

Dry-mix dispersion can be used when a formulation requires both alginate and other dry ingredients such as sugars, starches, etc. The dry powders are mixed thoroughly so that the alginate particles are diluted and separated by the other ingredients. This mixture is slowly added to well stirred water, preferably with a vortex as before, and the other ingredients, often in a ratio of 5:1 to 10:1, help to keep the alginate particles apart as they are wetted.

An even more efficient method of diluting the alginate particles is to use liquid-mix dispersion in which they are wetted with a non-solvent. This can be either a water-miscible non-aqueous liquid (such as ethanol or glycerol) or a water-immiscible liquid (such as a vegetable oil). Enough liquid is needed to give a pourable slurry and this is poured into the water, well agitated as before. The particles are dispersed and the rate of hydration, and solution, will depend on the time taken for the non-solvent liquid to diffuse from the surface of the particles.

D. PRESERVATION OF SOLUTIONS

Microorganisms will grow in solutions of commercial alginates because they usually contain sufficient nitrogenous compounds and salts. Bacterial or mould growth may cause depolymerization and loss of viscosity of the alginate as well as contamination and spoiling of any product in which the alginate is used. Food and cosmetic products are protected by their traditional preservatives such as sorbic acid, potassium sorbate, benzoic acid, sodium benzoate and the methyl or ethyl ester of p-hydroxybenzoic acid. For other uses less expensive, and sometimes more effective, preservatives are available such as formaldehyde and sodium pentachlorophenate and other phenol derivatives. Compounds of copper and zinc, and quaternary ammonium salts, should not be used because they will react with the alginate.

VISCOSITY

Many of the uses of alginates depend on their thickening effect, their ability to increase the viscosity of aqueous systems using relatively low concentrations. At the concentrations used in most applications, the viscosity behaviour of alginate solutions is pseudoplastic, the solution flows more readily the more it is stirred or pumped (the viscosity decreases as the rate of shear increases). This effect is reversible except at very high rates of shear (Glücksman, 1969). It is most marked with high molecular weight alginates, with sodium alginate solutions which contain calcium ions, and with propylene glycol alginate above 1% concentration; some of these solutions can also be thixotropic, that is they show a time-dependent thinning at constant shear rate and their recovery to the initial viscosity is time dependent. Reproducible viscosity measurements are made using a rotational type of viscometer, such as the Brookfield Synchro-Lectric.

Several factors influence the viscosity of alginate solutions.

A. MOLECULAR WEIGHT

The higher the molecular weight of a soluble alginate, the greater the viscosity of its solution. Manufacturers can control the molecular weight (degree of polymerization, DP) by varying the severity of the extraction conditions and they offer products ranging from 10-1 000 mPa.s (1% solution) with a DP range of 100-1 000 units. Sodium alginate of viscosity 200-400 mPa.s, "medium viscosity", probably finds the widest application.

B. CONCENTRATION

There is no simple relationship between concentration and viscosity for alginate solutions but McDowell (1960,1977) found a useful empirical equation which applied to a wide variety of alginates over a range of at least a hundredfold change in viscosity:

$$\log_{10} \text{viscosity} = a \sqrt{(\text{concentration}) - b}$$

where a is a constant related to the DP of the alginate, b is a constant for a particular type of alginate. Graphs or tables of viscosity versus concentration are available from manufacturers for their particular products (Kelco, 1976; McDowell, 1977; Protan, 1986) and some typical figures are shown in Table 3.

C. TEMPERATURE

Viscosity decreases as temperature increases, at a rate of about 2.5% per degree Celsius (Figure 7). Viscosity usually returns to a little less than the original value on cooling. However if alginate solutions are kept above 50°C for several hours, depolymerization may occur giving a permanent loss of viscosity.

Alginate solutions can be frozen and thawed without change of viscosity, as long as they are free of calcium (less than 0.5%); if calcium is present the viscosity will increase and a gel may even form and these changes will not reverse.

D. pH

The viscosity of alginate solutions is unaffected over the range of pH 5-11. Below pH 5, the free -COO ions in the chain start to become protonated, to -COOH, so the electrostatic repulsion between chains is reduced, they are able to come closer and form hydrogen bonds, producing higher viscosities (King, 1983). When the pH is further reduced, a gel will form, usually between pH 3-4; however if the alginate contains residual calcium this gelation may occur about pH 5. If the pH is reduced quickly from pH 6 to pH 2, a gelatinous

Viscosity (mPa.s)

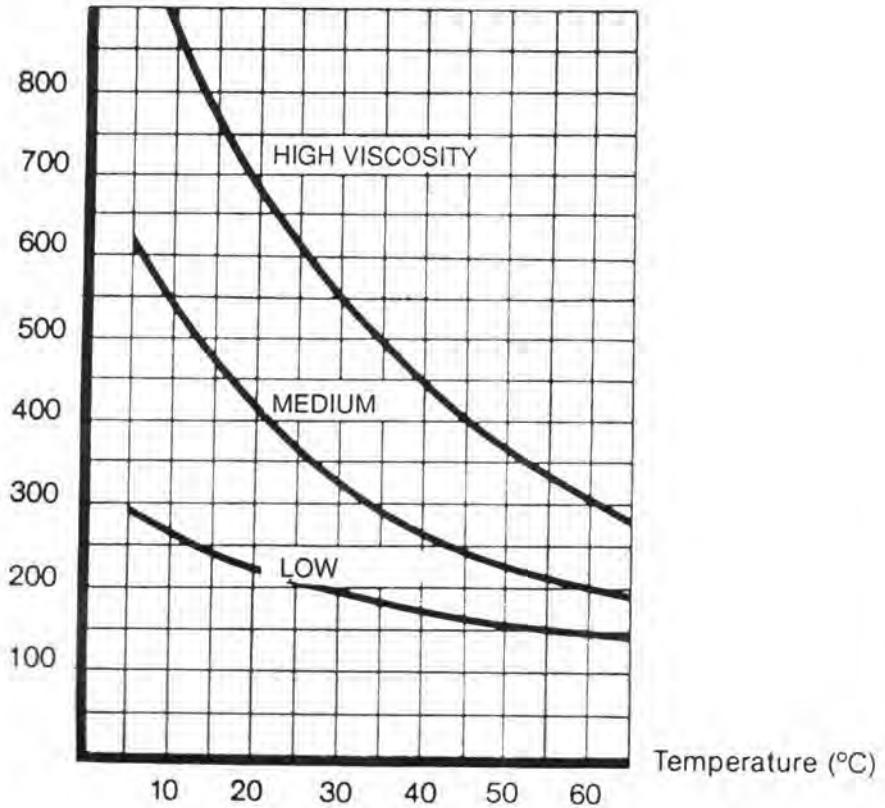


Figure 7 Viscosity in 1% solutions at different temperatures.
(Source: Protan, 1986a)

precipitate of alginic acid will form. Above pH 11, slow depolymerization occurs on storage of alginate solutions, giving a fall in viscosity.

Propylene glycol alginate has fewer $-\text{COO}^-$ ions and is less affected by increasing acidity. Its solutions remain unchanged to about pH 3; below this value precipitation and gel formation occur. Above pH 6.7, hydrolysis of the ester groups occurs slowly with consequent loss of viscosity.

E. CALCIUM IONS

The presence of low concentrations of calcium ions in an alginate solution will increase its viscosity and larger amounts will cause the formation of a gel. The addition of Ca^{++} is therefore a way of increasing the viscosity of a solution without having to increase either the amount of alginate dissolved or the molecular weight of the alginate being used. It also allows the flow properties of solutions to be adjusted (reduced) by adding sequestering agents such as calgon and EDTA. The disadvantage is that alginate solutions with calcium ions show a greater loss of viscosity with stirring (are more shear sensitive) than alginates with no calcium. As the concentration of calcium ions and viscosity increase, the solutions change from pseudoplastic to thixotropic, that is they take some time to recover their original viscosity after being stirred. The way in which calcium reacts with alginate is discussed in the later section on "Gels".

Most commercial alginates made by the calcium alginate process contain residual quantities of calcium; for example the usual food grades of sodium alginate from Kelco contain 1.2% calcium and in special low-calcium grades this is reduced to 0.2% (Kelco, 1976). 1.2% calcium represents 17% substitution of calcium for sodium in the sodium alginate and this is sufficient to increase the viscosity; thickened, flowable solutions result from 7-20% substitution by calcium ion while gels form with about 30%. There is a region of calcium addition, just before gel formation, where very thick solutions result which are thixotropic. The range of calcium concentration over which this occurs is much greater for alginates with a high content of mannuronic acid (high M/G ratio). Alginates with a high guluronic acid content show a more abrupt transition from solution to gel. The effect of calcium on the viscosity of a calcium-containing alginate can be estimated by measuring the viscosity of a solution before and after the addition of a sequestering agent, such as sodium hexametaphosphate, which removes the calcium ions in a complexed phosphate ion.

Alginates made by the alginic acid process contain negligible amounts of calcium so that if an increase in viscosity is required at a fixed alginate concentration, a small amount of a sparingly soluble

calcium salt such as calcium sulfate or calcium citrate may be added. The effect of calcium on the viscosity of an alginate is difficult to predict and is usually found by experimentation. It will depend on the uronic acid composition and degree of polymerization of the alginate; alginates with higher molecular weights and/or higher M/G ratios give greater viscosity changes (McDowell, 1960). The way in which the solution is made also affects the final viscosity; for example a 1% solution prepared by dilution of a 3% solution will differ in viscosity from a 1% solution prepared directly; the type and duration of the stirring used in preparation will also affect the result.

Propylene glycol alginate with 85% of the carboxylic acid groups esterified is hardly affected by the presence of calcium ions. At the other extreme, 60% or less esterification gives an ester which behaves similarly to sodium alginate except that thixotropic effects are much more evident.

GELS

The polysaccharides derived from seaweeds - alginates, agars, carrageenans and furcelleran - can all be induced to form gels under certain conditions. A better understanding of the structure of these gels has developed in recent years and useful reviews have been written by Rees (1972) and Morris (1985).

Solutions of alginate will react with many di- and trivalent cations to form gels; the gels will form at room temperature, or any temperature up to 100°C, and they do not melt when heated. They find applications in various industries, particularly when calcium is used as the divalent ion. Alginate solutions will also form gels if they are carefully acidified; these gels are generally softer than calcium gels and, unlike calcium gels, give the feel of melting in the mouth so they find many applications in the food industry.

CALCIUM GELS

Those who are seriously interested in formulating calcium gels should refer to the work by King (1983, pp. 141-173) which is the most thorough discussion available of the variables which should be considered, the practical systems commonly used and some examples of actual applications.

Calcium has found greatest popularity as the divalent ion for gel formation because its salts are cheap, readily available and non-toxic. If a calcium chloride solution is stirred into an alginate solution, a precipitate of calcium alginate results; it may be stringy or gelatinous. To obtain a smooth gel, the calcium must be released slowly into the alginate solution. This is done by using a calcium salt with a low solubility (such as calcium citrate) which slowly

releases calcium ions. An alternate method is to use a calcium salt which is practically insoluble in neutral solution but dissolves as the pH falls (such as dicalcium phosphate); when an acid of low solubility (such as adipic acid) is added, it gradually lowers the pH, calcium ions are released and a gel forms. The time needed for a gel to form can be controlled by the solubilities of the calcium salt and acid, their particle size and the operating temperature. Retarding agents can also be used, such as sequestrants which complex the calcium ions and make them unavailable until all the sequestrant has reacted; when a dry powder product contains both alginate and a calcium salt, the addition of sufficient sequestrant will delay the availability of calcium ions until the alginate is dispersed and hydrated. Details of calcium salts, acids and sequestrants which are used have been discussed by Littlecott (1982) and more detail by King (1983).

The gel strength depends on the source (algal species) of the alginate, the concentration of alginate, its degree of polymerization and the calcium concentration. Alginates from different seaweeds can have differing ratios of mannuronic acid to guluronic acid in their structures and different proportions of M, G and MG blocks (see "Structure of alginic acid" section). This ratio, and the way in which the acids are distributed in the alginate chains, have a marked effect on gel formation and gel strength. Alginates with a high proportion of G blocks form rigid gels; they form fairly suddenly as calcium ion concentration is steadily increased. The opposite holds for alginates with mainly M blocks; they form gradually and are softer and more elastic. This behaviour is related to the molecular structure of the gels.

The early hypotheses for gel formation was that calcium ions displaced hydrogen ions on the carboxylic acid groups of adjacent chains and formed simple ionic bridges between the chains. Rees (1969) argued why that was unlikely and later he put forward the "egg-box model" (Grant et al., 1973), now generally accepted. This requires the cooperative mechanism of binding, of two or more chains, shown in Figure 8. The buckled chain of guluronic acid units is shown as a two-dimensional analogue of a corrugated egg-box with interstices in which the calcium ions may pack and be coordinated. "The analogy is that the strength and selectivity of cooperative binding is determined by the comfort with which 'eggs' of the particular size may pack in the 'box', and with which the layers of the box pack with each other around the eggs" (Grant et al., 1973). The model can be extended to be three-dimensional. While calcium helps to hold the molecules together, their polymeric nature and their aggregation bind the calcium more firmly; this has been termed "cooperative binding". The structure of the guluronic acid chains (Figure 4) gives distances between carboxyl and hydroxyl groups which allow a high degree of coordination of the calcium. The strontium ion is larger and even more firmly bound; magnesium ions are smaller and are not held, so magnesium alginate does not form gels (McDowell, 1977).

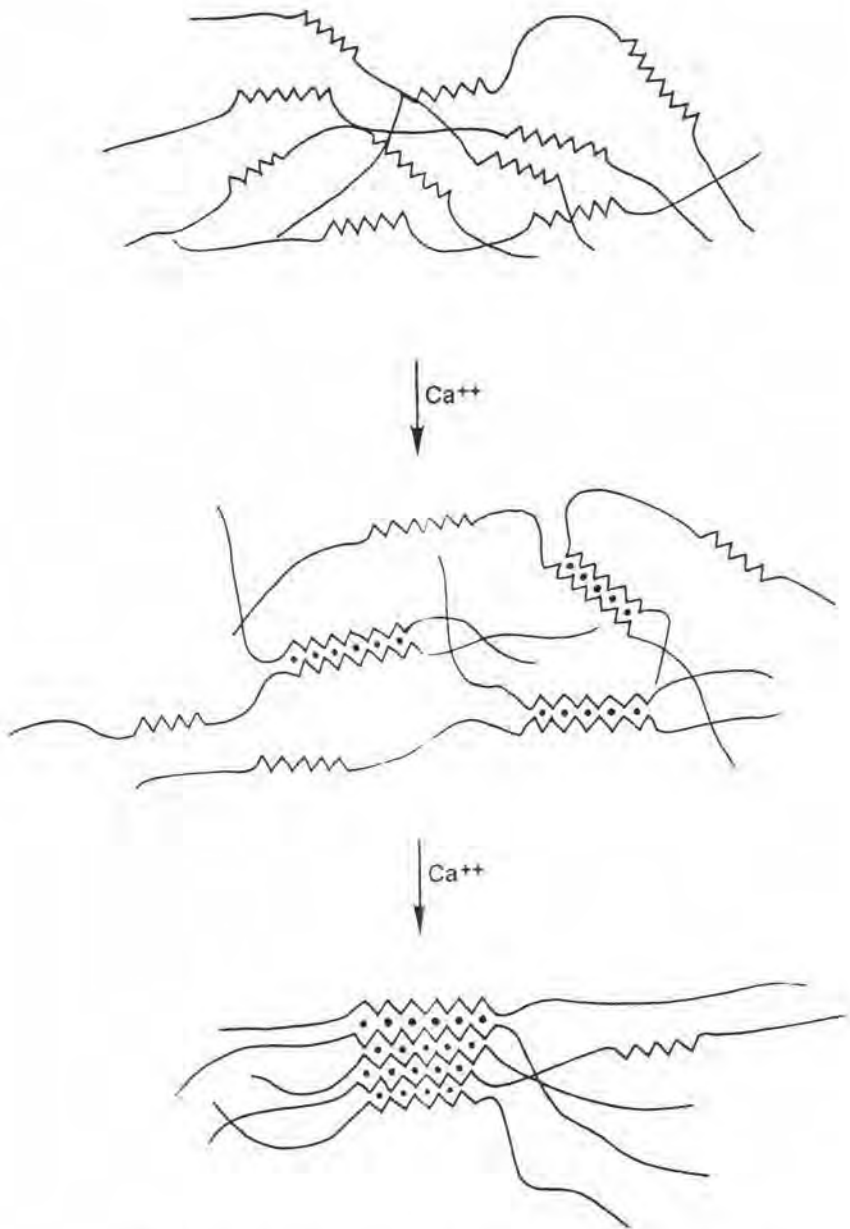


Figure 8 Gel formation via G blocks: egg box model

Propylene glycol alginate, with a low degree of esterification (below 60%) and a high degree of polymerization, can form soft gels with calcium salts. As the degree of esterification is raised to about 85%, it is hardly affected by calcium (McDowell, 1977). It is also more tolerant of calcium at lower pH values (Steiner and McNeely, 1950).

ACID GELS

The structure of these gels has not been studied as comprehensively as calcium gels, probably because they are more limited in their application. A steadily increasing number of carboxyl ions on the alginate chains become protonated as the pH falls, reducing the electrical repulsion between chains. The chains can then move closer together which allows hydrogen bonding to be more effective. At first this produces a higher viscosity and eventually, at pH 3.5-4.0, a gel forms. Small amounts of calcium (less than 0.01%) must be present; the reason is not known.

King (1983) has outlined the useful characteristics of acid gels. For equivalent alginate concentrations, acid gels have about half the strength of calcium gels and they do not show any syneresis. This softness, when combined with their feeling of melting in the mouth, means they are useful in some food applications where they can imitate the effect of gelatin; calcium gels, even soft ones, still feel lumpy in the mouth. They can be made so that they can be stirred or pumped and then reset to a gel, a valuable property when manufacturing some processed foods. However acid gels are not stable when heated and become softer with time even at room temperature, as the alginic acid depolymerizes; they are stable for about a year if refrigerated (5°C). Propylene glycol alginate is not suitable for making acid gels.

FILM FORMATION

Alginates can be made into two types of film which have different properties: water-soluble films (usually from sodium alginate) and oil-soluble films (usually from calcium alginate).

Water-soluble films can be made by evaporation of a solution of alginate or by extrusion of an alginate solution into a non-solvent which mixes with water, such as acetone or ethanol. These films are impervious to grease, fats and waxes but allow water vapour to pass through. They are brittle when dry but can be plasticized with glycerol, sorbitol or urea. They have good non-stick properties and are useful as mould release agents, for example in the manufacture of fibreglass plastics. Where a high solids film is needed, a very low viscosity alginate can be used. Self-supporting films need greater strength and require the use of higher viscosity alginates with a greater degree of polymerization. Triethanolamine alginate is used to form soft flexible films.

Water-insoluble films can be made by treating a water-soluble film with a di- or trivalent cation (Ca^{++} is the most frequently used one) or with acid. They can also be made by extrusion of a solution of a soluble alginate into a bath of a calcium salt. Some alginates, such as zinc alginate, are soluble in excess ammonia solution; if the NH_3 is evaporated from a film of such a solution, an insoluble film of zinc alginate remains. These films of insoluble alginate are not water-repellent and will swell on prolonged exposure to water.

FILAMENT FORMATION

If a solution of sodium alginate is forced through fine holes into a solution of a calcium salt, filaments of calcium alginate will be formed. Much research went into the development of alginate yarns (Steiner and McNeely, 1954; Maass, 1959) but they are not resistant to alkaline soaps. This, plus the relative cost of alginate and the development of many synthetic fibres, led to a loss of interest until quite recently when a new commercial product appeared. Made in the United Kingdom, it is a bandage-type material which is used as a dressing on wounds. When it comes into contact with sodium salts in the body fluids, some of the calcium ions are exchanged for sodium ions so that a thin soft gel forms at the interface of the dressing and the wound and the dressing never sticks to the wound.

GENERAL COLLOIDAL PROPERTIES

General colloidal properties is the term used to explain why alginate is successful in some applications where the reasons are not fully understood and where the alginate has been chosen on an empirical basis. These applications have been discussed by McDowell (1960) and Leigh (1979). They include the use of: sodium alginate, as a flocculant, as a suspending agent, and as a stabilizer in ice cream; propylene glycol alginate, in acidic frozen products such as ice sherberts, in fruit squash containing fruit solids, and in stabilizing beer foam.

SAFETY IN FOODS

The Food Chemical Codex gives specifications for alginic acid, its propylene glycol ester and its ammonium, calcium, potassium and sodium salts. These four salts have been granted GRAS status (generally recognized as safe) in the USA and propylene glycol alginate has been approved as a food additive for use as an emulsifier, stabilizer or thickener. The joint Expert Committee of Food Additives of the Food and Agriculture Organization of UN/World Health Organization has also issued specifications for alginates and recommended an Acceptable Daily Intake, for alginic acid salts of 50 mg per kg body weight per day, for propylene glycol alginate of 25 mg/kg/day. King (1983) has listed 39 countries which permitted alginate salts as at January 1982; three of them had not approved the

propylene glycol ester. Food additive laws differ from country to country, even from state to state within a country, and are constantly being revised. Therefore users must acquaint themselves with the latest information in their relevant countries and cannot rely on the information given here and in the references cited.

USES

Earlier reviews on the uses of alginates include those by Steiner and McNeely (1954), Maass (1959), McDowell (1960), Glicksman (1969a), McNeely and Pettitt (1973), Cottrell and Kovacs (1980), and King (1983).

Not all reviews cover all uses; for example while most give lengthy treatments to food uses, few say much about textile printing and paper applications. Reviews which are more specific to a particular use are listed in the following subsections. The main uses of alginates are shown in Table 4.

TEXTILE PRINTING

In textile printing, alginates are used as thickeners for the paste containing the dye. These pastes may be applied to the fabric by either screen or roller printing equipment. An excellent review by Hilton (1969) discusses the role of the thickener in the printing of fabrics and the advantages/disadvantages of sodium alginate in different printing processes. Alginates became important thickeners with the advent of reactive dyes which combine chemically with cellulose at its hydroxyl groups. Many of the standard thickeners, such as starch, also react with these dyes and this leads to lower colour yields and sometimes insoluble products which are not easily washed out and which can result in a fabric with poor handle. Alginates react minimally with reactive dyes, they wash out of the finished textile readily and are the best thickeners for these dyes. They are also used with other types of dyes.

The viscosity of the paste can be varied according to the application and the equipment. Thick pastes with short flow characteristics are useful when the extent of penetration into the fabric must be limited but thinner pastes with long flow are required for fine-patterned prints. For alginates containing small quantities of calcium, viscosity can be controlled by adding sequestering agents such as polyphosphates. However these pastes are more likely to lose viscosity as shear rate increases and a paste which is less shear sensitive can be made using a high concentration of a lower viscosity alginate. This latter kind of paste is especially useful for printing disperse dyes on synthetic fibres. Most alginate manufacturers can supply basic recipes for the different types of dyes and printing processes (for example, Protan, 1985) which are a useful starting point; the quantities of alginate can vary from 1.5% of high viscosity alginate to 5% of low viscosity alginate.

Table 3

Variation of viscosity (mPa.s) with concentration for sodium alginate solutions at 20°C

<u>Type of alginate</u>	<u>Concentration</u>				
	<u>1%</u>	<u>1.5%</u>	<u>2%</u>	<u>3%</u>	<u>4%</u>
Very low viscosity	10	20	45	130	350
Low viscosity	20	60	180	650	2200
Medium viscosity	350	1800	6000	not measurable	
High viscosity	800	4000	9000	not measurable	

Table 4

Principal uses of alginate

<u>End-uses</u>	<u>Percentage of the quantity of total demand</u>
Textile printing	50
Food	30
Paper	6
Welding rods	5
Pharmaceuticals	5
Others	4

Source: ITC (1981).

Alginates are normally incompatible with cationic dyes. However Racciato (1979) has reported that premixing the cationic dye with selected surfactants before addition to the thickener will allow the use of many cationic dyes. He claims that compatibility with almost every cationic dye can be obtained if either xanthan gum or algin is used. In the printing of cotton cloths using reactive dyes, Prelini (1982) suggests ways of obtaining good colour value and of avoiding colour bleeding, using alginates and other thickeners. Rompp, Axon and Thompson (1983), discuss the use of alginates with reactive dyes on cotton, viscose rayon and cotton-synthetic blend fabrics.

Ramakrishnan (1981) deals with the principles of reactive printing and the problems which arise in rotary printing machines, and the use of sodium alginate in the processing. Obenski (1984) has discussed the US printed fabric market, the use of alginates and guar gum as thickening agents and their share of the US market.

General reviews of thickeners in printing pastes, which include the use of alginates, have been made by Christie (1976), Shenai and Saraf (1981), Narkar (1982) and Teli, Shah and Sinha (1986).

Reviews dealing more specifically with the use of alginates in textile printing can be found in Ornaf (1969), Hilton (1972), Iwahashi (1975), Shah (1975), Balassa (1977), Khairiowala and Afrin (1984), Hebeish et al. (1986) and Teli and Chiplunkar (1986).

FOODS

Alginates have a long history of use in foods and these uses are based mainly on their thickening, gelling and general colloidal properties. Thickening is useful in sauces, syrups and toppings for ice cream, etc., pie fillings (it reduces moisture retention by the pastry), cake mixes (it thickens the batter aids moisture retention), and canned meat and vegetables (it can give either temporary or delayed-action thickening). Gel formation leads to uses in instant milk desserts and jellies, bakery filling cream, fruit pies, animal foods and reformed fruit. General colloidal properties are difficult to define but are illustrated by the results obtained by adding sodium alginate to ice cream and water ices, or propylene glycol alginate to stabilize beer foam or the suspended solids in fruit drinks (Leigh, 1979). Details of these and other applications can be found in some of the more recent reviews which have been written by Glicksman (1969a), McNeely and Pettitt (1973), McDowell (1975), Lawrence (1976), Cottrell and Kovacs (1980), Littlecott (1982), King (1983) and Sime (1984); their content is summarized below.

McNeely and Pettitt (1973) is well referenced to the general literature and much of the material is still useful. McDowell (1975) classifies uses according to the relevant alginate property such as thickening, gelling, film formation and stabilizing; it is a general

review of value to a new user of alginates. Lawrence (1976) surveyed those US patents since the early 1960's that deal with gels for edible purposes and he includes a lengthy section on alginate. The four most recent reviews are all written by personnel from Kelco, the largest world manufacturer of alginate. Littlecott (1982) gives a good explanation of how to form food gels with alginates and provides many examples and formulations. Sime (1984) also deals with food gelling systems and is a useful addition to Littlecott's review; after discussing the general principles, he gives details for making reformed pimiento strips for olives, and structured fruits from fruit puree. Cottrell and Kovacs (1980) relate food applications to properties of alginate and give a wide variety of sample formulations for various food products; they give few literature references.

The review by King (1983) is excellent; he draws on the material used by Cottrell and Kovacs for describing the properties of alginates and then provides a thorough and well documented description of the food uses of alginates up to 1981-82.

One of the more recent developments is the use of alginates in restructured meat products. The US Department of Agriculture approved the use of alginate as a binder in these products last year (September, 1986) and this should lead to a new market for alginate. Restructuring is the process of taking flaked, sectioned or chunked meat and binding the pieces to resemble intact cuts of meat. The final products can be shaped as nuggets, roasts, loaves and steaks. Until now most restructured products have been sold frozen or cooked, so they could retain their shape. With the use of binders, the restructured products can be sold fresh or raw. The binder is a powder of sodium alginate, calcium carbonate, lactic acid and calcium lactate. When mixed with the raw meat, they form a calcium alginate gel which binds the meat. This binding mixture can be used to replace the sodium chloride and phosphate salts commonly used, thereby reducing the sodium level in the restructured products. Up to 1% sodium alginate is permitted. A patent has been assigned to the developers of the process, Colorado State University Research Foundation (Schmidt and Means, 1986), the inventors have published the information separately (Means and Schmidt, 1986) and the application is outlined by Andres (1987).

Alginates have been used for other re-formed foods. Morimoto (1984) patented a process for making shrimp or crabmeat analogue products using alginate and proteins such as soy protein concentrate or sodium caseinate. A mixture of the two is extruded into a calcium chloride bath to form edible fibres which are then frozen, thawed, chopped, coated with sodium alginate and formed in an appropriately shaped mould. After further freezing and thawing a product analogous to natural shrimp is obtained. Wylie (1976) described the manufacture of analogue fish fillets (sole) using minced white fish and a calcium alginate gel; the products could be grilled or cooked with sauce. A meat substitute has been formed from an aqueous mixture of protein and

alginate by a process of freezing, slicing, gelling and heat setting; a well defined fibre structure results (Shenouda, 1983).

The principles used for making structured fruit products have been extended to making structured potato products such as croquettes and french fries (Anon., 1983). A synthetic potato skin shell containing alginate can be filled with mashed potato and browned to produce 'baked potatoes' in the fast-food market (Ooraikul and Aboagye, 1986). A patent by Cox (1982) for forming simulated, shaped, edible products includes the production of caviar and cottage cheese as examples.

There has been an increased interest in the use of alginate-pectin mixed gels with potential for use in jams, fruit flans and mayonnaise (Thom et al., 1982; Toft, 1982; Morris and Chilvers, 1984). New dessert gels from alginate have been reviewed by Kelco (1983) while Protan (1986a) has discussed alginates as stabilizers in bakery creams, jams and jellies.

The reasons for the effectiveness of alginate as a stabilizer in ice cream have never been fully understood; Muhr and Blanshard (1984) have studied the mechanism for the reduction of crystal growth but their work is not yet conclusive.

A moisture barrier which allows breaded or batter-covered products to come in contact with a sauce or filling can be made using a coating of soluble alginate which is then treated with calcium chloride (Earle and McKee, 1986). Alginates are being used to make improved rice pasta and vegetable pasta (Hsu, 1985, 1985a). Calcium alginate can be formed as a fibrous precipitate and is used to simulate the texture of natural fruit and vegetables (Anon., 1980).

IMMOBILIZED BIOCATALYSTS

Many commercial chemical syntheses and conversions are best carried out using biocatalysts such as enzymes or whole cells. Examples are (a) the use of enzymes for the conversion of glucose (40% of the sweetness of sucrose) to fructose (about 150% of the sweetness of sucrose), the production of L-amino acids for use in foodstuffs, the synthesis of new penicillins after hydrolysis of penicillin G, (b) the use of whole cells to promote the conversion of starch to ethanol, for beer brewing, for the continuous production of yoghurt. To carry out such processes on a moderate to large scale, the biocatalysts need to be in a concentrated form and to be recoverable from the process for reuse. This can be achieved by "immobilizing" the enzymes or cells; they can be fixed to the surface of an insoluble solid or entrapped in a polymeric material. In the 1970s many single enzymes were isolated, immobilized and used, but more recently it has been found that it is easier, more economical and often more effective to immobilize whole cells, which contain multi-enzyme systems. An added advantage of immobilizing cells is the increased stability often

found; it is not unusual for a half-life of one day for ordinary suspended cells, to be increased to 30 days for immobilized or resting cells. A good introduction to the reasons for using biocatalysts and for their immobilization is given by Tramper (1985); he also describes the use of alginate for immobilization. Bucke and Wiseman (1981) give a more detailed background and review the developments to the early 1980s.

Alginate gels have proved to be a very successful medium for entrapping biocatalysts, especially when formed as beads of gel. The cell suspension is mixed with sodium alginate solution (2-4%) and this is extruded as drops into calcium chloride solution (0.05-0.1M). An immediate skin forms around the drop and as calcium ions gradually diffuse inwards, a gel forms. The size of the beads can be regulated from the size of the needle or nozzle, usually 0.2-1.0 mm but up to 5 mm. The fresh beads can be separated and used or they can be dried; drying increases their strength and reduces their ability to swell so they contain, when rewetted, more cells per unit volume.

Alginates which form strong gels are best for this application; the alginate should therefore contain a high proportion of guluronic acid, such as that extracted from the stipes of Laminaria hyperborea, a species particularly abundant in the cold waters of Norway. There have been recent reports of high guluronic acid contents in seaweeds from warmer waters, Sargassum species from Sabah, Malaysia (Wedlock, Fasihuddin and Phillips, 1986) and Sargassum, Turbinaria and Cystoseira from Sri Lanka (Shyamali, de Silva and Savitri Kumar, 1984).

Details of methods are available from manufacturers (such as Protan, 1987) or from the literature (Dallyn, Falloon and Bean, 1977; Klein and Wagner, 1982; Klein, Stock and Vorlop, 1983; Tanaka, Matsumura and Veliky, 1984; Rehg, Dorger and Chau, 1986).

Johansen and Flink (1985, 1986, 1986a, 1986b) have applied the internal gelation principles, developed for food gels, to immobilization techniques using yeast cells for their studies. Sodium alginate, an insoluble calcium salt and D-glucono-1,5-lactone are mixed in water; the lactone slowly hydrolyses, lowers the pH, releases calcium ions and gelation occurs gradually, from within the solution. The resulting immobilizates have particles of higher strength, with at least equal fermentation rates, when compared to externally gelled material. Rochefort, Rehg and Chau (1986) have stabilized calcium alginate gels by washing with 0.1M aluminium nitrate. Burns, Kvesitadze and Graves (1985), produced dried spheres of calcium alginate containing magnetite and found they have good potential as a support for enzyme immobilization.

Cell immobilization with alginate can be done under mild conditions with little loss of activity of the cells and the activity

is often stable for extended periods of time. Temperatures can be 0-100°C and the pH neutral but any buffers used must not contain citrate or phosphate. These anions will remove calcium ions from the gel and can lead to its breakdown, although Birnbaum et al. (1981), have developed methods for stabilizing alginate gels in phosphate-containing media. The cell-gel entrapment can be done under sterile conditions and the alginate gel is stable (0-100°C) and non-toxic. The cells can be recovered if necessary by adding a sequestering agent for the calcium ions (such as polyphosphate or EDTA); once the calcium ions are removed from the gel, its structure is lost and it changes to a liquid with the cells suspended in it.

The number of processes in which alginate has been used for cell or enzyme immobilization, on laboratory and larger scales, has increased dramatically in the last few years. Good sources of these works are journals such as *Biotechnology Letters*, *Biotechnology and Bioengineering* or the abstracts available from data bases such as *Biobusiness* (Dialog Information Services) and *Current Biotechnology Abstracts* (Pergamon Infoline). Some examples are:

- (a) production of ethanol from starch (McGhee, Carr and St. Julian, 1984);
- (b) beer brewing with immobilized yeast (Onaka et al., 1985);
- (c) production of citric acid (Lim and Choi, 1986);
- (d) continuous yoghurt production (Prevost, Divies and Rousseau, 1985);
- (e) fermentation to produce butanol and isopropanol (Schoutens et al., 1986);
- (f) continuous acetone-butanol production (Frick and Schuegerl, 1986);
- (g) pilot-plant production of prednisolone from hydrocortisone (Kloosterman and Lilly, 1986);
- (h) glycerol production from the marine alga, Dunaliella tertiolecta (Grizeau and Navarro, 1986).

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Until the late 1950s the main use for alginate in the paper industry was in surface sizing. Its addition to the normal starch sizing gives a smooth continuous film and a surface with less fluffing. The oil resistance of alginate films give a size with better oil resistance so an improved gloss is obtained with high gloss inks. If papers or boards are to be waxed, alginate in the size will