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# Coarse dispersed systems as ultrasound contrast media

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The objective of this *in vitro* study was to develop and characterise coarse dispersions as ultrasound contrast media for incontinence diagnostics. Suspensions were formulated by adding 1% (w/w) of water insoluble particles (barium sulphate for suspension, compound tragacanth powder, light kaolin) and different concentrations (0.05–1.5% (w/w)) of the sugar ester Crodesta® F10 (sucrose distearate) to 5.5% glucose-solutions. Milk and Intralipid® were used as emulsions. The dispersions (100 ml) were filled into balloons and insonated in a waterbath. Video images were analysed for echogenicity of the dispersions in the balloons. Particle size measurements of the dispersions and true density measurements of the particles were performed. Suspensions of barium sulphate (mean particle size $x = 8.1\ \mu m$, relative density $\varrho_{rel} = 4.5$) initially produced high echogenicity. Echogenicity rapidly decreased to produce a non-echogenic dispersion after approximately 10 min, due to sedimentation of the solid particles. Light kaolin suspensions ($x = 2.7\ \mu m$, $\varrho_{rel} = 2.73$) were only weakly echogenic. Compound tragacanth suspensions produced strong echogenicity, but sedimentation of the relatively large particles ($x = 150.0\ \mu m$, $\varrho_{rel} = 1.6$) occurred within 10 min. The emulsions were non echogenic. Crodesta F10 ($x = 45.5\ \mu m$, $\varrho_{rel} = 1.04$) produced suspensions of high and sustained (>30 h) echogenicity in concentrations between 0.01 and 1.5% (w/w) because of their sufficiently high particle size and very low sedimentation velocity. Coarse suspensions (but not emulsions) may produce quantitatively and qualitatively good and lasting echogenic properties. The formulation of a technologically simple, low-cost ultrasound contrast medium for incontinence diagnostics seems feasible.

## 1. Introduction

A variety of different dispersed systems with microbubbles as dispersed phase in a continuous aqueous phase are currently used as ultrasound contrast media (Echovist, Levovist, Albunex, EchoGen, aerosomes (gasfilled liposomes)) [1–5]. These ultrasound contrast media are mostly used vascularly. The particle size of the dispersed particles therefore must be small. In order to achieve sufficient echogenicity from systems with small dispersed particles, the difference in the acoustic impedance (Z) between the dispersed phase and the continuous phase of the ultrasound contrast medium must be high ($Z = \varrho \cdot c$, with $\varrho$ = density of the phase and c = speed of sound in the phase). The acoustic impedance of air (gas) is $4.3 \times 10^2\ kg \cdot m^{-2} \cdot s^{-1}$, of water $1.5 \times 10^6\ kg \cdot m^{-2} \cdot s^{-1}$ and of an oil approx. $1.3 \times 10^6\ kg \cdot m^{-2} \cdot s^{-1}$. Clearly, the difference in impedance between both phases in a gas-in-water dispersion is much bigger than in an oil-in-water emulsion.

In dispersed systems however, besides the differences in the acoustic impedance of both phases, the size of the dispersed phase is of prime importance for the backscattering intensity of the dispersion.

The backscattering intensity of dispersions depends on a number of factors which can be divided into two main groups: those dependent on experimental conditions and those dependent on the physical properties of the dispersed and the continuous phase [6]:

Assuming a spherical shape of the scatterer (dispersed particle), the size of the scatterer can be expressed as its radius (r).

The power of the scattered beam ($P_s$), i.e. the power of the ultrasound beam that comes back from the scatterer to the transducer of the ultrasound scanner, is proportional to the intensity of the incident beam ($I_i$). The proportionality constant is called the 'scattering cross-section' ($\sigma$) of the scatterer:

$$P_s = \sigma I_i = I_s \cdot 4\pi R^2 \qquad (1)$$

with R being the distance of the transducer from the scatterer and $I_s$ being the intensity of the scattered beam. If R is much bigger than the radius of the receiving transducer ($r_t$), the power that is received by the receiving transducer from the scattering particle ($P_r$) is:

$$P_r = I_i \sigma r_t^2 / 4R^2 = I_s r_t^2 \pi \qquad (2)$$

For the intensity of the scattered beam follows:

$$I_s = I_i \cdot \sigma \cdot 1/(4\pi R^2) \qquad (3)$$

Eq. (3) demonstrates that the backscattered power received by the transducer is proportional to the scattering cross-section of the scatterer. It can also be seen from eq. (3) that the experimental conditions include the intensity of the incident beam ($I_i$) and the distance of the scatterer from the receiving transducer (R). In all ultrasonic measurements of contrast media these conditions have to be standardised.

Factors depending on the physical properties of the dispersion are 'hidden' in the scattering cross section ($\sigma$) which is defined as:

$$\sigma = \{4/9\pi r^2((2\pi/\lambda)\ r)^4\} \times \{[(\varkappa s - \varkappa)/\varkappa]^2 + 1/3[(3(\varrho s - \varrho))/(2\varrho s - \varrho)]^2\} \qquad (4)$$

Eq. (4) shows that $\sigma$ strongly depends on the radius of the particle r: $\sigma \propto r^6$ [7]. Additionally, the differences in the compressibilities of the scatterer ($\varkappa s$) and the embedding medium ($\varkappa$) come into the equation. Compressibility is related to the speed of sound (v) and subsequently to the impedance [$v = (1/\varkappa\varrho)^{1/2}$]. $\lambda$ is the wavelength of the ultrasound.

Finally, the difference in the densities of the scatterer ($\varrho s$) and the embedding medium ($\varrho$) influence the value of $\sigma$.

Compressibility and density represent the influence of the impedance of the two phases in a dispersion on the backscattering intensity of the dispersed particles.

For non-vascular use, such as the use of ultrasound contrast agents in the bladder, e.g., for imaging in urogynaecology [8] and the investigation of female urinary incontinence [9], the dispersed particles may be much larger than for vascular applications. Coarse solid or liquid dispersed systems (i.e. suspensions and emulsions) therefore might be useful as contrast agents, although the differences in

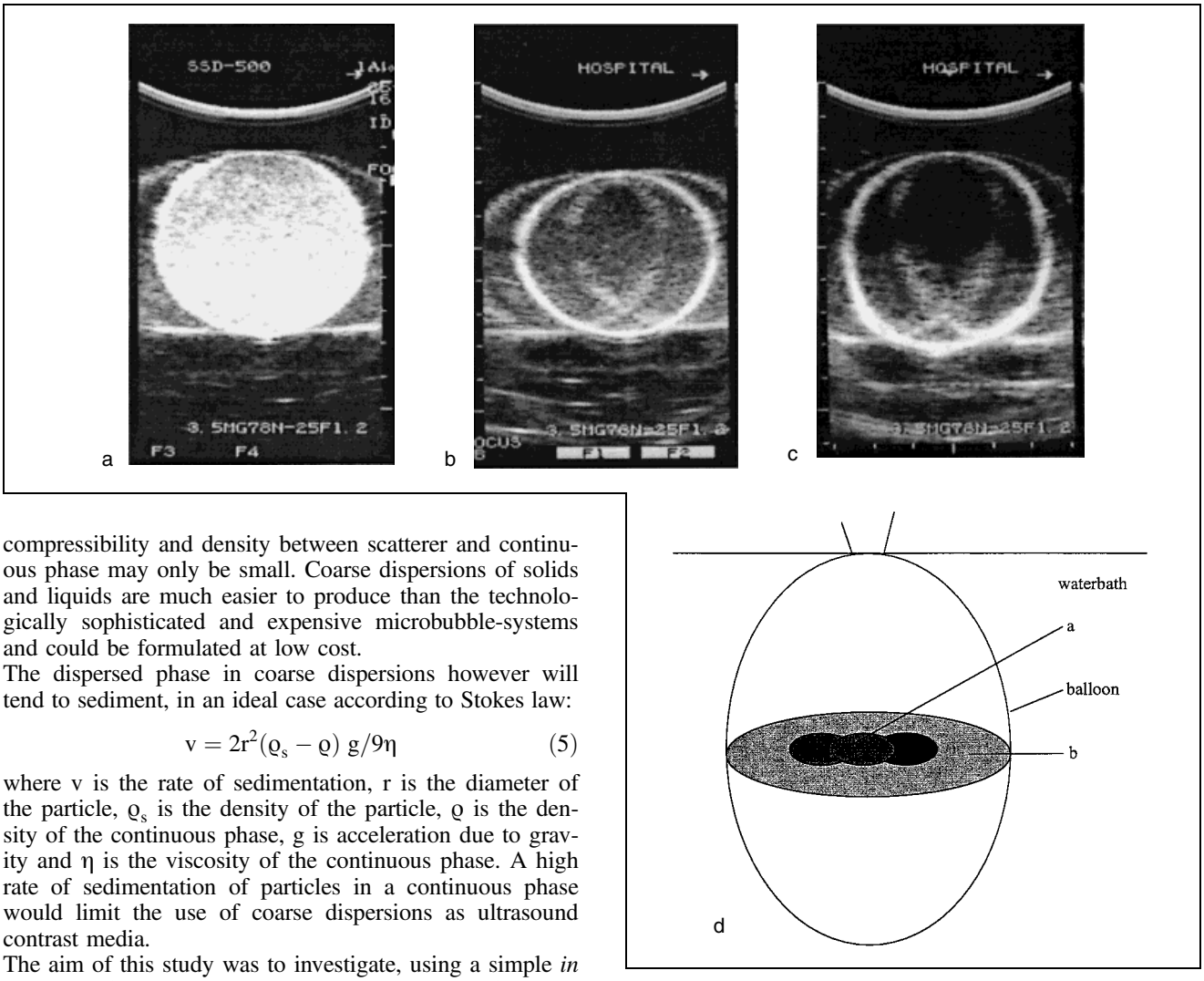


Fig. 1: Video printouts of different formulations: a) Crodesta F10 (1%) in 5.5% glucose solution, b) light kaolin (1%) in 5.5% glucose solution, c) 5.5% glucose solution, d) schematic representation of the filled balloon showing the scanning area inside the balloon (a) and the areas on the video printout used to assess echogenicity using the reflectance spectrophotometer (b)

compressibility and density between scatterer and continuous phase may only be small. Coarse dispersions of solids and liquids are much easier to produce than the technologically sophisticated and expensive microbubble-systems and could be formulated at low cost.

The dispersed phase in coarse dispersions however will tend to sediment, in an ideal case according to Stokes law:

$$v = 2r^2(\varrho_s - \varrho)\, g/9\eta \qquad (5)$$

where v is the rate of sedimentation, r is the diameter of the particle, $\varrho_s$ is the density of the particle, $\varrho$ is the density of the continuous phase, g is acceleration due to gravity and $\eta$ is the viscosity of the continuous phase. A high rate of sedimentation of particles in a continuous phase would limit the use of coarse dispersions as ultrasound contrast media.

The aim of this study was to investigate, using a simple *in vitro* experimental set-up, whether coarse suspensions and emulsions may be used as ultrasound contrast media for non-vascular, intravesical use, e.g. in incontinence diagnostics in the bladder, due to their ability to exhibit sufficient and persistent echogenicity.

## 2. Investigations, results and discussion

To predict the usefulness of a dispersion as ultrasound contrast medium for incontinence diagnostics, it is important to calculate the backscattering cross section ($\sigma$) and the sedimentation velocity of the dispersed particles in the continuous phase. To determine $\sigma$, the particle size of the dispersed particles and the density of the dispersed phase must be measured. The density of the continuous phase (5.5% glucose solution) is $1.02\ g \cdot cm^{-3}$ [10]. The compressibility of the dispersed particles ($\varkappa_s$) is difficult to measure, but can be estimated based on the typical speed of sound values for lipids and solid substances.

To determine the velocity of sedimentation, additionally the viscosity ($\eta$) of the continuous phase must be known: $\eta_{5.5\%\ \text{glucose solution}} = 1.164$ mPas [10]. The Table summarises the results of these measurements and calculations.

**Table: Particle size, density, sedimentation rate and backscattering intensity of dispersed particles**

| Dispersed phase | Volume Diameter (μm) | Density ($\varrho$) ($g/cm^3$) | Sedimentation time per meter (s/m) | $\sigma 1$ ($m^2$) | $\sigma 2$ ($m^2$) |
|---|---|---|---|---|---|
| Tragacanth Comp. | 150.0 | 1.58 | 169 | $4.10 \cdot 10^{-9}$ | $1.26 \cdot 10^{-8}$ |
| Barium Sulphate | 8.1 | 4.48 | 9,406 | $3.37 \cdot 10^{-16}$ | $4.35 \cdot 10^{-16}$ |
| Crodesta F10 | 45.5 | 1.04 | 49,126 | $5.56 \cdot 10^{-14}$ | $7.53 \cdot 10^{-12}$ |
| Light Kaolin | 2.7 | 2.73 | 171,230 | $3.37 \cdot 10^{-19}$ | $5.40 \cdot 10^{-19}$ |
| Lipid droplets in milk | 1.2 | 0.92 | −16,368,698** | $3.22 \cdot 10^{-21}$ | $9.64 \cdot 10^{-20}$ |
| Lipid droplets in Intralipid | 1.0 | 0.92* | −23,570,925** | $2.85 \cdot 10^{-23}$ | $8.54 \cdot 10^{-22}$ |

$\sigma 1$: scattering cross section of dispersed particles ($m^2$) for a speed of sound value in the particle of 1500 m/s
$\sigma 2$: scattering cross section of dispersed particles ($m^2$) for a speed of sound value in the particle of 5000 m/s
*: estimated value
**: a negative value indicates that the dispersed particles float to the top of the dispersion

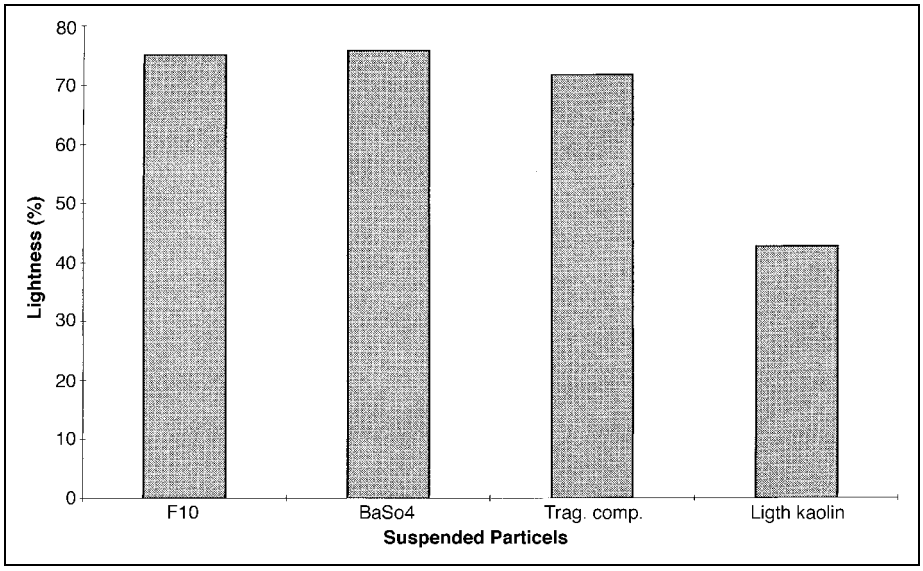


Fig. 2:
Lightness (%) of different suspensions (1%) in 5.5% glucose solution, approx. 30 s after the balloon have been shaken

Tragacanth compound has the highest value for the backscattering intensity, followed by Crodesta F10 and barium sulphate. These dispersions should result in high echogenicities. The values for light kaolin, milk and Intralipid are much lower, so the echogenicity of these dispersions should be lower too.

The influence of different values for the speed of sound in the dispersed phase on σ is small. The uncertainty about $\varkappa_s$ therefore seems to be of minor importance for the estimation of the backscattering intensity.

The sedimentation rate of the dispersed particles follows the same order, with tragacanth coumpound having the highest and Intralipid having the lowest sedimentation rate.

Fig. 1 shows video printouts of a dispersion of Crodesta F10 in glucose solution, having a high echogenicity and of a dispersion of light kaolin showing only weak echogenicity. A pure glucose solution was nonechogenic.

In Fig. 2 the lightness values of the four suspensions immediately after the balloons have been shaken, to achieve a homogeneous dispersion of the solid particles, are shown. Although the theory predicted a higher backscattering intensity for tragacanth compound dispersions than for Crodesta F10 and barium sulphate suspensions, the echogenicities of these three dispersions were nearly identical, whilst the value for light kaolin was much lower. Milk, Intralipid and pure glucose solution all gave lightness values around 25%, and were non-echogenic.

The nearly identical lightness values of the tragacanth compound, Crodesta F10 and barium sulphate suspensions may be explained by a saturation effect for the echogenicity in the video printouts, due to the concentration of the suspended particles being too high. For a cloud of small scatterers with individual scattering cross-section (σ), the effective scattering cross section ($\sigma_{eff}$) is:

$$\sigma_{eff} = \sigma \cdot m \qquad (6)$$

with m being the number of scatterers [6]. With increasing concentration of the scatterer the backscattering intensity should increase linearly. To investigate the effect of the concentration of the scatterer on the echogenicity of Crodesta F10 suspensions, these were investigated over a range of concentrations (Fig. 3). The results of these measurement indicate that a minimum concentration of suspended particles of approximately 0.01% (w/w) still resulted in a high echogenicity. These measurements confirm the interpretation that too high a concentration of scattering particles leads to a saturation of the lightness in the video output. The minimal concentration of the scatterer that is necessary to achieve sufficient echogenicity therefore must be determined experimentally.

In order to allow sufficient time for the investigation (e.g. of the bladder in incontinence diagnostics) the suspended particles should not settle too fast. In Fig. 4 the lightness of barium sulphate dispersions at the same position in the

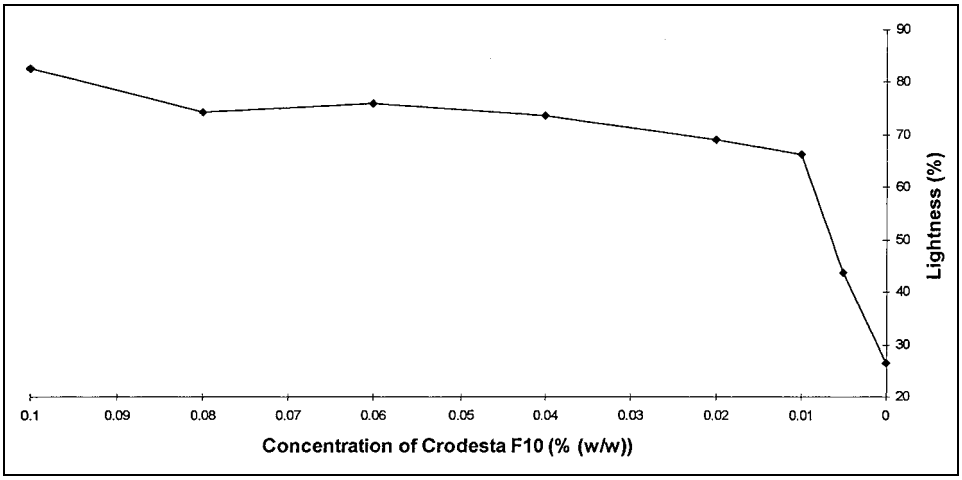


Fig. 3:
Lightness (%) of suspensions of Crodesta F10 with different concentrations of Crodesta® F10 (0–0.1%) in 5.5% glucose solution, approx. 30 sec. after the balloon have been shaken

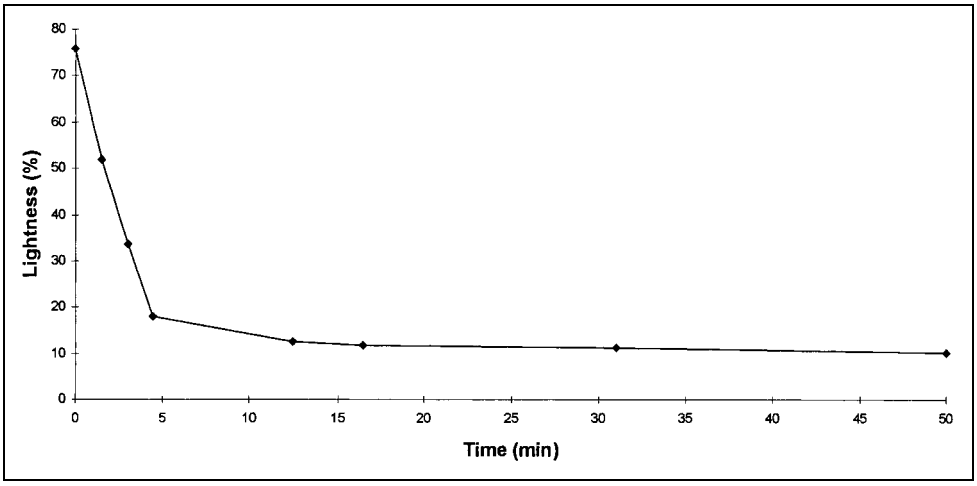


Fig. 4:
Lightness (%) of barium sulphate suspensions (1%) in 5.5% glucose solution as a function of time after shaking (t = 0 approx. 30 s after shaking)

balloon is determined as a function of time. A fast decrease of the echogenicity was found. After 10 min the sample was non-echogenic at the position in the balloon that was scanned (same position as shown in Fig. 1). Fig. 5 shows that for a 1% (w/w) tragacanth compound suspension the echogenicity in the top part of the balloon decreased rapidly, in the middle somewhat slower and in the bottom of the balloon basically stayed constant. This experiment shows that the decrease of echogenicity in the dispersions is due to sedimentation. Fig. 6 shows a video printout of the tragacanth dispersion after 1 h, showing that sedimentation of the dispersed particles has occurred.

As expected from Stokes law and confirmed experimentally, sedimentation is fast for tragacanth compound and barium sulphate suspensions. Dispersions of Crodesta F10, which has a density of 1.04, i.e. very close to that of the continuous phase, exhibited high and very long lasting echogenicity, and hence are the best candidates for the proposed purpose of all the dispersions investigated (Fig. 7).

The first account of results presented in this study shows that coarse suspensions may produce quantitatively and qualitatively good and lasting echogenic properties, depending on their particle size and density. Even with very small differences in the densities between the dispersed and the continuous phase and at very low concentrations of the dispersed phase high echogenicity could be produced. This is advantageous in diagnostics as a small difference in density leads to a small sedimentation velocity of the dispersed particles and results in a longer investigation time.

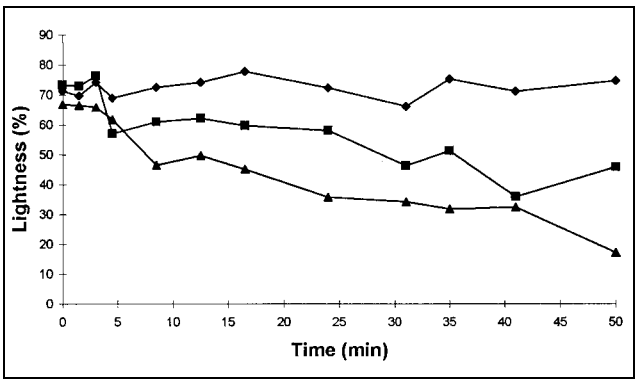


Fig. 5: Lightness (%) of tragacanth compound suspensions (1%) in 5.5% glucose solution as a function of time after shaking the balloon (t = 0 approx. 30 sec after shaking) in different parts of the balloon: top (▲), middle (■), and lower part ◆)

Future studies must investigate the dependency of echogenicity on the use of narrow particle size classes, a potential irritancy of the dispersed particles to the epithelium of bladder and urethra, the microbiological status and possible ways of sterilisation of the dispersed systems.

However, the formulation of a technologically simple, low-cost ultrasound contrast medium seems feasible. Po-

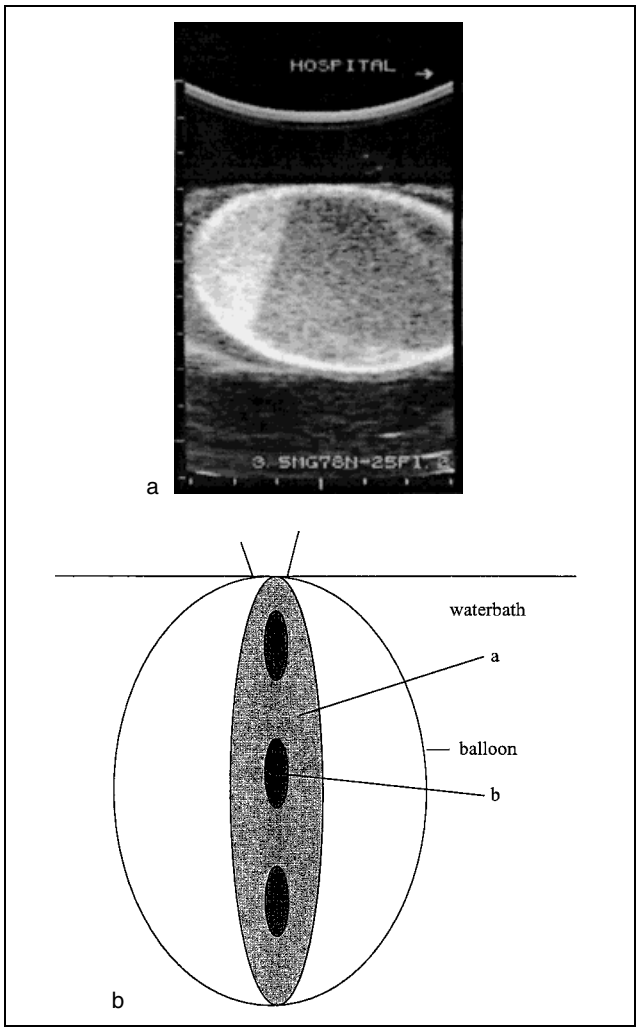


Fig. 6: a) Video printouts of tragacanth compound (1%) in 5.5% glucose solution, b) schematic representation of the filled balloon showing the scanning area inside the balloon (a) and the areas on the video printout used to assess echogenicity using the reflectance spectrophotometer (b)

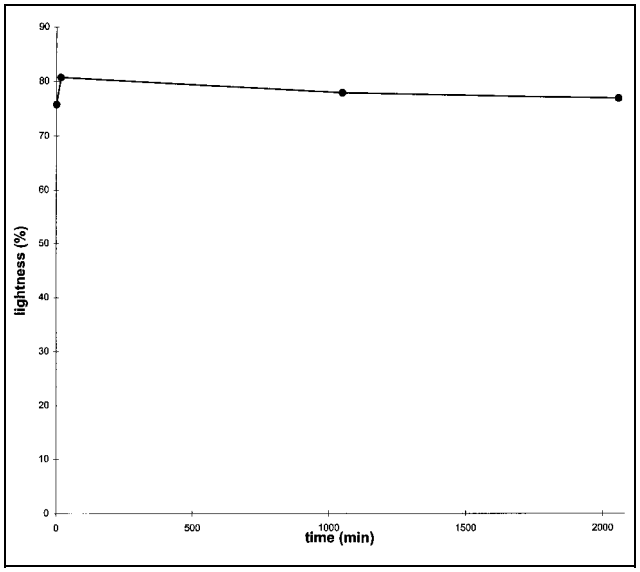


Fig. 7: Lightness (%) of Crodesta F10 suspensions (1%) in 5.5% glucose solution as a function of time after shaking (t = 0 approx. 30 s after shaking)

tential candidates for the dispersed phase can be chosen on the basis of calculated backscattering intensity and sedimentation velocity values and the actual echogenicity can be assessed using the lightness value of the video printout.

## 3. Experimental

### *3.1. Formulations*

Suspensions were formulated in triplicate by adding different amounts (0.05–1.5% (w/w)) of Crodesta F10 (sugar ester saccharose distearate), supplied as free sample by Croda Surfactants, Auckland, NZ and 1% (w/w) of barium sulphate for suspension (E-Z-HD, E.Z.-EM Inc.), compound tragacanth powder (BP 1980, contains starch as insoluble particulate component), and light kaolin (BP 1993) to aqueous 5.5% glucose solution (intravenous infusion, BP 1993, Baxter).
Standardised milk (3.3% fat) and Intralipid 10%, fat emulsion (Baxter) were used as emulsions.

### *3.2. Echogenicity assessment*

The contrast media (100 ml) were filled into balloons and insonated in a waterbath at room temperature using an Aloka SSD 500 scanner with a 3.5 MHz probe. The distance of the transducer to the balloon was kept as constant as possible by the operator. A videoprinter (Sony UP 890 CE) was used to obtain printouts under standardised ultrasound and videoprinter settings (US: 3.5M G 78 N 25 F1.2, Videoprinter: neutral for brightness, contrast and magnification). The images of the balloons were analysed for echogenicity measuring the $l^*$-value (lightness) using a Labscan 6000 reflectance spectrophotometer (Hunter Associates Inc.). The spectrophotometer was calibrated by measuring the lightness of parts of the video printouts that were 'pure' black and 'pure' white. This allowed expression of the values of lightness in % in areas of the printout that represented the inside of the balloon. Each sample was measured at three positions of the printouts that represented slightly different positions in the inside of the balloon (see Fig. 1). Standard deviations of the measurements were smaller than 1.5% lightness. It should be noted that this method analyses the printout from the scanner and it is not actually directly measuring the physical parameter backscattering intensity (σ) but rather represents a simple practical way to assess the echogenicity of an ultrasound dispersion.

### *3.3 Particle size*

Particle size measurements of the dispersions were performed using a Master Sizer X (Malvern Instruments).

### *3.4. Density*

True densities of the particles were determined in triplicate using a Beckmann Luftvergleichspyknometer 930 (Beckmann Instruments). CV of the measurements were smaller than 1%.

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