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The influence of L-amino acid molecular structure on the phase transition temperature of hydroxypropyl methylcellulose

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Abstract

The influence of molecular structure of small ionised molecules on the thermo-reversible sol:gel phase transition of a hydroxyalkyl cellulose ether has been investigated with respect to an L-amino acid series. The concentration dependency of the phase transition temperature of 1% w/w hydroxypropyl methylcellulose solutions in the presence of different amino acids was studied turbidimetrically. The effects of different amino acids ranged from -33.54 to $+45.52\,^{\circ}\mathrm{C\,M^{-1}}$. Correlations between the effect on phase transition temperature and amino acid molecular properties showed the strongest relationships with amino acid hydrophobicity. Small hydrophilic amino acids lowered the transition temperature, whereas large hydrophobic aromatic amino acids increased this temperature. The effect of different amino acids was proposed to be a balance between the ability of their hydrophilic regions to dehydrate and disrupt the polymer hydration sheath, and the ability of their hydrophobic regions to associate with and solubilise the methoxyl-dominated regions of the polymer.

Keywords: Hydroxypropyl methylcellulose; Phase transition; Gelation; Amino acids; Cloud point; Hydrophobic

1. Introduction

Water-soluble cellulose ethers have a diverse array of applications in the pharmaceutical, cosmetic and food industries, where they are employed as thickening, binding, film and matrix-forming agents, playing a key role in maintaining product integrity and functionality (Doelker, 1993; Henderson, 1988; Melia, 1991).

Aqueous solutions of hydroxypropyl methylcellulose (HPMC) and certain other non-ionic hydrophobically-modified cellulose ethers, undergo a thermo-reversible sol:gel phase transition on heating, and it has been proposed that this results from dehydration of hydrophobically substituted regions of the polymer chain, and the formation of a 3D network structure stabilised by hydrophobic interactions (Doelker, 1993; Sarkar, 1979). Haque & Morris (1993); Haque, Richardson, and Morris (1993) have applied a range of investigative techniques to further probe this phenomenon, and have proposed an elegant detailed model of the gelation mechanism. At low temperatures, native cellulose interactions between unsubstituted regions allow HPMC chains to aggregate into discrete cellulosic bundles, with methoxyl-substituted regions in each bundle forming hydrophobic clusters. On heating, they suggest that thermogelation proceeds via a two-stage mechanism where, in the early stages, the hydrophobic clusters partially dissociate and, upon exposure to the aqueous environment, then become sheathed in a structured water 'cage'. On further heating, thermal disruption of the water 'cages' allows association between the hydrophobic regions of different cellulosic bundles, and results in the formation of a stable network structure.

The sol:gel phase transition manifests physically as gel formation or the appearance of cloudiness which can be detected rheologically or turbidimetrically (Heyman, 1935). As this phase change occurs over a narrow temperature range, the sol:gel transition is usually described as a specific temperature; the thermal gelation temperature (TGT) or cloud point temperature (CPT). It has long been recognised that dissolved ions can modulate the transition temperature, increasing or decreasing TGT or CPT (Touitou & Donbrow, 1982). Salts

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commonly depress the phase transition temperature to an extent dependent on their type, valency and charge, and this can influence polymer properties as diverse as solution viscosity, dispersability and drug release (Alderman, 1984; Mitchell et al., 1990; Touitou & Donbrow, 1982). In contrast, surfactants and other amphiphilic molecules can raise the phase transition temperature and recent studies have provided evidence that this may arise from micellar solubilisation of hydrophobic regions (Nilsson, 1995; Riddell, Evertsson, Nilsson, & Sundelof, 1999). Much of our knowledge has been derived from studies of methylcellulose and ethylhydroxyethylcellulose, but HPMC is perhaps now a more important polymer because of its wide application.

HPMC and other cellulose ethers are often co-formulated with water-soluble excipients; for example HPMC may be co-formulated with amino acids in foods and dietary supplements, or in pharmaceuticals when amino acids are used as taste masking agents, counter ions, osmotic agents or buffers. Amino acids have also been used in HPMC tablets to modulate drug solubility and permeability, and improve in vivo bioavailability (Wu, Brunelle, & Turner, 2005). It is therefore essential to understand potential interactions between HPMC and excipients that may influence polymer functionality. The purpose of this study was to investigate if the molecular properties of L-amino acids influence the thermal gelation properties of HPMC solutions. L-amino acids are particularly suited to this study as they provide us with a series of highly-characterised molecules with a range of molecular structures and varied side chains with which to probe the effects of small molecules on the HPMC sol:gel phase transition.

2. Materials and methods

2.1. Materials

Hydroxypropyl methylcellulose (HPMC) USP type 2208 (Methocel K4M, BNOG04012N11, Hydroxypropyl substitution: 8.4%, Methoxyl substitution: 22.4%) was a kind gift from Colorcon Ltd, Dartford, UK. Other materials were obtained from Sigma-Aldrich Co Ltd, Dorset, UK. Water was Maxima HPLC grade (USF Elga, Buckinghamshire, UK) with maximum conductance 18.2 MΩ/cm.

2.2. Preparation of solutions for turbidimetry

One percent w/w HPMC solutions were prepared containing a single free base L-amino acid at a range of concentrations up to maximum solubility at room temperature. This was achieved by mixing separately-prepared concentrated HPMC and amino acid solutions with continuous stirring until visually homogenous. The solutions were then allowed to equilibrate for 16 h at 2–8 °C prior to experimental work. To confirm that amino acids were present in their zwitterionic form, the pH of each HPMC:amino acid mixture was measured (Hanna HI8424 pH Meter, Bedfordshire, UK) and the degree of ionisation calculated from published pKa values for amino acids at room temperature (Martin, 1993). These calculations confirmed that, except for amino acids with an ionisable side chain group, all amino acids were present with >99.9% ionisation of both amino and carboxyl groups.

2.3. Measurements of phase transition temperature

Phase transition temperatures were measured turbidimetrically by cloud point determinations in triplicate on HPMC:amino acid mixtures using a temperature-ramped white light turbidimeter (C.Washington, Nottingham, UK) and 10 mm pathlength cells. Cloud point temperature was defined as the temperature at which light transmission was reduced by 50% (Sarkar, 1979). The gradient of the fitted linear regression line (ΔCPT) of cloud point temperature against amino acid concentration was used to compare the relative influence of each amino acid on HPMC phase transition.

2.4. Modelling of amino acid molecular parameters

Amino acids were modelled using computational chemistry methods in order to determine certain molecular parameters. Semi-empirical quantum mechanical geometry optimisation was performed using the AM1 Hamiltonian within the program Spartan PCPro (Wavefunction, Inc., Irvine, California, USA). From these optimised geometries, a range of molecular parameters were determined including dipole moment, SP energy, molecular area and volume. Other parameters were taken from published data and are referenced accordingly.

2.5. Correlation of ΔCPT with amino acid molecular parameters

Linear correlation analyses at a significance level of $\alpha = 0.01$ were undertaken between ΔCPT and the different parameters using SigmaPlot 2002, v.8.0 (SPSS, Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Influence of amino acids on the phase transition temperature

In the absence of amino acids, the phase transition temperature of 1% w/w HPMC solutions was 74.3 ± 0.5 °C

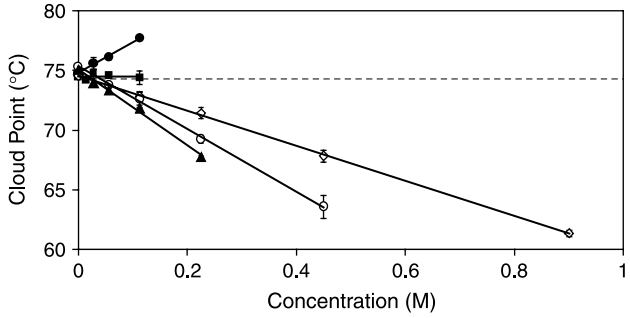
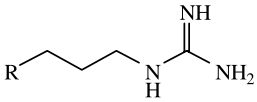
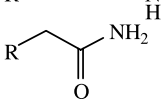
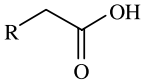
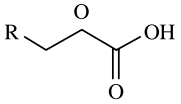
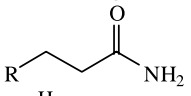
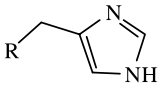
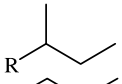
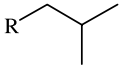
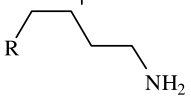
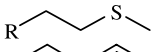
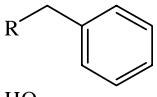
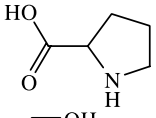
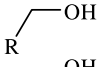
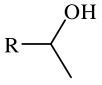
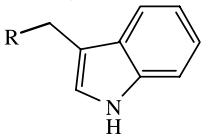
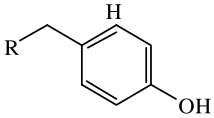


Fig. 1. The effect of concentration on the phase transition temperature of 1% w/w HPMC (Methocel K4M) solutions for selected L-amino acids: (●) Phe; (■) Ile; (◇) Pro; (○) Thr; (▲) Gln. Dotted line represents the cloud point temperature of HPMC in the absence of amino acids. Mean ($n = 3$) ± 1SD.

Table 1
Values of ΔCPT determined by linear regression analysis

Amino acid	Abbreviation	Side chain structure	ΔCPT[a] (°C M^{-1})	Standard deviation of residuals from line (S*y*.*x*) (°C)
Alanine	Ala		−18.17	1.36
Arginine	Arg		−5.20	1.81
Asparagine	Asn		−33.54	0.43
Aspartic acid	Asp		−30.57	0.46
Glutamic acid	Glu		−23.78	0.28
Glutamine	Gln		−31.84	0.31
Glycine	Gly		−20.93	1.31
Histidine	His		−8.79	0.58
Isoleucine	Ile		−0.03	0.58
Leucine	Leu		−2.52	0.51
Lysine	Lys		−30.29	0.60
Methionine	Met		−1.695	0.50
Phenylalanine	Phe		25.70	0.31
Proline[b]	Pro		−14.68	0.39
Serine	Ser		−35.23	2.19
Threonine	Thr		−26.15	0.54
Tryptophan	Trp		45.52	0.22
Tyrosine	Tyr		40.43	0.31
Valine	Val		−11.77	1.14

[a] ΔCPT was the gradient of the fitted line in plots of cloud point against amino acid concentration.
[b] Full structure shown.

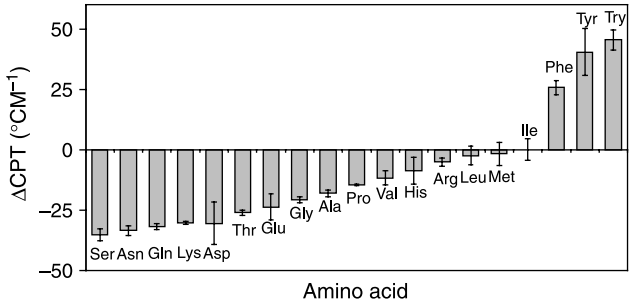


Fig. 2. The rank order of L-amino acids in their effect on the phase transition temperature of 1% w/w HPMC solutions.

($n=3$) correlating well with typical published values for Type 2208 HPMC (Alderman, 1984). Fig. 1 illustrates how this value was changed in the presence of increasing concentrations of different amino acids. The apparently linear relationships allowed the gradient of the fitted regression line (ΔCPT) to be used as measure of the relative molar potency.

Table 1 shows ΔCPT values obtained for the full range of amino acids examined. The low values obtained for the standard deviation of the residuals from the line ($Sy.x$), confirm the appropriateness of a linear regression fit. ΔCPT values were found to vary widely, with extreme values ranging from serine (-35 °C M^{-1}) to tryptophan ($+45$ °C M^{-1}). Arranged in rank order the range of amino acids studied formed a useful series which show a clear gradation in their effect (Fig. 2).

3.2. Correlation of amino acid properties with phase transition modifying behaviour

Table 2 shows the results of linear correlation analyses of ΔCPT with published and calculated molecular parameters

Table 2
Linear correlations of ΔCPT with amino acid molecular parameters

Parameter	Pearson's correlation coefficient (r value)
Walden product (classical)[a]	−0.450
Dipole moment[b]	−0.257
Isoelectric point[a]	0.057
SP energy[c]	0.436
Enthalpy of formation[c]	0.580
Molecular weight[c]	0.647
Molecular area[b]	0.663
Molecular volume[b]	0.693
Solvation free energy[d]	0.735
Log P[e]	0.730
Hydrophobic contribution constant[f]	0.723
Solvation free energy[d]	0.939[g]
Log P[e]	0.873[g]
Hydrophobic contribution constant[f]	0.920[g]

[a] Values taken from Lutz, Vrachopoulou, and Groves (1994).
[b] Values calculated in this study by molecular modelling.
[c] Values taken from Lide (2001).
[d] Values taken from Sandberg & Edholm (2001).
[e] Values calculated using Log P Predictor, Interactive Analysis, Bedford, MA, USA.
[f] Values taken from Palekar, Shine, and Lien (1996).
[g] Values calculated with amino acids with ionisable side chains excluded.

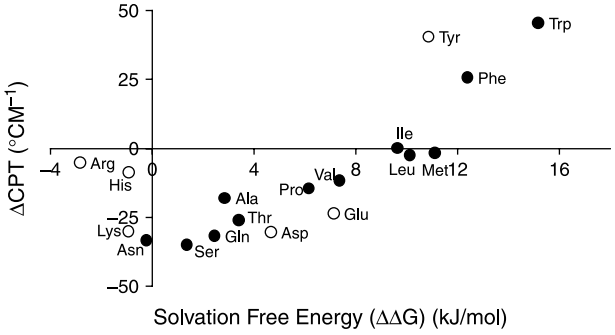


Fig. 3. Relationship between the solvation free energy of amino acid residues and their ability to modulate the HPMC phase transition temperature. (●) Non-ionisable side chain; (○) Ionisable side chain.

describing different properties of the amino acid series. This was done in an attempt to identify if particular molecular features or properties of the amino acids might correlate with their phase transition modifying behaviour. Significant correlations ($r>0.549$, $\alpha<0.01$) were obtained with molecular area, weight and volume, but the highest correlation values were obtained with solvation free energy, log P, and hydrophobic contribution constant. These latter parameters are those associated with the hydrophobic character of the molecule, which, as all amino acids were in a zwitterionic form, relates to their side chain groups. Plots of these parameters against ΔCPT (Figs. 3–5) show how the majority of amino acids follow a main sequence, whilst a few appear to be outliers. Different amino acids were outliers in different plots, but all were amino acids with a further ionisable group on the side chain (Asp, Glu, Arg, His, Lys, Tyr). There are several arguments for considering these amino acids separately; they can adopt a non-standard zwitterionic state, not all groups are fully ionised, they have less hydrophobic/hydrophilic separation and they are capable of ion-pairing (Greenstein & Winitz, 1961; Gao, Wang, & Lien, 1995). Improved correlations are obtained when these amino acids were excluded from the linear correlation analyses (Table 2) and whilst the relationship between ΔCPT and solvation free energy (Fig. 3) appears almost linear, closer examination of Figs. 4 and 5 suggests, for log P and the hydrophobic contribution constant, a biphasic relationship with different slopes above and below 0 °C M^{-1}.

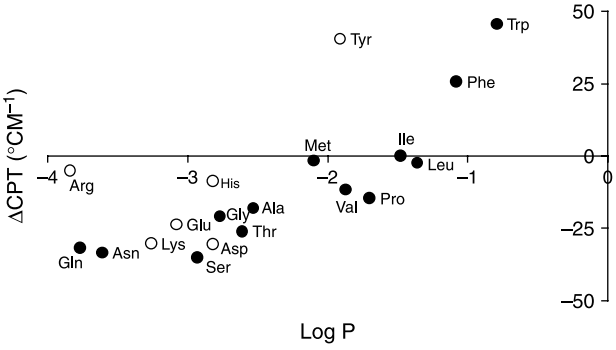


Fig. 4. Relationship between log P values for amino acid residues and their ability to modulate the HPMC phase transition temperature. (●) Non-ionisable side chain; (○) Ionisable side chain.

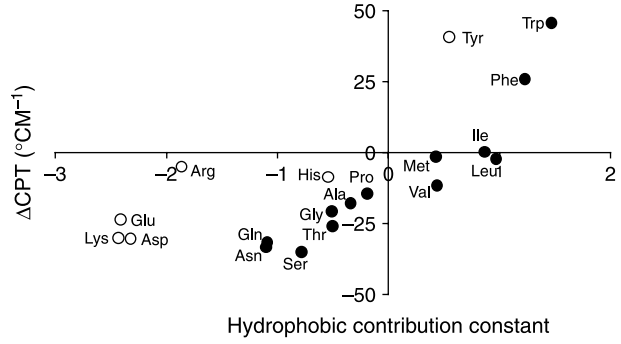


Fig. 5. Relationship between the hydrophobic contribution constant of amino acid residues and their ability to modulate the HPMC phase transition temperature. (●) Non-ionisable side chain; (○) Ionisable side chain.

This entices us to postulate that different mechanisms might be involved in the depression and elevation of phase transition temperature, by amino acid species.

3.3. Prospective mechanisms by which amino acids may depress or elevate HPMC phase transition temperature

The ability to modulate the phase transition temperature of non-ionic hydrophobically-modified cellulose ethers is not exclusive to amino acids. It is well known that other inorganic and organic ions can influence the cloud point temperature of cellulose ether solutions. Mitchell et al. (1990); Touitou & Donbrow (1982) have shown how molecules that depress cloud point temperatures and 'salt-out' the polymer, tend to be small hydrophilic ionic species with a high charge density. They suggest that these molecules reduce the phase transition temperature by removing water from the polymer hydration sheath and lowering the temperature at which hydrophobic interactions between polymer chains becomes favourable. In the current study, the amino acids are present as zwitterions, and this mechanism may explain the effect of the smaller, more hydrophilic amino acids in depressing phase transition temperatures. Glycine for example, is effectively a 'pure' zwitterion, and has a significant depressant effect on cloud point ($\Delta CPT = -20.93\ ^{\circ}C\ M^{-1}$). The depressant effect seems to increase with hydrophilicity of the amino acid. Fig. 5, which relates ΔCPT to the hydrophobic contribution constant of the side chain, shows how amino acids which are more potent than glycine (Gly) in lowering ΔCPT, are also those which have less hydrophobic character. These are the amino acids with side chains containing non-ionisable hydrophilic groups such as hydroxyl (Ser, Thr) or amide (Asn, Gln). Conversely, amino acids that are less potent than glycine appear to possess more hydrophobic side chain structures. Fig. 5 shows that as the hydrophobicity of the side chain is increased, the effect on the phase transition is reduced. For example, if we increase the length of the aliphatic side-chain from –H (Gly), $–CH_3$ (Ala), $–C_3H_7$ (Val) through to $–C_4H_9$ (Ile, Leu), there is increasingly less effect on the phase transition temperature. Thus, we might postulate that the propensity of the amino acid zwitterion to dehydrate HPMC, is in some way being moderated by an effect from the hydrophobic regions of the amino acid molecule.

The only amino acids that caused elevations in HPMC phase transition temperature were Phe, Trp and Tyr. These are molecules which possess a hydrophobic side chain incorporating an aromatic ring. Ridell et al. (1999) have reported how sodium ibuprofen, a molecule with some structural similarity to these amino acids, also elevated ΔCPT. Sodium ibuprofen combines an ionised carboxyl group with a pendant aromatic hydrocarbon chain, and it can form micelle-like aggregates in solution. When added to HPMC solutions, there was evidence that sodium ibuprofen was adsorbed to HPMC polymer chains, forming mixed drug:polymer micelles which solubilised the hydrophobic regions of the polymer dominated by methoxyl substitution. If we adopt the gelation mechanisms proposed by Haque et al. (1993); Sarkar (1979), then solubilisation of these regions would strengthen the solvation sheath of the polymer and raise phase transition temperatures.

Further evidence for the micellar solubilisation of HPMC hydrophobic regions comes from studies of HPMC:sodium dodecyl sulphate mixtures. Surfactant molecules were shown to interact with hydrophobic regions in the HPMC chain in a co-operative manner, and following micellisation, increased both polymer solubility and the sol:gel phase transition temperature (Holmberg & Sundelof, 1996; Kjoniksen, Nystrom, & Lindman, 1998; Nilsson, 1995).

The ability of amphiphilic molecules to associate with and solubilise hydrophobic regions of HPMC may therefore be an important mechanism in increasing the phase transition temperature. It is interesting to postulate that the aromatic amino acids (Phe, Trp, Tyr) may increase the phase transition temperature through this mechanism as they are known to participate in hydrophobic interactions and self-associate during the formation and stabilisation of tertiary protein structures (Cserhati & Szogyi, 1995; Wilce, Aguilar, & Hearn, 1995).

The varied effect of aliphatic amino acids on the phase transition temperature may also be explained by this mechanism. The results described previously show how increasing the length of the aliphatic side chain reduces the propensity of the amino acid to depress the phase transition temperature, and we can speculate that extending the aliphatic chain may facilitate hydrophobic interactions which counterbalance the negative effect of the zwitterion on the phase transition. A reasonable conclusion would then be, that the effect observed for a particular amino acid is a balance between the ability of the zwitterion and other hydrophilic regions of the amino acid to dehydrate and disrupt the hydration sheath, and the ability of the hydrophobic regions of the amino acid to associate with and solubilise the methoxyl-dominated regions of the polymer.

4. Conclusions

L-amino acid molecules modulate the HPMC sol:gel phase transition but differ widely in their effects, which correlated strongly with molecular parameters associated with the hydrophobic character of the amino acid side chain. Small hydrophilic amino acids reduced the HPMC phase transition

temperature, whereas large aromatic amino acids increased the transition temperature. In common with other amphiphilic molecules, there has been evidence that amino acids may associate with and solubilise hydrophobic regions of HPMC, and increase the temperature required for gelation. The effect observed for a particular amino acid may therefore arise from a balance between (i) the ability of the zwitterion to remove water of hydration and disrupt the structured water that sheaths the hydrophobic substituents of the polymer and (ii) the ability of the side chain to undergo hydrophobic association and solubilise the methoxyl dominated regions of the polymer. The effect of amino acids on the phase transition temperature of HPMC solutions may have an impact on polymer functionality and should be considered in applications where HPMC and amino acids are used together.

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