Properties of polysaccharides in sol and gel states.

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Biomaterials Applications
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Program


7.- Physical Gels based on cooperative interactions. Structure of junction zones. Mechanisms of gelation with one and two polysaccharides.

8.- Processing and applications of few polysaccharides: hyaluronan, chitosan, alginate....
Fourth part

-Viscosity in dilute and semi-dilute solution

-Influence of MW and polymer concentration

-Shear rate effect and salt influence

-Dynamic rheology (sol & gel)
The experimental conditions to characterize a charged polymer are the following:

- dilute aqueous solution \((C<C^*)\)

- control of the counterions (only monovalent if possible)

- presence of 1-1 external salt, at least 0.05M \((\kappa^{-1}\sim1.3\text{nm})\) or better 0.1M \(\rightarrow\) nearly same analysis than for neutral polymers (viscosity, SEC, LS etc...) \(\rightarrow\) \(M_w\), molar mass distribution and radius of gyration and intrinsic persistence length \((\text{SEC, LS})\) \(\rightarrow\) Intrinsic viscosity
Influence of polymer concentration on the solution structure

\[ C^* \] is the overlap concentration (isolated molecules ↔ entangled molecules)

Dilute regime
(necessary for characterization)

Semi dilute regime
Viscosity

\[ \frac{F}{A} = \sigma = \eta \frac{dv}{dx} = \eta \dot{\gamma} \]

- Shear stress
- Rate of shear

yield stress
Newtonian
pseudoplastic

\[ \log \eta = k \log \dot{\gamma} \]

log
log
VISCOSITE DES SOLUTIONS DILUEES DE POLYMÈRES

Relation entre l'accroissement de viscosité du solvant et les caractéristiques des polymères

\[ \eta_{\text{rel}} = \frac{\eta - \eta_0}{\eta_0} \]
\[ \eta = \eta_0 + k' \eta_0^2 c \] \hspace{2cm} (1)
\[ k' \approx 0.3 - 0.5 \]

\[ \frac{\eta - \eta_0}{\eta_0} = \eta \left( \eta \right) + k' \left( \eta \right)^2 c + B \left( \eta \right)^n \] \hspace{2cm} (2)

(1) régime dilué
(2) régime dilué → concentré (plot en log-log)
Low Shear viscometer $\rightarrow \eta (\gamma)$ at low viscosity (low concentration-low MW)
Dynamic and flow rheology experiments with temperature control at larger viscosity.

Solution or gel $\eta(\gamma)$ & $G', G''$ moduli ($\omega$)

$\Rightarrow$ chain dynamic / entanglements
*Viscosité complexe*

\[ |\eta^*| (\omega) = \omega^{-1} (G'^2 + G''^2)^{1/2} \]

Viscoelastic solution behaviour

Gel like behaviour
Xanthan, the first bacterial industrial polysaccharide (TOR)

Intrinsic viscosity is very high and stable in the presence of external salt ($\leq 0.125 \text{ g/L}$)

($M_w = 7 \times 10^6$, $[\eta] = 10.500 \text{ mL/g in } 10^{-1}M \text{ NaCl}$).
Influence of the weight-average molar mass on the viscosity.

Xanthan

Graph showing the influence of weight-average molar mass on viscosity.
Influence of the molar mass and shear rate on the viscosity of a 2g/L solution (in the ordered conformation)
Influence of stiffness and the salt concentration on the intrinsic viscosity.

Xanthan
Lp≈450Å
Slope ≈ 1

Comparison between xanthan and PSS-Na
Conformational stability

Role of temperature and ionic concentration on the xanthan conformation

NMR signals as a function of the temperature.
Influence of salt concentration on the xanthan conformation.

a) Water
b) 5.6 mM NaCl
c) 10.8 mM NaCl
d) 15.8 mM NaCl
e) 28 mM NaCl
f) 0.1M NaCl

Tm is the half transition

helix

coil
Influence of calcium addition ($\text{Cl}_2\text{Ca}$) on the conformational transition ($T_m$). $C_p=1\ \text{g/L}$ under Na$^+$ form.
Influence of the valency of counterions on the conformational stability (Cp=1g/L).

Under Cl⁻ form.
Xanthan forms a left-handed cholesteric in semi-dilute solution (7.5% v/v).
Rheological behaviour of Xanthan in concentrated solution.

Reduced viscosity and cholesteric volume fraction as a function of xanthan concentration. $M_w=235,000, 0.1\text{M NaCl}, \gamma=1 \text{ s}^{-1}$

Relative viscosity of a xanthan solution as a function of temperature. $C=110 \text{ g/L}, \gamma=2.37 \text{ s}^{-1} 0.1\text{M NaCl}$ $M_w=235,000$. 
Flow experiments on HA.

[\eta] must be determined from viscosity in the Newtonian plateau.

Viscosity as a function of the shear rate for HA $M_w = 1.3 \times 10^6$, $[\eta] = 2092 \text{ mL/g}$ in 0.1M NaCl. Influence of the polymer concentration.

$[\eta] = K M^a \rightarrow \text{direct relation with the molar mass } MW$  

$[\eta]_0 = \lim_{c,\gamma \to 0} \frac{\eta_0 - \eta_s}{\eta_s C} \quad (\eta_0 - \eta_s) / \eta_s = C[\eta] + k' ([\eta]C)^2 + B (C[\eta])^{3.4-4}$

$[\eta](\text{mL/g}) = 0.0336 M^{0.79}$
Specific viscosity is imposed by $[\eta]$ $(L_p,M)$ and $C$.

(see example of HA)

Importance of $[\eta]$:  
-if determined at zero shear rate, it is directly related to the value of $M$ or $M_v$ 
-empiric relation $[\eta] = K M^a$

Relation between viscosity of a polymeric solution and polymer characteristics.

In the dilute regime:

\[ \frac{\eta_{sp}}{C} = [\eta] + k' [\eta]^2 C \]

In the semi-dilute regime:

\[ \eta_{sp} = C[\eta] + k'(C[\eta])^2 + B(C[\eta])^n \]

with $n \sim 3.4-4$

On HA, we found $k' \sim 0.4$ $B \sim 7.77 \times 10^{-3}$

$C[\eta]$ is the overlap parameter (reflects the role of $C$ and $M$)

\[ \eta_{sp} = C[\eta] \{ 1 + k_1 (C[\eta]) + k_2 (C[\eta])^2 + k_3 (C[\eta])^3 \} \]

with $k_1 = 0.4$

\[ k_2 = (k_1)^2 / 2 = 0.08 \]

\[ k_3 = (k_1)^3 / 3 = 7.1 \times 10^{-3} \]

(T.K.Kwei, M.Nakazawa, S.Matsuoka, M.K.Cowman, Y.Okamoto)
-all the polymers in « good » solution (synthetic, natural, charged or not, stiff or flexible) whatever their Mw and C give specific viscosity at zero shear rate on the master-curve
Specific viscosity at zero shear rate as a function of the overlap parameter ($C[\eta]$).

→ single curve independently of the polymer concentration and molar mass.
Comparison between predicted and experimental values.

Dependence of the specific viscosity at zero shear rate as a function of the overlap parameter $C[\eta]$: ▲ relation; □ Hyaluronan in 0.1N NaCl; ◆ Xanthan in 0.1N NaCl

M.Rinaudo, Food hydrocolloids 15,433 (2001)
### Molar mass determination from intrinsic viscosity?

#### Mark–Houwink relationship:

\[
[\eta] = K \cdot M^a
\]

K, a depend on the salt concentration and temperature

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<th>Poly(acrylamide)</th>
<th>water</th>
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<th>(1.0)</th>
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<td>(0.1 M)</td>
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<td>(0.005 M)</td>
<td>25</td>
<td>2.3</td>
<td>0.93</td>
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<tr>
<td></td>
<td>aqueous KCl (3.1 M)</td>
<td>25</td>
<td>20.4</td>
<td>0.60</td>
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</table>
$M/G \sim 1$
Interchain interactions give abnormal viscosity \( \rightarrow \) cannot be used to determine \( L_p \) or to predict the rheological behavior.

V.P. Kapoor et al.
Slope of viscometry (concentration) curve increases when \( M/G \) increases → interacting systems when the slope is larger than 3.4-4 (see our development of viscosity)
Chain dimensions with local stiffness (worm like chain).

A. Radius of gyration

Neutral and ionic polymers in $\theta$-conditions ($C_s \to \infty$)

assuming a worm like chain:

$$\langle S^2 \rangle_\theta = \frac{1}{3} L L_p (...)$$

Ionic polymers $\rightarrow$ e.s contribution

$$L_T = L_p + L_e$$

$$\alpha_s^2 (e.l) (L, L_p, \lambda, \mu)$$

(1)

$$\langle S^2 \rangle_\mu = \frac{1}{3} L \ L_T \ \alpha_s^2 (e.l) \ (\text{for } L \gg L_T)$$

(2)

$$\langle S^2 \rangle_\mu^{1/2} = K' M^v$$

(2')

B. Intrinsic viscosity

In $\theta$-conditions (or $C_s \to \infty$)

$$[\eta]_\theta = \phi (L_r, dr) \left\{ \frac{M_l}{2L_p} \right\}^{-3/2} M^{1/2}$$

$$L_r = L/2L_p$$

$$dr = d/2L_p\} \rightarrow \phi (L_p, M)$$

(3)

$$a_\theta = d \log [\eta]_\theta / d \log M$$

$$a_\theta \geq 0.5$$

For ionic polymers:

$$[\eta]_\mu = [\eta]_\theta \left( \frac{L_T}{L_p} \right)^{3/2} \alpha_\eta \alpha_\eta \eta_\mu \phi_{\eta}$$

(4)

$$[\eta]_\mu = KM^a$$

(5)

(5')

C. Second virial coefficient

$$A_{2,\mu} = A_{2,hs} + A_{2,es}$$

$$A_{2,es} = (N_A N_k^2 \beta/2M^2) h_o (z)$$

$$C_s \to \infty$$

Experimental determination from SEC multidetection analysis $\rightarrow [\eta] (M), R_g (M) \rightarrow L_p$
Conclusions

- For same $L_p$, $M \rightarrow [\eta]_\theta \uparrow$ when $M_L \downarrow$

- $\Phi < 2.8 \times 10^{23}$ (Flory C$^S_t$)

but $\Phi \uparrow$ when $M \uparrow$

- $a\eta_\theta > 0.5$ but $\downarrow$ when $M \uparrow$ (limit 0.5)

$a\eta_\theta \rightarrow 0.5$ when $N_k \rightarrow \infty$

$\rightarrow$ * non gaussian chain

* low hydrodynamic interaction

* low (or no) excluded volume ($L_r < 100$)

(for ionic polysaccharide in salt excess to suppress e.s)
Dynamic experiment on Xanthan

Reduced $G'$ (a) and $G''$ (b) moduli as a function of the generalized frequency $\omega\tau_r$. Xanthan concentrations 0.16 to 3 g/L.
Steady shear viscosity, $\eta(\gamma)$ and modulus of the complex viscosity, $|\eta^*| (\omega)$, as a function of shear rate and frequency ($T=25 \, ^\circ\text{C}; \text{solvent}, \text{NaCl 0.1 M}$).

$$|\eta^*| = \left[ (G'^2 + G''^2)^{1/2} \right] / \omega$$

Dynamic experiments at different polymer concentrations.

When $c$ increases, $G'$ & $G''$ increase but $\omega_p$ decreases.
Master curve obtained for dynamic measurements.
Cox Merz representation: complex viscosity and flow viscosity can be compared.

Exemple for a linear polymer perfectly soluble
Fifth part

Gelation mechanisms

Specific interchain interactions

- junction zones usually with stereoregular polysaccharides

- association with the sol-gel transition
Mechanism of Gelation of different systems based on cooperative interactions (junction zones → physical gels)

-one polysaccharide

- thermogelation of amphiphilic cellulose derivative (methylcellulose → blockwise methyl distribution) or grafted derivative (alkylated HA or chitosan)

- thermoreversible gels (H-bonds; gellan, carrageenan, agarose....)

- ionic gel (alginites, pectins)

-two polysaccharides

- synergistic system (xanthan/GM)
Schematic representation of

(a) a chemical gel made of flexible polymers (entropic elasticity)

(b) a physical gel based on junction zones (enthalpic) involving cooperative interactions

Original behaviour of polysaccharides is to form gels in given thermodynamic conditions (T°c, pH, ionic concentr...)
Gelation involving a single polysaccharide

- Thermogelation (hydrophobic) (gel when $T$ increases)
- Thermoreversible (H bonds) (gel melts when $T$ increases)
- Ionic gelation
1- Hydrophobic gelation or thermogelation

- Hyaluronan

- Chemical Modification on carboxylic acid group at C-6 position

- Introduction of very reactive dihydrazide groups via peptidic coupling in aqueous solution

\[
\begin{align*}
\text{HA} & + \text{ADH} \\
& \xrightarrow{\text{EDC, H}_2\text{O, pH 4.75}} \text{HA-ADH}
\end{align*}
\]

- Reductive aminations on pendant hydrazide groups
Experimental conditions.  
Second step.

\[
\text{HA-ADH} + \text{Aldehyde} + C_{10}H_{20}O + NaCNBH3 \rightarrow \text{HA-C10}
\]

R = ONa ou R' = C10

Yield \approx 90\% \text{ (after precipitation)}

DS = 5\% \rightarrow \text{HA-5C10}
Alkylation of Hyaluronan →

side chain mechanism of gelation

→ Balance between electrostatic repulsions and hydrophobic interactions

→ C10 and C12 look the more efficient substituents for 4-5% substitution

→ Conditions for chemical reaction allow to control the degree of substitution and the distribution of substituents (high solubility → random distribution)
Viscometric behaviour of alkylated HA.
Methylcellulose: $0 < DS < 3$
Water soluble for $1.3 < DS < 2.5$

$^{13}$C NMR in DMSO $d_6$
Temperature dependence of dynamic viscoelasticities for methylcellulose A4M at 10 g/L in water.

Constant frequency of 0.5 Hz, strain of 1% and temperature rate of 0.5 °C/min. ■, □ heating; ▲, △ cooling. G’ full symbols and G” open symbols. Inter. J. Polym. Anal. Ch. 2015, 20, 1–9.
Schematic drawing showing gelation through the hydrophobic effective units of MC

At lower temperatures, the hydrophobic association is possible from the hydrophobic effective units. During heating, the gel is formed with the hydrophobic junctions consisting of such hydrophobic effective units and the mean length $M_e$ between two junctions remains constant when the gelling temperatures are higher than 42.5 °C. Reproduced from Li, *Macromolecules* **1999**, *32*, 7070-7077.

Blockwise gelation

Phase separation over 60°C
2- H-bond gelation-thermoreversible gel
Agarose and carrageenans

A

K, l

B

λ

Agarose

(R=H or $-\text{SO}_3^-$)
Agarose competes with gelatin to prepare culture medium.
K- carrageenan melting at Tm by optical rotation and conductivity
Results obtained on κ-carrageenan to propose the two-steps mechanism of gelation

- Existence of hysteresis
Phase diagram for kappa carrageenan. Influence of the counterion.

\[ C_T = C_p + C_s \]

* \( C_T \) is the temperature for conformational transition.

* Same selectivity on coil and gelation.

Influence of the counterion.
Double-helix stability. Ionic selectivity

→ Ionic selectivity for K-carrageenan in monovalent 0.1M salt.
Anionic selectivity in kappa-carrageenan (K\(^+\) salts; 0.1M) → I\(^-\) stabilises the double helix (and prevents gelation)
Dynamic measurements on kappa-carrageenan as a function of the frequency in excess salt (0.1M). $G'$ □ and $G''$ ◯ for $C_p = 1 \text{g/L}$ in KCl; $G'$ ◇ and $G''$ × for $C_p = 10 \text{g/L}$ in NaI.

→ in presence of NaI, no gelation but double helix
Mechanism of gelation proposed for carrageenan.
Ionic selectivity and ion pair formation
→ helix-coil
→ sol-gel transitions

Stereoregularity → cooperativity
→ Helical conformation & gelation
Molecular structure of the bacterial polysaccharide Gellan.

(a) Native form (b) Commercial NaOH treated Gellan
Molecular structure of the bacterial polysaccharide Gellan.

(a) Native form   (b) Commercial NaOH treated Gellan

\[
\text{L-Glyceric} \\
\text{1} \\
\downarrow \\
\text{2} \\
\left[\rightarrow 3\right] \beta-D-\text{Glc}-\left(1\rightarrow 4\right) \beta-D-\text{Glc}A-\left(1\rightarrow 4\right) \beta-D-\text{Glc} \left(\rightarrow 4\right) \alpha-L-\text{Rhap}-\left(1\rightarrow\right)_n \\
\text{6} \\
\uparrow \\
\text{Ac}
\]

(b)

\[
\text{D-Glc} \quad \text{D-GlcA} \quad \text{D-Glc} \quad \text{L-Rhap}
\]

\[
\begin{array}{l}
\text{CH}_2\text{OH} \\
\text{HO} \\
\text{OH} \\
\text{COOH} \\
\text{OH} \\
\text{CH}_3
\end{array}
\]
Gellan. Influence of temperature on conformation stability.

Huggins constant

![Graph showing the influence of temperature on conformation stability of Gellan.](image)
Deacylated gellan. Influence of T on the properties.

Evolution of the reduced viscosity indicating the conformational change and aggregation depending on the counterion (Cp=3g/L; 0.025 M XCl).
Influence of salt concentration on conformational transition ($T_m$).

$CT = C_p + C_s$, the total salt concentration.

$T_m$ is the helix-coil transition temperature.
Extended phase diagram for deacylated gellan.

\[ T_m \] is the helix-coil transition and \( T_G \) is the temperature of gelation independent on the type of monovalent counterion (low charge parameter)

\( T_F \) is the melting temperature of the gel depending on the cation
Ionic selectivity and gel strength at 25°C.

(K⁺ > Na⁺ > Li⁺; 0.1M XCl )

Elastic modulus of gellan gels.

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<td>E</td>
<td>Fm</td>
<td>E</td>
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* E (N/m²x10⁴ Pa); F(N)
Deacylated gellan in 0.1M NaCl: Gel at 20°C; Sol at 80°C
Double helix of deacylated gellan

- K⁺ counterion

R. Chandrasekaran (Purdue Univ., USA)
Rheological behaviour of gellan. Role of substituents.

(a) 40% glyceryl & 3% acetyl
(b) 52% glyceryl & 50% acetyl.

- acetyl groups prevent gelation
- glyceryl groups stabilise double helix
Schematic representation of the position of acetyl and glyceryl substituents on the double helix structure (3-fold helix).

Glyceryl stabilizes the double helix; acetyl prevents gelation.

E.R. Morris (UK)
Cooling DSC scans (0.3°C/min.) for native gellan. 10g/L

(a) 10⁻² M NaCl
(b) 5x10⁻² M NaCl
(c) 10⁻¹ M NaCl

ΔH & Tm independant on excess salt due to the role of glyceryl groups (reversible transition).
Cooling DSC scans at 0.3°C/min. on gellan (10g/L) in 0.01M NaCl

(a) Native gellan

(b) Deacetylated gellan

(c) Deacylated gellan

→ Glyceryl groups stabilize the double helix.
DSC heating curves obtained on deacylated gellan (C=10 g/L; 0.3 K/min.)

(a) in 10^{-2} M NaCl
(b) in 5\times10^{-2} M NaCl
(c) in 10^{-1} M NaCl

Aggregates are formed when ionic concentration increases; the melting of aggregated helices occurs at a higher temperature.
Mechanism of gelation proposed for gellan, agarose & carrageenan.

Blockwise gelation
3- Ionic gelation