surfactant as estimated from surface tension measurements.

Despite the fact that both SDS and SHS bear a charged head group, the association of these two surfactants to AM was found to produce two distinctive effects on the solution stability of the AM molecule. In fact, the AM solutions in the presence of SHS exhibited poorer solution stability than in the absence of surfactant. This was evidenced by an increase in the turbidity of the solutions over time. As observed in Figure 29, the degree of instability of these solutions was found to depend on the concentration of SHS. Below 0.06 mM SHS, the complex was essentially stable but an increase in SHS concentration (i.e. an increase in the extent of binding of the surfactant to the polymer) was found to confer certain instability to the AM molecule. At SHS concentrations ≥ 0.3 mM the AM-surfactant complexes are much more unstable than at lower concentrations. Interestingly, by increasing the SHS concentration within this concentration region, the solubility of the complex increased in terms of reduced rate of aggregation. The aggregates increased in size and began to settle after 10, 15 and 20 hours at SHS concentrations of 0.3, 0.4 and 1 mM, respectively.

From our investigations on the solubility of different AM-surfactant systems it is clear that the structural changes that AM undergoes upon association with surfactants affect the solubility of the resulting AM-surfactant complexes, as well as the rate of aggregation of these into larger aggregates. In this respect, there seems to be at least two opposing effects determining the solubility and stability of the AM-surfactant complex. Firstly, the conformational changes associated with the formation of helical inclusion complexes confer poor stability of the complexes. The degree of insolubility associated with the conformational change will be higher the longer the chain length of the surfactant. Secondly, the polyelectrolyte character of the AM-surfactant complex, provided by association of charged surfactants to AM, favours the solubility of the complexes. For nonionic surfactants only the first effect is present. On the contrary, the stability of the complexes formed with ionic surfactants will be determined by a balance between the two opposing effects.
Figure 29. Transmittance of (A) AM-SDS solutions (0.2% w/v AM, 25°C) and (B) AM-SHS solutions (0.2% w/v, 50°C) as a function of time. The reported values are averages of the transmittance along the whole sample height.

The mechanism that favours the solubility of complexes formed between AM and ionic surfactants, is likely related to the polyelectrolyte character of the resulting polymer-surfactant complexes. Electrostatic repulsions may keep the AM molecule in a more expanded conformation which in turn might hinder intra- and intermolecular association thus delaying (or even preventing) the phase separation process. This view is supported by our findings on the stability of AM-SDS solutions in the presence of 100 mM NaCl. In the presence of electrolyte, AM solutions became instantaneously cloudy upon addition of SDS.

In the absence of stabilising charges, the conformational change induced by surfactant upon complexation is enough to cause the AM to adopt a more compact conformation, which in turn favours molecular association and a rapid growth of the molecular aggregates, thus causing the AM to precipitate readily. In this respect, we propose that the lower solubility of the complexes formed between AM and long-chain surfactants may result from the formation of stiffer helical segments upon complexation. This view is based on the results from studies of complexes of photoreactive dyes (a chromophore attached to a linear alkyl chain) with AM, which found that the rigidity of the dye in the complexed state increased by increasing the alkyl chain length. The formation of stiffer helical segments may in turn allow the AM molecule to adopt a more rigid conformation which would favour intra-molecular association and a rapid growth of the aggregates. In the case of a charged surfactant such as SHS, further association of the surfactant to AM beyond the point where helical inclusion complexes are formed would further increase the charge density of the complexes and counteract the destabilising effect induced by the complexation. This is indeed found to be the case for the SHS-AM system, for which an increase in the surfactant concentration beyond 0.3 mM SHS confers more stability to the complexes.
5. CONCLUSIONS

In this study the interactions between surfactants and starch have been investigated within different contexts. Systematic investigations on the effect of the surfactant structure (head group and chain length) on the temperature-induced changes of wheat starch granules in excess water revealed that:

i) The ability of surfactants to restrict or enhance the swelling of a starch with a normal AM content (~ 30% w/w) is affected by the length of the surfactant alkyl chain. With the only exception of the cationic surfactants alkyl ammonium bromides, short-chain (C\textsubscript{10}, C\textsubscript{12}) anionic and non-ionic surfactants favour granule swelling at lower temperatures whereas the long-chain surfactants (C\textsubscript{14}, C\textsubscript{16}) have the opposite effect.

ii) Calorimetric evidence indicates that the chain length-dependent effect of surfactants on the swelling properties of normal starch is correlated with the dissociation of the AM-surfactant complexes formed simultaneously as the granules gelatinise.

iii) Differences in the surfactant chain length (C\textsubscript{12} vs. C\textsubscript{16}) affect the accessibility of surfactants to normal starch granules during gelatinisation, the diffusion of the longer chain surfactant into the granule matrix being more restricted.

Investigations on the characteristics of the association process between short- and long-chain surfactants to AM and the solution properties of the resulting complexes showed that:

iv) Changes in surfactant chain length (C\textsubscript{12} vs. C\textsubscript{16}) and head group structure (sodium sulphates vs. maltosides) do not effect significantly the overall binding capacity of the AM helix in neutral aqueous solution.

v) Beyond the concentration at which the AM helix is expected to be saturated, the interactions between AM and the two alkyl sulphates, SDS and SHS, result in systems that exhibit surface activity.

vi) The solubility and stability of the AM-surfactant complexes is suggested to be determined by a balance between two opposing effects. Firstly, the conformational changes associated with the formation of helical inclusion complexes confer poor stability to the complexes. The degree of instability associated with the conformational change will be higher the longer the alkyl chain length of the surfactant. Secondly, the polyelectrolyte character of the AM-surfactant complex, provided by association of ionic surfactants to AM, favours the stability of the complexes.
6. FUTURE WORK

As it seems to be the case in any research project, our investigation has provided answers but it has also given rise to more questions. This section summarises the areas where it would be interesting to carry out further work to address some of these questions.

A good complement to our studies on the effect of surfactant structure on the swelling properties of starch would be the investigation on the ability of different surfactants to interact and solubilise granule surface proteins and lipids. Removal of these components is known to enhance the rate and extent of swelling in cereal starch granules. Furthermore, surfactants like SDS have been reported to be very effective in the removal of these starch granule components. Differences in the ability of different surfactants to solubilise surface proteins and lipids may explain, for example, the special capacity of cationic surfactants to enhance granule swelling or the fact that, in general, short-chain anionic surfactants are more effective than their nonionic counterparts in enhancing granule swelling. In this respect, it would also be interesting to explore whether the swelling enhancing effect of the alkyl ammonium bromides applies to all cationic surfactants or is specific for this particular group of surfactants.

Our investigations on the interactions between AM and surfactants in solution, in particular the sodium alkyl sulphates, were restricted by the surface activity of the resulting AM-surfactant complexes, the presence of a butanol impurity in the AM sample and the detrimental H$_2$O/D$_2$O effects on the stability of AM. The extent of binding of these surfactants to AM in solution, especially at elevated temperatures, may be more conveniently determined by means of other techniques such as surfactant selective electrodes and dynamic surface tension. In combination with optical rotation studies, these investigations may provide a clearer picture of the type of interactions and extent of association of alkyl sulphates to AM in bulk.
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This thesis compiles the results of several years of work. A work that was carried out with the intention of making a contribution to a research area that is both fascinating and overwhelmingly vast. What was scientifically achieved as a result of these years of work is described in the thesis. The awful lot that I learnt along the process, as a researcher and as a person, is not. All the accomplishments that were made during these years’ work, both the ones that are described in the thesis and those that are not, were possible thanks to the direct and indirect contribution of many people. To all of them I am very grateful. However, among them, there are some people in particular that I would like to take the opportunity to express my gratitude to:

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8. REFERENCES


Interactions between Surfactants and Starch: from Starch Granules to Amylose Solutions


