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Coacervation of Gelatin–Gellan Gum Mixtures and Their Use in Microencapsulation

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SUMMARY

Complex coacervation has been observed in gellan gum–gelatin mixtures at low total polymer concentrations. Coacervation was restricted to the pH range ≈ 3·5–5·0. Procedures are described for the microencapsulation of oils and solid particles.

INTRODUCTION

Gellan gum is the generic name for the extracellular polysaccharide produced by the bacterium *Pseudomonas elodea* (Kang & Veeder, 1982). The polymer is a linear anionic heteropolysaccharide (Jansson *et al.*, 1983; O'Neill *et al.*, 1983) composed of tetrasaccharide repeat units (Fig. 1). The native form is acetylated, containing, on average, one O-acetyl substituent per chemical repeat unit. The position of the acetyl substituent is not known with certainty but is believed to be attached at C(6) on one or both of the (1→3) and (1→4) linked glucose residues (Jansson *et al.*, 1983). Partial or complete deacetylation may be achieved by allowing the pH of the fermentation medium to rise naturally when the carbohydrate substrate is exhausted or by a subsequent heat treatment at alkaline pH.

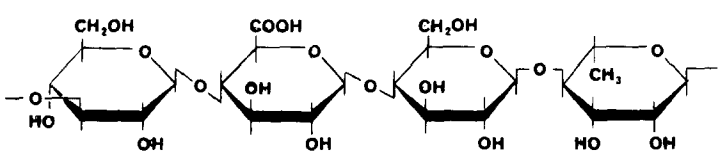

Fig. 1. Repeat unit of gellan gum.

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Gellan gum is an extremely good gelling agent. The acetylated polymer disperses readily in cold water to form viscous thixotropic samples and at higher concentrations elastic gels (Moorhouse *et al.*, 1981). Progressive deacetylation results in increasing brittleness of the gels (Moorhouse *et al.*, 1981). Gelation is sensitive to the type, valency and concentration of cations present in salts added to the dispersion prior to gelation (Moorhouse *et al.*, 1981). Physical–chemical studies (Carroll *et al.*, 1982; Brownsey *et al.*, 1981; Miles *et al.*, 1984; Attwool *et al.*, 1986) suggest that the junction zones of the gels arise due to the association and possibly crystallisation of sections of the polymer chain. Acetyl groups inhibit and cations promote such intermolecular association (Carroll *et al.*, 1982; Miles *et al.*, 1984; Attwool *et al.*, 1986).

The major industrial use of gellan gum would appear to be as a gelling or thickening agent. Gellan gum is highly resistant to enzymic breakdown and can withstand autoclaving. Uses have been suggested as an agar substitute with potential applications as a microbial growth medium or for plant tissue cultivation (Cottrell, 1980; Moorhouse *et al.*, 1981; Lin & Casida Jr, 1984; Shungu *et al.*, 1983; Harris, 1985). The sensitivity of gelation to acetyl content and salt composition of the gelation media permit use as a thermo-setting, cold-setting, thermo-reversible or thermo-irreversible gelling agent. The versatility suggests applications as a broad spectrum gelling agent. Potential food applications have been investigated (Sanderson & Clark, 1983, 1984), and on the basis of successful toxicity studies, Kelco have announced that they intend to seek approval for use of gellan gum as a food additive (Sanderson & Clark, 1983; Pettitt, 1986). Synergistic interactions have been reported between gellan gum and gelatin resulting in high strength gels (Shim, 1985). Multicomponent mixtures of gellan gum, xanthan gum and galactomannans provide a basis for varying the hardness, elasticity and cohesiveness of gels (Pettitt, 1986). Gellan gels prepared in the presence of high levels of glycols have been proposed as a medium for the controlled release of fragrances, insecticides, herbicides, pheromones, etc. (Kelco Commercial Leaflet; Sandford *et al.*, 1984). A less explored avenue for industrial usage is the potential use of gellan gum for microencapsulation. Gellan gum has been reported to form rigid coacervate gels in the presence of gelatin (Shim, 1985). In a commercial development leaflet CD-31 Kelco have reported several recipes for the microencapsulation of dibutyl phthalate as a core material. In this article we report a more detailed study of coacervation in gelatin–gellan gum mixtures at relatively low total polymer concentrations. The effect of pH upon coacervation has been examined to try and distinguish between simple and complex coacervation. These data have been used to investigate potential uses for encapsulation of oils or solid core materials.

EXPERIMENTAL

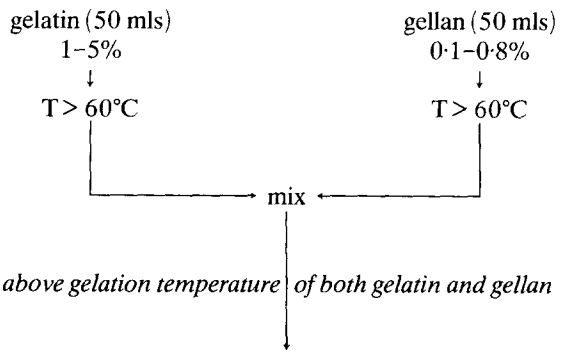
Materials

The gelatin was extracted from pig skin and supplied by Sigma Chemical Co. (Bloom 175, batch no. G 2625). The isoionic point was measured by the method of Janus *et al.* (1951) and found to be at pH 8. This value is typical of an acid-treated pig skin gelatin. Gellan gum is sold by Kelco-Alginate Industries Ltd under the trade name Gelrite. This is a fully deacetylated form and has been prepared as the potassium salt. The material has been clarified to remove bacterial cell debris. The oils used in the encapsulation were sunflower oil and colourless paraffin. Solid materials used for encapsulation included aluminium powder (Aldrich Chemicals) and 200–400 mesh Dowex ion-exchange resin (50W-X8) (BDH).

Methods

The description of the experimental work can be divided into three sections. Firstly, studies of gellan–gelatin mixtures to determine the region in which liquids containing coacervate droplets were obtained free from precipitation or flocculation; secondly, studies on the effect of pH on coacervation; and thirdly, the use of these data to encapsulate oils and solid particles.

Gellan–geletin mixtures were prepared according to Scheme 1. Studies were confined to low total polymer concentrations at which the mixtures remained liquid on cooling. Cooling was often accompanied by the formation of a white precipitate or floc which then sedimented upon standing. Those samples which became translucent upon cooling were



Scheme 1

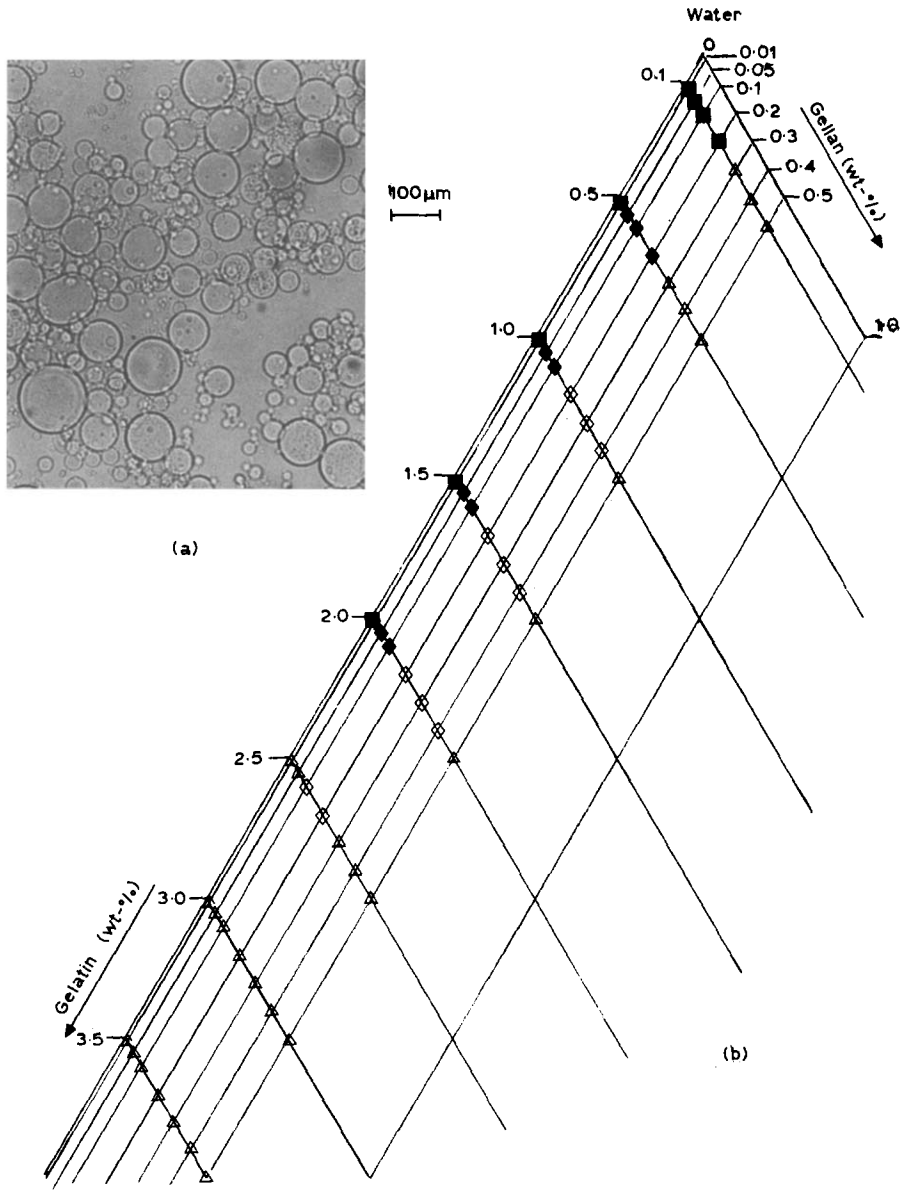


Fig. 2. Gellan–gelatin coacervates. (a) Typical coacervate obtained after mixing and cooling. (b) Ternary diagram illustrating the coacervation region observed after mixing and cooling: ■, indicates liquid medium, no coacervate; ◆, coacervate; ◇, coacervate plus flocs; △, flocculation.

examined under a microscope for evidence of coacervation or flocculation. A typical coacervate is shown in Fig. 2a. The coacervates contained a range of droplet diameters (1–100 μm). In Fig. 2b the region in which coacervation, essentially free of flocculation or precipitation, occurs is indicated on a section of the gellan–gelatin–water ternary diagram. This method of displaying the experimental data is convenient but is strictly an oversimplification. The use of a ternary diagram is really only allowed if the phase rule holds for phase equilibria involving polymers and if the number of components is in fact three. The first condition will probably be satisfied provided the system is allowed to reach equilibrium before lowering the temperature beneath the gelation thresholds for both polymers. Strictly speaking the second condition will not be met because both the gellan and gelatin are polydisperse with respect to molecular weight. However, despite these reservations Fig. 2b provides a useful representation of the experimental data.

Coacervation may be broadly divided into two types (de Jong, 1949). A simple coacervation is largely independent of the charge on the polymer(s) and complex coacervation requires an adequate opposition of charges on the two polymers. Thus complex coacervation should be restricted to a pH range in which one polymer is essentially positively charged and the other possesses a net negative charge. The effect of pH was investigated by modifying Scheme 1. The pH of samples of gellan (0·02–1·5%) and gelatin (0·02–5%) was adjusted to between 6 and 9 using 0·1 N NaOH prior to mixing. After mixing the pH was readjusted using 0·1 N HCl prior to cooling. Coacervation only happened in the pH range 3·5–5·0 where the gelatin will have a net positive charge and gellan a net negative charge. Slow reduction in pH plus thorough mixing made it much easier to control the coacervation. The coacervation region is shown on a section of the gellan–gelatin–water ternary diagram (Fig. 3). Controlling the pH extends the region within which coacervation will occur.

Coacervates produced by Scheme 1 show a broad size distribution ranging from 1 to 100 μm (Fig. 2a). Varying the ratio of the two polymers within the coacervation region led to no obvious change in the mean size or distribution of droplet sizes. For pH between 3·5 and 4·0 the droplets became distorted on stirring, and upon cooling gelled into ellipsoidal shapes. For pH $\approx$ 4·0–5·0 there was no noticeable change in shape and no significant change in yield or size distribution. The gelatin employed in the present studies was Bloom 175. Preliminary studies with acid-treated pig skin gelatin (Bloom 300) suggest similar behaviour. No studies have been made of the effects of cation type or ionic strength on the coacervation.

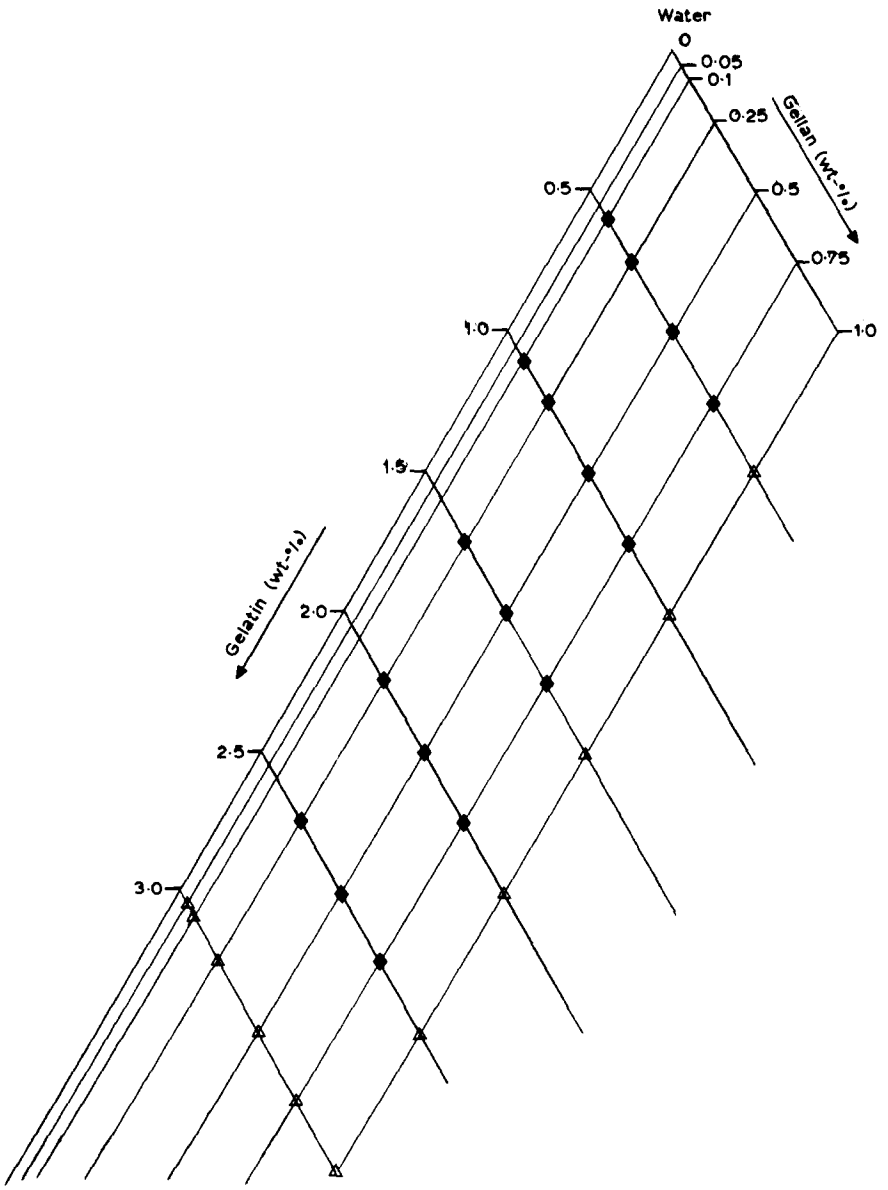
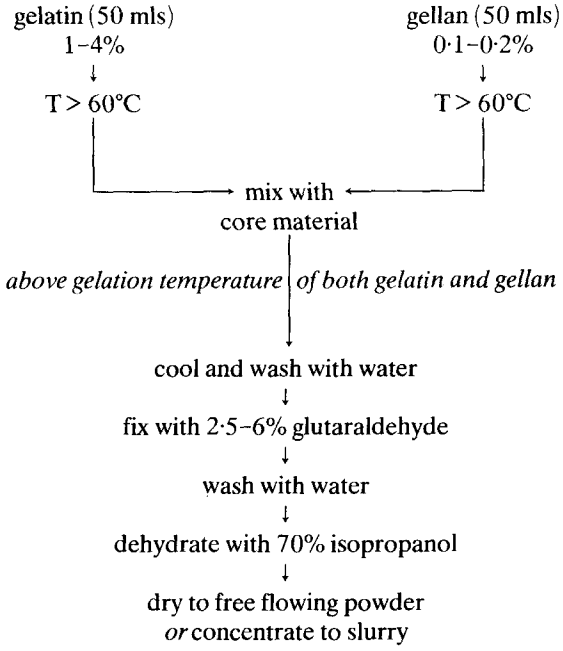
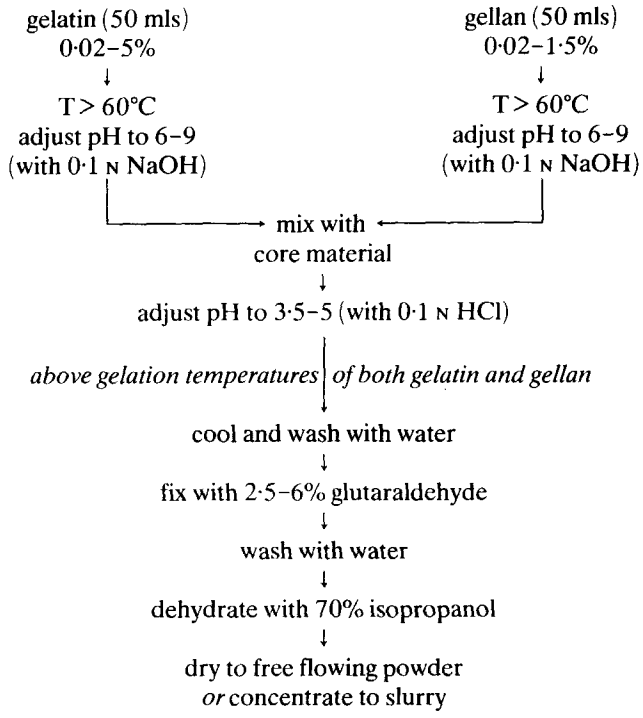


Fig. 3. Gellan–gelatin coacervates. Ternary diagram illustrating the coacervation region observed after mixing, adjusting the final pH ≈3·5–5·0, and cooling: ◆, coacervate; △, flocculation.



Scheme 2



Scheme 3

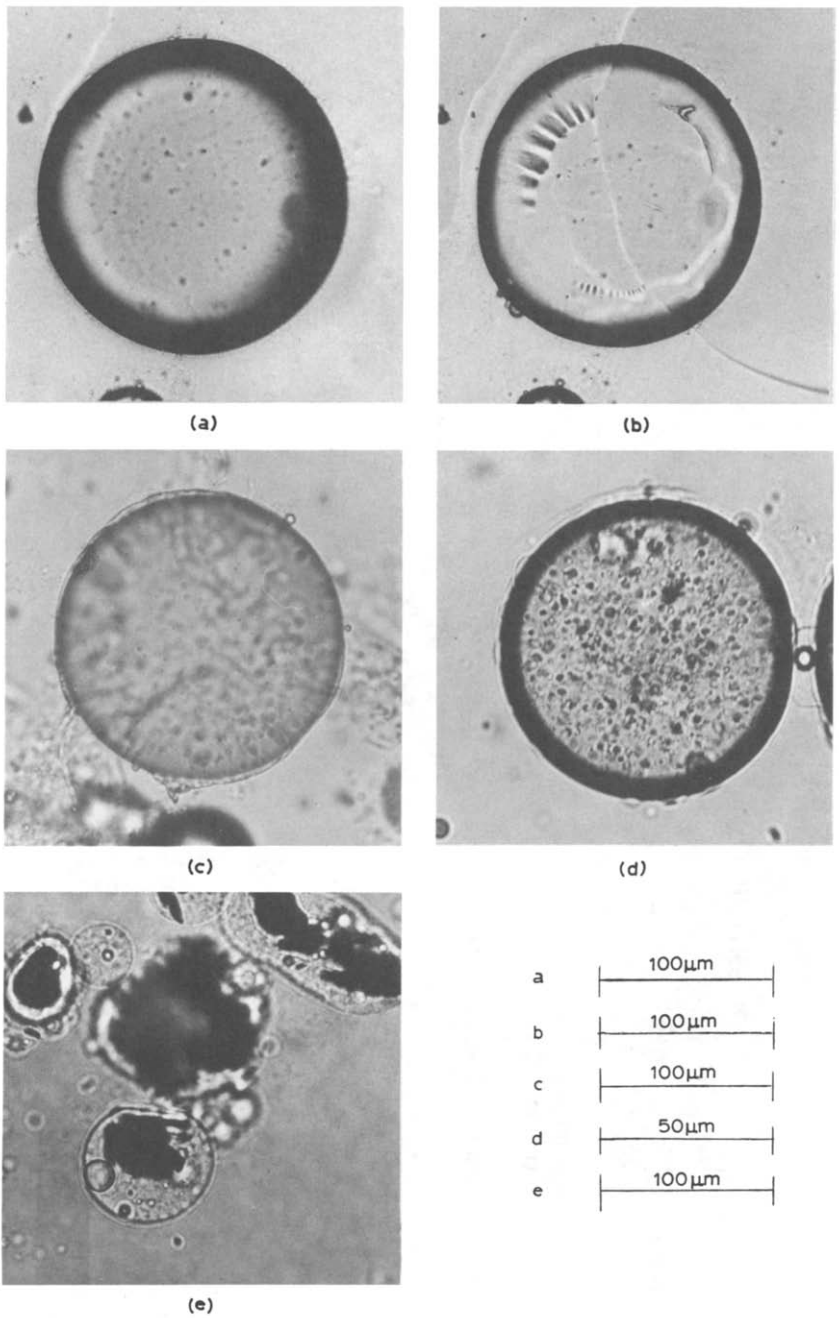


Fig. 4. Photographs of encapsulated materials. (a) Encapsulated paraffin oil, (b) encapsulated paraffin oil droplet shown in (a) after puncturing the gellan–gelatin skin, (c) encapsulated sunflower oil, (d) encapsulated aluminium powder, (e) encapsulated dowex resin.

Two schemes (2 and 3) were employed to encapsulate oils and solid particles (Fig. 4). Scheme 3 was preferable because controlling the pH extended the useful range within which coacervation and microencapsulation occurred. All samples could be concentrated to wet slurries. Whereas the encapsulated solids could be dried to free-flowing powders, drying of encapsulated oils often resulted in the capsules bursting.

CONCLUSIONS

At fairly low total polymer concentrations gellan–gelatin mixtures form liquids containing coacervate droplets. Coacervation only occurs over the limited pH range $\approx 3{\cdot}5-5{\cdot}0$ suggesting, at least at low total polymer concentrations, the process of complex coacervation. The coacervation can be controlled by manipulating the pH of the system and used to encapsulate oils or solid particles.

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