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POLYSACCHARIDE CONFORMATION IN SOLUTIONS AND GELS – RECENT RESULTS ON PECTINS

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Ca^{++} binding to poly-D-galacturonate (the main backbone sequence of the polysaccharide pectin from plant cell walls) has been investigated by equilibrium dialysis and circular dichroism (c.d.) to elucidate the nature of conformational ordering and chain association in the sol-gel transition. Of the total stoichiometric requirement of bound calcium, only $50 \pm 5\%$ is resistant to displacement by swamping concentrations of univalent counterions (Fig. 1). Closely similar behaviour has previously been

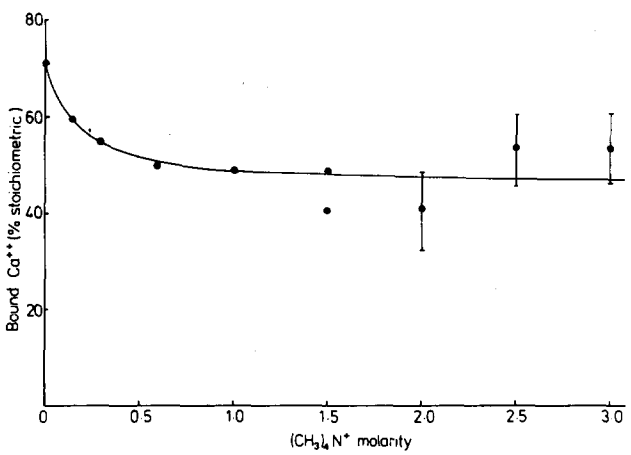

Fig. 1. Equilibrium study of Ca^{++} binding to polygalacturonate chains. A constant calcium ion concentration of 6 mM was used in competition with varying concentrations of tetramethylammonium chloride, as shown. The level of bound calcium not available for equilibration across the dialysis membrane is expressed as a percentage of the total stoichiometric requirement of polygalacturonate present.

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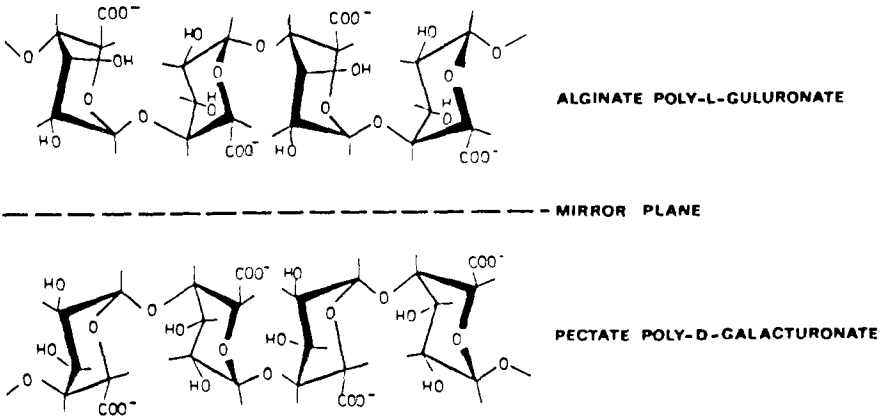

Fig. 2. Structural analogy between poly-L-guluronate (I) and poly-D-galacturonate (II).

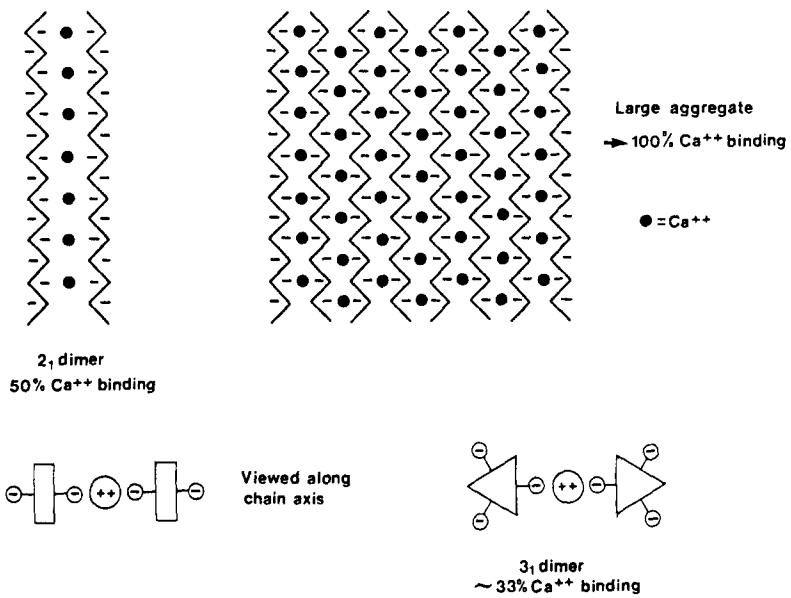

Fig. 3. Stoichiometry of Ca^{++} binding by polyuronate chains.

reported for the stereochemically analogous (Fig. 2) poly-L-guluronate (derived from the polysaccharide alginate) and was attributed to site-binding of Ca^{++} within dimers of chains of 2_1 helical symmetry (cf. Fig. 3). The c.d. changes which accompany Ca^{++} binding are also closely similar for the two polymers when allowance is made for their

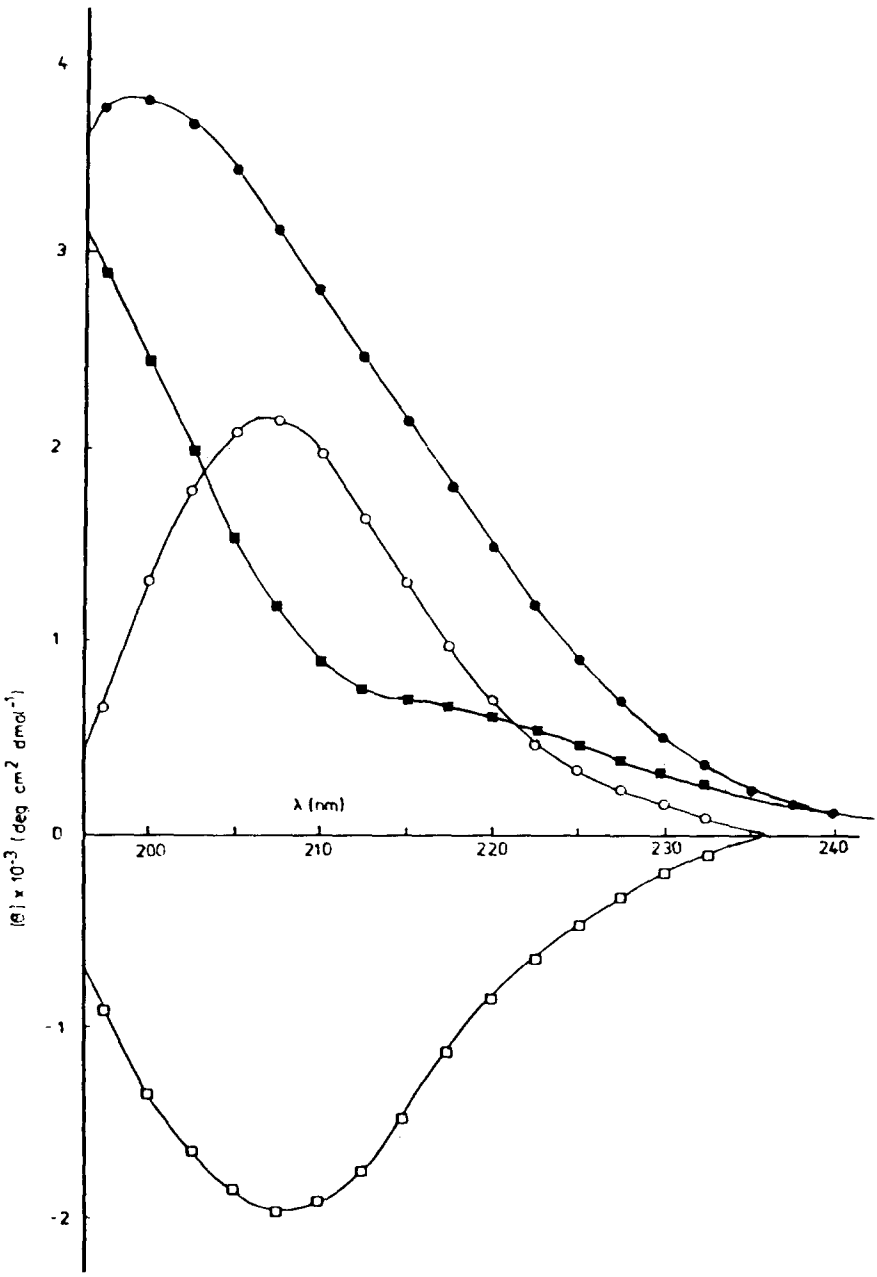

Fig. 4. Changes in polyuronate circular dichroism behaviour with cation binding. Spectral changes (○) between Na^+ solution (●) and Ca^{++} gel (■) for polygalacturonate chains are similar in form and magnitude but opposite in sign to those observed for polyguluronate (□).

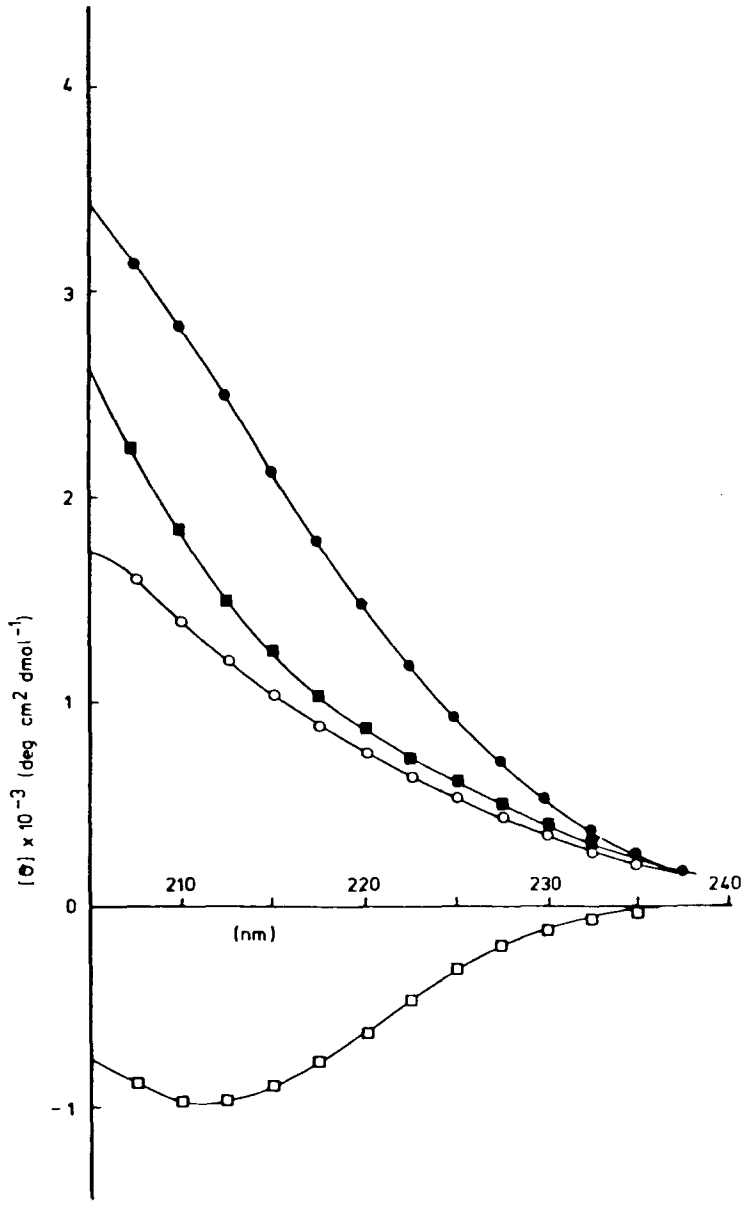

Fig. 5. Ca^{++} binding to polygalacturonate chains. Spectral changes (□) between Na^{+} polygalacturonate solution (●) and Ca^{++} gels (■) dialysed extensively against 0·5 M NaCl are of approximately half the magnitude of those observed in the presence of excess Ca^{++} (see Fig. 4). Solid films prepared after similar dialysis to displace all but the most tightly bound calcium ions show similar c.d. behaviour (○) to the gel state.

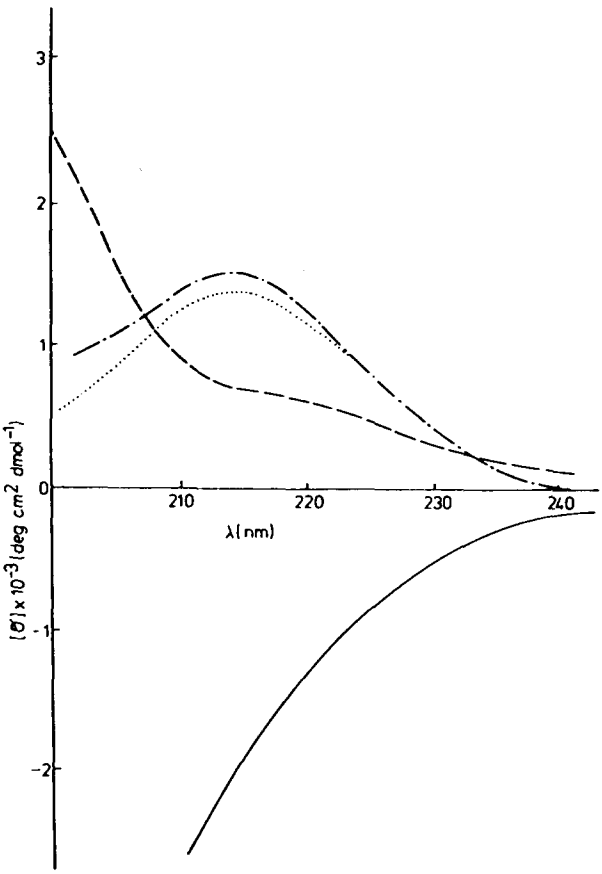

Fig. 6. Comparison of polyuronate c.d. behaviour in gels and in the solid state. Our previous c.d. studies indicated little change in conformation or packing of polyguluronate between hydrated gels (....) and solid films (-·-·-), whereas calcium polygalacturonate gels (– – –) show massive c.d. differences compared with films (——).

near mirror-image stereochemistry by inversion of sign (Fig. 4). When polygalacturonate gels, with calcium as sole or principal counterion, are dried to solid films, very profound c.d. changes occur, suggesting that the chain conformation and/or packing undergoes some modification during interconversion between gel and solid states (Figs 5 and 6). No such c.d. changes are seen for polyguluronate, consistent with the persistence of similar (2_1) chain conformations and packing for this polysaccharide in both the gel and solid state. On the basis of all this evidence we propose:

(i) the cooperative binding of Ca^{++} in polyguluronate and polygalacturonate gels is through 'egg-box' complexes with the polysaccharide chains in analogous 2_1 conformations;
(ii) drying of calcium polygalacturonate gels, but not polyguluronate gels, is associated with a polymorphic phase transition and it is for this reason that diffraction studies on dried films show the 3_1 helix.

We next investigated the role in calcium pectate gel networks of other structural features, in particular methyl esterification and 1,2-linked L-rhamnosyl residues in the polymer backbone. Acid hydrolysis of citrus, apple and sunflower pectins gave polygalacturonate blocks with a narrow molecular weight distribution, and average chain-length of ≃25 residues in each case (Fig. 7). Since the known relative stabilities of glycosidic linkages would lead to chain cleavage predominantly at L-rhamnose, this result indicates that the length of polygalacturonate sequences between rhamnose interruptions is approximately constant within and between the pectins studied. Calcium pectate gel strength is reduced dramatically by the incorporation of these

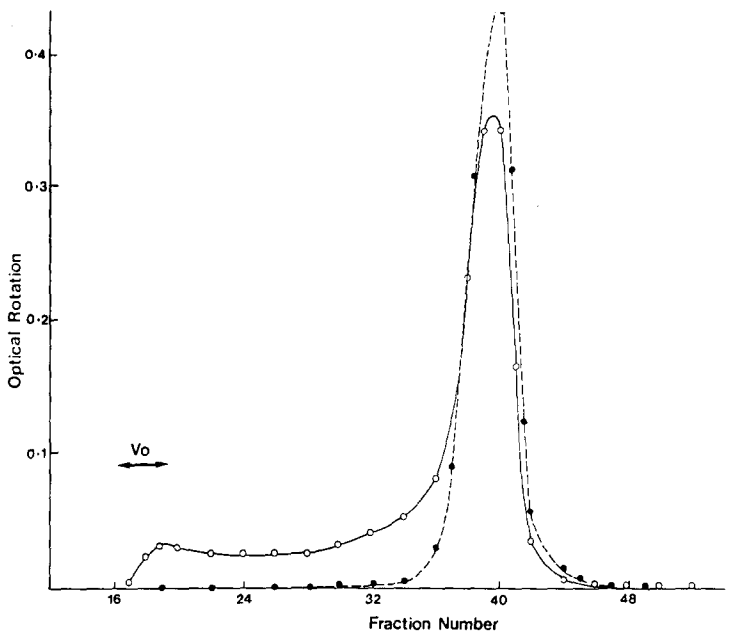

Fig. 7. Sephadex G-50 fractionation of neutralised acid insoluble (○) and soluble (●) blocks from pectin I. The column (400 × 26 mm) was thermostatted at 20°C, and eluted with distilled water at a flow rate of 9 ml/h. Column loading ≈ 0·4 g of dialysed and freeze-dried hydrolysate. Fractions of 4·5 ml were collected, and monitored by optical rotation (578 nm; 10 cm pathlength). $V_0 \approx 80$ ml; $V_t \approx 330$ ml; elution volume at band centre ≈ 174 ml. Similar elution profiles were obtained for the soluble and insoluble fractions of all pectins studied.

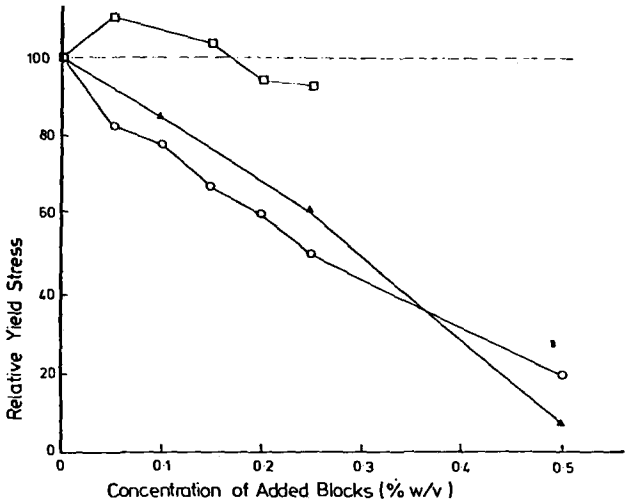

Fig. 8. Effect of added pectate blocks on calcium pectate (1% w/v gel strength). (○) De-esterified blocks (slow release of Ca^{++}); (□) 84% esterified blocks (slow release of Ca^{++}); (▲) de-esterified blocks (gelation from heated solution).

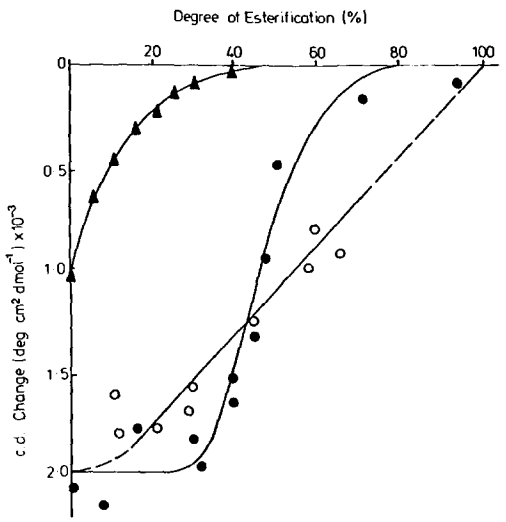

Fig. 9. Effect of degree and pattern of esterification on the calcium binding capacity of pectin, as monitored by circular dichroism. (○) Enzymically de-esterified pectin – free availability of Ca^{++}; (●) alkaline de-esterified pectin – free availability of Ca^{++}; (▲) alkaline de-esterified pectin – Ca^{++} binding in competition with 0·5 M Na^{+}.

In the latter two cases, the solid lines show the best fit obtained from comparison of the primary sequence requirements for cooperative binding of Ca^{++} postulated in the text, and statistical calculations of the distribution of esterified residues.

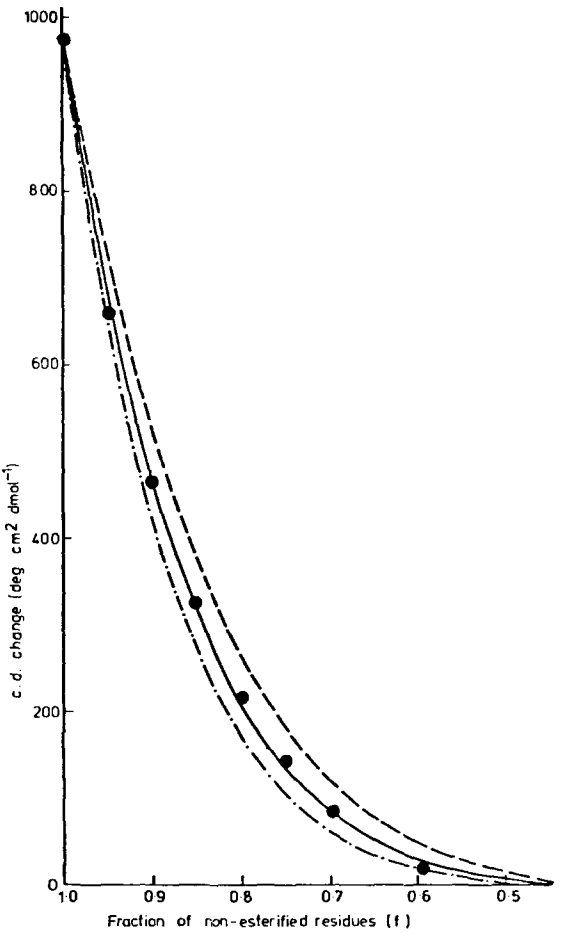

Fig. 10. Variation in cation binding to polygalacturonate with degree of random esterification. The bound calcium which is resistant to displacement by swamping levels of monovalent counterion (0·5 M Na^+), as monitored by c.d. change (•) between solution and gel, is compared with the statistical probability of occurrence of 6(– – –), 7(——) or 8(-·-·-) consecutive non-esterified residues along one chain face, for a range of methyl polygalacturonates.

chain segments when they are de-esterified (Fig. 8), but not when they are esterified. This interference with the development of a network structure which resists applied stress, provides further support for our model of junction zone formation from sequences of contiguous de-esterified residues, with Ca^{++}-mediated chain dimers providing the primary associations which can offer resistance to deformation.

Samples with different levels and patterns of esterification were prepared by enzymic (blockwise) and chemical (random) de-esterification of almost fully methyl

esterified pectin. In the former series the extent of Ca^{++} binding (as monitored by circular dichroism) increased almost linearly with the fraction of free carboxyl groups, whereas the latter showed a non-linear relationship of a form consistent with the requirement of this binding for blocks of contiguous non-esterified residues and, in the presence of excess univalent cations, binding was negligible when more than ~40% of the carboxyl groups were esterified (Fig. 9). Statistical calculations of sequence length distribution at different degrees of random de-esterification show the best fit with experimental data when binding is assumed to require sequences with seven or more consecutive free carboxyl groups along the participating face of the chain (Fig. 10). For 2_1 chain symmetry this corresponds to a sequence length of 14 residues, in excellent agreement with previous independent studies of Ca^{++} binding to oligogalacturonates.

In the absence of competing univalent counterions, c.d. changes are similar in form but so large in magnitude that site-binding of Ca^{++} must now go beyond the half-stoichiometry at which it is arrested in their presence. Ca^{++} binding monitored by c.d. and gel strength (yield stress, Fig. 11) measured mechanically both show a similar dependence on the pattern as well as the level of esterification, as expected for network formation by cooperative binding of Ca^{++} within interchain junction zones.

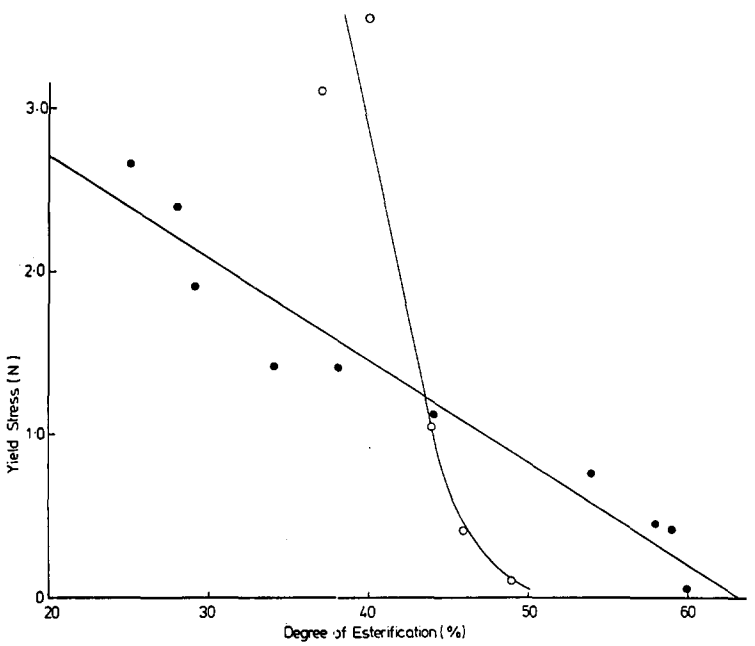

Fig. 11. Gel strength as a function of degree of esterification for: (•) enzymically de-esterified pectin; (○) alkaline de-esterified pectin.

To fit this binding data quantitatively, it is necessary to postulate a two-stage process: (i) initial dimerisation, probably corresponding to the 'strong associations' indicated by evidence from competitive inhibition (see above), for which a critical minimum sequence of seven residues is again required but esterified residues can now be accommodated within individual sites provided that they are paired with a free carboxylate on the complementary chain; (ii) subsequent Ca^{++}-induced aggregation of these preformed dimers which can occur irrespective of the pattern of esterification on the external faces; the evidence from mechanical measurements shows that these contribute little to gel strength at high stress.