ELSEVIER

International Journal of Pharmaceutics 170 (1998) 179–185

international journal of pharmaceutics

The use of image analysis as a means of monitoring bubble formation in alginate rafts

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Received 24 February 1998; received in revised form 31 March 1998; accepted 17 April 1998

Abstract

The use of image analysis as a means of measuring the dimensions of bubbles produced using a range of alginate raft formulations is described. Raft-forming mixes containing three sodium alginate samples (LFR 5/60, LF 120M and LF 10/40RB) were prepared, using sodium and potassium bicarbonate as gas-generating agents, and calcium and zinc carbonates as sources of divalent cations. The perimeters, areas and mean diameters of the bubbles were ascertained and an index described whereby the sphericity of the bubbles may be quantified. Systems produced using LFR 5/60 were found to produce the largest bubbles overall. Comparison with rheological studies indicated that this alginate sample produced the lowest viscosity mixes, suggesting a correlation between the viscosity of the medium in which the bubbles form and the subsequent bubble size.

Keywords: Alginates; Alginate rafts; Bubbles; Gel; Image analysis; Reflux esophagitis; Viscosity

1. Introduction

Alginate raft products are used in the treatment of reflux oesophagitis and act by forming a precipitated alginic acid gel in the stomach. This gel is then rendered buoyant by the inclusion of bicarbonates. In the acid conditions of the stomach, carbon dioxide bubbles are generated which become entrapped within the gel, causing it to float on the stomach contents. This floating gel then forms a barrier which protects the oesophagus from the acid contents of the stomach, either by physical containment of the acid or preferential reflux of the non-irritant gel (Washington, 1990).

In a previous investigation (Johnson et al., 1997a), we described studies whereby texture analysis has been used to characterise the strength of

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0378-5173/98/$19.00
PII S0378-5173(98)00143-4

rafts formed using alginates of known chemical composition (Johnson et al., 1997b). It was demonstrated that the guluronic–mannuronic acid content of the alginates, the polysaccharide molecular weight, the choice of bicarbonate salt and the choice of divalent cation may all have a profound effect on raft characteristics. However, the relationship between the bubble characteristics and raft properties has not been studied to date, at least partially because of the difficulties associated with measuring bubble size in a complex physical structure such as a raft. Methods which have been used in the past for other applications include that described by Ronteltap and Prins (1989), involving the use of an opto-electronic fibre which can be moved at a known rate through a foam. The tip of the fibre monitored the refractive index of the medium, with the probe connected to an analogue-digital converter to aid data acquisition. Other methods involve measurement of bubble dimensions taken from photographs (Bee et al., 1989). The mechanics of bubble formation has been extensively described in the chemical engineering and fluid mechanics literature (e.g. Plesset and Prosperetti, 1977; Blake and Gibson, 1987; Lubetkin, 1995; Feng and Leal, 1997). The essential steps in bubble formation include nucleation, growth, detachment, rise and bursting. Nucleation may be homogeneous or heterogeneous, depending on whether or not an additional interface is involved in the bubble formation process; in the case of heterogeneous nucleation, consideration must also be given to the detachment process. Growth of the bubbles will depend primarily on the extent of supersaturation of the gas in the liquid phase; in the early stages of bubble formation, when inertial and surface tension forces are dominant, the growth rate may be described by:

$$R(t) = At \quad (1)$$

where $R(t)$ is the radius of the bubble at time t and A is a constant. When the growth is determined by the rate at which gas may diffuse across the interface, the Scriven equation may be used (Scriven, 1959):

$$R(t) = 2\beta[Dt]^{1/2} \quad (2)$$

where β is a constant and D is the diffusion coefficient which will vary inversely with the viscosity of the medium across which the material is diffusing. The rate of rise of the bubbles is described by the Stokes law for rigid spheres and the Hadamard–Rybczynski solution for non-rigid systems; in both cases, the rate of rise will be inversely related to the viscosity of the surrounding medium. When the bubble reaches the surface of the liquid, bursting is governed by the rate of thinning of the upper bubble surface which, in the absence of surfactants, will be determined by inertial and viscous forces and will tend to be rapid. In the presence of molecules with surface activity, however, the thinning process may be slowed considerably and the bubbles may persist at the surface of the liquid for prolonged periods (Akers, 1976).

In this investigation, we report a novel method of assessing the characteristics of the alginate raft systems using an image analysis technique. This has been performed in conjunction with rheological studies on the raft-forming mixes themselves, as, for reasons described above, the viscosity of the medium may be expected to have an effect on bubble size. However, during the formation process, the bubbles will form both within and external to the raft; hence, the relationship between the 'viscosity' of such a multi-phase system and bubble formation is likely to be highly complex. Nevertheless, it was considered worthwhile to investigate any possible trends between the properties of the raft-forming mix and bubble dimensions, as any such information is potentially highly useful in choosing and designing raft formulations.

2. Materials and methods

2.1. Materials

Three sodium alginate samples (Pronova Biopolymers A/S) were chosen for investigation (LFR 5/60, LF 10/40RB and LF 120M); these represent a range of uronic acid compositions which were found to be 67.2, 50.9 and 42.4% guluronic acid by nuclear magnetic resonance

(NMR) imaging (Johnson et al., 1997b). In addition, LFR 5/60 has a lower molecular weight than the other two samples, as estimated using intrinsic viscosity measurements (12000, 124000 and 139000, respectively). Raft-forming mixtures for each of the alginates (2.5% w/w) were prepared containing either sodium or potassium bicarbonate (2.5% w/w) with or without the addition of calcium carbonate or zinc carbonate (0.75% w/w), as described in a previous study (Johnson et al., 1997a). All carbonates used were obtained from BDH.

2.2. Image analysis

A Seescan Image Analyser was used throughout the study. A clear plastic petri dish containing 5 ml of 0.1 M HCl (kept at 37°C) was placed on the light source and a single drop of the raft forming mixture was introduced onto the surface of the acid using a 5 ml plastic syringe. This quantity of the raft-forming mixture was used in order to produce a single layer of bubbles, as the image analyser does not take the depth of field into account. The bubbles were allowed to form for 30 s before the image was captured. The captured image was then thresholded to give the optimum resolution of the objects. The image could then be manipulated before the objects were counted. 'Kill small' was used to remove single pixels which did not have neighbouring pixels. Certain objects were discarded where, for example, two bubbles were so close that the image analyser could not resolve them into discreet objects, or where portions of the raft-forming mixture had not reacted with the acid. At least 50 data points were taken for each system, with fields of view being selected in accordance with the availability of areas in which single layers were apparent.

The 'fill holes' command was used to produce objects which appeared to be coloured circles rather then rings of colour. The area and perimeter of the bubbles were then automatically measured; this enables both the size of the bubbles and also the deviation of the shape of the bubbles from spherical to be estimated. It should be noted that the measurements made are two-dimensional, when in reality the raft and the bubbles trapped within it are three-dimensional. The shape of the bubbles is therefore seen by the instrument as neo-circular rather than neo-spherical.

2.3. Viscosity measurements

Flow rheology measurements were made on the alginate solutions using a Carrimed CSL500 controlled stress rheometer (version 5.0, TA Instruments). The rheometer was used with a cone and plate measuring geometry; the cone had a diameter of 4.0 cm and a cone angle of 2°. The temperature of the plates was maintained at 20°C using the Peltier temperature control system housed in the rheometer. A sample of approximately 0.6 ml was placed on the bottom plate using a syringe. Each solution was measured at least three times, with the plates being cleaned and fresh sample loaded each time. The stress on each sample

Table 1
The effect of alginate raft formulation on bubble diameter (calculated from perimeter values)

Additions to alginate solution	Mean diameter (mm)		
	LFR 5/60	LF 10/40RB	LF 120M
$NaHCO_3$	0.396 (0.243)	0.316 (0.057)	0.235 (0.058)
$KHCO_3$	0.255 (0.160)	0.263 (0.068)	0.267 (0.043)
$NaHCO_3+CaCO_3$	0.592 (0.176)	0.156 (0.029)	0.242 (0.047)
$KHCO_3+CaCO_3$	0.414 (0.140)	0.324 (0.066)	0.300 (0.062)
$NaHCO_3+ZnCO_3$	0.362 (0.192)	0.206 (0.069)	0.268 (0.047)
$KHCO_3+ZnCO_3$	0.553 (0.221)	0.294 (0.060)	0.251 (0.035)

Standard deviations given in parentheses.

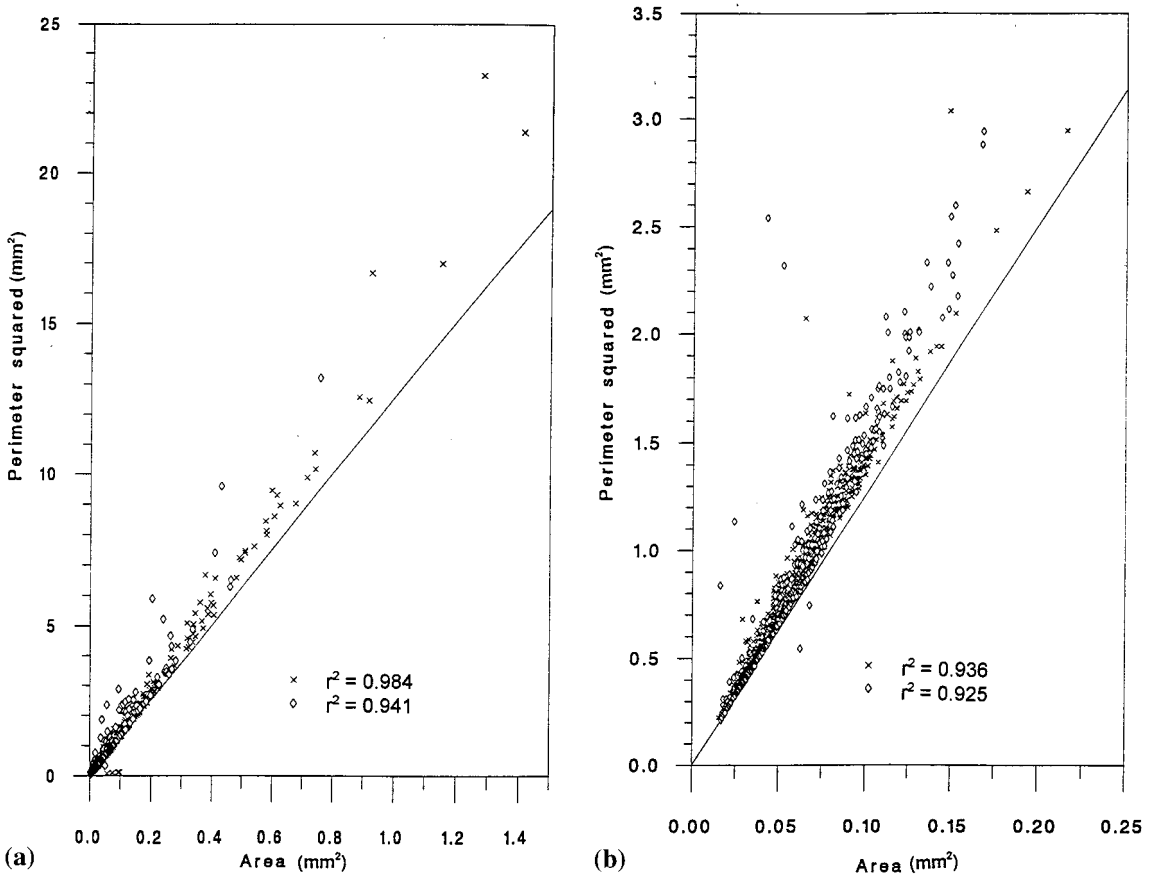

Fig. 1. Bubble perimeter squared against bubble area for: (a) LFR 5/60, (b) LF 10/40RB, and (c) LF 120M rafts with added bicarbonate. (x) Sodium bicarbonate, (◇) potassium bicarbonate. Correlation coefficients for the linear relationship between perimeter squared and bubble area given in each diagram.

increased from 0 to 15 N/m^2 over a period of 2 min. Solutions (2.5% w/v) of each alginate were measured in distilled water, with repeat measurements giving standard deviations within 5% of the mean.

3. Results

3.1. Image analysis

The diameter values (calculated from the perimeter) for the various raft-forming mixes are shown in Table 1. In general, the mean bubble diameters were greater for the LFR 5/60 than for the LF 10/40RB or the LF 120M, both of which yielded similar values. This indicates that either the low molecular weight or high guluronic acid content of the LFR 5/60 samples may be the overriding factor in determining the bubble dimensions, although the similarities in both molecular weight and bubble dimensions of LF 10/40RB and LF 120M would indicate that the molecular weight may be the predominant consideration. However, given the variability of the data, particularly for the LFR 5/60 systems, it is not possible to ascertain any more than broad trends in the figures.

Fig. 1 shows scatter plots of P^2 against A for the formulations of each of the alginates without added carbonates. Plotting P^2 against A for a perfect circle gives a straight line with a gradient

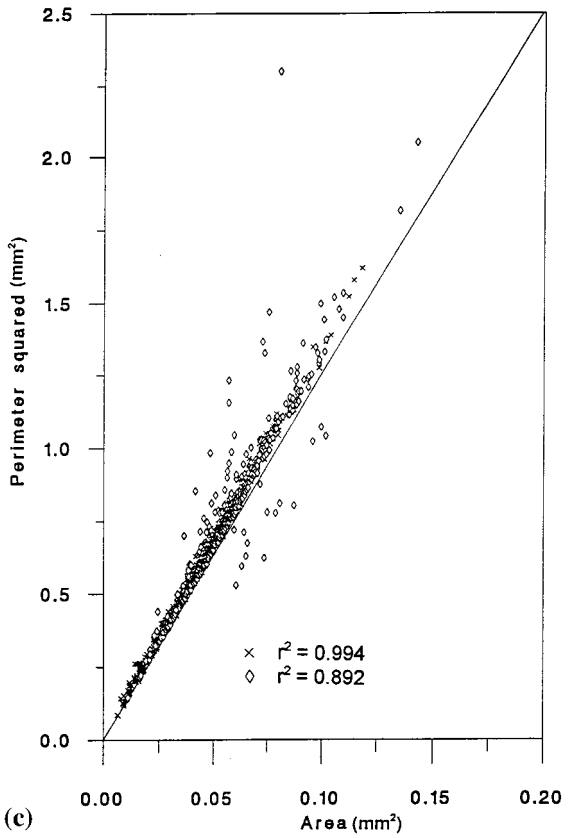

Fig. 1. (*Continued*)

of 4π ($= 12.566$); this line is also included on the plots in Fig. 1 for reference. Using the line of best fit through the experimental points, the gradient can be calculated, and hence the deviation from circularity can be quantified. Table 2 shows the gradients for each of the formulations and the gradient divided by 4π which gives an idea of deviation from a perfect circle. The positive deviation indicates that the perimeter values are greater than would be expected for perfectly spherical bubbles, indicating the extent of shape distortion. While the method provides a potentially useful means of determining bubble sphericity in raft systems, no definite pattern could be ascertained which related the circularity of the bubbles to the formulation.

3.2. Viscosity studies

In all cases, the rheological behaviour of the solutions was found to be Newtonian; hence, single values of viscosity are given, calculated from the slopes of the curves. The mean viscosity was 0.014 Pa·s for the LFR 5/60, 0.608 Pa·s for the LF 10/40RB, and 0.871 Pa·s for the LF 120M; hence, as expected, the lower molecular weight grade (LFR 5/60) yielded solutions with the lowest viscosity.

4. Discussion

The trend to emerge from the work is that the low-molecular-weight alginate, LFR 5/60, pro-

important single factor in determining bubble dimensions for a given bicarbonate concentration. Over and above the relevance to raft formation, it is hoped that the methodology described here may be of use for the characterisation of other gas-generating systems such as effervescent dosage forms.

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Table 2
The gradients of squared bubble perimeter (P^2) against bubble area A for a range of alginate raft formulations

Additions to alginate solution	Gradient			Gradient/4π		
	LFR 5/60	LF 10/40RB	LF 120M	LFR 5/60	LF 10/40RB	LF 120M
$NaHCO_3$	15.195	13.986	13.278	1.209	1.113	1.057
$KHCO_3$	16.130	15.952	13.459	1.284	1.269	1.071
$NaHCO_3+CaCO_3$	14.047	13.392	15.285	1.118	1.066	1.216
$KHCO_3+CaCO_3$	13.925	15.479	14.011	1.110	1.232	1.115
$NaHCO_3+ZnCO_3$	14.328	14.291	14.110	1.140	1.137	1.123
$KHCO_3+ZnCO_3$	14.701	14.970	14.511	1.170	1.191	1.155

duces larger bubbles that the other two higher-molecular-weight alginates under study. This is in itself of interest for understanding the mechanism of raft formation and also is in agreement with the available theoretical models for bubble generation. However, the absence of a clear correlation between factors such as divalent cation inclusion and choice of bicarbonate is also of interest, as previous studies (Johnson et al., 1997a) have shown these parameters to have a profound effect on raft strength. The implication is, therefore, that for a given bicarbonate concentration the bubble dimensions are not as important as the inter-alginate bonding in determining raft strength. Given the well-known relationship between the rheological properties of a suspension and the characteristics of the suspended particles (or in this case bubbles), the above finding is of some relevance in the rational development of raft-forming systems.

If the bubble size is indeed dictated by the viscosity of the surrounding medium, the observation that only the choice of alginate has a discernible influence on bubble dimensions rather than divalent cation inclusion has several implications. The formation of alginate rafts may be considered to be a kinetic process, with the precipitation of the alginic acid, the interaction with the divalent cations, the generation of the bubbles, the floating of the precipitated gel and the dispersion of the raft occurring to some extent simultaneously. The relative rates of these processes and the importance of their interaction is not yet understood. However, if bubble formation is directly related to the viscosity of the medium in which gas generation takes place, the lack of any substantial effect of the presence of divalent cations, which are well known to increase alginate solution viscosity (e.g. Haug and Smidsrød, 1968; McDowell, 1986), implies that the chelation process occurs after or physically separated from bubble formation. This is of interest in terms of understanding the raft formation process, but may also have implications for the development of other floating dosage forms based on similar principles.

5. Conclusions

The study has demonstrated the use of image analysis as a means of characterising the bubble dimensions in alginate rafts. By necessity, the methodology adopted represents a compromise in that the rafts under examination were unilayered and that it was assumed that the two-dimensional image of the bubbles could be related directly to the three-dimensional structure. However, extensive preliminary experimentation indicated that the formation of unilayered rafts was essential for the analysis; furthermore, the assumption that such rafts are representative of the more complex situation appears to be reasonable. Similarly, the near-sphericity of the bubbles renders the use of the second assumption, regarding the three-dimensional nature of the bubbles, acceptable. While the observed differences in bubble size were small, the data has demonstrated that the nature of the alginate, and in particular the molecular weight of that alginate, appears to be the most