

New approach to the formulation of hydrogel beads by emulsification/thermal gelation using a static mixer

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Abstract

A new approach in the formulation of hydrogel beads by emulsification/in situ thermal gelation using static mixer technology is described. κ -Carrageenan was selected as the model hydrogel. The emulsion generated by a Sulzer SMX6 static mixer consisted of warm κ -carrageenan sol (1.5% w/w in water or 10 mM CaCl₂) as the dispersed phase, and ambient temperature sunflower seed oil as the continuous phase. Dispersion followed by in situ gelation of κ -carrageenan droplets was possible within a short residence time (1–2 s) in the static mixer, under defined operational conditions, known as the feasibility region. This region was defined as the zone of operation conditions necessary to obtain discrete gel beads, within a defined range of κ -carrageenan solution injection temperature, volume fraction and total flowrate. The temperature boundaries of the feasibility region were determined by the κ -carrageenan gelation temperature and solution viscosity. The resulting beads had a Sauter mean diameter ranging from 350 to 200 μ m, which decreased with the increase of κ -carrageenan injection temperature, total flowrate and/or the number of static mixer elements. Theoretical values of maximal bead diameter and Sauter mean diameter were calculated on the base of critical Weber number, which was demonstrated through good agreement with the experimental values. It was demonstrated that an existing model for the prediction of gel bead diameter in a SMX static mixer is applicable for the new procedure described in this study.

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1. Introduction

The immobilization of biocatalysts, pharmaceuticals and other active reagents in hydrogel beads is important for many industrial and biotechnology applications. Among numerous gel-forming systems, natural polysaccharide hydrogels such as carrageenans, alginate and agarose provide interesting choices because they are non-toxic, and easily form gels under mild conditions, which is particularly important for the encapsulation of many labile biologicals.

Several encapsulation protocols for immobilization in polysaccharide hydrogels, are based on the emulsification of the aqueous gel-forming solution phase, into an hydrophobic phase. The emulsified solution droplets are then caused to gel in a separate step by dropping the temperature

(thermal gelation with carrageenan, gellan or agarose) (Audet and Lacroix, 1989) or by the addition or through triggering the release of interacting ions (ionotropic gelation with alginate) (Poncelet et al., 1995).

The droplet size of the dispersed solution in the emulsion stage, effectively determines the resulting gel bead diameter. Emulsified solution droplet diameter is defined by the ratio of inertia, viscosity and interfacial tension forces. Emulsification is generally carried out on a batch basis in a standard baffled turbine reactor (Audet and Lacroix, 1989; Poncelet et al., 1995). However, for larger scale, the production of microbeads may be also realized by passing both phases (oil and aqueous) through the static mixer (Poncelet et al., 1993).

A static mixer consists of a series of specially designed stationary elements placed transversely in a tube. These elements form crossed channels that promote division and longitudinal recombination of the liquid flowing through the static mixer. For a two-phase system, the two fluids are

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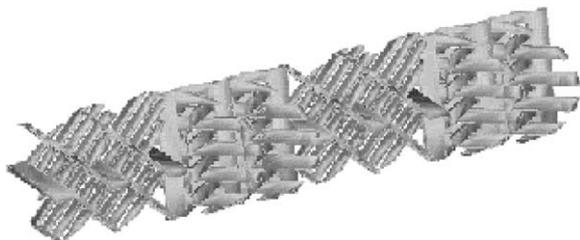


Fig. 1. The Sulzer SMX static mixer (4 mixing elements).

emulsified. A Sulzer SMX mixer was used in the present study, and consists of a framework of intersecting baffles or strips, mounted both perpendicular and at an angle to the pipe axis as illustrated in Fig. 1. The only power required for static mixers is the external pumping power that propels the fluids through the mixer, in contrast to the motor of the turbine mixer which drives the mixing impellers. The benefits of static mixers include low maintenance costs, potential for continuous processing, compact installation, very low residence time (near 1 s) and low shear forces, relative to the turbine mixer (Strieff, 1977; Pahl and Muschelknautz, 1982). In addition, large shear gradients are observed in turbine mixers, which give rise to excessive size dispersion in the resulting emulsion. More uniform shear encountered in the static mixer could be expected to reduce the droplet size distribution. Secondly, reduced shear, minimizes shear damage to fragile encapsulants such as cells and large molecular weight biologicals. There are a large variety of static mixer designs, making it possible to make a selection, appropriate to a particular application. The use of static mixers for microencapsulation and the influence of operational parameters and mixing element design on the resulting droplet size distribution has been described in several previous studies (Poncelet et al., 1993; Maa and Hsu, 1996,1997; Legrand et al., 2001).

For the production of hydrogel beads, one of the most widely used materials is κ -carrageenan. This natural polymer is a linear, sulfated polysaccharide extracted from marine red algae. The primary structure is made up of alternating $\alpha(1,3)$ -D-galactose-4-sulphate and $\beta(1,4)$ -3,6-anhydro-D-galactose residues. κ -Carrageenan will form gels, when it undergoes a coil (disordered sol state) to helix (ordered state) transition, triggered by a reduction in temperature and/or through ionic interactions. The junction zones formed between the helices of κ -carrageenan chains lead to the formation of a three-dimensional network or gel (Hermansson et al., 1991; De Ruiter and Rudolph, 1997; Michel et al., 1997).

The production of κ -carrageenan hydrogel beads by emulsification/thermal gelation with the use of the static mixer was first proposed by Neufeld and co-workers (Neufeld et al., 1999) (initial patent application in 1994). The procedure involves heating both the dispersed κ -carrageenan solution and continuous oil phases before mixing, as illustrated

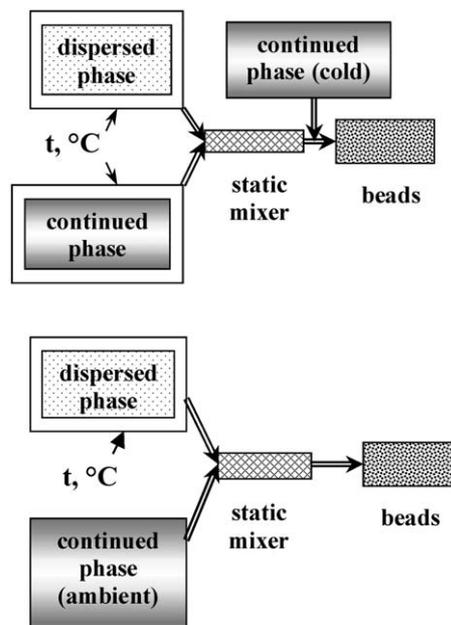


Fig. 2. Standard (upper) and new (lower) approach to hydrogel bead production by emulsification/thermal gelation.

in Fig. 2. Cold oil was injected in the outlet of the static mixer to initiate gelation. In the present study, an improved alternative to this procedure is proposed. The principal difference is that a non-heated, ambient temperature continuous oil phase is used to disperse warm κ -carrageenan solution as illustrated in Fig. 2. In this case, emulsion droplet formation and gelation takes place almost simultaneously. The cooling of the emulsion at the outlet of the static mixer is not necessary in this case. The installation is simpler, and only the κ -carrageenan solution need to be heated. The other advantage of this procedure is the possible application in the immobilization to temperature-sensitive materials.

The objectives of the present study were to demonstrate that hydrogel beads produced by emulsification/thermal gelation via a static mixer can be realized with the use of ambient temperature continuous phase and to establish the operational parameters (temperature, flow rate and the κ -carrageenan volume fraction) needed for bead formation. The effect of the operational parameters on the microbead size was examined, together with a known correlation for predicting bead diameters in static mixers.

2. Experimental

2.1. Materials

κ -Carrageenan in the form of potassium salt was kindly provided by SKW Biosystems (Baupre, France) and was used without additional purification. Sunflower oil (Alba, Lesieur, France) was a commercial grade product. Calcium chloride (Sigma-Adrich Chemie GmbH, Steinheim,

Germany) and potassium chloride (Pancreas Quimica CA, Barcelona, Spain) were of analytical grade.

2.2. Preparation of κ -carrageenan solution

κ -Carrageenan powder was suspended in the distilled water or 10 mM CaCl_2 solution (1.5% w/w) at room temperature, heated to 70°C, stirred and held for 20 min to dissolve the polymer. The κ -carrageenan solution was then cooled to the desired temperature for use in subsequent measurements or in microbead production.

2.3. Viscosity measurements

Viscosity measurements were performed with the controlled-stress rheometer AR 1000-N (TA Instruments, UK) using a cone-plate geometry. Hot (near 60°C) κ -carrageenan solution was poured directly on the rheometer plate, which was pre-equilibrated at the same temperature. The sample was immediately covered by a thin layer of paraffin oil to avoid solvent evaporation during the measurements. The samples were then cooled to the required temperature (from 28°C to 60°C). The viscosity for shear rates ranging from 1 to 600 s^{-1} was determined.

2.4. Differential scanning microcalorimetry (DSC) measurements

DSC measurements were performed with a Micro-DSCIII calorimeter from SETARAM (France) equipped with 1 cm^3 sample and reference cells. A known weight (about 600 mg) of κ -carrageenan solution was transferred to the DSC sample cell and an equivalent weight of pure water added to the reference cell. The cells were cooled to 10°C, equilibrated for 10 min, then heated to 60°C. After heating and equilibrating at 60°C for 10 min, the samples were cooled to 10°C. The rate of heating and cooling was varied from 0.5°C/min to 3°C/min. The gelation temperature of κ -carrageenan solution was determined from the peak of endothermic cooling curve.

2.5. Interfacial tension measurement

The interfacial tension between κ -carrageenan solution and sunflower oil was determined in the temperature range from 30°C to 50°C with a Processor Tensiometer K-12 (KRUSS GmbH, Hamburg, Germany) equipped with a Wilhemy platinum plate.

2.6. Production of κ -carrageenan microbeads with a static mixer

The experimental setup is shown schematically in Fig. 3. κ -Carrageenan solution was held at controlled temperature with continuous agitation (magnetic stirrer with tempera-

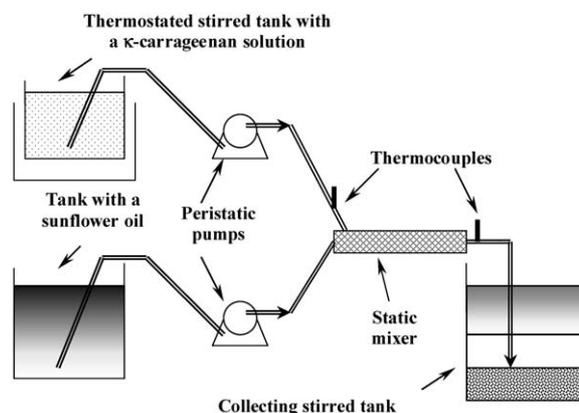


Fig. 3. Scheme of the experimental installation used for κ -carrageenan microbead production.

ture control AM 3003 Control, Bioblock Scientific, France) and the oil was held at room temperature (22°C). The static mixer was fed by two peristaltic pumps (Masterflex, Cole-Parmer Instrument Company, USA).

Mixing elements of type SMX DN6 with the hydraulic diameter 1.59 mm manufactured by Sulzer Chemtech AG (Switzerland) designed for laminar flow conditions were used (Fig. 1). A series of 5 or 8 mixing elements (diameter 6 mm, length of each element 6 mm) were inserted into a plexiglass tubular housing with diameter and length of 6 and 50 mm, respectively.

At the outlet of the static mixer, the liquid–liquid dispersion was collected in a stirred tank, containing cold (10°C) 0.2 M KCl solution.

The total volume flowrate Q_t was varied from 0.2 to 0.4 dm^3/min , corresponding to linear velocities (U_0) from 0.118 to 0.236 m/s. The κ -carrageenan volume fraction (α) in oil was 0.05 or 0.1. The temperature of κ -carrageenan solution at the point of injection (T_{inj}) and of final liquid–liquid dispersion obtained (T_{exit}) was measured by two thermocouples (PreciCal, AOiP, France) positioned near the injection and exit points, as illustrated in Fig. 3. The temperature of κ -carrageenan solution at the point of injection was varied from 30°C to 55°C.

During phase separation, the resulting gel beads were stored in 0.2 M KCl at room temperature for 14–16 h in order to harden the gel and to complete transfer of beads to the aqueous phase. The microbeads were then collected by decantation, and residual traces of oil removed with absorbant paper. Microbeads were recovered on a nylon sieve (10 μm) and used for size analysis.

2.7. Microbead size analysis

Particle size analysis was performed using a laser diffraction particle sizer (Malvern MasterSizer, Malvern Instruments S.A., France). The Sauter mean diameter (d_{32}) and the maximal bead diameter (d_{max}) were determined. Reported

values represent the average of at least three replicates for each sample.

3. Results and discussion

3.1. Feasibility region

The temperature of κ -carrageenan gelation (T_{gel}) and, consequently, the temperature required to form gel beads depends on the salt concentration. The T_{gel} value rises with either κ -carrageenan concentration or external salt content (Hermansson et al., 1991; De Ruiter and Rudolph, 1997; Michel et al., 1997). Therefore all experiments were carried out with κ -carrageenan in distilled water (identified below as carr/water) or in 10 mM CaCl₂ (identified below as carr/salt).

The feasibility region was identified according to combination of operating parameters which gives rise to fully discrete and spherical gel beads. Outside of the feasibility region, non-spherical beads, bead agglomerates or beads containing oil inclusions were observed. In addition, conditions leading to a premature gelation of the κ -carrageenan giving rise to plugging of the static mixer assembly was judged to be outside of the feasibility region.

The values of κ -carrageenan solution injection temperature (T_{inj}) and the resulting emulsion temperature (T_{exit}) were determined to be the main parameters giving rise to gel beads of high quality. The T_{inj} and T_{exit} values were strictly related by thermodynamic. To delineate the feasibility region, it was appropriate to examine the relationship between T_{exit} and T_{inj} , plotted for different values of total volume flowrate Q_t and κ -carrageenan volume fraction as seen in Fig. 4. Three operating regions may be identified in Fig. 4. The first and most important region was that giving rise to discrete and spherical gel beads, which was identified as the feasibility region. The second region was that in which the mixer becomes blocked by a premature gelation of the κ -carrageenan. The third region describes an operating zone where bead aggregates formed due to partial or complete droplet coalescence or the oil inclusions in the beads were observed.

The boundaries of these operating regions could be explained as follows. The lower temperature boundary of the inlet κ -carrageenan solution (for T_{inj}) was determined by the viscosity which raised when the temperature approached the gelation point of the polymer as seen in Fig. 5. When T_{inj} was lower than 30°C for carr/water and lower than 37°C for carr/salt, the solution viscosity increased, making the solution more difficult to pump, but more importantly, causing a clogging of the static mixer due to premature polysaccharide gelation at the contact with the oil of ambient temperature.

Once the dispersion is formed, it is then important that the κ -carrageenan gels rapidly, otherwise the emulsion will be unstable once it exits the mixer assembly. Therefore, the T_{exit} value is also of importance for successful microbead forma-

tion. To avoid coalescence, T_{exit} must be close to 29°C and 32°C, which are the T_{gel} values for carr/water and carr/salt, respectively. However, as can be seen from Fig. 4, the feasibility region had the following temperature boundaries for T_{exit} : about 24°C for carr/water and 27°C for carr/salt.

The dispersion process in the static mixer, accompanied by cooling the κ -carrageenan solution occurs very rapidly, within the range of 1–2 s (according to the residence time in static mixer). As can be seen from Fig. 6, an increase in the cooling rate actually led to reduction in T_{gel} . This might be caused by the development of an irregular conformation of the κ -carrageenan helix, causing the gel to form at a lower temperature. A limitation in the cooling rate (no more than 3°C/min) for the Micro-DSCIII calorimeter used for T_{gel} determination did not allow to obtain the T_{gel} for the rapid cooling (about 10°C/min) taken place during dispersion process. However, an extrapolation of data in Fig. 6 shows that T_{gel} for both solutions at higher cooling rates could be very close to the values shown in Fig. 4 as critical for T_{exit} .

Most of the data points for κ -carrageenan volume fractions of 0.1 were outside of the feasibility region. This was due to the coalescence of dispersed κ -carrageenan droplets, as T_{exit} was greater than T_{gel} . Also, with the high volumetric flowrates (0.4 dm³/min) the increase of the inertial forces acting on the dispersed droplets, led to the entrainment and capture of continuous phase oil droplets into the κ -carrageenan beads. Increasing the number of mixing elements from five to eight did not have an appreciable effect on the feasibility region boundaries (Fig. 4).

In summary, the feasibility region was primarily determined by the properties of the κ -carrageenan. The minimum T_{inj} value was defined by the solution viscosity, and the maximum T_{inj} value was defined by the gelation temperature ($T_{\text{exit}} \leq T_{\text{gel}}$). It should be noted that the general approach established for κ -carrageenan bead formation (the dependence of the feasibility region from the solution properties) can be applied to the preparation of other types of hydrogel beads by emulsification/thermal gelation using static mixer technology.

3.2. Dependence of bead size from operating parameters

The size distributions for the beads obtained were sharp and their form did not depend from T_{inj} , Q_t and number of mixing elements.

The bead size (d_{32}) as a function of T_{inj} and Q_t was represented in Fig. 7. An increase in the injection temperature led to a reduction of the bead diameter. The dispersion process was promoted by a decreasing in interfacial tension with an increase in temperature (from 0.027 to 0.021 N/m², with temperature increase from 30°C to 50°C) as well as by the decrease in κ -carrageenan solution viscosity.

The reduction in microbead diameter with increase of Q_t value (see Fig. 7) may be explained by enhancing of shear

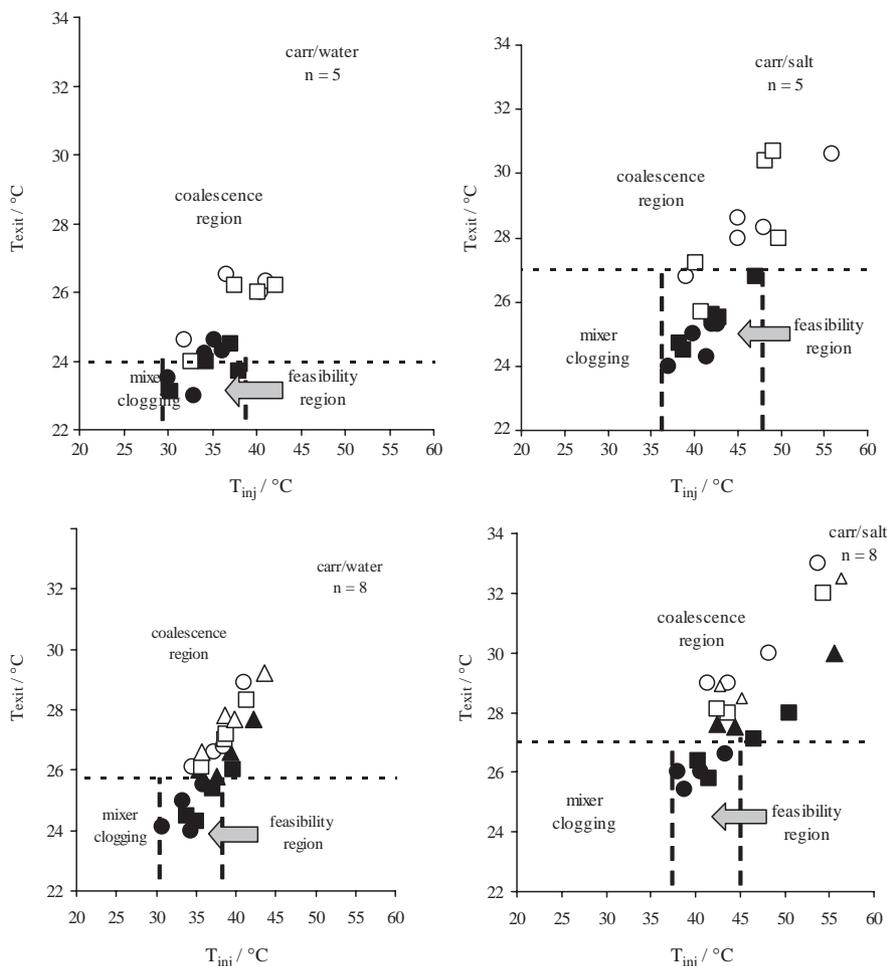


Fig. 4. Relationship between the κ -carrageenan solution injection temperature (T_{inj}) and κ -carrageenan/oil dispersion temperature after passage through the static mixer (T_{exit}) for $\alpha = 0.05$ (closed symbols) and $\alpha = 0.1$ (open symbols) for $Q_t = 0.2$ (circles), 0.3 (squares) and 0.4 (triangles) dm^3/min .

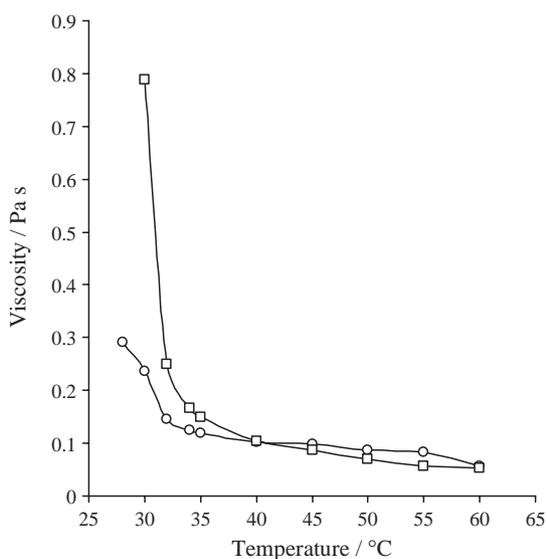


Fig. 5. Dependence of κ -carrageenan water (circles) and 10 mM CaCl_2 (squares) solutions viscosity from temperature (at shear rate 100 s^{-1}).

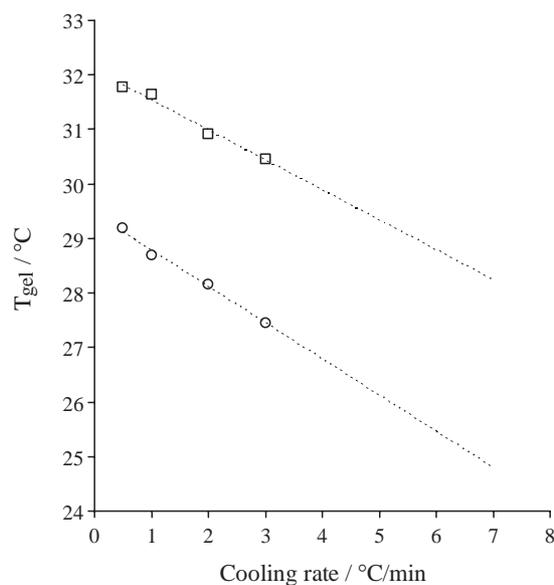


Fig. 6. Changes in the gelation temperature at different cooling rates for κ -carrageenan water (circles) and 10 mM CaCl_2 (squares) preparations.

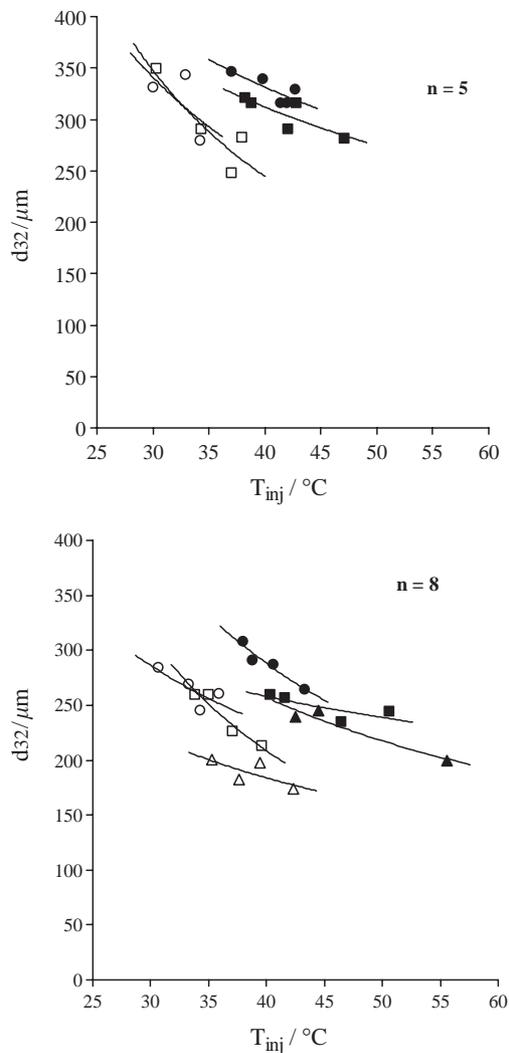


Fig. 7. Evolution of the Sauter mean bead diameter d_{32} with the T_{inj} : carr/water (open symbols), carr/salt (closed symbols), $Q_t = 0.2$ (circles), 0.3 (squares), 0.4 (triangles) dm^3/min ($\alpha = 0.05$), for $n = 5$ (upper) and 8 (lower) elements in the static mixer.

and elongation forces acting on the drop. The energy dissipation of processed fluid, and as a consequence, the energy expended in the process of phase dispersion, enhanced proportionately with the flowrate, giving rise to droplet disintegration (Al Taweel and Walker, 1983). However, the overall influence of the linear fluid velocity on the microbead mean diameter was relatively low for the levels of Q_t tested.

The increase of the number of mixing elements from five to eight led to a reduction in bead size as seen in Fig. 7. It can be seen that for beads obtained under similar conditions of Q_t and T_{inj} , a decrease of d_{32} in the range of 40–50 μm was observed. This effect of additional elements reducing the bead size was a consequence of dispersion kinetics where a longer residence time in the mixer gave rise to smaller droplet size.

It should be noted that large increases in temperature and/or flowrate were not the best methods to reduce bead

size for the procedure described. As it was indicated above, significant changes in these parameters led to coalescence and/or appearance of oil inclusions in the beads. Using the procedure of the κ -carrageenan with the ambient continuous phase, it is preferable to reach out the beads size diminishing by the increasing in the mixing elements number.

3.3. Correlation with the dispersion models

The dispersion process in different static mixers may occur either the laminar or the turbulent flow regime. In the present study, Reynold's number (Re) values, which defined the hydrodynamic regime, were varied from 2.9 to 5.8, which were consistent with the laminar flow regime for the SMX static mixer (Pahl and Muschelknautz, 1982). The Re value is representative of the ratio between the inertial and viscous forces acting in the flow:

$$Re = \frac{\rho_c U_0 d_h}{\mu_c} \quad (1)$$

The dispersed phase droplets are extended by shear and/or elongation forces in the fluid stream in the static mixer, while interfacial forces act against the droplet division. The ratio of viscous shear forces to interfacial tension forces is given by the Weber number for the laminar flow regime:

$$We = \frac{\mu_c G d_h}{2\sigma} \quad (2)$$

If the ratio of forces exceeds the critical value, We^{crit} , disintegration of the droplet takes place. For the laminar flow regime, and under condition that $\mu_c \approx \mu_d$, the We^{crit} may be described by the following equation (Grace, 1982):

$$We^{crit} = \frac{\mu_c G d_{max}^{theor}}{2\sigma} \simeq 1. \quad (3)$$

Rheologically, the viscosity of the κ -carrageenan solution diminishes with increasing shear rate. In estimating the shear gradient in the static mixer like $G = 8U_0/d_h$, the κ -carrageenan viscosity was deduced from the rheogram and found to be very close to that of the oil. Thus, for each group of experimental parameters (such as μ_c , G and σ) the theoretical maximum droplet diameter d_{max}^{theor} obtained by shearing, might be calculated from Eq. (3).

Fig. 8 is a plot of the experimentally determined maximum bead diameter (d_{max}), as it varied with d_{32} for various bead size distributions. The slope was found to be equal 4.8 ($R = 0.79$). Using the correlation from Fig. 8 and the d_{max}^{theor} values calculated from Eq. (3), the theoretical d_{32}^{theor} were found and compared with the experimental d_{32} and d_{max} values (Fig. 9). It can be seen from the Fig. 9 that the d_{32}^{theor}/d_h values were very close to the experimentally determined d_{32}/d_h values. Since the d_{max}^{theor}/d_h and d_{32}^{theor}/d_h magnitudes were calculated assuming a principal role of shear forces in the dispersion process, close correlation between experimental and theoretical values would suggest that the dispersion of κ -carrageenan in oil arising in the SMX static mixer occurred principally by the action of shear forces.

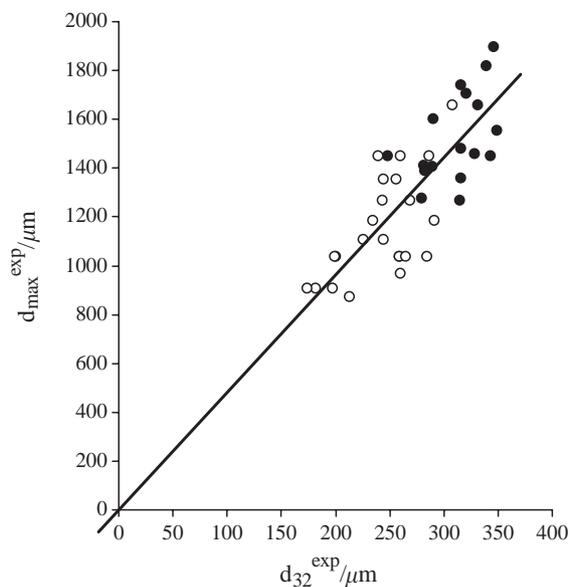


Fig. 8. Correlation between maximum bead diameter (d_{\max}) and mean diameter (d_{32}) values obtained from the bead size distribution ($\alpha = 0.05$) for $n = 5$ (closed circles) and 8 (open circles).

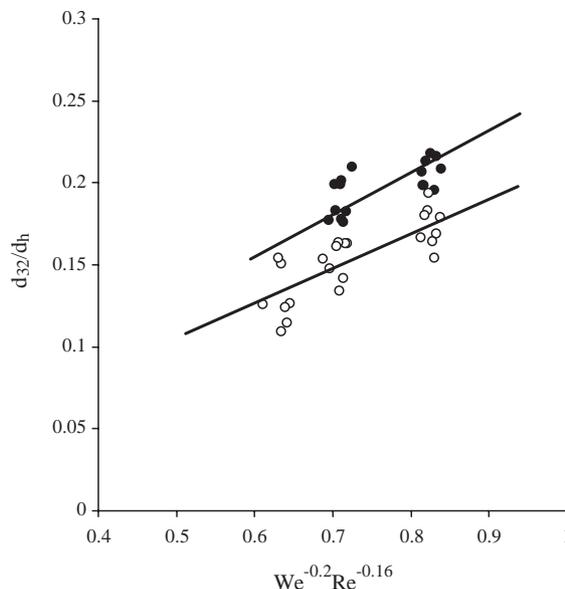


Fig. 10. Experimental data linear regression ($\alpha = 0.05$) as function of $We^{-0.2} Re^{-0.16}$ for $n = 5$ (closed circles) and 8 (open circles).

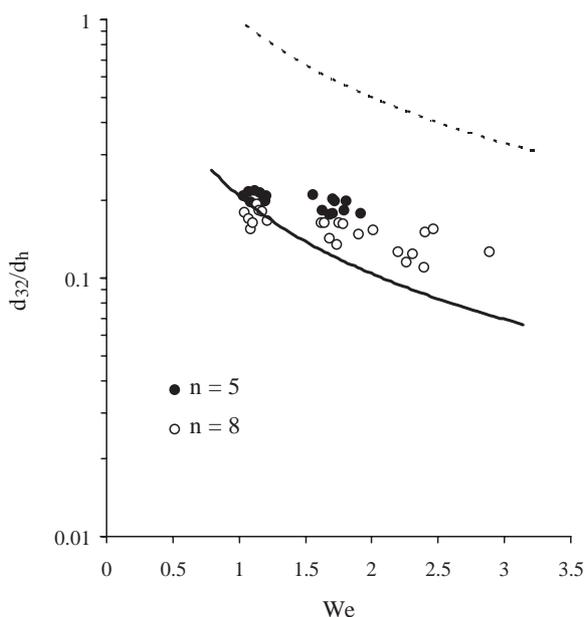


Fig. 9. Dependence of d_{32}/d_h (circles), $d_{\max}^{\text{theor}}/d_h$ (dotted line) and $d_{32}^{\text{theor}}/d_h$ (solid line) on the Weber number ($\alpha = 0.05$).

Many different models to predict the mean diameter of liquid–liquid dispersions in static mixer have been described in the literature. Most had been developed for the turbulent flow regime, such as those proposed for the Sulzer SMV static mixer (Strieff, 1977) or the Kenics static mixer (Haas, 1987).

For the SMX static mixer, the correlation proposed by Legrand et al. (2001) was

$$\frac{d_{32}}{d_h} = 0.29 We^{-0.2} Re^{-0.16}. \quad (4)$$

The dispersion process regime in the SMX static mixer was considered to be in a transient state between laminar and turbulent flow (Legrand et al., 2001). The numerical value of the proportionality constant was defined by the static mixer design (type), number of mixing elements, dispersed phase volume fraction and the ratio of viscosities of the respective phases.

The dimensionless experimentally determined bead diameter is plotted in Fig. 10 as a function of $We^{-0.2} Re^{-0.16}$, according to the Eq. (4). The numerical proportionality constants were 0.26 in the case of 5 mixing elements ($R = 0.58$), and 0.21 in the case of 8 mixing elements ($R = 0.79$). Thus, the mean diameter of the beads, obtained by emulsification in the static mixer with continuous phase at ambient temperature, can be predicted by the existing standard correlations.

4. Conclusions

A new, simpler approach to the procedure for the production of κ -carrageenan gel beads by emulsification/thermal gelation, using the static mixer, was developed. This process was realized with a continuous phase at ambient temperature, so that dispersion formation is followed instantly by κ -carrageenan gelification. The feasibility region was found in which fully intact, discrete and spherical beads could be formed, determined by the κ -carrageenan solution properties (viscosity and gelification temperature) and the flow rates of the respective fluids. It was shown that the bead size diminished with the increase in the κ -carrageenan injection temperature, the total flowrate and/or the number of mixing elements. It was found that the theoretically d_{\max}^{theor} and d_{32}^{theor} values, calculated with the assumption that the

κ -carrageenan/oil dispersion in the SMX static mixer occurs under the action of shear forces, are in good agreement with the experimental values. An existing model for the SMX static mixer can be used to predict the beads size for the procedures described.

Notation

d_{32}	mean beads diameter (diameter by Sauter), μm
d_{32}^{theor}	theoretical mean beads diameter, μm
d_h	static mixer hydraulic diameter, mm
d_{max}	maximal beads diameter, μm
$d_{\text{max}}^{\text{theor}}$	theoretical maximal beads diameter, μm
G	shear gradient in the static mixer, s^{-1}
n	mixing elements number
Q_t	total volume flowrate, dm^3/min
Re	Reynolds number, dimensionless
T_{exit}	temperature of κ -carrageenan/oil dispersion obtained, $^{\circ}\text{C}$
T_{gel}	temperature of κ -carrageenan gelification, $^{\circ}\text{C}$
T_{inj}	temperature of κ -carrageenan in the point of injection, $^{\circ}\text{C}$
U_0	linear fluid velocity, m/s
We	Weber number, dimensionless
We^{crit}	critical Weber number, dimensionless

Greek letters

α	κ -carrageenan volume fraction, dimensionless
μ_c	continuous phase viscosity, Pa s
ρ_c	continuous phase density, kg/m^3
σ	interfacial tension, N/m^2

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