OPIC
Office de la propriété intellectuelle du Canada

CIPO
Canadian Intellectual Property Office

(12) (19) (CA) **Demande-Application**

(21) (A1) **2,243,174**
(86) 1997/01/16
(87) 1997/07/24

(72) BERGMANN, Martina, DE
(72) HELDMANN, Dieter, DE
(72) WEITSCHIES, Werner, DE
(72) FRITZSCH, Thomas, DE
(72) SUDMANN, Violetta, DE
(71) SCHERING AKTIENGESELLSCHAFT, DE

(51) Int.Cl.^{6} A61K 49/00
(30) 1996/01/18 (196 02 930.9) DE

(54) **SUSPENSIONS DE BULLES DE GAZ ET LEUR UTILISATION SOUS FORME D'AGENTS DE CONTRASTE ULTRASONORES**

(54) **GAS BUBBLE SUSPENSIONS AND THEIR APPLICATION AS AN ULTRASOUND CONTRAST MEDIUM**

(57) L'invention concerne des matrices poreuses constituées de substances de faible poids moléculaire servant à générer des suspensions de bulles de gaz stables, leur utilisation sous forme d'agents de contraste ultrasonores et des procédés de production de ces matrices et agents de contraste.

(57) The invention concerns porous matrices of low molecular substances for generating stable gas bubble suspensions, their application as an ultrasound contrast medium and the process for producing the matrices and medium.

PCT WELTORGANISATION FÜR GEISTIGES EIGENTUM
Internationales Büro

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 6 :		(11) Internationale Veröffentlichungsnummer: **WO 97/26016**
A61K 49/00	**A2**	(43) Internationales Veröffentlichungsdatum: 24. Juli 1997 (24.07.97)

(21) Internationales Aktenzeichen: PCT/EP97/00208

(22) Internationales Anmeldedatum: 16. Januar 1997 (16.01.97)

(30) Prioritätsdaten:
196 02 930.9 18. Januar 1996 (18.01.96) DE

(71) Anmelder *(für alle Bestimmungsstaaten ausser US)*: SCHERING AKTIENGESELLSCHAFT [DE/DE]; Müllerstrasse 178, D-13353 Berlin (DE).

(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: BERGMANN, Martina [DE/DE]; Kurfürstenstrasse 15, D-12249 Berlin (DE). HELDMANN, Dieter [DE/DE]; Krefelder Strasse 3, D-10555 Berlin (DE). WEITSCHIES, Werner [DE/DE]; Greisenaustra[illegible] 10961 Berlin (DE).

(81) Bestimmungsstaaten: AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, europäisches Patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Veröffentlicht
Ohne internationalen Recherchenbericht und erneut zu veröffentlichen nach Erhalt des Berichts.

(54) Title: GAS BUBBLE SUSPENSIONS AND THEIR APPLICATION AS AN ULTRASOUND CONTRAST MEDIUM

(54) Bezeichnung: GASBLASENSUSPENSIONEN UND DEREN VERWENDUNG ALS ULTRASCHALLKONTRASTMITTEL

(57) Abstract

The invention concerns porous matrices of low molecular substances for generating stable gas bubble suspensions, their application as an ultrasound contrast medium and the process for producing the matrices and medium.

(57) Zusammenfassung

Die Erfindung betrifft poröse Matrices aus niedermolekularen Substanzen zur Generierung stabiler Gasblasensuspensionen, deren Verwendung als Ultraschallkontrastmittel sowie Verfahren zur Herstellung der Matrices und Mittel.

WO 97/26016 PCT/EP97/00208

GAS BUBBLE SUSPENSIONS AND THEIR USE AS ULTRASONIC CONTRAST MEDIA

The invention relates to the subject that is characterized in the claims, i.e., porous matrices for generating stable gas bubble suspensions, their use as ultrasonic contrast media, and a process for the production of the matrices and media.

Ultrasonic diagnosis offers the ability to diagnose physiologic and pathophysiologic conditions without burdensome ionizing rays such as those associated with x-ray or radionuclide studies and to do so relatively economically in comparison with magnetic resonance imaging. Ultrasound waves are reflected or absorbed depending on the acoustic properties of the tissue. For imaging, differing acoustic properties of tissues and bodily fluids are used. Owing to the large difference in density between body tissue or bodily fluids, on the one hand, and gas bubbles, on the other hand, gases in the form of microbubbles are especially well-suited as contrast media for ultrasound. Ultrasonic contrast media are therefore studied and developed essentially based on gas bubbles and/or gas-containing substances.

The simplest kind of ultrasonic contrast medium can be obtained by methods such as shaking, acoustic irradiation, or recycling of an aqueous suspension medium between two syringes. The bubbles that are introduced can be stabilized by suitable additives such as surfactants and/or viscosity-enhancing

substances. Such contrast media are described in, e.g., EP 0 077 752. The strong dependency of the number and size of the gas bubbles on the type, duration, and intensity of agitation is a problem. This makes the production difficult to reproduce and makes it impossible to control the risk of an embolism owing to overly large gas bubbles.

A similar type of contrast medium is described in WO 93/05819, whereby instead of the gases found in the atmosphere, such as air, nitrogen, carbon dioxide and noble gases, however, gases with a specific Q-factor are proposed for the production of bubble suspensions. In this case, these are generally halogenated hydrocarbons, which are distinguished by low solubility in physiological media. Especially perfluorinated compounds are suitable as exchange gases. Since, however, even in this case -- as described previously -- the bubble suspensions are introduced by recycling between two syringes via a three-way cock into the suspension medium, these media have a heterogeneous and poorly reproducible bubble size distribution. The risk of an embolism caused by overly large gas bubbles is thus increased.

In addition to the possibility of producing a gas bubble suspension immediately before use by agitating the medium, solid vehicles can also be formulated, from which bubbles are released after resuspension in a suitable diluent. For administration, a microparticle suspension is used here. Such solid vehicles can be microparticles that consist of, for example, a mixture of at least one surface-active substance with at least one non-surface-active solid, such as are disclosed in EP 0 365 467.

In addition to the substances mentioned in EP 0 365 467, non-surface-active solids can also be substances that are used as x-ray contrast media (WO 93/00930 and WO 92/21382), whereby in the case of the last-mentioned publication, the x-ray contrast media are crosslinked with one another via functional groups and crosslinkers.

The above-mentioned microparticle suspensions are ultrasonic contrast media with which contrast effects can be achieved in the arterial system. Contrast intensity and duration seem to be in need of improvement, however.

Other examples of microparticulate ultrasonic contrast media are disclosed in WO 95/21631. Here, water-insoluble wall-formers are dissolved in an organic solvent (toluene), then emulsified in an aqueous surfactant solution and freeze-dried. Resuspension yields a microparticle suspension that shows in vivo ultrasonic contrast. The use of specific fluorinated substances is also described for the microparticulate ultrasonic contrast medium type.

EP 0 554 213 thus discloses microparticles based on galactose that contain SF_6 instead of air. The contrast-prolonging effects of these media are weak, however.

WO 95/22994 also discloses microparticles that contain fluorinated substances. The bubble size distribution is standardized, and the number of bubbles is considerably increased, so that the dose that is necessary for ultrasonic contrast medium administration can be considerably reduced.

In WO 95/03835, ultrasonic contrast media that are based on particles that contain defined gas mixtures are claimed. The gas mixtures consist of at least one fluorinated, gas-osmosis-action component and at least one conventional gas, such as nitrogen, oxygen and/or carbon dioxide. The particles are composed of proteins, dextrins, starches and starch derivatives, such as, e.g., hydroxyethyl starches. As indicated in the publication, among the above-mentioned substances, those with a high molecular weight (> 500,000 dalton) are preferred since otherwise the stabilization of the gas bubbles is inadequate. Such substances cannot be filtered renally, however, and must, i.a., be catabolized via the liver. As a result, the retention time in the body is increased. During catabolization, the above-mentioned hydroxyethyl starch is cleaved into substituted oligosaccharides, which are renally eliminated primarily after failing to reach the renal threshold. As a possible problem, the storage of the hydroxyethyl starch in the cells of the reticuloendothelial system is discussed. In this case, the ethyl ether bond is not accessible for enzymatic catabolization. The metabolism and elimination of hydroxyethylglucose, as well as their possible pharmacological effect, have not yet been clarified [Krech, I.; Wind, S. (1995) Krankenhauspharmazie [Hospital Pharmaceutics] 16(2), 62-63]. Moreover, owing to their poor solubility, high-molecular substances involve the danger that, after the contrast medium preparation is resuspended, particulate portions of an uncontrolled size will be administered. This puts the patient at risk of embolism.

Particles that contain fluorinated gases and air are also claimed in WO 95/16467, whereby the proportion of fluorinated components is limited here to 41%.

WO 94/09829 describes liposome-containing ultrasonic contrast media. As surfactants that stabilize gas bubbles, phospholipids, but also other surfactants that are sparingly water-soluble, are mentioned. The production of such liposomal systems is generally done by freeze-drying solvents that can be freeze-dried, such as, e.g., tert-butanol or $C_2Cl_4F_2$. The use of organic solvents for the production of these media involves high expense for solvent recovery and necessitates a careful examination of the product with respect to residual solvent content. Moreover, because of their ozone-depleting potential, the halogenated hydrocarbons that are used as solvents, especially fluorinated chlorinated hydrocarbons (FCKW) such as $C_2Cl_4F_2$, have to be viewed with an extremely jaundiced eye.

The object of this invention was therefore to provide compounds for the production of ultrasonic contrast media that overcome the drawbacks of the prior art, i.e., ones which

- overcome existing pharmaceutical and pharmacological problems,
- can be produced easily and without using organic solvents,
- generate reproducible bubble sizes and numbers,
- result, in dissolved form, in high contrast intensity,
- lead to long-lasting contrast effects,
- exhibit high bubble stability and which, moreover

- can be filtered renally and thus can be quickly extracted.

This object is achieved by this invention.

It has been found that preparations that consist of a porous, solid, water-soluble matrix that contains a low-molecular skeleton former, a surfactant and a gas, whereby the gas is enclosed in the pores of the matrix, are extremely well suited for the production of a preparation for ultrasonic diagnosis.

Unlike the microparticulate contrast media of the prior art, the contrast media according to the invention are generated from a particle-free, porous, solid structure, which is referred to below as a porous matrix. To illustrate the basic structural differences, Figures 1 and 2 can be cited. Figure 1 shows a scanning electron microscope recording of a microparticulate preparation of the prior art (EP 0 365 467); Figure 2 shows a recording of a preparation according to the invention that is produced according to Example 19 at the same magnification (1 cm in the figures corresponds to 1.1 μm in reality). The uniform pores, from which gas bubbles are released after the matrix is dissolved, are readily visible. The sizes and numbers of pores exhibit high reproducibility. In this case, the bubble size is essentially limited by the pore size. Also determined by the porosity of the matrix is the number of bubbles that can be released from the matrix. The above-mentioned parameters (bubble size and number) can easily be controlled by various production parameters and have a major influence on the effectiveness of the contrast medium.

The formation of the gas bubbles also is not tied to agitation of the medium before administration, so that the gas bubbles can be released in unaltered form. It is particularly advantageous to be able to stabilize the released gas bubbles with the aid of surfactants, which optionally are components of the matrix. Thus, in the case of the media according to the invention, a stabilized gas bubble suspension is generally used for administration.

Owing to the high reproducibility of the generated bubbles, the danger of embolization of the lung by overly large bubbles is also minimized. Moreover, the use of low-molecular and thus readily soluble substances for matrix formation lowers the risk of administering undissolved, particulate formulation components.

The porous matrices according to the invention are composed of a water-soluble skeleton former, which generally has a molecular weight of < 15,000 dalton and a surfactant that dissolves quickly and readily in water, whereby the proportion of surfactant in the matrix is 0.01 to 10% (m/m). Since the skeleton former does not necessarily have to be soluble in an organic solvent, a broader selection of readily compatible skeleton formers becomes available.

Suitable skeleton formers are:

Amino acids, polyamino acids, peptides, proteins, mono-, di-, tri-, tetra-, oligo- and polymeric saccharides, as well as their derivatives, synthetic saccharides and saccharide derivatives. As examples, there can be mentioned L-glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-

proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-asparagine, L-glutamine, L-arginine, L-histidine, glycyl-glycine, glycyl-glycyl-glycine, glucose, galactose, fructose, mannose, sorbose, saccharose, lactose, maltose, trehalose, gentiobiose, lactulose, turanose, maltotriose, melibiose, melezitose, maltotetraose, maltopentaose, stachyose, arabinose, xylose, ribose, dulcitol, xylitol, mannitol, ribitol, inositol, sorbitol, α, ß, γ-cyclodextrins, hydroxypropyl-ß-cyclodextrin and other derivatives as well as polymeric saccharides with a molecular weight of < 15,000 dalton, such as, e.g., dextran 8, dextrins or synthetic saccharide polymerizates, such as, e.g., ficoll.

X-ray contrast media and contrast media for magnetic resonance imaging are also suitable as skeleton formers. There can be mentioned as examples iopromide, iotrolan, iopamidol, iohexol, as well as Gd-DTPA (gadopentetic acid), Gd-DTPA-dimeglumine salt (Magnevist), Gd-EOB-DTPA (gadoxetic acid, disodium salt) and gadobutrol.

According to the invention, substances that can be renally filtered unimpeded with a molecular weight of < 15,000 dalton (Silbernagl S., Despopoulos A., Taschenatlas der Physiologie [Paperback Atlas of Physiology], p. 132) are preferred, whereby saccharides with at least two sugar units, x-ray contrast media and contrast media for magnetic resonance imaging are especially suitable. The use of substances with a molecular weight of < 15,000 dalton provides a clear advantage compared to media of the prior art since renal elimination is ensured for such

substances. Stressing of the body by long retention times of a contrast medium component, as well as by metabolites, is thus avoided. Since water solubility generally increases with decreasing molecular weight, the danger of administering non-dissolved formulation components is also reduced.

Water-soluble, nonionic surfactants are suitable as surfactants, whereby those with a perfluorinated hydrocarbon component and/or with a molecular weight of < 15,000 dalton are preferred. There can be mentioned as examples sorbitan fatty acid ester, polyoxyethylenesorbitan fatty acid ester, polyoxyethylene sorbitol fatty acid ester, polyoxyethylene fatty acid ester, glycerine poloxyethylene fatty acid ester, ethoxylated mono-, di-, triglycerides, which can be optionally partially hydrogenated, as well as ethoxylated mixtures of the latter, ethoxylated castor oils, ethoxylated phenols, polyoxyethylene fatty alcohol ether, polyglycerol fatty acid ester, sorbitan perfluorofatty acid ester, polyoxyethylene sorbitan perfluorofatty acid ester, polyoxyethylenesorbitol perfluorofatty acid ester, polyoxyethylene perfluorofatty acid ester, polyoxyethylene perfluorofatty alcohol ether, polyglycerol perfluorofatty acid ester, polyoxyethylene polyoxypropylene polymers and/or fluoroalkyl poly(ethylenoxide) alcohols such as, e.g., Zonyl$^{(R)}$, saccharide fatty acid ester, saccharide fatty acid ether, ethoxylated saccharide fatty acid ester, ethoxylated saccharide fatty acid ether, fatty acid ethanolamides and/or ethoxylated fatty acid ethanolamide.

As surfactants, phospholipids, especially hydrogenated phosphatipylcholine, are also suitable.

As gases, in addition to the gases that have already been established in ultrasonic diagnosis, such as nitrogen, oxygen, CO_2 or air, fluorinated gases are also especially used. Surprisingly enough, even when "standard" gases are used, stronger and longer-lasting in vivo contrast effects are observed than are achieved with the particulate preparations of the prior art.

When fluorinated gases are used, surprisingly enough, the use of gas mixtures, such as are necessary with the preparations of the prior art, is no longer necessary.

According to the invention, the following substances which are gaseous at room temperature and normal pressure are preferred:

Tetrafluoroallenes, hexafluoro-1,3-butadiene, decafluorobutane, perfluoro-1-butenes, perfluoro-2-butenes, perfluoro-2-butine, octafluorocyclobutane, perfluorocyclobutene, perfluorocyclopentane, perfluorodimethylamine, hexafluoroethane, tetrafluoroethylenes, pentafluorothio(trifluoro)methane, tetrafluoromethane, perfluoropropane and perfluoropropylene.

Among the above-mentioned, the perfluorinated substances are especially preferred.

Another aspect of the invention relates to a process for the production of matrices according to the invention. The matrices according to the invention can be produced at lower expense, under aseptic conditions, by first an aqueous skeleton former

solution being made available, to which it is especially advantageous to add gas-bubble-stabilizing surfactants. The separated or combined solutions can be sterilized by filtration first, and the mixture thus produced is then quickly frozen. The removal of water is done under conditions that allow a direct transition of the ice into the gaseous state, bypassing the liquid aggregate state. Possible suitable conditions are shown in the phase diagram of water (see Figure 3). A porous matrix remains, which is aerated with the gas desired in each case. To ensure complete gas exchange (i.e., for removal of the residual air that is contained in the pores of the matrix, or the water vapor), repeated evacuation with subsequent pressure compensation is advisable. To obtain a gas phase that is as pure as possible, a vacuum of < 0.1 mbar should prevail immediately before pressure compensation. It is appropriate to carry out production in containers that can be sealed during the desired gas phase; said containers can later be used directly as part of a kit.

A special advantage of the process according to the invention is that the use of an organic solvent is no longer necessary. Technologically demanding processing, such as would be necessary in the case of sparingly soluble or slowly soluble or only water-dispersable substances, is also unnecessary. Residual solvent contents as a critical quality feature thus play no role for the media according to the invention. This is of considerable advantage both from an ecological standpoint and for purposes of product safety. Thus, many organic solvents

themselves, even in extremely small amounts or concentrations, are suspected of being carcinogens and/or mutagens.

From the matrices according to the invention, the desired particle-free ultrasonic contrast media can easily be produced by adding an aqueous medium. The addition of the aqueous medium is done by the attending physician immediately before use. The aqueous medium can contain the adjuvants that are commonly used in galenicals, such as, e.g., isotonizing and viscosity-increasing additives. Shaking the matrix that is mixed with the fluid is not necessary. The contrast media thus produced are distinguished in that they are blood-isotonic or almost blood-isotonic. They can be injected immediately after resuspension. The concentration of the contrast media is 10 to 600 mg, preferably 50 to 400 mg of matrix material per milliliter of suspension. Depending on use, the media are administered at a dose of 0.01 to 0.20 ml/kg of body weight.

The media according to the invention are equally suitable for all imaging modes of sonography, such as, e.g., M-, B-Doppler mode but also for modes in which nonlinear effects are used, such as, e.g., harmonic and harmonic power mode, and they can be produced with high reproducibility.

The numbers of bubbles that are generated from the matrix lie considerably above those of the media of the prior art, and the media therefore manifest clearly improved contrast effects; the time window that is available for the study has also been lengthened considerably (see also, in this regard, in-vivo tests 41-52).

The following examples are used to provide a more detailed explanation of the subject of the invention, without intending to be limited to this object.

Example 1

20 g of dextran (molecular weight: ~1200 g/mol) is mixed with 0.2 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 80 g of water. It is stirred until completely dissolved. The solution is decanted in 5 g portions and frozen with liquid nitrogen. After the water is removed under conditions which make possible a direct transition from the solid state to the gaseous state, bypassing the liquid aggregate state, gas exchange is carried out with decafluorobutane. A porous matrix remains, from which after resuspension in 10 ml of water per gram of matrix material, 12.3 x 10^9 bubbles in the range of 0.56-7.46 µm can be generated. The determination of the numbers and sizes of the bubbles was done with a laser diffractometer from the Melvern Instruments Company, type Master Sizer 1000.

Example 2

10 g of dextran (molecular weight: ~1200 g/mol) is mixed with 0.1 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 90 g of water. Then, the procedure is as described under Example 1. After resuspension in 10 ml of water, 3.7 x 10^9 bubbles in the range of 0.56-7.46 µm are released per gram of substance.

Example 3

20 g of dextran 8 (molecular weight: ~8000 g/mol) is mixed with 0.2 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 80 g of water. Then, the procedure is as described under Example

1. After resuspension in 10 ml of water, 10.5 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 4

30 g of raffinose (molecular weight: 594 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. Then, the procedure is as described under Example 1. After resuspension in 10 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 262 mosmol. 14.6 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 5

20 g of trehalose (molecular weight: 342 g/mol) is mixed with 0.2 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 80 g of water. Then, the procedure is as described under Example 1. After resuspension in 10 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 272 mosmol. 9.4 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 6

20 g of maltose (molecular weight: 342 g/mol) is mixed with 0.2 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 80 g of water. Then, the procedure is as described under Example 1. After resuspension in 10 ml of water, a preparation is obtained that is isotonic at an osmolality of 281 mosmol. 8.2 x 10^9

bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 7

40 g of maltooligosaccharide (molecular weight: ~684 g/mol) is mixed with 0.4 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 10 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 314 mosmol. 30.0×10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 8

20 g of maltooligosaccharide (molecular weight: ~684 g/mol) is mixed with 0.2 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 80 g of water. It is stirred until completely dissolved. The solution is decanted in 10 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 10 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 310 mosmol. 19.1×10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 9

30 g of melezitose (molecular weight: 522 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. Then, the procedure is as described under Example

1. After resuspension in 10 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 315 mosmol. 4.3×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 10

30 g of melibiose (molecular weight: 360 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 4 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 8 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 306 mosmol. 4.3×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 11

30 g of maltotriose (molecular weight: 504 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 3 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 296 mosmol. 3.6×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 12

30 g of Gd-EOB-DTPA (molecular weight: 726 g/mol) (gadoxetic acid, disodium) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 3 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 10 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 344 mosmol. 24.6×10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 13

30 g of gadobutrol (molecular weight: 605 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 3 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 269 mosmol. 20.1×10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 14

30 g of gadopentetic acid (molecular weight: 548 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 3 g portions and frozen

with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 7 ml of water, a preparation is obtained that is isotonic at an osmolality of 282 mosmol. 29.1 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 15

30 g of iopamidol (molecular weight: 777 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 4 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 268 mosmol. 26.3 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 16

15 g of iopamidol (molecular weight: 777 g/mol) is mixed with 0.15 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 85 g of water. It is stirred until completely dissolved. The solution is decanted in 8 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 343 mosmol. 16.9 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 17

30 g of iohexol (molecular weight: 821 g/mol) is mixed with 0.3 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 70 g of water. Then, the procedure is as described under Example 1. After resuspension in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 264 mosmol. 24.4 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 18

20 g of iotrolan (molecular weight: 1,626 g/mol) is mixed with 0.2 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 80 g of water. It is stirred until completely dissolved. The solution is decanted in 10 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 3 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 256 mosmol. 10.3 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 19

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 289 mosmol. 22.4 x

10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 20

20 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.2 g of Zonyl(R) FSO-100 (molecular weight: ~725 g/mol) and 80 g of water. It is stirred until completely dissolved. The solution is decanted in 10 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 291 mosmol. 21.9 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 21

23 g of Magnevist(R) (molecular weight: 938 g/mol) is mixed with 0.23 g of Zonyl(R)-FSO-100 (molecular weight: ~725 g/mol) and 77 g of water. It is stirred until completely dissolved. The solution is decanted in 3 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 342 mosmol. 6.16 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 22

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Triton$^{(R)}$-X-100 (molecular weight: ~874 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 290 mosmol. 9.56 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 23

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Tween$^{(R)}$ 20 (molecular weight: 718 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 289 mosmol. 16.55 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 24

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Cremophor$^{(R)}$ RH 40 (molecular weight: ~2,700 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 291 mosmol. 11.05 x 10^9 bubbles in a range of 0.56-7.46 μm are released per gram of substance.

Example 25

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Rewoderm(R) Li 48-50 (molecular weight: ~3,800 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 288 mosmol. 24.05 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 26

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Solutol(R) HS 15 (molecular weight: ~1,000 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 289 mosmol. 13.46 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 27

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Lutrol(R) F 68 (molecular weight: ~8,600 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 290 mosmol. 17.68 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 28

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Span(R) 85 (molecular weight: 1,028 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After resuspension in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 291 mosmol. 20.45 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 29

30 g of iohexol (molecular weight: 821 g/mol) is mixed with 0.3 g of Zonyl(R)-FSN (molecular weight: ~950 g/mol) and 70 g of water. Then, the procedure is as described under Example 1. After resuspension in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 269 mosmol. 23.81 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 30

30 g of gadobutrol (molecular weight: 605 g/mol) is mixed with 0.3 g of Solutol(R) HS 15 (molecular weight: ~1,000 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 3 g portions and frozen with liquid nitrogen. After the water is completely removed, a porous matrix remains. When resuspended in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 271 mosmol.

6.64×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 31

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Zonyl(R)-FSO-100 (molecular weight: ~725 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 289 mosmol. 4.07×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 32

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Triton(R)-X-100 (molecular weight: 874 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 290 mosmol. 3.01×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 33

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Tween(R) 20 (molecular weight: 718 g/mol) and 60 g of water. Then, the procedure is as described under Example 1.

After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 289 mosmol. 4.27 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 34

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Cremophor(R) RH 40 (molecular weight: ~2,700 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 291 mosmol. 1.76 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 35

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Rewoderm(R) Li 48-50 (molecular weight: ~3,800 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 288 mosmol. 9.58 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 36

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Solutol(R) HS 15 (molecular weight: ~1,000 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 289 mosmol. 2.52×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 37

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Lutrol(R) F 68 (molecular weight: ~8,600 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 290 mosmol. 4.32×10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 38

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of Span(R) 85 (molecular weight: 1,028 g/mol) and 60 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 6 ml of water, a preparation is obtained that is isotonic at an osmolality of 291 mosmol. 6.65 x

10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 39

30 g of iohexol (molecular weight: 821 g/mol) is mixed with 0.3 g of Zonyl(R)-FSN (molecular weight: ~950 g/mol) and 70 g of water. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 269 mosmol. 8.25 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 40

30 g of gadobutrol (molecular weight: 605 g/mol) is mixed with 0.3 g of Solutol(R) HS 15 (molecular weight: ~1,000 g/mol) and 70 g of water. It is stirred until completely dissolved. The solution is decanted in 3 g portions and frozen with liquid nitrogen. Then, the procedure is as described under Example 1. After drying is completed, gas exchange with hexafluoroethane is carried out. When resuspended in 5 ml of water, a preparation is obtained that is almost isotonic at an osmolality of 271 mosmol. 2.11 x 10^9 bubbles in a range of 0.56-7.46 µm are released per gram of substance.

Example 41

In-vivo use of a medium according to the invention that is produced according to Example 22:

A beagle [female, 11.2 kg of body weight (hereinafter: body weight)] is anesthetized (inhalation anesthesia about 2/3 oxygen, about 1/3 N_2O, 1.5-2% enflurane; spontaneous respiration) and prepared for a sonographic study of the heart. The study is carried out with an ultrasound system of the HP trademark (type 77020 E, 5 MHz transducer) in the B-mode. The test animal receives the test substance administered intravenously (a medium according to the invention that is produced according to Example 22).

A contrast medium, which was produced analogously to WO 95/22994 (Example 12), is used as a reference substance.

The doses used are 0.1 ml/kg of body weight both for the medium according to the invention and for the reference substance. The results are depicted in the form of the intensity-time plots in Figure 4. In this case, the upper (more gently downward sloping) curve corresponds -- as in Figures 5-8 below -- to the preparation according to the invention. It is clearly evident that the medium according to the invention manifests a longer-lasting contrast after intravenous injection than the medium from the prior art. These contrast properties allow the physician longer examination times (study window). In addition, the need for a possible secondary injection is considerably reduced, which reduces the stress on the patient and contributes to an advantageous cost/benefit ratio.

Example 42

In-vivo use of a medium according to the invention that is produced according to Example 27:

The procedure is as described in Example 41. A preparation that is produced as described in Example 27) is used as a test substance; a medium from WO 95/22994 (Example 12) is used as a reference. The doses for the reference and the test substances were identical and were 0.1 ml/kg of body weight in each case.

The intensity-time plots are depicted in Figure 5.

Example 43

In-vivo use of a medium according to the invention that is produced according to Example 28:

The procedure is as described in Example 41, whereby the test animal, however, had a body weight of 11.9 kg. A preparation that is produced as described in Example 28) is used as a test substance; a medium from EP 0365467 (Example 1) was used as. The doses used were 0.05 ml/kg of body weight for the medium of the invention according to Example 28 as well as 0.2 ml/kg of body weight for the reference. The results of the study are depicted in the form of the intensity-time plots in Figure 6. Also in this case, despite a low dose, a more intensive and longer-lasting contrast is observed for the preparation according to the invention.

Example 44

In-vivo use of a medium according to the invention that is produced according to Example 29:

The procedure is as described in Example 43. A preparation that is produced as described in Example 29) is used as a test substance; a medium from EP 0365467 (Example 1) was used as a reference.

The doses used were 0.05 ml/kg of body weight for the medium according to the invention as well as 0.2 ml/kg of body weight for the reference. The results of the study are depicted in the form of the intensity-time plots in Figure 7.

Example 45

In-vivo use of a medium according to the invention that is produced according to Example 19:

A beagle (female, 9.7 kg of body weight) is anesthetized; in addition, the procedure is as described in Example 41. A preparation that is produced according to Example 19 is used as a test substance; a medium from WO 95/22994 (Example 12) is used as a reference. The doses for the reference and the test substance were 0.1 ml/kg of body weight each.

The intensity-time plots are depicted in Figure 8.

Example 46

In-vivo use of a medium according to the invention that is produced according to Example 5:

The procedure is as described in Example 45. A preparation that is produced as described in Example 5 is used as a test substance; a medium from WO 95/22994 (Example 12) was used as a reference. The doses for the reference and the test substance were identical and were 0.1 ml/kg of body weight.

The results of the study are depicted in Figure 9. The heart of the dog was imaged. There are shown in detail:

(a) "Before" contrast

(b) maximum contrast of the reference substance

(c) maximum contrast of the test substance

(d) contrast 1 minute after maximum contrast (reference)

(e) contrast 1 minute after maximum contrast (test substance)

As the figure shows, the superiority of the preparations according to the invention is manifested especially in the case of a long examination time.

Example 47

In-vivo use of a medium according to the invention that is produced according to Example 11:

The procedure is as described in Example 41. A preparation that is produced as described in Example 11) is used as a test substance; a medium from WO 95/22994 (Example 12) was used as a reference. The doses for the reference and the test substance were identical and were also 0.1 ml/kg of body weight here.

The results of the study are depicted in Figure 10. The meanings of items (a) to (e) correspond to those of Figure 9.

Example 48

In-vivo use of a medium according to the invention that is produced according to one of Examples 31, 35 and 39.

A beagle (female, 9.6 kg of body weight) is anesthetized (inhalation anesthesia about 2/3 oxygen, about 1/3 N_2O, 1.5-2% enflurane; spontaneous respiration) and prepared for a sonographic study of the heart. The study is carried out with an ultrasound system of the HP trademark (type 77020 E, 5 MHz transducer) in the B-mode. In each case, the test animal receives an intravenous administration of a medium according to the invention that is produced according to one of Examples 31, 35 and 39 as well as, as a reference, an injection of a contrast medium according to prior art, which has been produced analogously to WO 95/11994 (Example 12).

The doses used were 0.1 ml/kg of body weight for the medium according to the invention and for the reference. The results are depicted in the form of intensity values in Table 1. It is also clearly evident here that the media according to the invention manifest a considerably higher level of contrast after intravenous injection than the reference medium.

Substance	Contrast Intensity in Density Units [DU] absolute	relative
produced according to WO 95/22994 Example 12	140	100
medium of the invention according to Example 31	160	114
medium of the invention according to Example 35	184	131
medium of the invention according to Example 39	177	126

Table 1

Example 49

In-vivo use of a medium according to the invention that is produced according to one of Examples 4, 8 and 9.

A beagle (female, 9.7 kg of body weight) is anesthetized (inhalation anesthesia about 2/3 oxygen, about 1/3 N_2O, 1.5-2% enflurane; spontaneous respiration) and prepared for a sonographic study of the heart. The study is carried out with an ultrasound system of the HP trademark (type 77020 E, 5 MHz transducer) in the B-mode. In each case, the test animal receives an intravenous administration of a medium according to the invention that is produced according to one of Examples 4, 8 and 9 as well as, as a reference, an injection of a contrast medium according to prior art, which has been produced analogously to WO 95/22994 (Example 12).

The doses used were 0.1 ml/kg of body weight for the medium according to the invention and for the reference. The results are depicted in the form of area values under the intensity-time curve (density units x sec) in Table 2. It is clearly evident that the media according to the invention manifest a higher level of area after intravenous injection.

Substance	Area Values under the Intensity-Time Curve (Density Units x Sec)	
	absolute	relative
produced according to WO 95/22994 Example 12	5890	100
medium of the invention according to Example 8	12337	209
medium of the invention according to Example 4	9697	164
medium of the invention according to Example 9	10258	174

Table 2

Example 50

In-vivo use of a medium according to the invention that is produced according to one of Examples 8 and 19

A beagle (male, 15.5 kg of body weight) is anesthetized (inhalation anesthesia about 23% oxygen, 1-3% enflurane; the remainder nitrogen; spontaneous respiration) and prepared to derive the spectral Doppler signal from the femoral artery. The study is carried out with the ultrasound system ATL UM-9 with a transducer such as L 10-5. The test animal receives an

intravenous administration of the test substance that is produced according to Example 8 or 19 as well as an administration of a contrast medium that is produced according to WO 95/22994 (Example 12) as a reference. The dose for all injections was 0.1 ml/kg of body weight.

The spectral Doppler signal is evaluated using an intensitometer and plotted against time. The resulting areas under the intensity-time curves are depicted in Table 3.

Substance	Area Values under the Intensity-Time Curve (AUC)	
	absolute	relative
produced according to WO 95/22994 Example 12	2079	100
medium of the invention according to Example 8	3638	175
medium of the invention according to Example 19	4318	208

Table 3

Example 51

A beagle (female, 9.7 kg of body weight) is anesthetized (inhalation anesthesia about 2/3 O_2; about 1/3 N_2O; 1.5-3% enflurane, spontaneous respiration) and the kidney is prepared for a perfusion study. The study is performed with an ultrasound system of the HP trademark (type Sonos 1000, 5 MHz) in the color Doppler mode. The test animal receives an intravenous administration of a medium of the invention according to Example

22 (0.1 ml/kg of body weight). After administration, the perfusion visualization of the organ is considerably improved in comparison with the visualization before administration.

Example 52

A beagle (female, 9.7 kg of body weight) is anesthetized (inhalation anesthesia about 2/3 O_2; about 1/3 N_2O; 1.5-3% enflurane, spontaneous respiration) and prepared for a sonographic study of the abdominal aorta. The study is performed with an ultrasound system of the ATL trademark such as UM9 with transducer C10-5 in the harmonic B-mode. The test animal receives an intravenous administration of a medium according to the invention that is produced according to Example 8 (dose 0.1 ml/kg of body weight). Immediately after the injection is completed, the vascular volume is labeled echogeneically. Before the injection, volume was not enhanced.

Example 53

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.4 g of ethoxylated fatty acid monoethanolamide (Aminol $N^{(R)}$, molecular weight: 480 g/mol) and 60 g of water. Then, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 20.34×10^9 bubbles in a contrast-relevant range of 0.56-7.46 μm are released per gram of substance.

Example 54

0.67 g of sucrose palmitate-stearate 7 is mixed with 100 g of water and heated in a microwave. After cooling, a slightly cloudy, stable solution is obtained. 60 g of this opalescent solution is mixed with 40 g of iopromide (molecular weight: 791 g/mol). Then, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 62.01 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 µm are released per gram of substance.

Example 55

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 60 g of a sucrose palmitate-stearate 15 solution (w = 0.67%). Then, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 38.97 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 µm are released per gram of substance.

Example 56

0.67 g of sucrose palmitate-stearate 7 is mixed with 100 g of water and heated in a microwave. After cooling, a slightly cloudy, stable solution is obtained. 60 g of this opalescent solution is mixed with 40 g of iopromide (molecular weight: 791 g/mol). Then, the procedure is as described in Example 1. Gas exchange is carried out with perfluorohexane. After resuspension

in 6 ml of water, an isotonic preparation is obtained. In this case, 2.82 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 µm are released per gram of substance.

Example 57

0.67 g of sucrose palmitate-stearate [one word or figure illegible] is mixed with 100 g of water and heated in a microwave. After cooling, a slightly cloudy, stable solution is obtained. 60 g of this opalescent solution is mixed with 40 g of iopromide (molecular weight: 791 g/mol). It is stirred until completely dissolved. The entire solution is frozen by addition in drops to liquid decafluorobutane. After water is removed under conditions that make possible direct transition from the solid state to the gaseous state, bypassing the liquid aggregate state, the product is decanted in 1 g portions, and a gas exchange with decafluorobutane is carried out. After resuspension in 3 ml of water, an isotonic preparation is obtained. In this case, 34.07 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 µm are released per gram of substance.

Example 58

0.4 g of hydrogenated phosphatipylcholine (Pro Lipo H (R)) is mixed with 60 g of water and then with 0.4 g of iopromide (molecular weight: 791 g/mol). Hereinafter, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 27.97 x 10^9

bubbles in a contrast-relevant range of 0.56-7.46 μm are released per gram of substance.

Example 59

0.4 g of hydrogenated, oil-free soybean lecithin (Epikuron 100H$^{(R)}$) is mixed with 60 g of water and then with 0.4 g of iopromide (molecular weight: 791 g/mol). Hereinafter, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 52.97 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 μm are released per gram of substance.

Example 60

1.5 g of sucrose palmitate-stearate 7 is mixed with 100 g of water and heated in a microwave. After cooling, a slightly cloudy, stable solution is obtained. 70 g of this opalescent solution is mixed with 30 g of maltooligosaccharide (molecular weight: 684 g/mol) and 0.1 g of PVA (molecular weight: 10,000 g/mol). The preparation is decanted in 4 g portions. Then, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 9.37 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 μm are released per gram of substance.

Example 61

0.6 g of hydrogenated phosphatipylcholine (Pro Lipo H$^{(R)}$) is mixed with 70 g of water and then with 30 g of

maltooligosaccharide (molecular weight: 684 g/mol). The preparation is decanted in 4 g portions. Hereinafter, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 21.08 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 μm are released per gram of substance.

Example 62

40 g of iopromide (molecular weight: 791 g/mol) is mixed with 0.04 g of polyoxyethylenesorbitan monolaurate Tween 21$^{(R)}$) and 60 g of water. It is stirred until completely dissolved. The preparation is decanted in 5 g portions and frozen in liquid propane. After the water is removed under conditions that make possible a direct transition from the solid state to the gaseous state, bypassing the liquid aggregate state, a gas exchange with decafluorobutane is carried out. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 3.77 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 μm are released per gram of substance.

Example 63

0.67 g of sucrose palmitate-stearate 7 is mixed with 100 g of water and heated in a microwave. After cooling, a slightly cloudy, stable solution is obtained. 60 g of this opalescent solution is mixed with 40 g of iopromide (molecular weight: 791 g/mol). The preparation is decanted in 5 g portions and frozen in liquid butane. After the water is removed under conditions

that make possible a direct transition from the solid state to the gaseous state, bypassing the liquid aggregate state, a gas exchange with decafluorobutane is carried out. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 34.16 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 µm are released per gram of substance.

Example 64

40 g of iopromide (molecular weight: 791 g/mol) is mixed with water until completely dissolved. 0.4 g of hydrogenated phosphatidylcholine consisting of soybean (Epikuron 200SH) is mixed with tert-butanol until completely dissolved. The solutions are combined and made up to 100 g of total weight with water. Then, the procedure is as described in Example 1. After resuspension in 6 ml of water, an isotonic preparation is obtained. In this case, 15.95 x 10^9 bubbles in a contrast-relevant range of 0.56-7.46 µm are released per gram of substance.

Example 65

20 g of stachyose (molecular weight: 739 g/mol) is mixed with 0.4 g of hydrogenated phosphatidylcholine (Pro Lipo $H^{(R)}$) and 80 g of water. The preparation is decanted in 2.5 g portions. Then, the procedure is as described in Example 1. After resuspension in 2.5 ml of water, an isotonic preparation is obtained.

Example 66

A beagle (female, 11.8 kg of body weight) is anesthetized (inhalation anesthesia 23% oxygen, 1-3% enflurane, the remainder nitrogen; spontaneous respiration) and prepared to derive the spectral Doppler signal from the femoral artery. The study is carried out with ultrasound system ATL UM-9 with transducer type L 10-5. The test animal receives an intravenous administration of the test substance that is produced according to Example 54 (0.05 ml/kg) as well as an administration of a contrast medium that is produced according to EP 0 365 467 (Example 1, 0.2 ml/kg) as a reference. Figure 11 shows the intensity-time plots of the two injections. The significantly more intensive and longer-lasting contrast of the preparations according to the invention is clearly evident.

Example 67

A beagle (female, 11.9 kg of body weight) is anesthetized (inhalation anesthesia 23% oxygen, 1-3% enflurane, the remainder nitrogen; spontaneous respiration) and prepared to derive the spectral Doppler signal from the femoral artery. The study is carried out with ultrasound system ATL UM-9 with transducer type L 10-5. The test animal receives an intravenous administration of the test substance that is produced according to Example 58 (0.05 ml/kg) as well as an administration of a contrast medium that is produced according to EP 0365467 (Example 1, 0.2 ml/kg) as a reference. Figure 12 shows the intensity-time plots of the

two injections. The significantly more intensive and longer-lasting contrast of the preparations according to the invention is clearly evident.

Example 68

A beagle (female, 12.1 kg of body weight) is anesthetized (inhalation anesthesia 23% oxygen, 1-3% enflurane, the remainder nitrogen; spontaneous respiration) and prepared to derive the spectral Doppler signal from the femoral artery. The study is carried out with ultrasound system ATL UM-9 with transducer type L 10-5. The test animal receives an intravenous administration of the test substance that is produced according to Example 59 (0.05 ml/kg) as well as an administration of a contrast medium that is produced according to EP 0365467 (Example 1, 0.2 ml/kg) as a reference. The area under the intensity-time curve is 252.2% of the reference.

Example 69

A beagle (female, 12.1 kg of body weight) is anesthetized (inhalation anesthesia 23% oxygen, 1-3% enflurane, the remainder nitrogen; spontaneous respiration) and prepared to derive the spectral Doppler signal from the femoral artery. The study is carried out with ultrasound system ATL UM-9 with transducer type L 10-5. The test animal receives an intravenous administration of the test substance that is produced according to Example 64 (0.05 ml/kg) as well as an administration of a contrast medium that is produced according to EP 0365467 (Example 1, 0.2 ml/kg)

as a reference. The area under the intensity-time curve is 223.[one number illegible]% of the reference.

Example 70

A beagle (male, 17.1 kg of body weight) is anesthetized (inhalation anesthesia 23% oxygen, 1-3% enflurane, the remainder nitrogen; spontaneous respiration) and prepared to derive the spectral Doppler signal from the femoral artery. The study is carried out with ultrasound system ATL UM-9 with transducer type L 10-5. The test animal receives an intravenous administration of the test substance that is produced according to Example 65 (0.05 ml/kg) as well as an administration of a contrast medium that is produced according to EP 0 365 467 (Example 1, 0.2 ml/kg) as a reference. The area under the intensity-time curve is 181.0% of the reference.

Claims

1. Preparations for the production of a preparation for ultrasonic diagnosis that consist of a porous, solid, water-soluble matrix that contains a low-molecular skeleton former, a surfactant and a gas, whereby the gas is enclosed in the pores of the matrix.

2. Porous matrix according to claim 1 that contains as a skeleton former a water-soluble skeleton former with a molecular weight < 15,000 dalton from the group of amino acids, polyamino acids, peptides, proteins, mono-, di-, tri-, tetra-, oligo- and polymeric saccharides, as well as their derivatives, synthetic saccharides and their derivatives.

3. Porous matrix according to claim 1 or 2 that contains as a skeleton former L-glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-asparagine, L-glutamine, L-arginine, L-histidine, glycyl-glycine, glycyl-glycyl-glycine, glucose, galactose, fructose, mannose, sorbose, saccharose, lactose, maltose, trehalose, gentiobiose, lactulose, turanose, maltotriose, melibiose, melizitose, maltotetraose, maltopentaose, stachyose, arabinose, xylose, ribose, dulcitol, xylitol, mannitol, ribitol, inositol, sorbitol, α, ß, γ-cyclodextrins, hydroxypropyl-ß-cyclodextrin, dextran 8, dextrins and/or ficoll.

4. Porous matrix according to claim 1 or 2 that contains as a skeleton former x-ray contrast media or contrast media for magnetic resonance imaging.

5. Porous matrix according to claim 4 that contains as a skeleton former iopromide, iotrolan, iopamidol and/or iohexol.

6. Porous matrix according to claim 4 that contains as a skeleton former Gd-DTPA (gadopentetic acid), Gd-DTPA-dimeglumine salt (Magnevist), Gd-EOB-DTPA (gadoxetic acid, disodium salt) and/or gadobutrol.

7. Porous matrix according to one of claims 1 to 6 that contains as a surfactant a water-soluble, nonionic surfactant, whereby the proportion of surfactant in the matrix is 0.01 to 10% (m/m).

8. Porous matrix according to one of claims 1 to 7 that contains as a surfactant sorbitan fatty acid ester, polyoxyethylenesorbitan fatty acid ester, polyoxyethylene sorbitol fatty acid ester, polyoxyethylene fatty acid ester, glycerine poloxyethylene fatty acid ester, ethoxylated mono-, di-, triglycerides, which can be optionally partially hydrogenated, as well as ethoxylated mixtures of the latter, ethoxylated castor oils, ethoxylated phenols, polyoxyethylene fatty alcohol ether, polyglycerol fatty acid ester, sorbitan perfluorofatty acid ester, polyoxyethylene sorbitan perfluorofatty acid ester, polyoxyethylenesorbitol perfluorofatty acid ester, polyoxyethylene perfluorofatty acid ester, ethoxylated castor oils, polyoxyethylene perfluorofatty alcohol ether, polyglycerol perfluorofatty acid ester, polyoxyethylene polyoxypropylene polymers, fluoroalkyl poly(ethylenoxide) alcohols, saccharide fatty acid ester, saccharide fatty acid ether, ethoxylated saccharide fatty acid ester, ethoxylated

saccharide fatty acid ether, fatty acid ethanolamides and/or ethoxylated fatty acid ethanolamide.

9. Porous matrix according to one of claims 1 to 7 that contains phospholipids as surfactants.

10. Porous matrix according to one of claims 1 to 9 that contains as a surfactant a surfactant with a perfluorinated hydrocarbon component and/or with a molecular weight of < 15,000 dalton.

11. Porous matrix according to claims 1 to 10 that contains as a gas nitrogen, oxygen, CO_2, air and fluorinated gaseous compounds.

12. Porous matrix according to claims 1 to 11 that contains as a gas tetrafluoroallenes, hexafluoro-1,3-butadiene, decafluorobutane, perfluoro-1-butenes, perfluoro-2-butenes, perfluoro-2-butine, octafluorocyclobutane, perfluorocyclobutene, perfluorocyclopentane, perfluorodimethylamine, hexafluoroethane, tetrafluoroethylenes, pentafluorothio(trifluoro)methane, tetrafluoromethane, perfluoropropane and/or perfluoropropylene.

13. Porous matrix according to claims 1 to 12 that contains as a gas a perfluorinated substance.

14. Ultrasonic contrast medium that is generated from a porous matrix according to one of claims 1 to 13 that contains, as a liquid carrier medium, water optionally with the additives that are commonly used in pharmaceutical technology.

15. Ultrasonic contrast medium according to claim 14 that contains as a carrier medium physiological electrolyte solution,

an aqueous solution of monovalent or multivalent alcohols or an aqueous solution of a mono- or disaccharide.

16. A kit for the production of an ultrasonic contrast medium that contains gas bubbles and that consists of

(a) a first container, equipped with a closure that makes it possible to remove the contents under sterile conditions and that is filled with a liquid suspension medium, and

(b) a second container, equipped with a closure that makes it possible to add the suspension medium under sterile conditions, filled with the porous matrix according to one of claims 1 to 13 and a gas, whereby the volume of the second container is calculated so that the suspension medium from the first container has plenty of room in the second container.

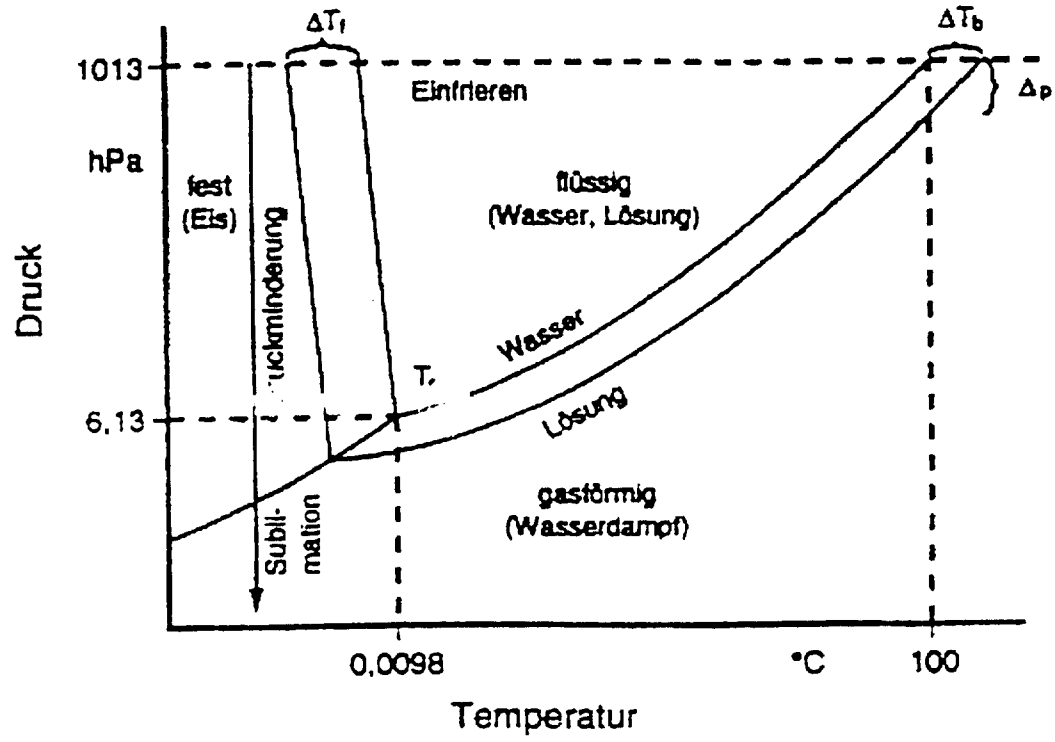
17. Process for the production of porous matrices according to one of claims 1 to 13, wherein first an aqueous solution of the desired skeleton former is produced, to which optionally gas-bubble-stabilizing surfactants are added, then the solution thus produced is freeze-dried and after drying is completed, the porous matrix is aerated with the gas desired in each case.

WO 97/26016 PCT/EP97/00208

Fig. 1

Fig. 2

Fig. 3

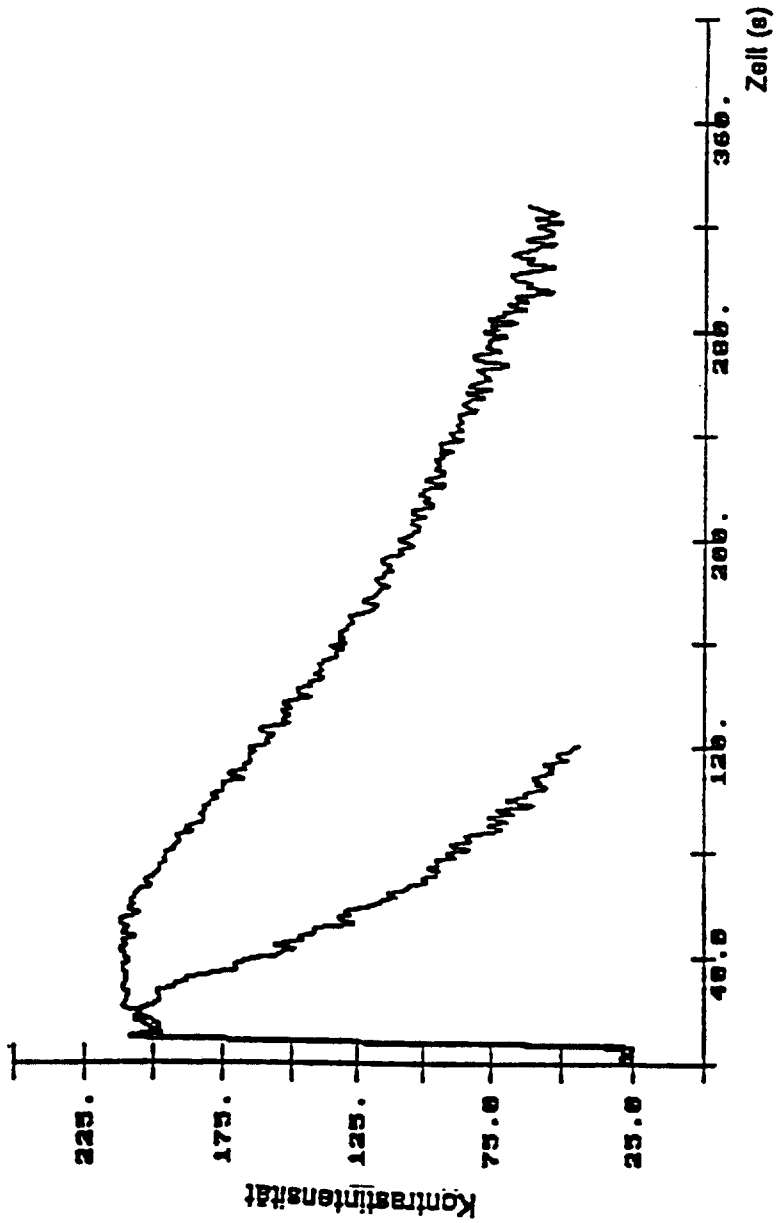


Zustandsdiagramm des Wassers und einer wäßrigen Lösung

ΔT_f = Gefrierpunktserniedrigung
ΔT_b = Siedepunktserhöhung
Δp = Dampfdruckerniedrigung
T_r = Tripelpunkt

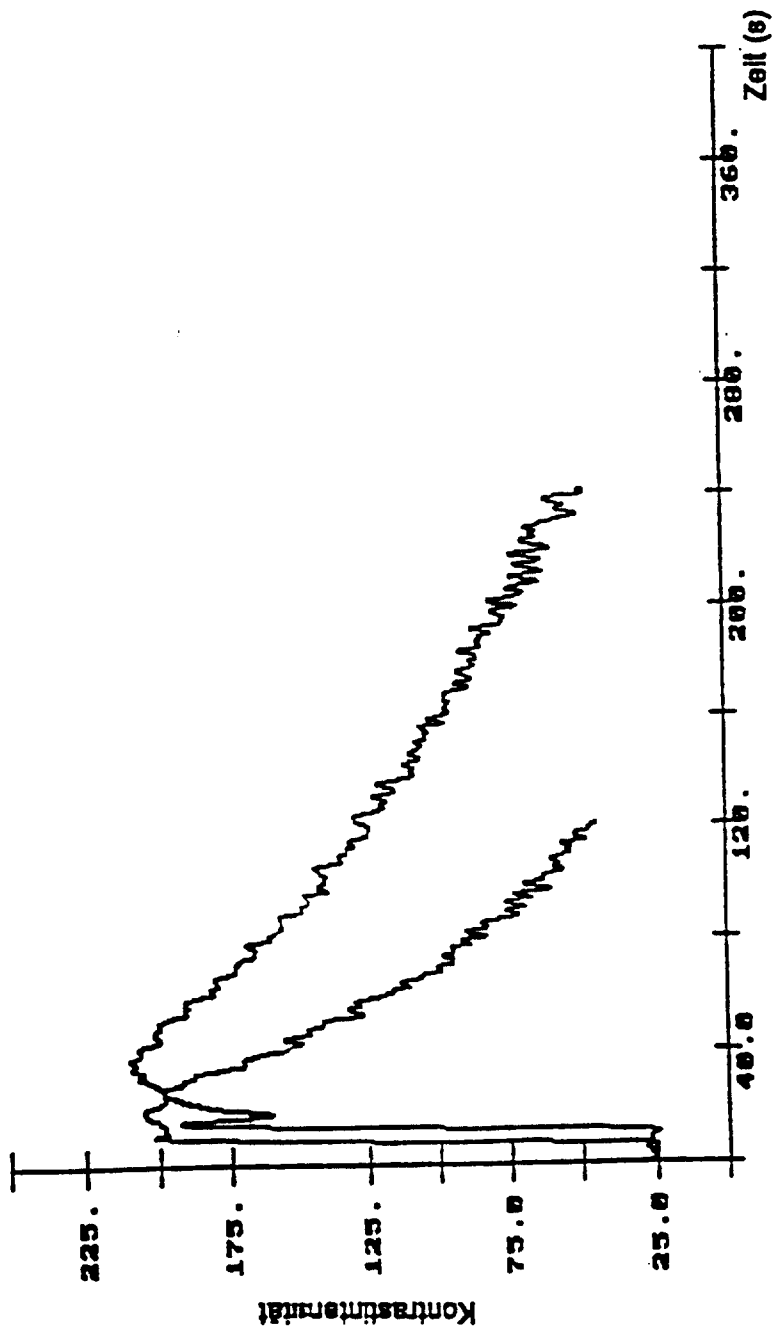
[Key:]
Druck = pressure
fest (Eis) = solid (ice)
Sublimation = sublimation
Druckminderung = reduction in pressure
Einfrieren = freezing
flüssig (Wasser, Lösung) = liquid (water, solution)
gasförmig (Wasserdampf) = gaseous (water vapor)
Temperatur = temperature
:Zustandsdiagramm des Wassers und einer wäßrigen Lösung =
:constitutional diagram of water and an aqueous solution
ΔT_f = reduction in freezing point
ΔT_b = increase in boiling point
Δp = reduction in vapor pressure
T_r = triple point

Fig. 4



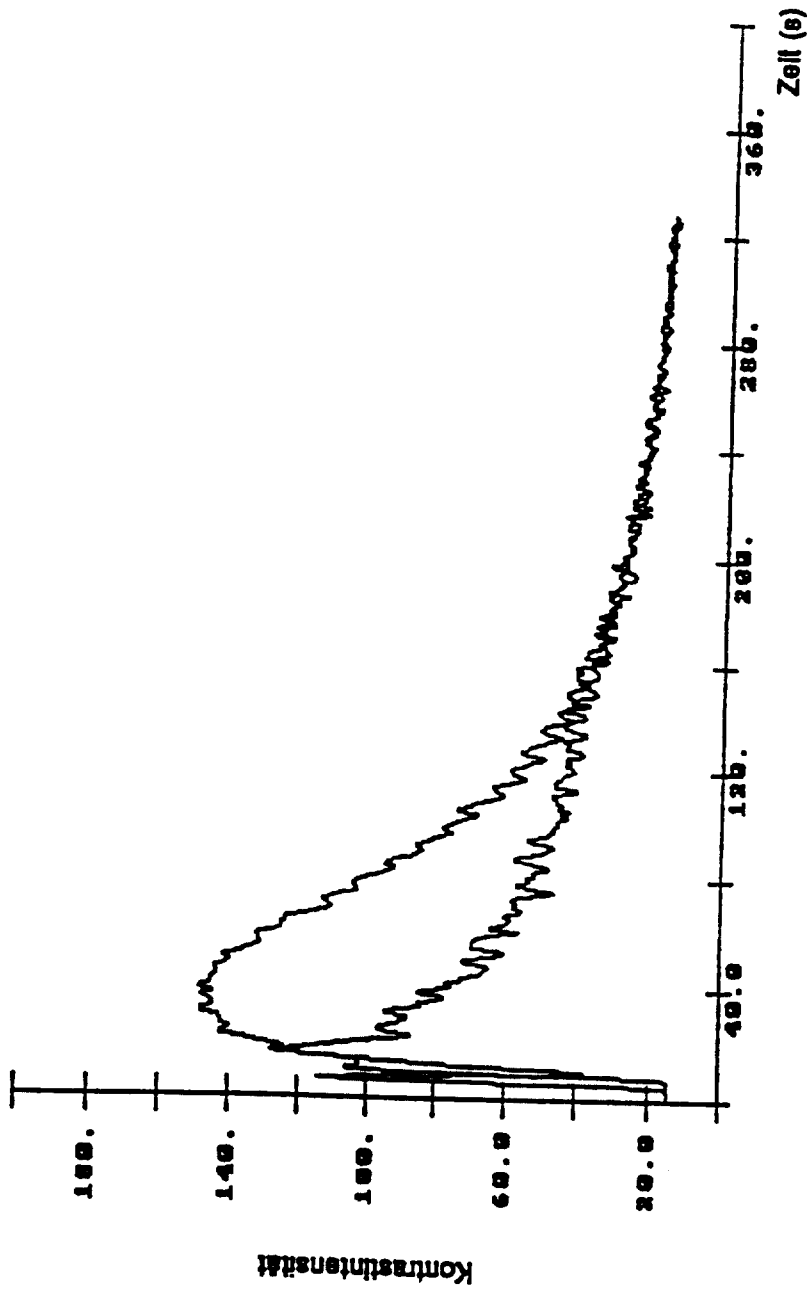
[Key:]
Kontrastintensität = contrast intensity
Zeit (s) = time (s)

Fig. 5



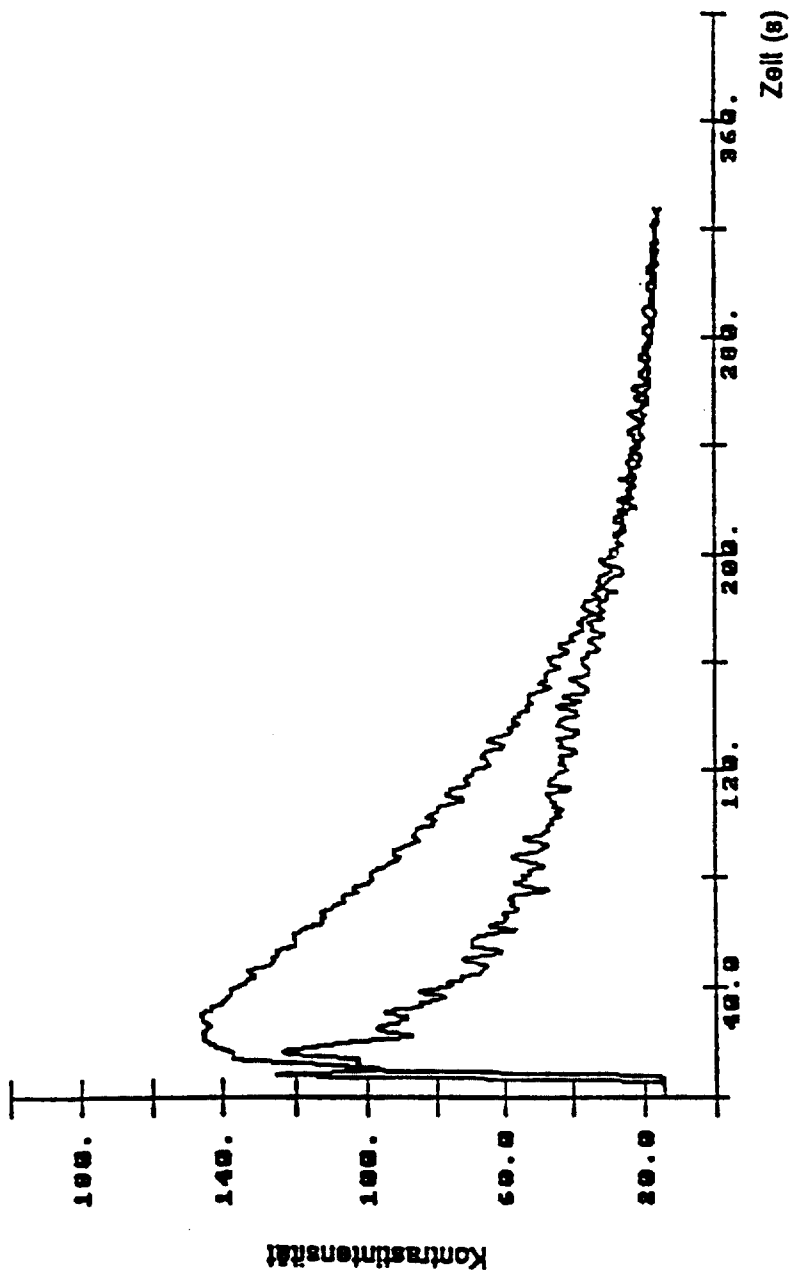
[Key:]
Kontrastintensität = contrast intensity
Zeit (s) = time (s)

Fig. 6



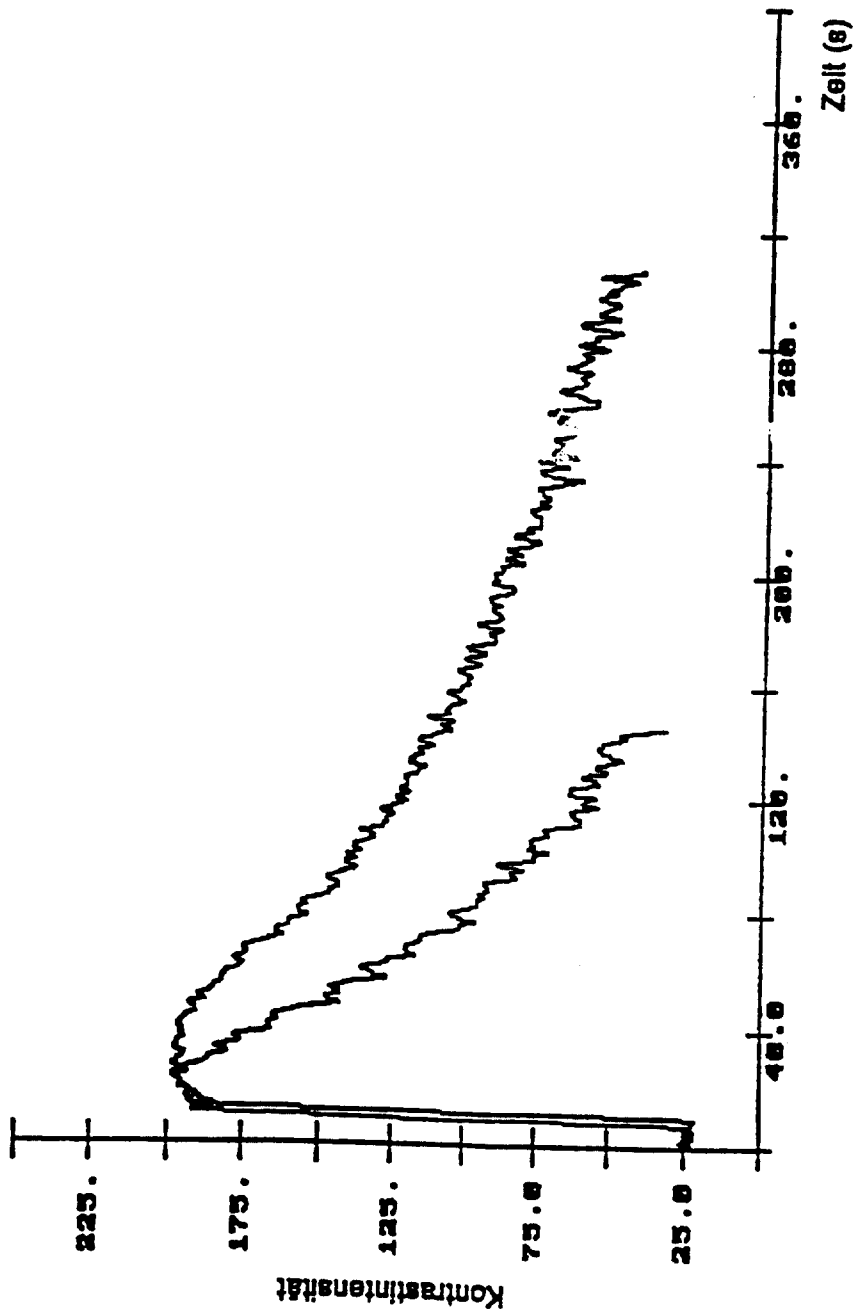
[Key:]
Kontrastintensität = contrast intensity
Zeit (s) = time (s)

Fig. 7



[Key:]
Kontrastintensität = contrast intensity
Zeit (s) = time (s)

Fig. 8



[Key:]
Kontrastintensität = contrast intensity
Zeit (s) = time (s)

Fig. 9

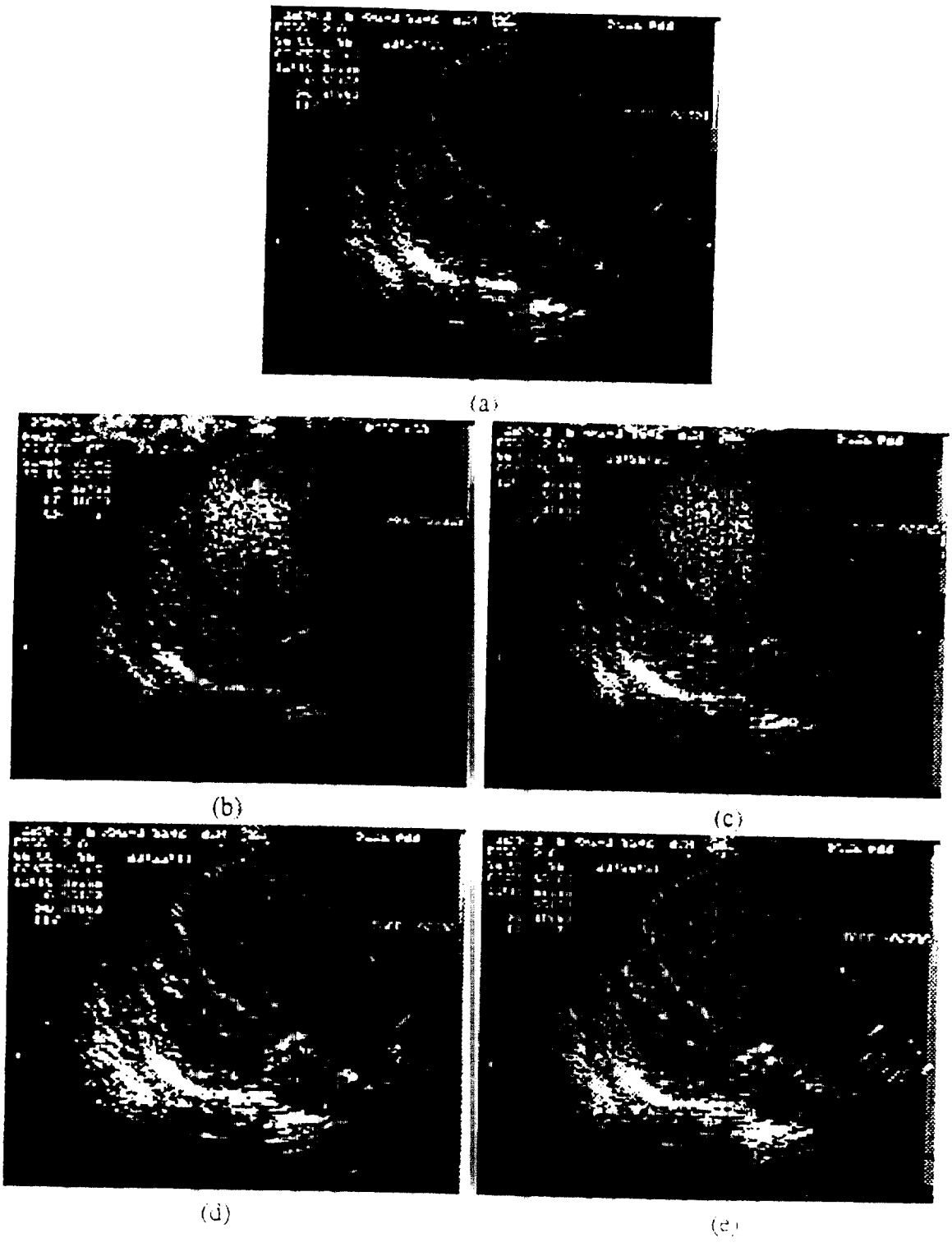


Fig. 10

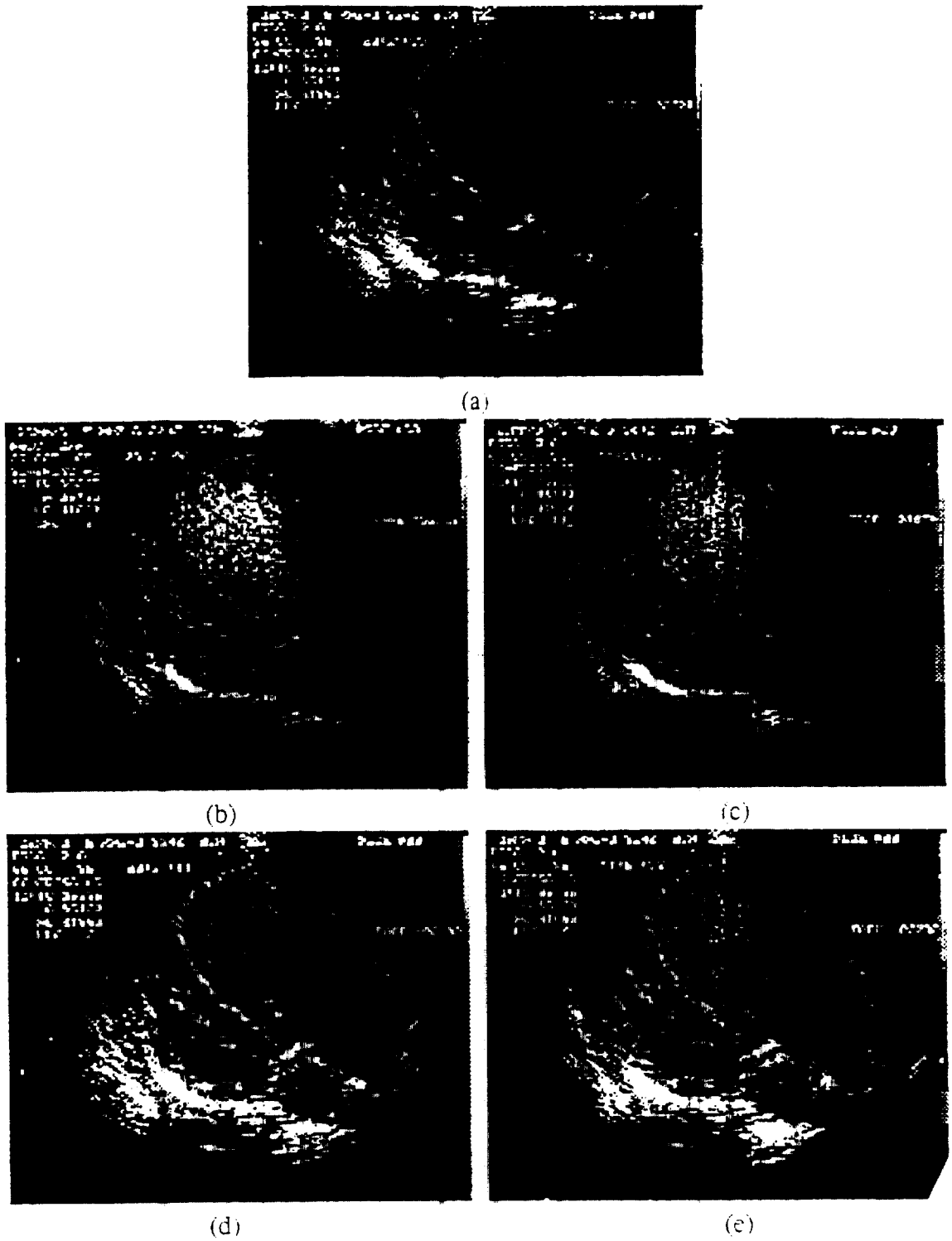
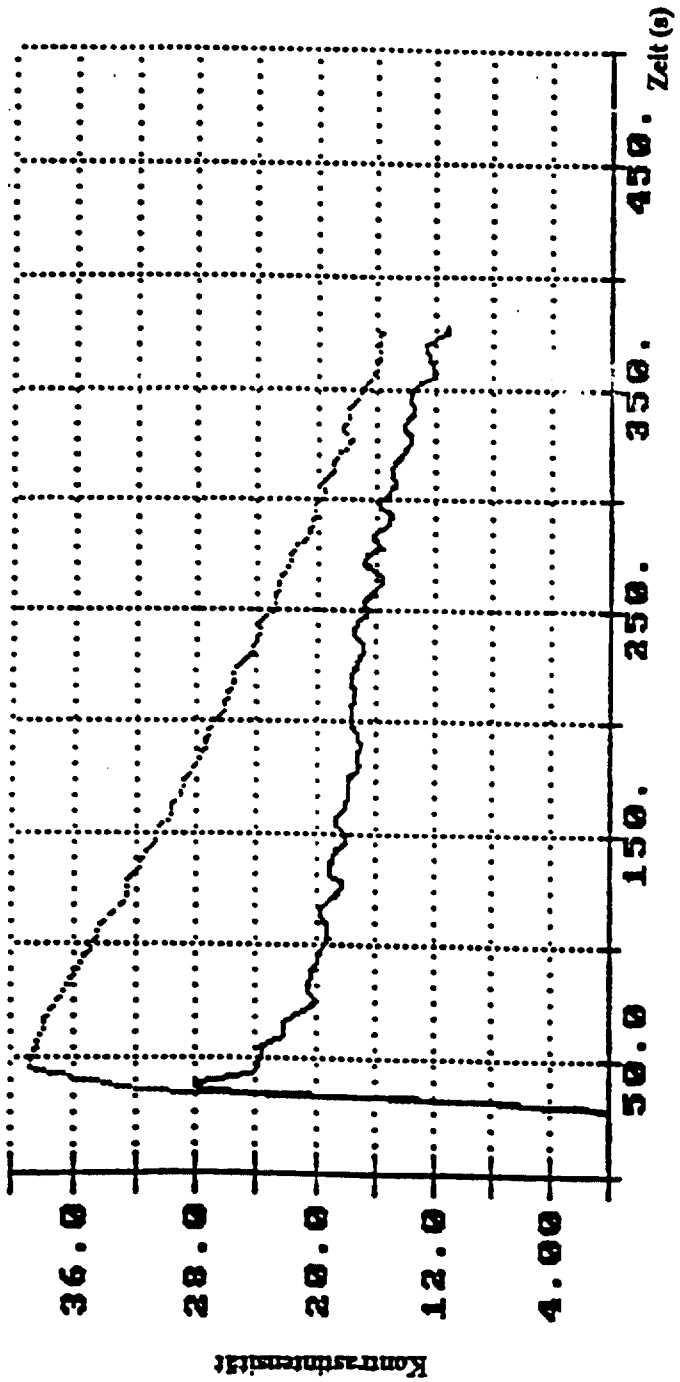
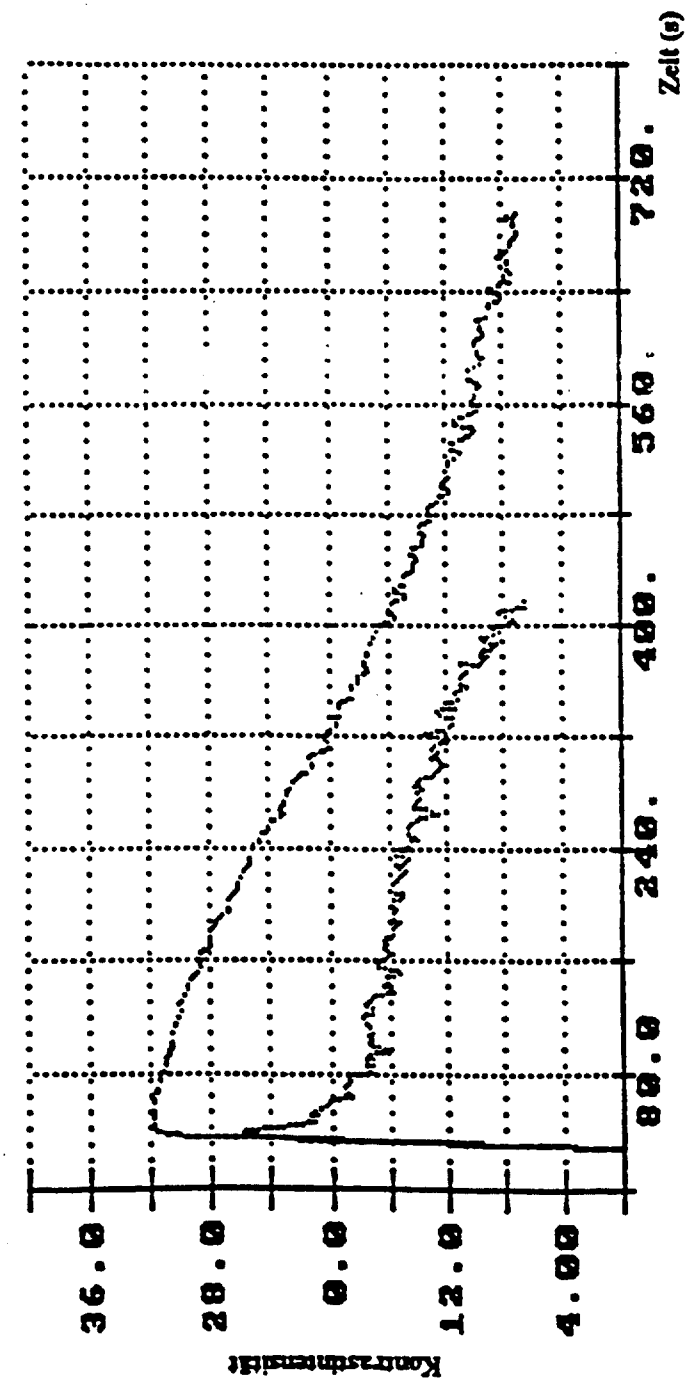


Fig. 11



[Key:]
Kontrastintensität = contrast intensity
Zeit (s) = time (s)

Fig. 12



[Key:]
Kontrastintensität = contrast intensity
Zeit (s) = time (s)