Shear Disruption of Soya Protein Precipitate Particles and the Effect of Aging in a Stirred Tank

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Summary

An improvement in the resistance of isoelectric soya protein precipitate to capillary shear disruption was achieved by aging in a stirred tank. The aggregate strength was found to depend on the extent and duration of agitation prior to exposure to shear. An optimum value of an aging parameter $Gt \sim 10^5$ was determined, where G is the rms velocity gradient in the aging vessel and t is the time of aging. The disruption of precipitate aggregates by exposure to high rates of shear for short times was dependent on the rate of shear and time of exposure. The dominant mechanism of aggregate breakup was fragmentation, with erosion occurring to a lesser extent. The size of the fragments produced by shear disruption was weakly dependent on the rate of shear, with higher rates of shear producing smaller fragments.

INTRODUCTION

Efficient centrifugal separation of biological solids such as precipitated protein is often limited by the small particle size, a small difference in density between the solids and their associated liquor, or the presence of the solid phase as fragile aggregates which break up under the shear fields present in processing equipment.

The effects of aging have been widely reported[1-3] as improving recoverability in centrifuges. However, little detailed evidence exists to identify the mechanisms by which this improvement has occurred and to define whether it is due to aggregate growth alone or a structural rearrangement and strengthening of the aggregate. Hoare[1] indicated that gravity sedimentation or low shear aging of precipitated casein resulted in aggregate growth but the resistance of these aggregates to shear was not evaluated. Edwards et al.[2] reported that the centrifugal recovery of heat-coagulated leaf protein concentrate was improved by aging but no analysis was given. The nature of blood plasma fractional precipitates is also known to be affected by aging.[3]

The purpose of this research was to investigate the effects of aging, and of exposure for short times to high rates of shear, on protein precipitates. This should result in a better understanding of factors affecting the solid–liquid separation process. An isoelectric soya protein precipitate system was chosen because it is biochemically well characterized and because of its technololgi-

cal significance. Rates of shear between 10^4 and 10^5 s^{-1} and times of exposure between 0.004 and 0.2 s were chosen as being typical of the feed zones of high-speed centrifuges.[4]

Fundamental Considerations

Tomi and Bagster[5] and Glasgow and Luecke[6] recently reviewed the behavior of aggregates in shear fields, with particular reference to the growth and breakup processes. Growth is considered to occur initially by Brownian motion bringing the small colloidal particles together and subsequently by the velocity gradient mechanism described by Smoluchowski, where the collision frequency between particles is given by

$$J_{ij} = \frac{1}{6}(d_i + d_j)^3 n_i n_j G \tag{1}$$

where the rms velocity gradient, per second, is

$$G = \sqrt{\epsilon/\nu} \tag{2}$$

and the terms are defined in the Nomenclature Section.

Under conditions of shear, particle growth is opposed by breakup processes which have been attributed to the following mechanisms: 1) deformation and rupture from dynamic pressures; 2) erosion of "primary" particles from the aggregate by aggregate–aggregate, aggregate–solid surface interactions or by hydrodynamic shear; and 3) fragmentation of groups of "primary" particles by mechanisms as in 2).

The final performance of any system is determined by the balance between the processes of aggregation and breakup, and ultimately a state of dynamic equilibrium is expected to exist. The limiting size of the aggregates is both a function of their strength, which depends on interparticle cohesion and the structural arrangement of the particles, and the intensity of agitation. Glasgow and Luecke,[6] having found that for the deaggregation of clay–polymer flocs both primary erosion and fragmentation were significant, suggested that deviations from theoretical descriptions at long aging times were due to an increase in the strength parameter of the floc. They indicated that the more easily dislodged fragments are removed first; after an extended residence time particles with a greater than average number of polymer bonds would tend to be retained selectively.

The strength of isoelectric soya protein precipitate depends on the bonding between individual molecules and between aggregates. Soya protein consists primarily of a mixture of 7S (mol. wt. 1.8–2.1 $\times$ 10^5) and 11S (mol. wt. 3.5 $\times$ 10^5) globulins. The 7S globulin is a quaternary trimeric protein in which the subunits are associated via hydrophobic and perhaps hydrogen bonding (Thanh and Shibasaki[7]). Kinsella[8] reviews the 11S protein structure as consisting of two opposed hexagonal-shaped rings, each containing six alternating acidic and basic subunits. The two hexagonal rings associate by electrostatic attraction and hydrogen bonds whereas the links between the individual acidic and basic subunits may involve disulfide bonds.

EXPERIMENTAL

Soya protein was precipitated from an aqueous protein solution (total water extract) by pH adjustment with sulfuric acid to the isoelectric point (pH 4.8). The resulting dispersion was subjected to capillary shear using an Instron 1141 food tester (Instron Ltd., High Wycombe, Bucks, England) modified to house a range of capillary tubes. Details of the capillary tubes and crosshead speeds used are given in Table I, along with the corresponding Reynolds numbers, average shear rates, and the ranges of average exposure times investigated. A diagram of the capillary system is shown in Figure 1. The tubes were mounted in the end of a reservoir barrel of constant bore (9.54 mm) and a piston was driven at a preselected crosshead speed, forcing the protein dispersion through the capillary tube.

Total Water Extract

A 10% w/w defatted soya flour [Soy Fluff 200 W, The Oppenheimer Casing Co. (UK) Ltd., London, England] dispersion in water (pH 9.0) was mixed for 30 min at 107 rad s^{-1} in a jacketed, baffled vessel with a six-bladed disk turbine impeller (vessel i.d. = 95 mm, impeller diameter = 36 mm). The resulting protein solution was clarified in a laboratory centrifuge (MSE, High Spin 21, 30 min at 8000g) and assayed by the Biuret method.[9] The protein was diluted to 30 kg m^{-3} with deionized water.

Precipitation

A sealed, jacketed, baffled vessel with a six-bladed disk turbine was used for precipitation (vessel i.d. = 95 mm, impeller diameter = 32 mm). The speed of the thyristor-controlled dc shunt motor was measured with an optical tachometer. Sulfuric acid (30% w/v) was added through a capillary tube close to the impeller at a rate of 0.02 mL s^{-1} using a pH titrator, Radiometer

TABLE I
Instron Capillary Tube Data[a]

Crosshead speed (mm s^{-1})	Capillary bore (μm)	Flow rate (m^3 s^{-1})	Reynolds number (N_{Re})	Average shear rate (s^{-1})	Range of average exposure times[b] investigated (s)
8.33	330	5.95×10^{-7}	1640	9×10^4	0.004–0.086
8.33	575	5.95×10^{-7}	940	1.7×10^4	0.011–0.065
6.67	330	4.76×10^{-7}	1320	7.2×10^4	0.005–0.027
3.33	330	2.38×10^{-7}	650	3.6×10^4	0.009–0.054
0.83	330	5.95×10^{-8}	160	9×10^3	0.036–0.22

[a]Capillary tubes: precision bore stainless steel. Tube length: 25–600 mm.
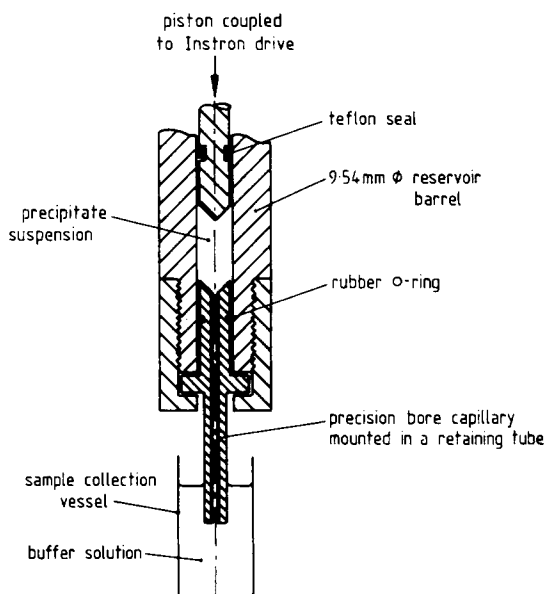[b]These times correspond to tubes of different lengths.

Fig. 1.   The capillary shear device.

type TTT lc. Between 84 and 87% of the measured protein was precipitated. All experiments were performed at $25 \pm 0.5°C$.

Aging Experiments

The influence of the conditions of aging on the resistance of the aggregated protein precipitate to shear disruption was evaluated by aging the dispersion in the reactor at constant stirrer speed and then subjecting a sample to a fixed level of capillary shear. The resulting change in the particle size distribution was measured by a Coulter counter. The aging was performed at the same stirrer speed as that used for the initial precipitation, which ranged from 15.7–67.9 rad s^{-1}. These stirrer speeds corresponded to initial mean particle sizes (before capillary shear disruption) of 53.5–8.8 μm, respectively. Aging times in the vessel of up to 2400 s were investigated. The condition of capillary shear used was an average rate of shear of 1.7×10^4 s^{-1} and an average exposure time of 0.065 s (575-μm bore tube) for all samples. A total protein concentration of 30 kg m^{-3} was used in all aging experiments.

Capillary Shear Disruption Experiments

Protein precipitate which had been aged for 1800 s was exposed to a range of high shear rates for short exposure times in the capillary tubes. The influence of shear conditions was determined by measuring the change in the

particle size distributions by Coulter counter. Initial experiments indicated that aggregate–tube wall interactions caused significant disruption when 10% (vol.) of the distribution was larger than approximately 8% of the tube bore. Subsequent experiments were performed with particles prepared below this limiting size. This was achieved by precipitation and aging at a stirrer speed of 52.2 rad s^{-1}.

The effect of particle concentration on shear disruption was assessed for total protein concentrations from 0.3-30 kg m^{-3}. The precipitate was prepared at 30 kg m^{-3} and diluted with sodium acetate buffer ($I = 0.07$, pH 4.8) to the required concentration. Samples were subjected to an average rate of shear of 9 × 10^4 s^{-1} for 0.043 s and the change in particle size distribution measured.

The extent of aggregate disruption for a range of shear rates and times of exposure was determined for 30 kg m^{-3} total protein dispersions. Mass-average rates of shear ranged from 9 × 10^3 to 9 × 10^4 s^{-1} with average exposure times between 0.004 and 0.22 s.

Capillary Shear—Theoretical Considerations

Dilute dispersions of soya protein precipitate (less than 3% w/v) exhibit Newtonian behavior at high shear rates. Assuming laminar isothermal flow of an incompressible fluid, the rate of shear at the wall is a maximum and can be defined by[10]

$$\dot{\gamma}_w = \frac{-du}{dr}\bigg|_w = \frac{4Q}{\pi R^3} \tag{3}$$

The rate of shear in the capillary tube varies linearly from a value of zero at the tube axis to the maximum value at the wall. However, the fluid velocity distribution used to derive eq. (3) is of a parabolic form; accounting for this velocity distribution, a mass-average shear rate is given by[4]

$$\bar{\gamma} = (8/15)\,\dot{\gamma}_w \tag{4}$$

This equation accounts for the majority of the particles traveling through the lower shear region near the tube center and was used for evaluating average rates of shear in this work. The possible occurrence of particle migration away from the wall[11] also supports the use of eq. (4).

The mean residence time in the tube is defined by

$$t = \pi R^2 L/Q \tag{5}$$

and was used to define the time of exposure to shear, t. The parabolic velocity distribution will result in a distribution of residence times depending on the radial position at which the particle enters the tube. The minimum residence time for any particle will be half the mean residence time.

Particle Size Analysis

Particle size distributions were measured on a model T_A Coulter counter. The samples were dispersed in sodium acetate buffer (pH 4.8, $I = 0.07$) to give a final dilution of 1 in 10,000 and were analyzed using 70-, 140-, and 280-μm-diam orifice tubes. The Coulter counter "half-count" calibration method[12] was used with DVB latex particles. Cumulative percent oversize distributions were recorded in duplicate for all samples and the data averaged. Total error for the determination of mean particle sizes was $\pm 5\%$.

The response of the Coulter counter to protein precipitates depends on both the aggregate shape and porosity, which will be determined by the conditions of preparation and handling. However, optical microscope studies of the suspension (Fig. 2) indicated that the Coulter counter mean diameters were similar to the physical dimensions observed. An investigation of the breakup occurring in the glassware by recycling the dispersion[13] showed this to be insignificant and it is assumed that any such breakup occurs in the orifice and results in a reading equivalent to the original due to coincidence effects.

RESULTS AND DISCUSSION

Aging in a Stirred Tank

Aging the precipitated protein by stirring for a fixed time at the precipitation speed results in an approach to a state of dynamic equilibrium at which
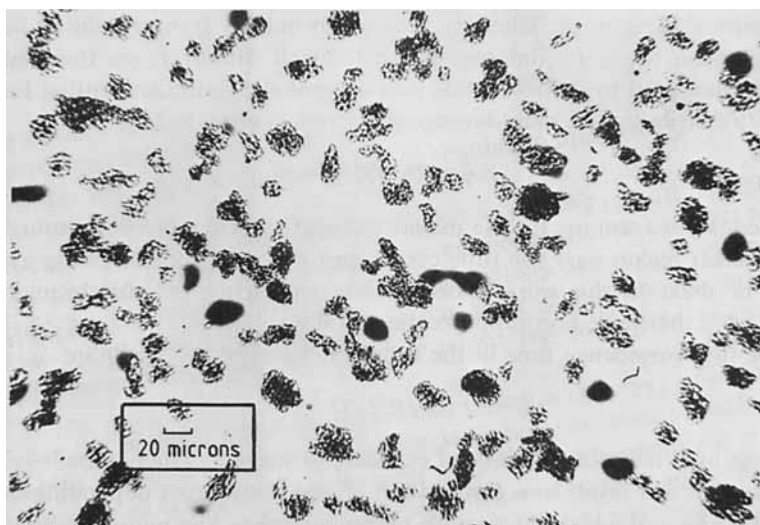

Fig. 2. The photomicrograph of isoelectric soya protein precipitate. Coulter counter size (μm): $d_m = 16.5$, $d_{90} = 9.9$, SD $= 10.0$.

the rate of formation of aggregates equals the rate of breakup. Figure 3 indicates for a typical aging experiment that a significant reduction in the number of small particles occurs during this period. This is accompanied by a relatively small increase in the number median value, which suggests a mechanism involving a "mopping-up" of the small aggregates by the larger aggregates. This is predicted by Smoluchowski's collision frequency theory, which states that the probability of collision between small and large particles is greater than the probability of collision between small particles, and so that large particles behave as nuclei around which small particles collect. The change in particle number frequency resulting from subsequent exposure to capillary shear is shown in Figure 4 for samples aged from 300 to 2400 s. Two mechanisms of breakup are indicated. Samples sheared after short aging times produced a wide range of fragment sizes, up to ca. 10 μm in diameter. As the time of aging increases, a distinct bimodal distribution occurs for the fragments produced by shearing. The peak between 4 and 8 μm may be associated with fragmentation and the increase in the number of particles less than about 3.5 μm with erosion mechanisms. However, the erosion breakup is insignificant in terms of the volumetric distribution change. The
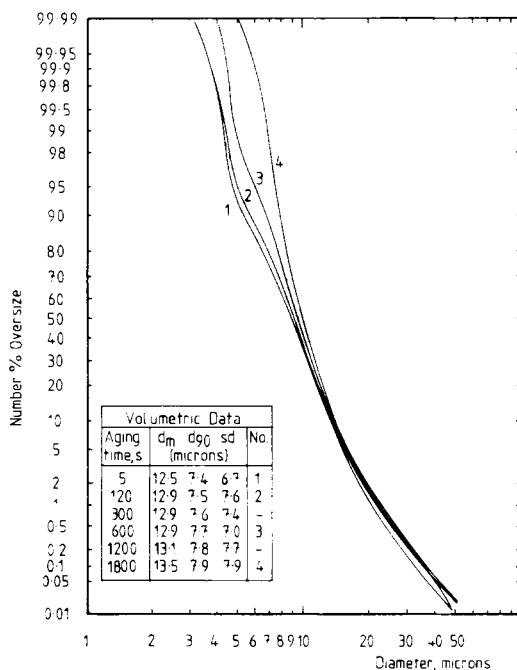

Fig. 3. The effect of aging in a stirred vessel on the particle size number distribution of isoelectric soya protein precipitate. Stirrer speed: 36.6 rad s^{-1}; aging times (s): (1) 5, (2) 120, (3) 600, (4) 1800.
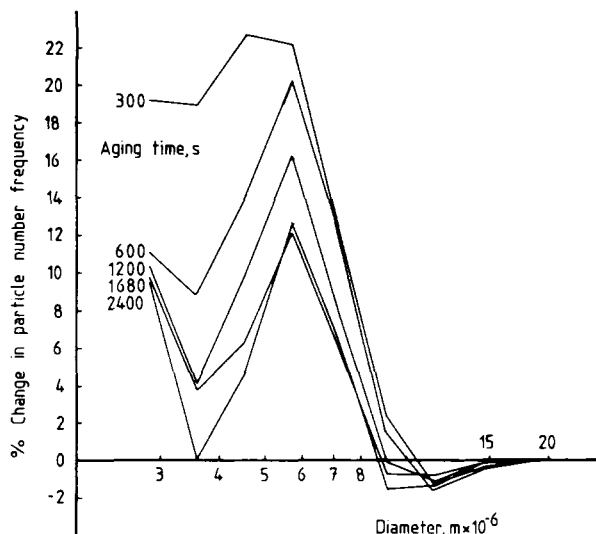
Fig. 4. The effect of aging on the change in particle number frequency resulting from capillary shear. Average rate of shear $= 1.7 \times 10^4$ s^{-1}; average time of exposure $= 0.065$ s; initial distributions (μm): $d_m^0 = 15.2 \pm 0.1$, SD$^0 = 9.5 \pm 0.2$. Tube diameter $= 575$ μm.

median size of the fragments produced (5.8 μm) is essentially unaffected by the time of aging and only the percent change in numbers decreases.

These results suggest that an improvement in aggregate strength occurs with increased aging time. This can be attributed to a denser aggregate formed by "infilling" of small "primary" particles, as shown by the reduction in the number of small particles in Figure 3, and to aggregate rearrangement. This rearrangement is probably caused by the processes of aggregate breakup followed by a more stable reaggregation, and by the deformation of the aggregate in the shear field in the stirred tank resulting in an increase in the interparticle contacts. The selective retention, on the aggregate, of particles with a higher number of interparticle bonds per unit cross-sectional area is indicated in Figure 4 by the reduction in the number of aggregates disrupted with increased aging and also by the increasing uniformity of the particle fragments that are broken off. Rearrangement by shear deformation will be assisted by the existence of a distribution of shear rates within the stirred tank. The pumping effect of the impeller causes an aggregate to pass from the relatively low-shear bulk conditions through the higher-shear impeller zone many times. The changing shear conditions will impose a dynamic stress on the aggregate and so may result in a shear-induced rearrangement. A similar effect was reported by Sherman[14] for globules in water-in-oil emulsions where under low-shear conditions an increase in shear rate packed the globules more tightly together until, at higher rates of shear, disruption occurred.

These proposed mechanisms of improvement in aggregate strength by increased aging in a stirred tank will depend on the effective pumping rate, the level of shear (stirrer speed), and the residence time in the tank. These variables can be incorporated in an aging parameter Gt for a given impeller and tank geometry. The rms velocity gradient G, from eq. (2) is given in the tank by[15]

$$G = (2\pi NT/V\mu)^{1/2} \qquad (6)$$

An empirical correlation was used to evaluate the torque[16]

$$T = 7.21 \times 10^2 N^2 D^5 \qquad (7)$$

The aging parameter Gt gives an indication of the amount of shear work performed on the aggregates in the tank and was used to correlate the ratio of final to initial mean aggregate diameters resulting from capillary shear exposure, for a wide range of particle sizes produced under different reactor shear conditions (Fig. 5). It should be noted that Gt does not account for the distribution of shear rates within the vessel. The data in Figure 5 indicate that an approximate value of $Gt = 10^5$ is necessary to ensure maximum aggregate strength. This suggests that large aggregates (produced under low reactor shear conditions) require longer aging times to achieve strengths equivalent to small aggregates produced under conditions of high reactor shear.
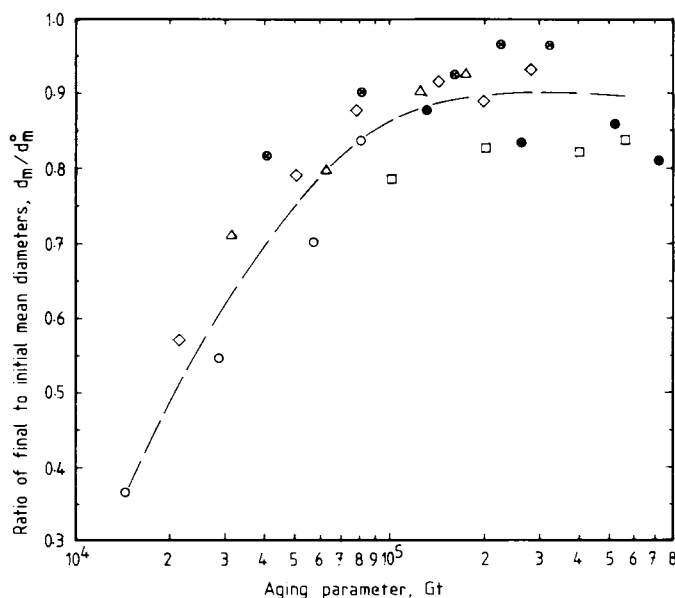

Fig. 5. The effect of aging on the change in mean diameter resulting from capillary shear. Tube diameter $= 575$ μm; average rate of shear $= 1.7 \times 10^4$ s^{-1}; average time of exposure $= 0.065$ s; initial mean diameters (μm): ( ) 53.5, ($\triangle$) 23.4, ($\diamond$) 19.5, ($\otimes$) 15.2, ($\square$) 10.2, ($\bullet$) 8.8.

This is supported by the long periods of aging often required to realize an improvement in the recoverability of protein precipitates aged under low-shear conditions.

Capillary Shear Disruption

The effect of concentration on the capillary shear breakup of aggregates was determined to establish the significance of particle–particle collisions as a breakup mechanism. Figure 6 shows that a small decrease in the ratio of final to initial mean diameters resulting from shear occurs with increasing concentration. The larger decrease in the ratio of final-to-initial d_{90} sizes indicates that more small particles are being broken off at increasing concentrations. This suggests that erosion due to particle–particle collisions is playing some part in the overall particle breakup and that an increase in concentration results in an increase in the frequency of such collisions. The lack of a significant trend for the ratio of standard deviations shows that the spread
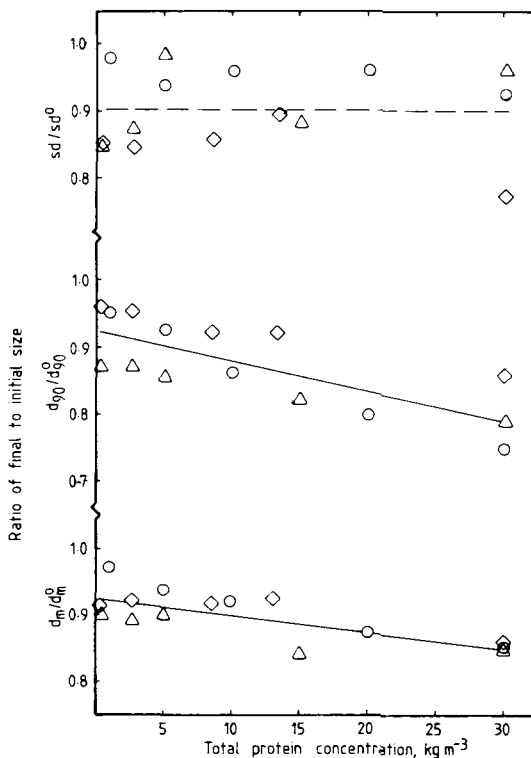

Fig. 6.   The effect of concentration on the change in particle size distribution resulting from capillary shear. Average rate of shear $= 9 \times 10^4$ s^{-1}; average time of exposure $= 0.043$ s; initial distribution data (μm): ($\bigcirc$) $d_m^0 = 11.4$, $d_{90}^0 = 8.0$, S.D.$^0 = 5.0$; ($\diamond$) $d_m^0 = 14.0$, $d_{90}^0 = 7.6$, S.D.$^0 = 8.4$; ($\triangle$) $d_m^0 = 14.8$, $d_{90}^0 = 9.3$ S.D.$^0 = 7.6$.

about the mean size does not change with increasing concentration. This is consistent with the removal of small particles from the surface of large aggregates, i.e., an erosion mechanism. The increase in breakup at higher concentrations may be equally attributed to the increased probability of particle-wall interactions. All subsequent shear breakup experiments were performed at 30 kg m^{-3}.

The change in the particle number distribution due to exposure to the same level of capillary shear for different lengths of time is shown in Figure 7. A bimodal distribution of fragment sizes was obtained for all times, indicating the existence of both erosion and fragmentation mechanisms, the fines being produced by the former and the large particles by the latter mechanism. For exposure times less than ca. 10^{-2} s, the number of particles produced by shear disruption was independent of time. The median sizes of the fragments produced are given in the table in Figure 7 for the range of exposure times investigated. Very short times (less than 10^{-2} s) resulted in approximately constant fragment size. For times longer than 10^{-2} s a reduction in fragment size resulted from an increase in exposure time.

Figure 8 shows the percent increase in the total number of particles larger than 3.6 μm in size, as a function of the time of exposure to rates of shear from 9×10^3 to 9×10^4 s^{-1}. For times less than 10^{-2} s a constant amount of breakup occurs which may be attributed primarily to the instantaneous rupture of loose bound aggregates, or to breakup due to extensional flow in the entrance zone of the capillary tube. However, entrance/exit and collection effects in the capillary system are not considered major factors since these data were obtained over a range of tube flow conditions (tube $N_{Re} = 160$–1640) in

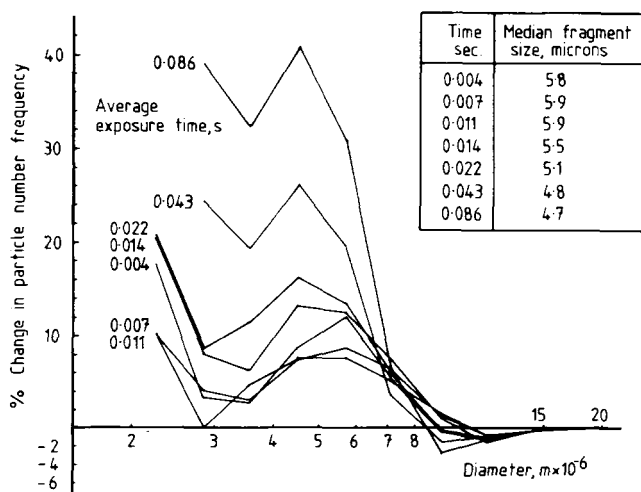| Time sec.	Median fragment size, microns
0·004	5·8
0·007	5·9
0·011	5·9
0·014	5·5
0·022	5·1
0·043	4·8
0·086	4·7

Fig. 7.   The effect of time of exposure to capillary shear on the particle number frequency distribution. Average rate of shear = 9×10^4 s^{-1}; tube diameter = 330 μm; initial distribution data (μm): $d_m^0 = 12.0$, $d_{90}^0 = 7.8$, S.D.$^0 = 6.2$.
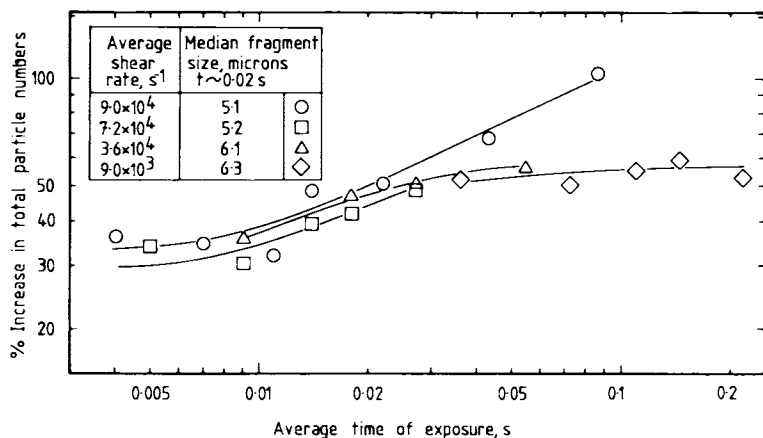
Fig. 8.   Increase in total particle numbers as a function of time. Initial mean diameter (μm) $d_m = 11.9 \pm 1.8$.

which the occurrence of end effects could be expected to result in varying amounts of breakup. For longer exposure times the rate of breakup is shown to be dependent on both the rate of shear and times of exposure. This suggests a breakup pattern in which the more readily separated fragments are removed initially with a gradual decrease in the rate of breakup until a dynamic equilibrium is attained at which the rate of breakup is equal to the aggregation rate.

Median sizes for the fragments produced follow a trend of reducing size with increasing rate of shear, as shown in the table in Figure 8, for a time of exposure of ca. 0.02 s. On the basis of the fragment size data given in Figures 7 and 8 it can be assumed that an equilibrium size exists for a given rate of shear. This has been confirmed in experiments of long exposure times in pumps and in a high-shear couette device.[17]

The change in mean volumetric size resulting from the same range of rates of shear and times as in Figure 8 is shown in Figure 9. Two tube sizes were used to indicate that tube wall effects are not significant for the initial sample size used. The rate of change in mean size is shown to be a function of both the rate of shear and time of exposure.

A significant aspect of these data is the relative stability of the aged aggregates at short times of exposure to capillary shear and the volumetrically insignificant amount of fines that are produced for aged dispersions.

CONCLUSIONS

Aging a dispersion of isoelectric soya protein precipitate in a stirred tank resulted in a reduction in the number of fine particles and an increase in the strength of the aggregates. The aggregate strength was found to be a function
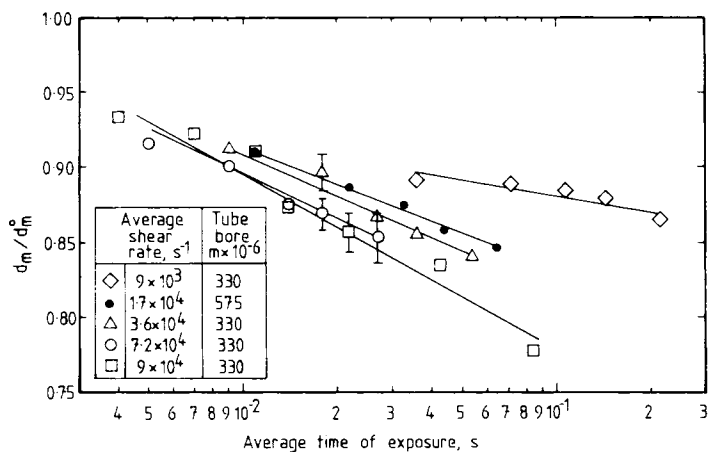
Fig. 9. The change in mean diameter resulting from exposure to capillary shear. Initial mean diameters (μm) $d_m^0 = 11.9 \pm 1.8$.

of the extent and time of agitation, and a value of an aging parameter, $Gt \sim 10^5$, was found to result in a maximum resistance to shear disruption.

The aging results indicate that consideration should be given to the reactor design and conditions of aging in the preparation of protein precipitates to maximize the aggregate strength characteristics. The data presented for iso-electric soya protein precipitate show that controlled aging can diminish the reduction in mean size resulting from exposure to a high shear rate, from 65% to about 10% of the initial mean size. Since centrifugal recovery is related to the square of the particle size this improvement in aggregate strength could result in a significant improvement in separation efficiency.

Among proposed mechanisms for the increase in aggregate strength with aging are the following: an infilling due to interaction of "primary" aggregates and large aggregates; shear-induced rearrangement resulting in closer packing; and the selective retention of particles with a high number of inter-particle contacts. These mechanisms suggest that aggregates formed under uniform low-shear conditions, such as in a continuous tubular reactor, would not exhibit the same structural stability as those formed in CSTR or batch tank configurations. However, the use of turbulence promotors[13] or acoustic conditioning systems[3] with tubular reactors may result in similar rearrangement effects.

The disruption of aggregates was found to depend on the rate of shear and the time of exposure. For the soya protein precipitate a 23% reduction in the mean size was observed for the most severe conditions of 9×10^4 s^{-1} and 0.086 s. This could result in a significant reduction in centrifugation efficiency.

The median size of the fragments produced by exposure to shear was found to be weakly dependent on the rate of shear, with higher shear rates producing small fragments. The mechanism of breakup due to exposure to cap-

illary shear can be attributed primarily to fragmentation, with erosion by particle-particle or particle-wall collisions being less significant on a volumetric basis.

These results emphasize the necessity of minimizing exposure to high shear in feed pumps and in centrifuge-entry zones to ensure maximum centrifugal recovery efficiencies.

Nomenclature

d	particle diameter (μm)
d_m	mean diameter (volume) (μm)
d_m^0	initial mean diameter (μm)
d_{90}	particle diameter for which 90% (volume) of the distribution is greater than (μm)
D	impeller diameter (m)
G	rms velocity gradient (s^{-1})
J_{ij}	collision frequency between particles of sizes d_i and d_j
L	tube length (m)
n_i, n_j	number of particles of sizes d_i and d_j
N	impeller speed (s^{-1})
Q	volumetric flow rate (m^3/s)
r	tube radial position (m)
R	tube radius (m)
SD	standard deviation
t	time of exposure to shear (s)
T	torque (N m)
u	velocity (m/s)
V	volume (m^3)

Greek

ϵ	rate of energy dissipation per unit mass (W/kg)
$\dot{\gamma}_w$	wall rate of shear (s^{-1})
$\bar{\dot{\gamma}}$	average rate of shear (s^{-1})
μ	dynamic viscosity (N s/m^2)
ν	kinematic viscosity (m^2/s)

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