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EP 0391741 A **US 20010051670 A**

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(54) Title of the Invention: **Radiopaque polymers**
Abstract Title: **Radiopaque polymers**

(57) Imageable polymers are formed by the acetalisation of a polymeric hydrogel containing 1,2- or 1,3-diol groups with a radiopaque species. In preferred embodiments the hydrogel is polyvinyl alcohol and the radiopaque species is an iodated phenyl aldehyde, iodated benzyl aldehyde or iodated phenoxy aldehyde. The hydrogels may be formed into microspheres which can be loaded with a therapeutic agent or drug to form a delivery system whence the active may be eluted in for example an electrolytic solvent or under acidic conditions.

Fig. 2A

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

Fig. 1A

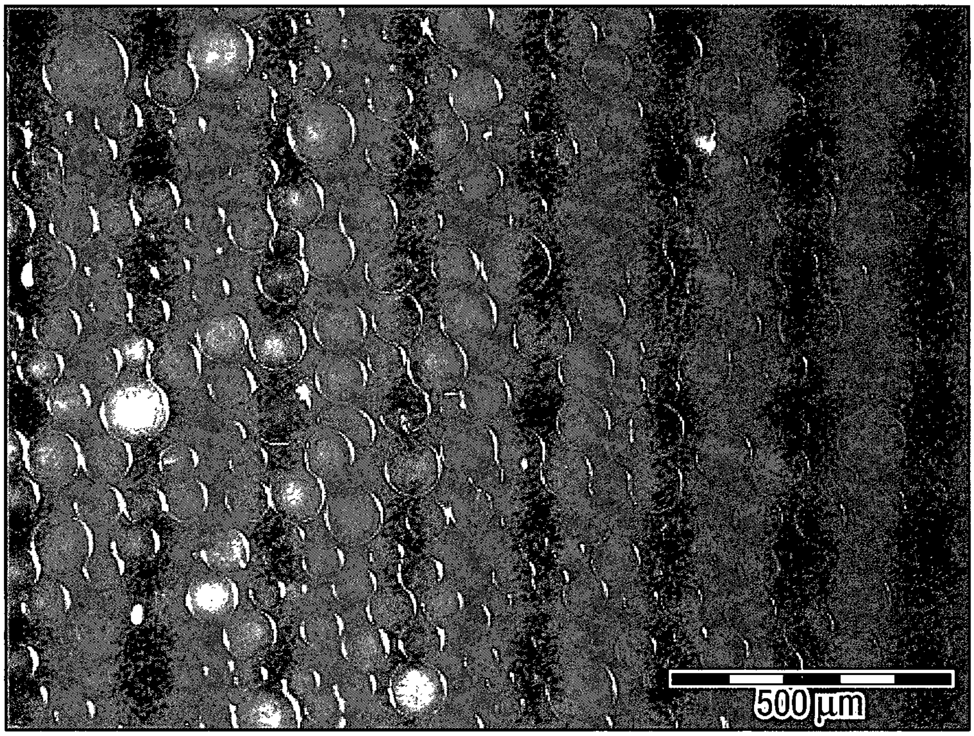


Fig. 1B

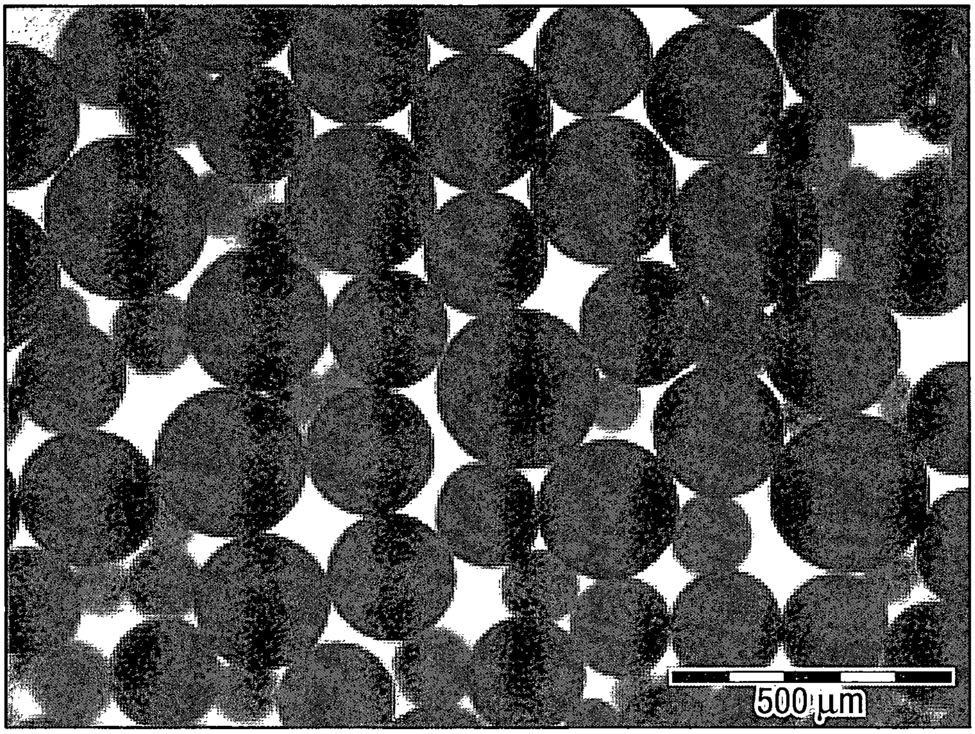


Fig. 2A

Fig. 2B

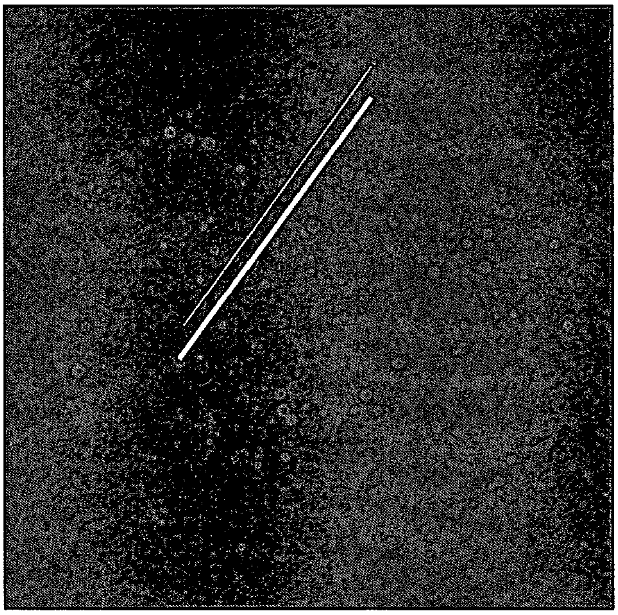


Fig. 2C

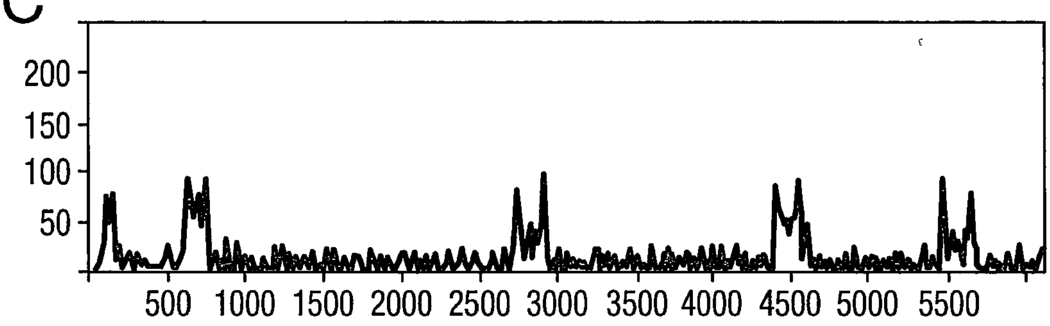


Fig. 3A

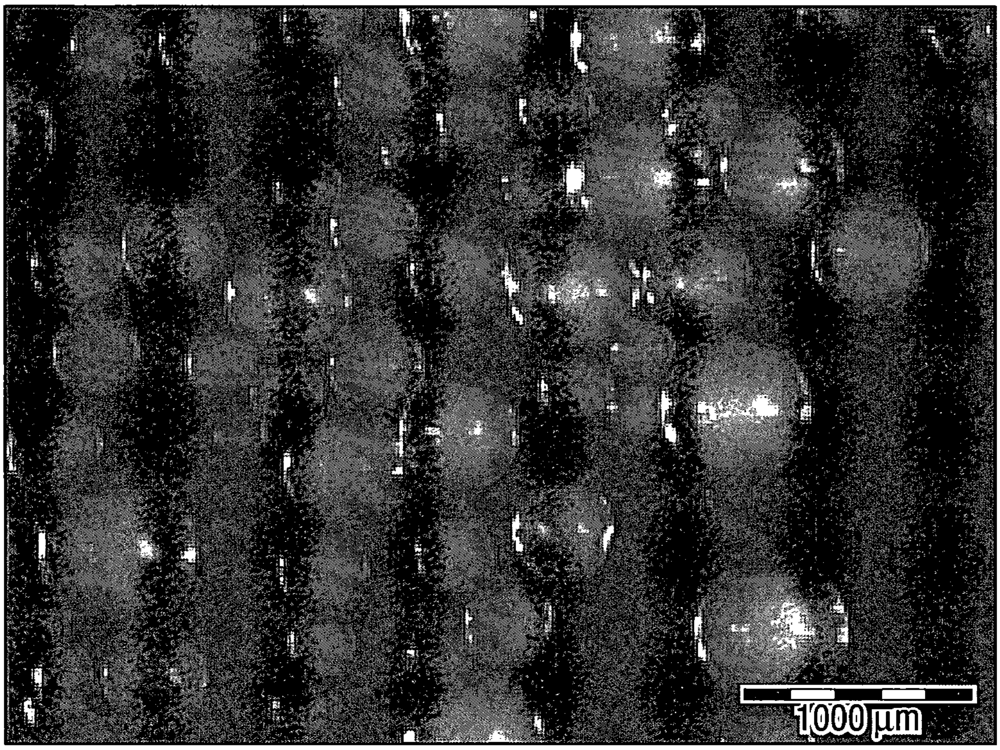
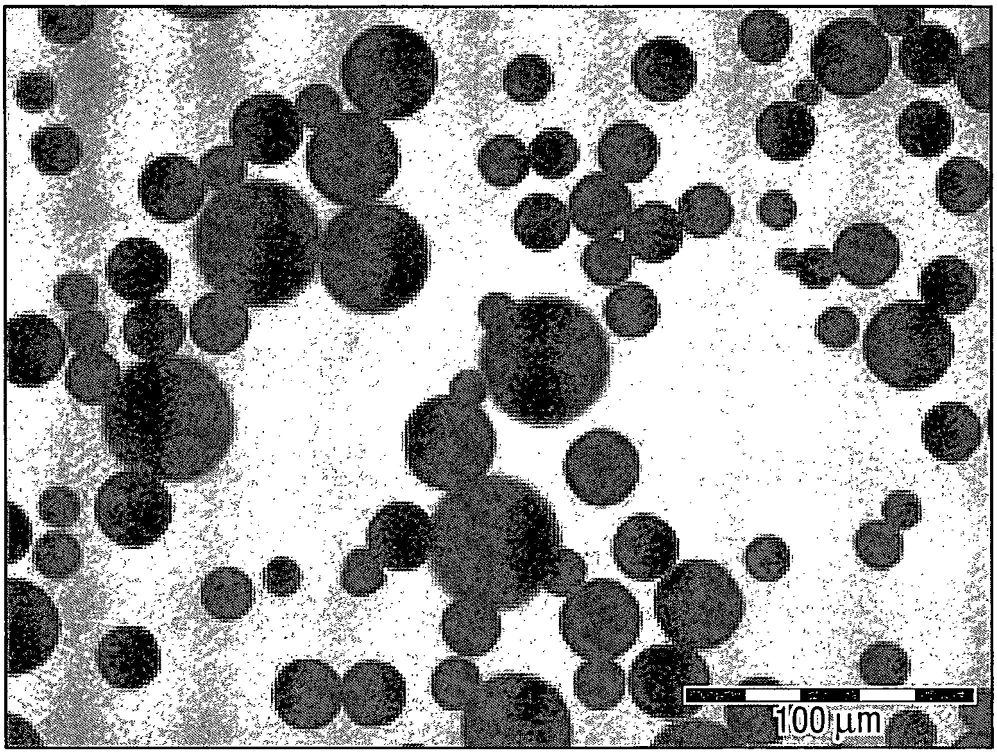


Fig. 3B



RADIOPAQUE POLYMERS

This invention relates to imageable, radiopaque polymers and to methods for making radiopaque polymers. The invention is suitable for making radiopaque hydrogels and, in particular, radiopaque hydrogel microspheres, which are imageable during embolization procedures. Such microspheres can be loaded with drugs or other therapeutic agents to provide an imageable drug delivery system.

Radiopacity, or radiodensity, refers to the property of obstructing, or attenuating, the passage of electromagnetic radiation, particularly x-rays. Radiopaque materials thus generally block radiation and are visible in x-ray radiographs or during x-ray imaging and under fluoroscopy. Radiopaque materials consequently find many uses in radiology and medical imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), fluoroscopy and other imaging techniques as are known in the art, including 3D versions of these such as SPECT (single-photon emission computed tomography).

Embolization of blood vessels is an important medical procedure in the treatment of tumors, fibroids and vascular malformations, in which an embolus is introduced or is formed in a blood vessel to reduce blood flow and induce atrophy of tumours and malformations. There is a range of embolic materials in clinical use that require transcatheter delivery to the site of embolization. This can be achieved with small particles such as poly(vinyl alcohol) (PVA) foam particles (e.g. Ivalon™). However, non-spherical particles suspended in a carrier fluid have a tendency to aggregate, making injection difficult and impractical.

The use of microspheres (also referred to herein as “beads”) as injectable biomaterials has become more and more popular over the last few decades. The tight control over the shape and dimension of injected particles makes them ideally suited for treatments in which the particle size is of critical importance. Microspheres have a controlled shape and size and behave very predictably during the injection procedure. For clinical use, microspheres need to possess a number of characteristics. The microspheres should be biocompatible, safe, stable, display desired functionality inside the patient, and should demonstrate desired and predictable degradation kinetics. All these parameters are determined by the physico-chemical nature of the synthetic microspheres.

Imaging of embolization procedures is important because it provides the clinician with visual feedback both during and after the procedure. In this way, the clinician can monitor the precise location of the embolic material and ensure that it is administered to and remains in the correct position in the vasculature, thus improving procedural outcomes and reducing procedural risk. However, imaging is currently only possible when using inherently radiopaque embolic materials or by mixing non-radiopaque embolic particles with radiopaque materials.

For example, iodinated polyvinyl alcohol (I-PVA) is a radiopaque embolic material in the form of a viscous liquid which precipitates in aqueous conditions such as those encountered *in vivo*. However, embolization with precipitating liquid can be inconsistent in terms of controlling the precise location at which the embolus is formed and there is always a risk of precipitation occurring in an undesired location outside the target area.

Contrast agents are inherently radiopaque. Common contrast agents include ethiodized oils, such as Ethiodol® (Guerbet Joint Stock Company, France; marketed in the EU under the trade name Lipiodol®). Ethiodol is an iodinated oily X-ray contrast medium composed of an iodinated poppy-seed oil (40% Iodine by weight). Ethiodol® may be used directly as an embolization agent. Due to its viscous nature, the ethiodized oil tends to accumulate in the capillary bed and slow down blood flow. It has thus been described as "microembolic". However, such use is contraindicated by the FDA and, in any event, it fails to provide a reproducible level of embolization. As a result, embolization with ethiodized oil is normally followed by conventional embolization with particles or microspheres to obtain the desired degree of antegrade blood blow. Soluble contrast medium, such as Isovue® (iopamidol, Bracco Diagnostics Inc.) and Omnipaque™ (iohexol, GE Healthcare), is often used in angiography to document the vascular anatomy and monitor blood flow. When soluble contrast medium is mixed with embolic particles, some "parenchymal contrast stain" may be visible for a short duration following the procedure, but this imaging feedback rapidly dissipates.

Contrast agents, such as Ethiodol® and Isovue® are, however, routinely mixed with embolic particles to impart radiopacity to an injectable composition. Although such compositions are useful, the different physical properties of the aqueous suspension of embolic particle and the contrast agent results in different in-vivo localisation. After administration, it is the contrast agent which is visible rather than the embolic particle since the contrast agent and the embolic particle may not reside at the same location in tissue.

It is desirable therefore to combine the predictability and reproducibility benefits of embolic microspheres with the radiopacity of contrast agents.

EP1810698 describes a process for forming stable radiopaque embolic beads in which PVA hydrogel embolic beads are loaded with iodinated oils to make them radiopaque. The process described in EP1810698 requires the beads to be dry or dried for loading with iodinated oil before recovering oil-loaded beads from any excess extra-particulate loading liquid, swelling the beads in an aqueous storage liquid and sterilizing the beads by heating to a temperature of at least 90°C.

In the process of EP1810698 it is an essential step that the final product is sterilized by a heating process in the presence of water. Sterilization is carried out by heating to a raised temperature of at least 90° but preferably to a temperature greater than 100°C under pressure. It is disclosed that the radiopaque oil is not adversely affected under the preferred sterilization conditions of reduced pressure, at a temperature of around 120°C. However, this approach does not provide control over elution of the contrast agent from the bead nor does it contemplate the impact of contrast agent on the loading and elution of drug.

WO2011/110589 describes synthesis of an iodinated poly(vinyl alcohol) by grafting iodobenzoyl chloride to poly(vinyl alcohol) via ester linkages. Whilst this polymer is demonstrated to be radiopaque, it results in a water insoluble polymer, which cannot then be formed into microspheres through the water-in-oil polymersation processes normally used to generate hydrogel microspheres with desirable embolization properties. The same publication mentions microspheres but contains no disclosure as to how this is achieved.

Mawad et al (Biomacromolecules 2008, 9, 263-268) describes chemical modification of PVA-based degradable hydrogels in which covalently bound iodine is introduced into the polymer backbone to render the polymer radiopaque. Iodine is introduced by reacting 0.5% of the pendent alcohol groups on PVA with 4-iodobenzoylchloride. The resulting polymer is biodegradable, embolizes via precipitation and is not formed into microspheres

There is clearly a need, therefore, for radiopaque embolics which combine the embolization efficiency and reproducibility of embolic beads with the radiopacity of contrast agents, such as ethiodized oils, in a single product. The ideal embolic particle is one which is intrinsically radiopaque and which is stable and reproducible, in size and physical properties such that the clinician can perform and image the embolization procedure with more certainty that visible contrast results from the embolic particle. Such radiopaque particles would allow for monitoring of injection and deposition of the embolic to the vascular site but would also be very useful for clinical follow-up to monitor the effects of embolization and ensure embolic remains in the desired location and to identify regions at risk for further treatment. The time window in which follow-up imaging can be obtained is increased significantly over existing methods.

Radiopacity (or radiodensity) can be quantified according to the Hounsfield scale, a principle which is central to X-ray computed tomography (CT scan) applications. On the Hounsfield scale, distilled water has a value of 0 Hounsfield units (HU), while air is specified as -1000 HU. To ensure good contrast, a radiopaque embolic would conveniently have radiopacity greater than +60 HU to enable visualization in the vasculature or within organs, such as the liver. Conveniently the embolic will have radiopacity which is, at least, 40HU greater than the tissue into which it is administered and preferably the radiopacity will be greater than 60HU, more preferably greater than 100HU and even more preferably greater than 500HU.

In addition to good radiopacity, the ideal embolic bead would have properties which enable efficient load drug loading and elution such that chemoembolization procedures may be monitored with confidence.

The applicants have established that by utilizing relatively straightforward chemistry, it is possible modify polymers to make them radiopaque. The chemistry may be performed on any diol-containing polymer and it is particularly suited to hydrogel polymers and pre-formed microspheres, such that non-radiopaque microspheres may be rendered intrinsically and permanently radiopaque, without adversely affecting the physical properties of the microsphere (i.e. size, spherical shape, high water content, swellability, and compressibility). The radiopaque microspheres have similar, or better, drug loading and elution properties as the non-radiopaque beads from which they are formed. The radiopacity of the microsphere is permanent or sufficiently long-lived to allow for monitoring during clinical follow up. The post-processing of pre-formed beads provides a degree of flexibility in terms of manufacturing in that the same manufacturing process can be used for radiopaque and non-radiopaque beads and size selection or sieving can be made prior to post-processing so that only a particular size or size range of beads may be made radiopaque.

Accordingly, in a first aspect, the present invention provides a polymer comprising 1,2-diol or 1,3-diol groups acetalised with a radiopaque species. The radiopacity of the polymer is thus derived from having a radiopaque material covalently incorporated into the polymer via cyclic acetal linkages.

As used herein, “radiopaque species” and “radiopaque material” refers to a chemical entity, or a substance modified by such a chemical entity, which is visible in X-ray radiographs and which can be resolved, using routine techniques, such as computed tomography, from the medium that surrounds the radiopaque material or species.

In this aspect the polymer can be any one which comprises 1,2-diol or 1,3 diol groups or a mixture thereof. Preferably the polymer comprises a high degree of diol groups throughout the polymer backbone, such as a polyhydroxypolymer. The polymer is suitably a hydrogel or other cross-linked polymer network. Particularly suitable polymers are those which comprise polyvinyl alcohol (PVA) or copolymers of PVA. PVA based hydrogels are particularly preferred as these are well known in the art and are used extensively in embolization procedures.

In a particular embodiment, the polymer comprises a PVA backbone which has pendant chains bearing crosslinkable groups, which are crosslinked to form a hydrogel. The PVA backbone has at least two pendant chains containing groups that can be crosslinked, such as acetates and acrylates. The crosslinkers are desirably present in an amount of from approximately 0.01 to 10 milliequivalents of crosslinker per gram of backbone (meq/g), more desirably about 0.05 to 1.5 meq/g. The PVA polymers can contain more than one type of crosslinkable group.

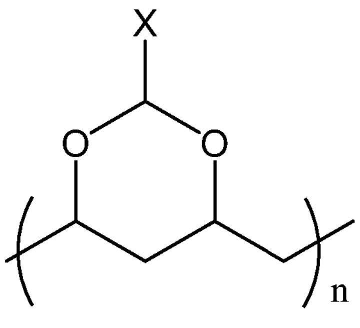
The pendant chains are conveniently attached via the hydroxyl groups of the polymer backbone are attached via cyclic acetal linkages to the 1,3-diol hydroxyl groups of the PVA

Cross linking of the modified PVA may be via any of a number of means, such as physical crosslinking or chemical crosslinking. Physical cross linking includes, but is not limited to, complexation, hydrogen bonding, desolvation, Van der wals interactions, and ionic bonding. Chemical crosslinking can be accomplished by a number of means including, but not limited to, chain reaction (addition) polymerization, step reaction (condensation) polymerization and other methods as will be routine for the polymer chemist.

The crosslinked groups on the PVA polymer backbone are suitably ethylenically unsaturated functional groups, such as acetates, which can be crosslinked via free radical initiated polymerization without requiring the addition of an aldehyde crosslinking agent. Preferably, the PVA polymer comprises pendent actetate groups formed from the acetalisation of PVA with N-acryloyl-aminoacetaldehyde dimethylacetal (NAADA). Such a modification of PVA is described in US patent number 5,583,163. An example of this type of modified PVA is Nelfilcon A.

The cross-linkable PVA is suitably cross-linked with an additional vinylic comonomer and, suitably a hydrophilic vinylic comonomer such as hydroxy-substituted lower alkyl acrylates and methacrylates, acrylamides and methacrylamides. In a particular embodiment the modified PVA as described above is crosslinked with 2-acrylamido-2-methylpropanesulfonic acid (AMPS® monomer from Lubrizol Corporation) to provide an acrylamido Polyvinyl Alcohol–co–acrylamido-2-methylpropane sulfonate hydrogel. In a preferred embodiment the cross-linking reaction is performed as am inverse emulsion polymerisation reaction to yield the acrylamido polyvinyl alcohol–co–acrylamido-2-methylpropane sulfonate hydrogel in the form of microspheres.

The polymer or hydrogel of the invention is radiopaque by virtue of a covalently attached radiopaque material throughout the polymer in the form of a cyclic acetal. Reactions for the formation of cyclic acetals are well known in organic chemistry and, thus, any radiopaque species which is able to form cyclic acetals is envisaged within the scope of the invention. Many materials are known to be radiopaque, such as Iodine, Bismuth, Tantalum, Gadolinium, Gold, Barium and Iron. Electron dense elements, such as the halogens, are particularly useful. Bromine, chlorine, fluorine and iodine can readily be incorporated into organic molecules which are able to form cyclic acetal linkages and provide a high degree of radiopacity. Consequently, in a particular embodiment the radiopaque polymer comprises a covalently attached halogen, preferably iodine. In this embodiment the radiopaque polymer has a structure according to General Formula I:



Wherein X is a group substituted by one or more halogens and preferably one or more iodine moieties

Preferably, iodine is provided in the form of an iodinated phenyl group. As above, the iodinated phenyl groups are incorporated into the polymer through cyclic acetal linkages.

Iodinated phenyl groups are useful because they can be mono, di, tri or even tetra-substituted in order to control the amount of iodine that is incorporated into the radiopaque polymer. It will be understood by the person skilled in the art that the amount of iodine in the polymer may also be controlled by controlling the degree of acetalisation in the polymer. In the present invention, the polymer comprises up to 50% of acetalised diol groups. Preferably at least 10% of the diol groups in the polymer are acetalised and more preferably at least 20% of the diols groups are acetalised. Whether the amount of iodine in the polymer is controlled by increasing the substitution on a phenyl ring or by controlling the degree of acetalisation of the polymer, the resulting polymer contains at least 10% iodine by dry weight. Preferably the polymer contains at least 20% iodine by dry weight and preferably greater than 30%, 40%, 50% or 60% iodine by dry weight. A useful contrast is obtained with polymers having between 30 and 50% iodine by dry weight.

The present invention is particularly suited to hydrogels and, in particular, hydrogels in the form of microparticles or microspheres. Microspheres are particularly useful for embolization as sizes of microsphere can be controlled and unwanted aggregation of embolic avoided. Microspheres can be made by a number of techniques known to those skilled in the art, such as single and double emulsion, suspension polymerization, solvent evaporation, spray drying, and solvent extraction.

In a particular embodiment the hydrogel microspheres are prepared from PVA modified with N-acryloyl-aminoacetaldehyde dimethylacetal (NAADA), as described above (and disclosed in US5,583,163) and cross-linked with 2-acrylamido-2-methylpropanesulfonic acid, as described above. Hydrogel microspheres of this type are described in US 6,676,971 and US 7,070,809.

Microspheres can be made in sizes ranging from about 10 μm (microns) to 2000μm. In most applications it will be desirable to have a small size range of microspheres. The process used to make the microspheres can be controlled to achieve a particular desired size range of microspheres. Other methods, such as sieving, can be used to even more tightly control the size range of the microspheres.

In a particular embodiment hydrogel microspheres according to the invention have a mean diameter size range of from 10 to 2000µm, more preferably 20 to 1500µm and even more preferably, 40 to 900 µm. Preparations of microspheres typically provide particles in size ranges to suit the planned treatment, for example 100-300, 300-500, 500-700 or 700-900 microns. Smaller particles tend to pass deeper into the vascular bed and so for certain procedures, particles in the range 40-75 and 70-150 microns are particularly useful.

In a particular embodiment, the polymer is a hydrogel microsphere with a net negative charge at physiological pH.

The hydrogel microspheres of this embodiment may be used in composition with suitable excipients or diluents, such as water for injection, and used directly to embolise a blood vessel. Consequently pharmaceutical compositions comprising radiopaque hydrogel microsphere which are formed from a polymer comprising 1,2-diol or 1,3-diol groups acetalised with a radiopaque species form a second aspect of the invention. In this aspect, it is preferred that the polymer comprises an iodinated aromatic group covalently bound to the polymer through cyclic acetal linkages as described above.

Alternatively, or additionally, an effective amount of one or more biologically active agents can be included in the embolic compositions. It may be desirable to deliver the active agent from the formed hydrogel. Biologically active agents that it may be desirable to deliver include prophylactic, therapeutic, and diagnostic agents including organic and inorganic molecules and cells (collectively referred to herein as an "active agent", “therapeutic agent” or “drug"). A wide variety of active agents can be incorporated into the radiopaque microspheres. Release of the incorporated active agent from the hydrogel is achieved by diffusion of the agent from the hydrogel, degradation of the hydrogel, and/or degradation of a chemical link coupling the agent to the polymer. In this context, an "effective amount" refers to the amount of active agent required to obtain the desired effect.

Accordingly in a further aspect the invention provides a pharmaceutical composition comprising a radiopaque hydrogel microsphere as described above and a therapeutic agent wherein the therapeutic agent is absorbed into the hydrogel matrix. Examples of active agents that can be incorporated include, but are not limited to, anti -angiogenic agents and chemotherapeutic agents, making the microspheres particularly useful for chemoembolization procedures.

In a particularly advantageous embodiment, the radiopaque hydrogel microspheres of the invention have a net charge such that charged drugs may be loaded into the microsphere by an ion exchange mechanism. As a result, the therapeutic agent is electrostatically held in the hydrogel and elutes from the hydrogel in electrolytic media or in-vivo to provide a sustained release of drug over several hours or even days.

In this embodiment it is particularly useful if the radiopaque hydrogel microspheres of the invention have a net negative charge such that positively charged drugs may be controllably and reproducibly loaded into the microsphere, and retained therein electrostatically, for subsequent prolonged elution from the hydrogel in-vivo. It will be understood that drugs without charge at physiological pHs may still be loaded into microspheres of the invention and this may be particularly advantageous when rapid elution or a “burst effect” is desired, for example, immediately after embolization or simply for rapid drug delivery to tissue in cases where embolization is not required or necessary.

Particularly preferred examples of drugs which may be loaded in this way include, but are not limited to, camptothecins (such as irinotecan) and anthracyclines (such as doxorubicin). Other preferred drugs include 5-FU, vandetanib, idarubicin, mitomycin, mitoxantrone, epirubicin, daunorubicin, bleomycin, topotecan, vinblastine, vincristine, vinorelbine, erlotinib, gefitinib, imatinib, laptinib, sunitinib, sorafenib, anastrozole, abiraterone, amifostine, buserelin, degarelix, folinic acid, goserelin, lanreotide, lenalidomide, letrozole, leuprorelin, octreotide, tamoxifen, triptorelin, bendamustine, chlorambucil, dacarbazine, melphalan, procarbazine, temozolomide, cytarabine, fludarabine, gemcitabine, methotrexate, pemetrexed and raltitrexed.

The radiopaque hydrogel microspheres are preferably water-swellable but water-insoluble.

In an embodiment the beads are water-swellable but have some solubility in water. In this embodiment, the extent of swelling may be controlled by the use of aqueous salt solutions or suitable solvents, as may be determined by routine experimentation. This may be particularly applicable to PVA polymers which are non-covalently crosslinked.

In another embodiment the beads are water and solvent-swellable but are also biodegradable. In this embodiment the beads biodegrade in-vivo over a period ranging from 4 weeks to 24 months.

As discussed above the radiopaque polymers of the invention may be made by utilizing straightforward chemistry to directly modify pre-formed microspheres to make them intrinsically radiopaque. Accordingly, in a further aspect, the invention provides a method of making a radiopaque polymer comprising reacting a polymer comprising 1,2- diol or 1,3-diol groups with a radiopaque species capable of forming a cyclic acetal with said 1,2-diol or 1,3 diols under acidic conditions.

The chemistry is particularly suited to polymers with a backbone of units having a 1,2-diol or 1,3-diol structure, such as polyhydroxy polymers. For example, polyvinyl alcohol (PVA) or copolymers of vinyl alcohol contain a 1,3-diol skeleton. The backbone can also contain hydroxyl groups in the form of 1,2-glycols, such as copolymer units of 1,2-dihydroxyethylene. These can be obtained, for example, by alkaline hydrolysis of vinyl acetate-vinylene carbonate copolymers. Other polymeric diols can be used, such as saccharides. In a particular embodiment, the polymer is cross-linked, such as crosslinked PVA or copolymers of PVA.

Polyvinyl alcohols that can be derivatized as described herein preferably have molecular weight of at least about 2,000. As an upper limit, the PVA may have a molecular weight of up to 1,000,000. Preferably, the PVA has a molecular weight of up to 300,000, especially up to approximately 130,000, and especially preferably up to approximately 60,000.

In a preferred embodiment, the PVA is a cross-linked PVA hydrogel, in which PVA modified with N-acryloyl-aminoacetaldehyde dimethylacetal (NAADA) is cross-linked with 2-acrylamido-2-methylpropanesulfonic acid, as described above, preferably in the form of microspheres, as described in US 6,676,971 and US 7,070,809

The radiopaque species is acetalised, and covalently attached to the polymer, through diol groups. Preferred radiopaque species are electron dense chemical moieties, such as simple organic molecules or organometallic complexes providing radiopacity greater than +1 HU, and which comprises a reactive moiety that enables formation of a cylic acetal with diol groups on the polymer. Particular reactive moieties include aldehydes, acetals, thioacetals and dithioacetals.

In a particular embodiment the radiopaque species comprises iodine. This is convenient because small organic molecules in which iodine has been substituted are commercially available or may be prepared using chemistry well known in the art. For example, iodinated aldehydes are radiopaque and are readily incorporated into diol-containing polymers using the method of the invention. Particularly useful radiopaque species include iodinated benzyl aldehydes, iodinated phenyl aldehydes and iodinated phenoxyaldehydes.

The reaction of radiopaque aldehydes with diol-containing polymers works surprisingly well with hydrogel polymers, which have been pre-formed into microspheres. Thus, in another aspect the invention provides a method of making a radiopaque hydrogel microsphere comprising the steps of:

(a) swelling a pre-formed hydrogel microsphere comprising a polymer with 1,2-diol or 1,3-diol groups in a solvent capable of swelling said microsphere; and (b) mixing the swollen beads with a solution of a radiopaque species capable of forming a cyclic acetal with said 1,2 or 1,3 diols under acidic conditions; and (c) extracting the microspheres.

The extracted microspheres may then be used directly, formulated into pharmaceutical compositions as described above or dried for long-term storage.

In a preferred embodiment, the reaction is performed on an acrylamido polyvinyl alcohol–co–acrylamido-2-methylpropane sulfonate hydrogel microsphere. Examples of such microspheres are described in US 6,676,971 and US 7,070,809.

The reaction is conveniently is conducted in polar organic solvent, and more particularly, polar aprotic solvents such as tetrahydrofuran (THF), Ethyl acetate, acetone, dimethylformamide (DMF), acetonitrile (MeCN) and dimethyl sulfoxide (DMSO), although suitable solvents will determined by the skilled person through routine experimentation and/or consideration of solvent properties such as boiling point, density etc.

The reaction is rapid and may be conducted at room temperature or at elevated temperature to improve yields and decrease reaction time. In a preferred embodiment the reaction is conducted at a temperature greater than 25°C and suitably greater than 40°C but less than 135°C and preferably less than 80°C. Reaction temperatures between 50 and 75°C are particularly useful. At elevated temperatures the conversion of hydrogel bead to radiopaque hydrogel bead can be accomplished in as little as 2-3 hours.

As above, the radiopaque species comprises a functional group selected from the group consisting of aldehydes, acetals, thioacetals and dithioacetals and comprises iodine. In this context, acetals and thioacetals may be considered to be protected aldehydes. Iodinated aldehydes, such as iodinated benzyl aldehyde, iodinated phenyl aldehyde or iodinated phenoxyaldehyde, are particularly useful and they are widely available and give good reaction yields.

Specific examples of radiopaque species that have been shown to produce radiopaque PVA hydrogel microspheres include 2,3,5-triiodobenzaldehyde, 2,3,4,6-tetraiodobenzyaldehyde and 2-(2,4,6-triiodophenoxy)acetaldehyde

In a further aspect the invention provides a radiopaque hydrogel microsphere obtained by the reaction of a polymer comprising 1,2-diol or 1,3-diol groups with an iodinated aldehyde, an iodinated acetal or an iodinated thioacetal.

In a preferred embodiment of this aspect the radiopaque hydrogel microsphere is obtained by the reaction of an acrylamido polyvinyl alcohol–co–acrylamido-2-methylpropane sulphonate hydrogel microspshere. These microspheres, examples of which are disclosed in US 6,676,971, US 7,070,809 and WO 2004/071495, have been found to react rapidly with iodinated aldehydes, iodinated acetals and iodinated thioacetals to yield radiopaque microspheres with high iodine content and provide good contrast in-vivo. The physical characterisatics (size, shape, charge, drug loading ability etc) are not adversely affected by iodination and in some cases are improved. Handling also appears to be largely unaffected. Mechanical robustness is preserved and the beads do not aggregate and suspend well in contrast agent and other delivery vehicles, such that delivery through a catheter may be achieved with relative ease. Delivery has been observed to be smooth and even, without any blocking of the catheter. Furthermore, the beads are stable to steam and autoclave sterilization methods.

Particularly suitable iodinated aldehydes include, but are not limited to, 2,3,5-triiodobenzaldehyde, 2,3,4,6-tetraiodobenzyaldehyde and 2-(2,4,6-triiodophenoxy)acetaldehyde

The radiopaque microspheres and compositions described above may be used in a method of treatment in which the microsphere or composition is administered into a blood vessel of a patient to embolise said blood vessel. The blood vessel is likely one associated with solid tumour, such as hypervascular hepatic tumors including hepatocellular carcinoma (HCC) and some other hepatic metastases including metastatic colorectal carcinoma (mCRC) and neuroendocrine tumors. The methods of treatment are imageable and provide the clinical with good visual feedback on the procedure in real-time or near real-time.

The microspheres described herein are surprisingly efficient in loading and eluting drugs. The microspheres readily load positively charged drugs, such as doxorubicin, epirubicin, daunorubicin, idarubicin and irinotecan. Experimental studies have shown that the ability of the microsphere to load and elute drug is the same before beads are rendered radiopaque using the chemistry of the invention as it is after reaction. In some cases, drug loading efficiency is surprisingly improved by more than 50%. In some cases, an increase of 100% in drug loading has been measured. In all cases, the extent of drug elution is unaffected, as compared to the non-radiopaque version of the beads, with substantially all of the drug eluted from the bead over a sustained period. In many cases the drug elution profile is improved in that the time over which drug is eluted from radiopaque microspheres is increased as compared to equivalent non-radiopaque microspheres. The microspheres of the invention thus, surprisingly provide increased drug loading efficiency and improved i.e. prolonged drug-elution over their non-radiopaque equivalents.

The polymers or microspheres of any of the above aspects and embodiments of the invention may be used in another aspect of the invention in which a method of imaging an embolization procedure is provided. In a further aspect a method of monitoring embolization after the completion of a procedure is provided. Depending on the permanent or rate of biodegradation of the radiopaque polymers of the invention, the post-procedural window in which the embolization may be monitored can be in the range of days, weeks or even months.

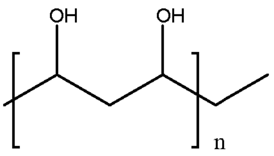
The invention will now be described by way of example with reference to the drawings in which

Figure 1 is micrograph of radiopaque hydrogel beads prepared according to the examples. The beads shown are 75-300μm, sieved after iodination, under different lighting conditions.

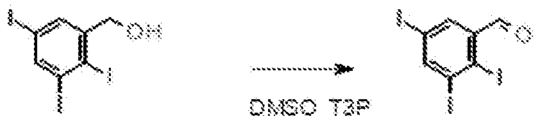
Figure 2 is microCT image of radiopaque beads prepared according to the invention. Figure 2A is a 3D radiograph of radiopaque beads. Figure 2B shows a 2D microCT image. The line profile (Figure 2C) shows: the x-axis (µm) is the length of the line drawn (shown in red across a section of the radiograph; and the y-axis indicates the level of intensity, using grey scale values, ranging from 0 (black) to 255 (white). T

Figure 3 shows light micrographs of sterilized radiopaque beads prepared according to the invention, before and after loading with doxorubicin. Figure 3A shows the radiopaque beads prior to loading and Figure 3B shows the drug-loaded beads.

Throughout these examples the structure of polymer comprising 1,2-diol or 1,3-diol groups is represented by the following structure :

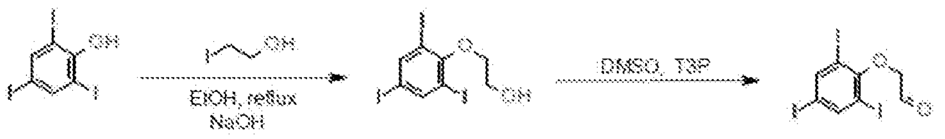


Example 1: Preparation of 2,3,5-triiodobenzaldehyde from 2,3,5-triiodobenzyl alcohol



In a 50ml three-necked round-bottomed flask fitted with a thermometer, a nitrogen bubbler and an air-tight seal, 10.2g of the alcohol was dissolved in 100ml of anhydrous DMSO under a nitrogen blanket and stirring conditions. Then, 1.0 molar equivalent of propane phosphonic acid anhydride, T3P, (50% solution in ethyl acetate) was added drop by drop over 5 minutes at 22°C to 25°C. The reaction solution was stirred at room temperature and monitored by high performance liquid chromatography. The conversion finished after 240 minutes. The yellow solution was poured into 100ml of deionised water while stirring, yielding a white precipitate which was filtered, washed with the mother liquors and 50ml of deionised water. The cake was slurried in 50ml of ethyl acetate, filtered and washed with 50ml of water again, dried sub vacuo at 40°C for 20 hours to yield 7.7g of a white solid. The structure and purity were confirmed by NMR analysis and high performance liquid chromatography.

Example 2: Preparation of 2-(2,3,5-triiodophenoxy)acetaldehyde



a) Synthesis of 2-(2,4,6-triiodophenoxy)ethanol from 2,4,6-triiodophenol

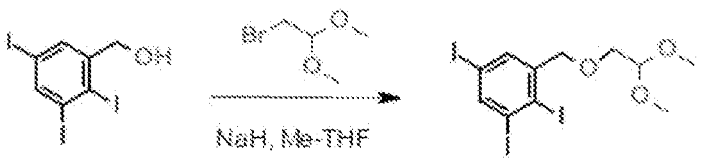
In a 500ml three-necked flat-bottomed flask fitted with a thermometer, a nitrogen bubbler and an overhead stirrer, 10g of phenol were dissolved in 100ml of ethanol, under a nitrogen blanked and vigorous stirring conditions at room temperature. 1.25 molar equivalent of sodium hydroxide pellets were added and the slurry was stirred under a nitrogen blanket for 30 minutes until complete dissolution of the pellets. Then, 1.1 molar equivalents of 2-iodoethanol were added, maintaining the temperature at 25°C and stirring for 15 minutes. The solution was heated to reflux of ethanol. The consumption of the phenol and formation of 2-(2,4,6-triiodophenoxy)ethanol were monitored by high performance liquid chromatography. After 25 hours, an additional 0.27 molar equivalents of 2-iodoethanol was added and the solution was stirred for a further 2 hours at reflux. After cooling the solution to room temperature, 150ml of deionised water were added quickly under vigorous stirring conditions. The resulting slurry was filtered under vacuum, washed with the mother liquors, three times 30ml of deionised water and finally with 5ml of ethanol.

The resulting pink cake was taken up into 100ml of ethyl acetate and the organic layer extracted with copious amounts of a sodium hydroxide solution, dried over magnesium sulphate and concentrated on a rotary evaporator to yield 5.9g of an off-pink solid, which was identified as 2-(2,4,6-triiodophenoxy)ethanol by comparative analysis with a commercial analytical standard from sigma-aldrich.

(b) Oxidation of 2-(2,4,6-triiodophenoxy)ethanol to 2-(2,3,5-triiodophenoxy)acetaldehyde:

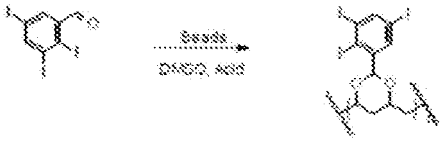
In a 500ml three-necked flat-bottomed flask fitted with a thermometer, a nitrogen bubbler and an overhead agitator, 5.9g of the alcohol was dissolved into 150ml of anhydrous DMSO under a nitrogen blanket. The solution was stirred and heated to 40°C, and 1.6 molar equivalents of T3P (50%w/w solution in EtOAc) were added slowly while maintaining the temperature at 40°C to 41°C. The consumption of alcohol and production of aldehyde was monitored by high performance liquid chromatography over time. After 24 hours, 150ml of water were added slowly to the reaction mixture over 2 hours using a syringe pump. An off-pink solid precipitated out of the solution and was filtered under vacuum to yield a pink cake which was washed with water. The resulting impure flocculate was taken up in ethyl acetate and hexane, then concentrated under vacuum at 40°C to yield an oil identified as 2-(2,3,5-triiodophenoxy)acetaldehyde by 1H NMR analysis.

Example 3: Synthesis of 1-(2,2-dimethoxyethoxymethyl)-2,3,5-triiodo-benzene from 2,3,5-triiodobenzyl alcohol and 2-bromo-1,1-dimethoxy-ethane (Example of a radiopaque acetal/protected aldehyde)



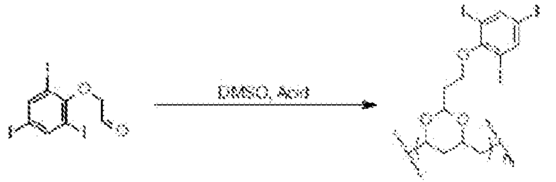
In a 50 ml three-necked flat-bottomed flask fitted with an overhead agitator, a thermometer, a nitrogen bubbler and a gas tight septum, 5.07g of the alcohol were dissolved in 55ml of anhydrous 2-methyltetrahydrofuran under a nitrogen blanket and stirring conditions. Then, 2.11g of the acetal followed by 0.540g of sodium hydride (60% dispersion in mineral oil) were added. The slurry was heated to reflux under a nitrogen blanket for 1010 minutes and monitored by high performance liquid chromatography. The reaction mixture was taken up into 50ml of dichloromethane and washed four times with 25ml of water. The organic layer was concentrated sub vacuo to yield a brown oil, which was identified as 1-(2,2-dimethoxyethoxymethyl)-2,3,5-triiodo-benzene by 1H NMR.

Example 4: Preparation of radiopaque microspheres from 2,3,5-triiodobenzaldehyde and a cross-linked PVA hydrogel microsphere



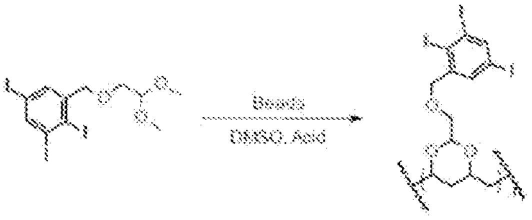
In a 50ml three-necked round-bottomed flask fitted with an overhead agitator, a thermometer and a nitrogen bubbler, 1.0g of dry PVA-based beads were swollen in an appropriate solvent (e.g. DMSO) under a nitrogen blanket and stirring conditions. Then, 0.40 to 1.5 molar equivalents of aldehyde (prepared according to example 1) were added to the slurry, immediately followed by 1.0 to 10 molar equivalents of acid (e.g. sulphuric acid, hydrochloric acid, methanesulfonic acid or trifluoroacetic acid). The reaction slurry was stirred at 50°C to 130°C for between 12 hours and 48 hours, while the consumption of aldehyde was monitored by high performance liquid chromatography. When required, a desiccant such as magnesium sulphate or sodium sulphate was added to drive the reaction further. When enough aldehyde had reacted on the 1,3-diol of the PVA-based hydrogel to render it sufficiently radio-opaque, the reaction slurry was cooled to room temperature and filtered. The cake of beads was washed with copious amount of DMSO and water, until free from any unreacted aldehyde, as determined by high performance liquid chromatography.

Example 5: Preparation of Radiopaque PVA hydrogel beads with 2-(2,4,6-triiodophenoxy)acetaldehyde.



2-(2,4,6-triiodophenoxy)acetaldehyde was prepared according to Example 2 and reacted with PVA-based hydrogel beads following the same method as Example 4 but with the temperature of the reaction maintained between 20°C and 50°C. The reaction time was also reduced to less than one hour.

Example 6: Preparation of radiopaque PVA hydrogels with 1-(2,2-dimethoxyethoxymethyl)-2,3,5-triiodo-benzene



In a 50 ml three-necked flat-bottomed flask fitted with an overhead agitator, a thermometer and a nitrogen bubbler, 1.0g of dry PVA-based beads were swollen in an appropriate solvent (e.g. DMSO) under a nitrogen blanket and stirring conditions. Then, 0.5 molar equivalents of aldehyde (1-(2,2-dimethoxyethoxymethyl)-2,3,5-triiodo-benzene, prepared according to Example 3) were added to the slurry, immediately followed by 163µl of methanesulfonic acid. The reaction slurry was stirred at 40°C for 80 minutes, and then heated to 80°C for 200 minutes, while the consumption of aldehyde was monitored by high performance liquid chromatography. As enough aldehyde had reacted on the 1,3-diol of the PVA-based hydrogel to render it sufficiently radio-opaque, the reaction slurry was cooled to room temperature and filtered. The cake of beads was washed with copious amounts of DMSO and water, until free from any unreacted acetal and aldehyde, as determined by high performance liquid chromatography.

Example 7: Preparation of Radiopaque PVA hydrogel microspheres from 2,3,4,6 tetraiodobenzaldehyde

2,3,4,6-tetraiodobenzyl alcohol was converted to 2,3,4,6 tetraiodobenzaldehyde using T3P and DMSO in a reaction as described in Example 1. 0.6 molar equivalents of 2,3,4,6 tetraiodobenzaldehyde was then added to 2.05g of PVA hydrogel microspheres (size 150-250µm) with DMSO under a nitrogen blanket. The reaction mix was heated to 50°C and stirred for several hours. The reaction was monitored with HPLC and when complete, the beads were filtered and washed with DMSO, water and then 0.9% saline. The radiopaque beads were then stored in a solution of 0.9% saline for analysis.

Example 8: Characterization of Radiopaque Beads

A light micrograph of the beads, typical of those produced by Example 4, 5 and 6 is shown in Figure 1. The dry weight of beads were measured by taking certain volume of beads with packing saline, and removing the free saline with tissue wicking. Then the beads were vacuum dried under 50°C overnight to remove water, and the dry bead weight and solid content (w/w %) of polymer were obtained from this.

The iodine content (w/w %) in dry beads were measured by elemental analysis.

For iodine content in wet beads, the calculation is

Bead solid content (%) x iodine content in dry beads (%)

The solid content of the hydrogel beads in a 0.9% saline solution was measured to be between 5% and 16%, while the weight/weight dry iodine content was measured to be between 5% and 56%, depending on the chemistry and the reaction conditions used. Beads prepared according to Example 4 had 55% iodine (dry).

Another way to express the iodine content in wet beads is mg I/mL beads, same as the unit used for contrast media. In this experiment the iodine content range was 26 mg I/ml beads to 214 mg I/ml beads.

Example 9 – MicroCT analysis of radiopaque beads

Micro-CT was used to evaluate the radio-opacity of samples of radiopaque embolic Beads prepared according to Example 4 above

The samples were prepared in Nunc cryotube vials (Sigma-Aldrich product code V7634, 48 mm × 12.5 mm). The beads were suspended in 0.5% agarose gel (prepared with Sigma-Aldrich product code A9539). The resulting suspension is generally referred to as a "Bead Phantom". To prepare these bead phantoms, a solution of agarose (1%) is first raised to a temperature of approximately 50°C. A known concentration of the beads is then added, and the two gently mixed together until the solution starts to solidify or gel. As the solution cools it gels and the beads remain evenly dispersed and suspended within the agarose gel.

Bead phantoms were tested for radio-opacity using micro-Computer Tomography (μCT) using a Bruker Skyscan 1172 μCT scanner at the RSSL Laboratories, Reading, Berkshire, UK, fitted with a tungsten anode. Each phantom was analysed using the same instrument configuration with a tungsten anode operating at a voltage of 64kv and a current of 155 μA. An aluminium filter (500μm) was used. Radio-opacity was reported in greyscale units from line scan projections across the bead. Values used for dynamic range for all samples in NRecon (thresholding): -0.005, 0.13 (minimum and maximum attenuation coefficient). A typical image and line scan is shown in Figure 2. The average radiopacity of the beads was found to be 109 greyscale units.

Example 10(a) Drug Loading of Radiopaque beads

1 mL of radio-opaque bead slurry was measured by using a measuring cylinder, followed by removal of liquid. 4 mL of doxorubicin solution (25 mg/mL) was mixed with the radio-opaque beads with constant shaking at ambient temperature. After 20 hr loading, the depleted solution was removed, and the drug-loaded beads were rinsed with deionised water 10 mL for 4-5 times. By measuring the doxorubicin

concentration of combined depletion solution and rinsing solution at 483 nm on a Varian UV spectrophotometer, the doxorubicin loaded was calculated as 80 mg/mL beads. The doxorubicin hydrochloride drug loading capacity of the radio-opaque beads was determined to be a non-linear function of the iodine content in the beads.

Example 10(b) Drug Loading of Radiopaque Beads

Radiopaque beads prepared according to Example 4 above were loaded with 37.5mg/ml of Doxorubicin solution as per the method of Example 10(a). Figure 3A shows the radiopaque beads prior to loading and Figure 3B shows the drug-loaded beads. Prior to drug loading the beads were observed as spherical microspheres with a pale to dark brown tinge. When the doxorubicin was loaded into the beads they turned a strong red colour. In this example, the beads were autoclaved to demonstrate to stability of the beads during sterilization. Bead integrity was preserved during autoclaving; the mean bead size during autoclaving reduced from 177μm to 130μm. Further shifts in the bead size distribution were observed when beads were loaded with Doxorubicin, which is consistent with drug-loading observed with non-radiopaque beads. In another example, the mean bead size reduced on drug loading at 51mg/ml, from 130μm to 102μm. The resulting beads remain within the range that is clinically useful, even after modification, sterilization, and drug-loading.

Example 11 – Radiopacity of Drug-Loaded Radiopaque Beads

An aliquot of the drug loaded beads prepared according to Example 10 were subjected to microCT analysis in the same way as described in example 9. The drug-loaded beads were found to be radiopaque. The average Bead Radio-opacity (Grey Scale) was determined to be 139 (n=3).

Example 12 – Preparation of Radiopaque PVA-AMPS Hydrogel Microspheres:

The method described in Example 4, above, except that: dry acrylamido polyvinyl alcohol–co–acrylamido-2-methylpropane sulphonate hydrogel microspsheres, of size range 100-300μm were swollen in DMSO and mixed with 0.65 molar equivalents (mole of aldehyde to mole of 1,3-diol) of triiodobenzaldehyde in the presence of acid and allowed to react for 24 hours at 60°C. The reaction was quenched and beads, filtered, washed and recovered, then stored in 0.9% saline for further analysis.

The iodine content of the beads was found to be 53% iodine (grams of iodine to grams of dried bead) which corresponds to a 183mg Iodine per ml of packed wet beads. Scanning electron microscopy, with EDX analysis, confirmed homogeneous distribution of iodine throughout the beads.

CLAIMS

1. A hydrogel comprising 1,2-diol or 1,3-diol groups acetalised with a radiopaque species.

2. A hydrogel according to claim 1 wherein the hydrogel is a cross-linked polymer network.

3. A hydrogel according to claim 1 or 2 wherein the hydrogel comprises polyvinyl alcohol (PVA) in which the PVA comprises pendent actetate groups formed from the acetalisation of PVA with N-acryloyl-aminoacetaldehyde dimethylacetal and which is cross-linked with 2-acrylamido-2-methylpropanesulfonic acid.

4. A hydrogel according to any of claims 1 to 3 wherein the radiopaque species is acetalised in the hydrogel in the form of a cyclic acetal.

5. A hydrogel according to any of claims 1 to 4 wherein the radiopaque species comprises iodine.

6. A hydrogel according to claim 5 wherein the radiopaque species comprises an iodinated phenyl group.

7. A hydrogel according to either of claims 5 or 6 wherein the hydrogel comprises greater than 10% iodine by dry weight.

8. A hydrogel according to any preceding claim wherein the hydrogel is in the form of microparticles or microspheres.

9. A hydrogel according to claim 8 wherein the hydrogel is in the form of microspheres with a mean diameter size range of from 10 to 2000μm.

10. A hydrogel according to any preceding claim wherein the hydrogel has a net charge at physiological pH

11. A composition comprising a hydrogel according to any preceding claim and a therapeutic agent wherein the therapeutic agent is absorbed into the hydrogel matrix.

12. A composition according to claim 11 wherein the therapeutic agent is electrostatically held in the hydrogel and elutes from the hydrogel in electrolytic media.

13. A method of making a radiopaque polymer comprising reacting a polymer comprising 1,2- diol or 1,3-diol groups with a radiopaque species capable of forming a cyclic acetal with said 1,2-diol or 1,3 diols under acidic conditions.

14. A method according to claim 13 wherein the polymer is cross-linked.

15. A method according to claim 13 or 14 wherein the polymer comprises polyvinyl alcohol (PVA) in which the PVA comprises pendent actetate groups formed from the acetalisation of PVA with N-acryloyl-aminoacetaldehyde dimethylacetal and which is cross-linked with 2-acrylamido-2-methylpropanesulfonic acid..

16. A method according to any of claims 13 to 15 wherein the radiopaque species is an organic molecule or organometallic complex, with radiopacity >1 HU, and which comprises a reactive moiety selected from the group consisting of aldehydes, acetals, thioacetals and dithioacetals.

17. A method according to any of claim 13 to 16 in which the radiopaque species comprises iodine.

18. A method according to claim 17 wherein the radiopaque species is an iodinated aldehyde.

19. A method according to claim 18 wherein the radiopaque species is an iodinated benzyl aldehyde, iodinated phenyl aldehyde or an iodinated phenoxyaldehyde

20. A method of making a radiopaque hydrogel microsphere comprising the steps of:

(a) swelling a pre-formed hydrogel microsphere comprising a polymer with 1,2-diol or 1,3-diol groups in a solvent capable of swelling said microsphere; and

(b) mixing the swollen beads with a solution of a radiopaque species capable of forming a cyclic acetal with said 1,2 or 1,3 diols under acidic conditions; and

(c) extracting the microspheres.

21. A method according to claim 20 which further comprises the step of drying the extracted microspheres

22. A method according to either of claims 20 or 21 in which the reaction is conducted in polar organic solvent and at elevated temperature.

23. A method according to any of claims 20 to 22 in which the radiopaque species comprises a functional group selected from the group consisting of aldehydes, acetals, thioacetals and dithioacetals.

24. A method according to any of claims 20 to 23 in which the radiopaque species comprises iodine.

25. A method according to claim 24 wherein the radiopaque species is an iodinated aldehyde.

26. A method according to claim 24 wherein the radiopaque species is an iodinated benzyl aldehyde, an iodinated phenyl aldehyde or an iodinated phenoxyaldehyde

27 A method according to claim 26 wherein the radiopaque species is 2,3,5-triiodobenzaldehyde, 2,3,4,6-tetraiodobenzyaldehyde or 2-(2,4,6-triiodophenoxy)acetaldehyde.

28. A method according to any of claims 20 to 27 in which the preformed hydrogel microsphere is comprises PVA with pendent actetate groups formed from the acetalisation of PVA with N-acryloyl-aminoacetaldehyde dimethylacetal and which is cross-linked with 2-acrylamido-2-methylpropanesulfonic acid

29. A method of treatment in which a hydrogel according to any of claims 1 to 10 or a composition according to either of claims 11 or 12 is administered into a blood vessel of a patient to embolise said blood vessel.

30. A method according to claim 29 in which the blood vessel is associated with solid tumour.

31. A method according to claim 30 in which the tumour is hepatocellular carcinoma.

32. A hydrogel according to any of claims 1 to 10 for use in embolization of a blood vessel.

33. A composition according to either of claim 11 or 12 for use in embolization of a blood vessel

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Application No:	GB1315936.3	**Examiner:**	Mr Jason Scott
Claims searched:	1-33	**Date of search:**	26 February 2014

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1, 2, 4, 5, 7, 10, & 11 at least	EP 0391741 A MEROCEL See whole document and especially example 15-17 teaching the complexation of iodine to acetalised PVA.
A	-	US 2001/051670 A BIOCURE See whole document and especially [0073] suggesting attaching a contrast agent to the polymer backbone.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

C08F; C08J

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, MEDLINE

International Classification:

Subclass	Subgroup	Valid From
C08J	0003/075	01/01/2006
A61K	0049/04	01/01/2006
A61K	0051/12	01/01/2006
C08F	0008/18	01/01/2006
C08J	0007/12	01/01/2006