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# Preparation of a radionuclide/gel formulation for localised radiotherapy to a wide range of organs and tissues

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Localised radiotherapy by instillation of radiolabeled particles is being used to treat rheumatoid arthritis and certain tumors. Such therapy is limited to organs and tissues capable of retaining the radioactive compound until the radioactivity is sufficiently low, and the leakage to other parts of the body is no longer unacceptable. In this study, radiolabeled particles, i.e. $^{90}$Y-silica colloid particles, were encapsulated in calcium alginate gels, and the leakage of radioactivity from the gels was monitored. The purpose of the study was to develop a formulation suitable for the localised delivery of radiation therapy to a wide range of organs and tissues. An injectable gel formulation was developed, liberating only small amounts of radioactivity into the surrounding medium. The formulation is a viscous liquid at room temperature and forms a gel on heating to normal body temperature. Thus, it should be suitable for the localised delivery of radiolabeled particles to a wide range of organs and tissues. The study also includes a formulation exhibiting time-dependent gelation. However, this formulation was not found to be suitable for the purpose.

## 1. Introduction

The use of radio labelled compounds for localised radionuclide therapy has been investigated for several decades. Such therapy has been delivered to the peritoneum, to the liver and to the knee joint (Lau et al. 1999; Leung et al. 1995; Rodriguez-Merchan and Wiedel 2001; Weiner and Spencer 2001; Westlin et al. 1997). In treatment of arthritis in the knee joint, the particulate nature of the $^{90}$Y compound ensures that the compound stays within the encapsulated compartment and thus makes possible the desired localisation of the radiation. This minimizes unwanted side effects due to normal tissue being exposed to the radiation. However, this strategy is limited to target organs and/or tissues capable of retaining the compound throughout the period of therapy. Encapsulating radio labelled particles into a gel formulation could extend the possible use of such products to a wider range of organs and tissues.

Alginate gels are suitable for injectable or implantable pharmaceutical formulations due to their biocompatibility and gentle conditions required for gelling (Skaugrud et al. 1999; Skjåk-Bræk and Espevik 1996; Smidsrød and Skjåk-Bræk 1990). The encapsulation efficacy of the gel is determined by the nature of the encapsulated material and gel porosity and stability. The porosity of a calcium alginate gel is dependent on gel composition and manufacturing conditions. Pore sizes of calcium alginate gels ranging from 20 nm to 200 nm have been reported in the literature (Fundueanu et al. 1999). Materials larger than the pore size will diffuse slowly or not at all through the gel matrix. Thus, particulate material in the micrometer size range should be efficiently encapsulated within a calcium alginate gel.

The homogenous radionuclide/gel mixture forms a continuous volume outside (but immediately adjacent to) the tissue to be treated. Therefore, a radionuclide suitable for this kind of delivery of radiotherapy should emit high energy $\beta^-$ particles, and have a suitable physical half-life. A short half-life will reduce the time needed to deliver the required radiation dose to the site of action. The leakage of radiation to other parts of the body could thus be reduced. However, preparations containing short-lived radionuclides may be difficult to transport from the manufacturer to the hospital, the radioactive decay being substantial before the delivery to the patient. Radionuclides emitting $\gamma$ radiation in addition to $\beta^-$ radiation enable continuous observation of the biodistribution of the radionuclide, but the $\gamma$ radiation may be harmful to the rest of the body and nearby people. Yttrium-90 ($^{90}$Y) is a pure $\beta^-$ emitter with a half-life of 64 hours and energy maximum 2.25 MeV. The half-life and relatively high $\beta^-$ energy of the nuclide makes it suitable for radiotherapy. The present work was therefore based on $^{90}$Y.

The gel containing the radioactive compound will form a separate compartment either within the tissue or at its surface. The calculation of the radiation dose delivered and the maximum range of the radiation in the tissue is complex, but could be calculated by Monte Carlo techniques (Kvinnsland et al. 2001). The dose distribution is dependent on the tissue composition and the gel volume. However, if the volume of the gel containing the radionuclide is larger than the range of the radiation in the tissue, the dose distribution in the tissue is dependent on the radioactive concentration of the radionuclide in the gel (MBq/ml), rather than the total radioactivity contained by the

gel. This facilitates the calculation of the dose distribution delivered to the tissue from a radionuclide matrix as compared with a thin layer of radionuclide positioned at the tissue surface.

Yttrium [$^{90}$Y] colloid suspension (YMM-1®) is composed of the radionuclide physically linked to the surface of silica particles. The mean diameter of the particles is approximately 2 μm (according to manufacturer). One established application of the product is the treatment of synovial hypertrophy in the knee- joints (Rodriguez-Merchan and Wiedel 2001).

The scope of the present study was to develop a pharmaceutical formulation suitable for the local delivery of an YMM-1®/gel mixture. Such a formulation should retain the colloid particles, thus avoiding adverse effects due to radioactivity being accumulated unintentionally in other parts of the body. The formulation should not destabilise the yttrium-silica complex, as liberated yttrium ions could diffuse freely out of the formulation and be distributed to the body. The formulation as such should tolerate the irradiation caused by the encapsulated radionuclide, it should be biocompatible, and not cause any undesirable side effects at the site of action.

Preferably, it should be possible to inject the formulation to a range of tissues and organs, followed by immediate gel formation *in situ*, the $^{90}$Y colloid suspension being retained in the resulting gel. Two calcium alginate formulations were investigated that are viscous liquids when injected, but quickly set to gels *in situ* after injection. One formulation displays time-dependent gel formation, whereas the other is forming a gel when heated from room temperature to body temperature.

## 2. Investigations, results and discussion

### *2.1. Effects of irradiation on the alginate samples*

The viscosities of alginate solutions before and after irradiation with a dose of 200 Gy are listed in Table 1. All samples displayed a marked decrease in viscosity after irradiation. The reduction of alginate viscosity after irradiation is probably due to a reduction in molecular weight of the polymer. It has previously been demonstrated that alginates are hydrolysed on exposure to ionizing radiation (Lee et al. 2003). The gel strengths of gels prepared from the irradiated alginate samples are compared with that of gels prepared from non-irradiated alginate samples in Table 2. It is found that the gel strength is reduced by the irradiation to a lesser degree than the viscosities of the alginate solutions. The radiation doses absorbed by these alginate solutions are larger than the doses absorbed when used in combination with therapeutic doses of $^{90}$Y. The reduction of polymer molecular weight caused by the irradiation is therefore not detrimental to the encapsulating properties of the gels.

**Table 1: Viscosities of alginate solutions before and after irradiation**

| Alginate sample | Viscosity (Pas) | | Relative reduction of viscosity |
|---|---|---|---|
| | Before irradiation | After irradiation | |
| 1% SF 120 | 0.06 ± 0.01 | 0.048 ± 0.004 | 20% |
| 1% LV 120D | 0.080 ± 0.005 | 0.060 ± 0.009 | 15% |
| 1% LF 120 L | 0.067 ± 0.006 | 0.04 ± 0.01 | 40% |
| 3% LF 120 L | 1.67 ± 0.01 | 1.103 ± 0.007 | 35% |
| 3% SF 120 | 2.287 ± 0.007 | 1.19 ± 0.01 | 48% |

Important features of the alginate qualities used are given in section 3.1. Viscosity values are the means of three experiments, error ranges are maximum deviations from mean values

**Table 2: Gel strength parameters of calcium alginate gels prepared from irradiated or non-irradiated alginate samples**

| Calcium alginate gel sample | | Gel strength (G′) (kPa) | | Relative reduction of gel strength |
|---|---|---|---|---|
| | | Non-irradiated aginate | Irradiated alginate | |
| 2% SF 120 | 15 mM $Ca^{2+}$ | 2.9 ± 0.2 | 2.9 | 0% |
| 2% LF 120 L | 60 mM $Ca^{2+}$ | 2.3 ± 0.2 | 1.8 | 22% |
| 2% LF 120 L | 15 mM $Ca^{2+}$ | 1.7 ± 0.2 | 1.4 | 18% |

Important features of the alginate qualities used are given in section 3.1. G′ values are the means of two experiments, error ranges are maximum deviations from mean values

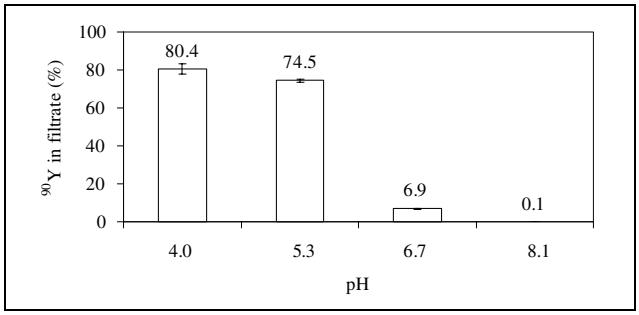


Fig. 1: Fractions of $^{90}$Y detached from the silica particles after exposure to media with different pH. Values are the means of two experiments as compared with the amount of $^{90}$Y found in an unfiltered $^{90}$Y-colloid suspension. Error bars are maximum and minimum values, respectively

### *2.2 Stability of the $^{90}$Y-silica complex as a function of pH*

The amounts of $^{90}$Y detected in the filtrates of the media of different pH are shown in Fig. 1. The results demonstrate a marked difference between the acidic and the alkaline media. The size of the silica particles are reported to be approximately 2 μm, they are thus retained during the filtration. $^{90}$Y detected in the filtrate must have been separated from the silica particles. On exposure to acidic media, the surface of a silica particle becomes uncharged, and $^{90}$Y is detached from particle. The results are in accordance with the results presented by Kosmulski (1997) on the adsorption behaviour of $Y^{3+}$ and $Gd^{3+}$ on silica surfaces. The experiment demonstrates that the $^{90}$Y-silica complex should not be exposed to acidic media when the particulate state of radiation source is desired.

### *2.3. Calorimetric analysis of the liposomes*

The DSC thermogram of the liposomes is shown in Fig. 2. The thermogram displays a small endothermic peak at around 34–36 °C and a larger peak at around 37–42 °C. The small peak is associated with the pretransition of the liposomes, and the larger is associated with the main phase transition of the liposomes from the crystalline to the liquid crystalline state. As the liposomes undergo phase transition, they are not capable of retaining the encapsulated calcium ions (Cevc 1993; Jacobson and Papadopoulos 1975). Due to a large concentration gradient over the liposome membrane, calcium diffuses out of the liposomes and into the surrounding medium.

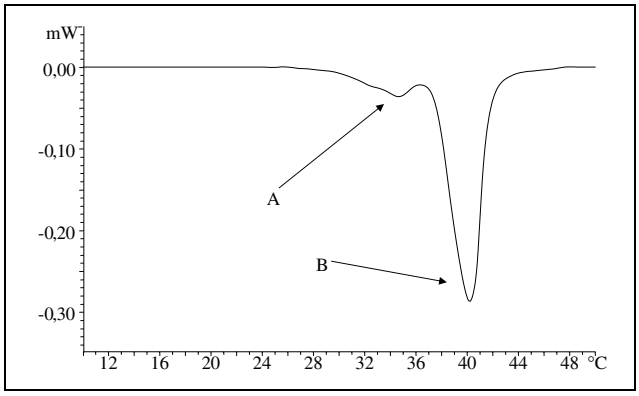


Fig. 2: DSC thermogram of liposomes. Sample size 0.25 mg. Heating rate 2.5 K/min. Pretransition (A) and main transition (B) are indicated with arrows

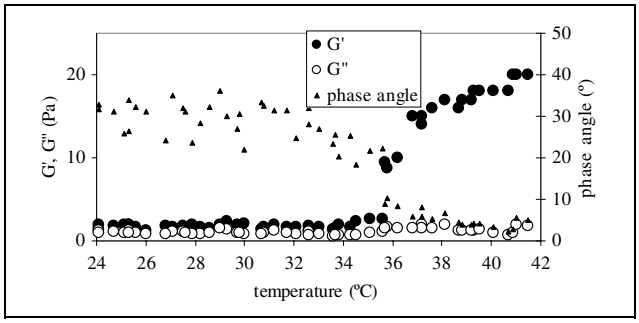


Fig. 3: Storage (G′) and loss moduli (G″) and phase angles of alginate/liposome sample as a function of temperature. Values of G′ and G″ are underestimated due to improper sample size

### 2.4. Study of the liposome-alginate gelation process

A gel sample was prepared by mixing equal amounts of liposome suspension and a 4% sodium alginate (SF 120) solution. Due to a limited supply of the sample, the amount used for the rheological study was smaller than normally required by the rheometer. The values of the storage (G′) and loss moduli (G″) reported are therefore underestimated, and should be interpreted semi-quantitatively. The values of G′ and G″ should not be interpreted as the true gel strength parameters of the gel, but they are still useful to identify the onset of the gelation process. G′, G″ and the corresponding phase angle of the sample as a function of temperature are presented in Fig. 3. A change from viscous to elastic behaviour and a corresponding marked increase in gel strength is observed at approximately 34–37 °C. The observed sol-gel transition occurs when the liposome phase transition temperature is reached, accompanied by the release of calcium from the liposomes to the surrounding alginate solution.

### 2.5. $^{90}$Y release from acid-mediated calcium alginate gels

All gel compositions displayed a first order release of $^{90}$Y, indicating free diffusion of $^{90}$Y through the gel and into the surrounding medium. After 2 days, 10 to 20 percent of encapsulated $^{90}$Y was released into the surrounding medium (data not shown). No significant difference in encapsulation efficiencies was observed among the different gel compositions. Although the pH of the final gel composition is neutral due to the dissolution of the calcium salt, the pH is temporarily lowered by the GDL during the gelation process, and this is probably destabilising the $^{90}$Y-silica complex. Therefore, acid-mediated gelling of calcium alginate was found to be an unsuitable technique for encapsulating a commercially available preparation of $^{90}$Y physically linked to silica particles (YMM-1®).

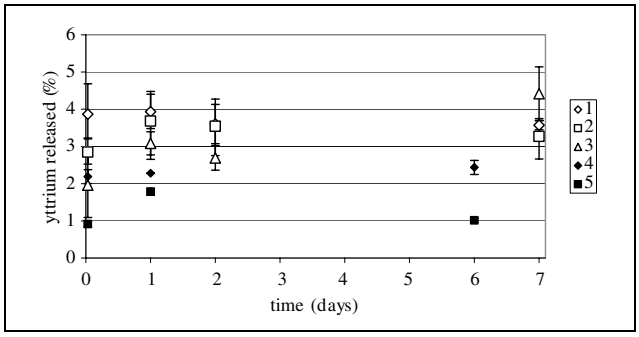


Fig. 4: Concentration profiles of $^{90}$Y in the release media. Values are the means of three experiments, compared with a theoretical 100% $^{90}$Y release. Error bars are the maximum and minimum values, respectively

### 2.6. $^{90}$Y release from liposome-mediated calcium alginate gels

The concentration profiles of $^{90}$Y in the water phase surrounding the gels are presented in Fig. 4. After a small initial burst release, little $^{90}$Y was further released from the gels. For all gel compositions, only a small proportion (<5%) of encapsulated $^{90}$Y had been released after one week. From the data presented, it is not possible to decide which of the five gel formulations that is best suited for encapsulating the $^{90}$Y-silica complex. The relative and absolute concentrations of alginate and calcium in the gel will determine the rheological properties and the pore structure of the gel, but it seems that all the gels investigated have gel structures suitable for efficient encapsulation of the YMM-1® particles. The burst release observed for all gel formulations is probably due to YMM-1® particles being situated very close to the gel surface. The gels investigated have small volumes (about 30–40 µl for gels 4 and 5). When used therapeutically, sample sizes in the millilitre to decilitre range will be more suitable. A 20-fold increase in sample size will reduce the surface to volume ratio by a factor of about 3, if a spherical geometry is assumed. This will probably reduce the burst release significantly. In contrast to the acid-mediated gels, heat-mediated gels of calcium alginate proved to be a suitable vehicle for encapsulating YMM-1®. Such gels can be obtained by mixing a sodium alginate solution with liposomally entrapped calcium chloride. The onset of gel formation is found slightly below normal body temperature, probably associated with the phase transition temperature of the liposomes. After a small burst release of $^{90}$Y, little $^{90}$Y release is observed from the gels during one week. The efficient $^{90}$Y entrapment and the mild gelling conditions enables the instillation of gel-entrapped radionuclide by minimal invasive injection techniques as gelling is achieved *in situ* during and after injection. The formulation should be suitable for radiotherapy of a wide range of tissues and organs.

## 3. Experimental

### 3.1. Chemicals

Sodium alginate samples (Protanal SF 120, lot number 477021, $F_G$ 0,694, MW 237000; Protanal LF 120 L, lot number 907788, $F_G$ 0,444, MW 221000; Protanal LV 120 D, lot number 940040, $F_G$ 0,349, MW 251000)

were supplied by FMC BioPolymer (Drammen, Norway). Yttrium [$^{90}$Y] colloid suspension (YMM-1®) was purchased from CIS bio international, Gif-sur-Yvette Cedex, France. Phospholipids (DPPC, lot number 563042-1/89; DMPC, lot number 5622191-1/20) were gifts from Lipoid, Ludwigshafen, Germany. Calcium carbonate and sodium chloride were purchased from NMD, Oslo, Norway. Calcium chloride, tetracemin sodium (EDTA) and glucono-δ-lactone (GDL) were purchased from Merck, Darmstadt, Germany).

### 3.2. Stability of alginate towards $\beta^-$ irradiation

Sodium alginate solutions were irradiated to a dose of 200 Gy using 16 MeV X-rays from a linear accelerator (Electa) normally used for radiation therapy. A 3 cm thick build-up layer was used in front of the gel. This ensures a homogenous dose throughout the gel. Rheological measurements were performed using a Bohlin VOR rheometer (Bohlin Reologi AB, Lund, Sweden). The viscosity of alginate solutions were measured before and after radiation by rotational viscosimetry using a C14 (cup) measuring geometry at 37 °C. Shear rates were 9.2–1500 Hz. Only results obtained in the linear viscoelastic region of each sample were used. The gel strength of calcium alginate gels were measured by oscillatory rheometry using an SP30 (serrated plate) measuring geometry at 37 °C (frequency 1 Hz, strain 10%). Homogenous calcium alginate gel samples were prepared as described by Draget et al. (1991). The samples were applied to the rheometer before the gels had set. Thus, the gel sample occupies the measuring geometry in the rheometer properly.

### 3.3. Detection and quantification of $^{90}$Y

The radioactivity of yttrium in samples was detected by liquid scintillation counting. Samples (0.1–0.2 µl) were diluted with a 3 ml Emulsifier-Safe scintillation cocktail and beta particle counts were obtained by a Packard Tri-carb 2100 TR Liquid scintillation analyzer. Detection range was 5–1700 keV, and counting time was 1 min per sample.

### 3.4. Stability of the $^{90}$Y-silica complex as a function of pH

YMM-1 was diluted with buffered solutions of pH 4.0, 5.3, 6.7 and 8.0, respectively. The suspensions were filtered through 0.45 µm membrane filters (MFS), and the activity of $^{90}$Y in the filtrates were determined as described in Section 2.3. The activities were compared with those of unfiltered samples that were used for reference.

### 3.5. Liposome preparation and characterisation

Liposomes were prepared by the film hydration method (Betageri and Kulkarni 1999). The phospholipids (90 mol% DPPC, 10 mol% DMPC) were dissolved in chloroform/methanol (9 : 1). A thin lipid film was prepared in a 500 ml round flask as the solvent was evaporated at 100 mbar and 50 °C using a rotavapor (Vacuubrand PC 511, Wertheim, Germany). The lipid film obtained was hydrated with 133 mM $CaCl_2$ at 50 °C for 2 h with occasional stirring. The liposomes were subjected to four freeze-thaw cycles employing liquid nitrogen and a water bath (50 °C) followed by subsequent extrusion (Lipex extruder, Biomembranes Inc., Vancouver, Canada) through 2 µm and 800 nm polycarbonate membranes (Nucleopore, Costar corp., Cambridge, USA). The liposomes were centrifuged for 20 min in an IEC Centra MP4 centrifuge (International Equipment, Needham Heights, MA, USA) (3000 rpm, rotor 224). The supernatant was removed and replaced with 200 mM NaCl. Centrifugation and supernatant replacement were repeated until the calcium concentration in the supernatant was sufficiently low (<5 mM). The temperature in the centrifuge did not exceed 30 °C at any time. The final concentration of phospholipids in the liposome suspension was 20.3 mM as determined by the phosphate analysis described by Rouser et al. (1970). The concentration of calcium in the liposome suspension was determined by titration of the suspension with 2 mM EDTA in the presence of Calcon colour indicator. The calcium concentration in the suspension was found to be less than 5 mM. After heating of the suspension to 37 °C, which is above the phase transition temperature of the liposomes, calcium was released from the liposome interior. The calcium concentration in the suspension after heating was 28 mM. Calorimetric measurements of the liposomes were performed with a Mettler Toledo DSC 822e differential scanning calorimeter.

### 3.6. Study of the liposome-alginate gelation process

200 mg of a 1 : 1 mixture of a 4% alginate solution (SF120) and liposome suspension was placed in a Bohlin VOR rheometer (Bohlin Reologi AB, Lund, Sweden) using an SP30 measuring geometry. The sample was covered with a low-viscous silicone oil to prevent evaporation of water from the sample during the experiment. The rheological properties of the sample were studied by oscillatory rheology (frequency 1 Hz, strain 10%) as the sample was heated (heating rate 1 K/2 min) from 24 °C to 41 °C.

**Table 3: Compositions of the acid-mediated calcium alginate gels**

| Alginate | | $CaCO_3$ | GDL |
|---|---|---|---|
| % | $F_G$ | (mM) | (g) |
| 2 | 0.349 | 60 | 0.534 |
| 3 | 0.349 | 15 | 0.134 |
| 3 | 0.349 | 60 | 0.534 |
| 4 | 0.349 | 60 | 0.534 |
| 3 | 0.694 | 15 | 0.134 |
| 3 | 0.694 | 60 | 0.534 |
| 4 | 0.694 | 60 | 0.534 |

YMM-1® corresponding to 10 MBq $^{90}$Y wass added to each gel

**Table 4: Compositions of the heat-mediated calcium alginate gels**

| Gel nr. | 3.3% alginate (g) | Liposome suspension (g) | YMM-1 (ml) | Alginate (%) | $CaCl_2$ (mM) | Number of samples | Sample weight (mg) |
|---|---|---|---|---|---|---|---|
| 1 | 0.124 | 0.362 | 0.2 | 0.60 | 15 | 3 | 220 |
| 2 | 0.136 | 0.264 | 0.2 | 0.75 | 12 | 3 | 200 |
| 3 | 0.056 | 0.057 | 0.05 | 1.14 | 10 | 2 | 80 |
| 4 | 0.099 | 0.102 | 0.05 | 1.30 | 11 | 2 | 125 |
| 5 | 0.079 | 0.145 | 0.05 | 0.95 | 15 | 2 | 135 |

Alginate SF120 was used in all the gels

### 3.7. $^{90}$Y release from acid-mediated calcium alginate gels

A mixture of sodium alginate solution, calcium carbonate powder and $^{90}$Y colloid suspension was mixed with a freshly prepared solution of glucono-δ-lactone (GDL). Upon hydrolysing, GDL lowers the pH of the mixture, making the calcium salt soluble, and gelation is initiated. A closer description of this method of homogenous gel formation is given by Draget et al. (1991). Gels (25 g) were prepared in cylindrical glasses, and water (25 ml) was added on top of the gels after setting of the gels. Small samples (0.2 ml) of the water phases above the gels were removed at predetermined times, and amounts of $^{90}$Y released from the gels were determined as described in Section 3.3. Gel compositions investigated are listed in Table 3.

### 3.8. $^{90}$Y release from heat-mediated calcium alginate gels

Liposome suspension was mixed with sodium alginate solution and $^{90}$Y colloid suspension before heating of the mixture. Calcium is released from the liposomes and exposed to the sodium alginate as the liposome transition temperature is reached. Calcium alginate gel formation is thus achieved. A closer description of this method of homogenous gel formation is given by Westhaus and Messersmith (2001). Gel compositions investigated are listed in Table 4. Two different procedures were performed to mediate heating and subsequent gelling of the samples. For gels 1–3, the alginate/liposome mixture was placed in a 20 ml cylindrical glass. The glass was slightly tilted, making the sample accumulate in one corner of the glass. 5 ml of water (38 °C) was added to the mixture, and the glass was immediately sealed and placed in a water bath (38 °C). A gel was formed in the bottom corner of each glass. The glasses were removed from the water bath after 1 h, and the remaining parts of the leakage experiments were performed at room temperature. For gels 4 and 5, the alginate/liposome mixture was added dropwise to a cylindrical glass containing 5 ml water (38 °C), resulting in the immediate formation of 3–5 gel droplets with diameters of approximately 3–4 mm in each glass.
Small samples (0.2 ml) of the water phase surrounding the gel droplets were removed at predetermined times, and amounts of $^{90}$Y released from the gels were determined as described in Section 3.3. After the final measurement, the gels were completely dissolved by adding EDTA to each glass, followed by occasional shaking. This procedure ensures complete gel dissolution as calcium in the gel is sequestered by EDTA. The activity (concentrations) of $^{90}$Y in the dissolved gels were determined as described in section 3.3, and interpreted as theoretical maximum release of $^{90}$Y.

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