1 General

1.1 Synthesis and characterization of iodixanol.

H. Priebe et al
*Acta Radiologica Supplement, **399**, 21-31 (1995)*

Iodixanol (Visipaque) is a new nonionic roentgen contrast medium intended for general use. Visipaque is a pharmaceutical formulation of iodixanol which is isotonic and isoosmotic with blood. Two synthetic routes from 5-nitro-isophthalic acid to iodixanol are described. The chemical structure is confirmed by spectroscopical data (^{1}H-NMR, ^{13}C-NMR, electrospray-MS, UV, IR and Raman). Chromatographic characteristics are related to the isomerism of iodixanol.

1.2 Physical properties of iodixanol.

Eivindvik, K. and Sjøgren, E.
*Acta Radiologica Supplement, **399**, 32-38 (1995)*

Iodixanol, the radiopaque in Visipaque, is a new nonionic, dimeric roentgen contrast medium for intravascular use. Compared to aqueous solutions of nonionic monomers, which have a higher osmolality than blood, aqueous solution of iodixanol have a lower osmolality due to the dimeric structure of the molecule. As a consequence of this advantageous property, solutions of all clinical concentrations of iodixanol can be made isotonic by the addition of salts of the key electrolytes sodium and calcium to the formulation. The viscosity of all iodixanol (Visipaque) solutions is less than or equal to that of iohexol (Nycodenz). Iodixanol itself is an amorphous and hygroscopic solid which is freely soluble in water. Partition coefficients show that iodixanol is even more hydrophilic than the nonionic monomers such as iohexol. The high hydrophilicity and the good aqueous solubility of iodixanol are due to the hydroxyl group in the dimer linkage and the hydrophilic amide side chains of the molecule.

1.3 Visipaque is isotonic to human and rat blood plasma.

Karlsson, J.O.G., Gregersen, M. and Refsum, H.
*Acta Radiologica Supplement, **399**, 39-42 (1995)*

Even at its highest concentration, 320 mgI/ml, Visipaque - based on the nonionic dimer **iodixanol** - is isoosmotic to blood plasma, whereas Omnipaque (300 mgI/ml) - based on the nonionic monomer iohexol - has an osmolality of about twice that of the plasma. However, the fact that a solution is isoosmotic to plasma does not necessarily mean that it is isotonic to plasma. An isoosmotic solution can still cause a net movement of water over the plasma membranes of, for example, erythrocytes and endothelial cells.

Determination of the tonicity of Visipaque 320 mgI/ml and comparison with that of Omnipaque 300 mgI/ml and hypertonic NaCl have been performed. No change in the water content of human erythrocytes was seen after mixing whole blood 10:1 with either Visipaque 320 mgI/ml or 155 mM NaCl, whereas a significant decrease in water content occurred with Omnipque 300 mgI/ml and 620 mM NaCl. No difference in the water content of rat erythrocytes was evident after mixing whole blood with Visipaque 320 mgI/ml or isotonic NaCl. However, as with human erythrocytes, a significant decrease in water content occurred after rat blood was mixed with Omnipaque 300 mgI/ml.

In conclusion, Visipaque 320 mgI/ml does not cause any net movement of water over the human or rat erythrocyte plasma membrane, i.e., Visipaque is isotonic to human and rat blood plasma.

1.4 Formulation, stability and compatibility of iodixanol.

Aars, E.V. and Eivindvik, K.
*Acta Radiologica Supplement, **399**, 50-58 (1995)*

Iodixanol (Visipaque) is an isotonic, electrolyte-balanced roentgen contrast medium for intravascular use. The patented and well-proven formulation and the rationale for it are described, and the efficacy and safety documented. The stability of iodixanol is well within the specifications under all relevant conditions, both in glass and polypropylene bottles; the product has a recommended shelf-life of at least 36 months when stored at room temperature and protected from light. Heating to body temperature before use is acceptable and recommendable, and storage at 37^{0}C for 1 month does not jeopardize product quality. Iodixanol has no apparent immediate *in vitro* incompatibility reactions with drugs used in connection with roentgen contrast examination.

1.5 Preclinical pharmacokinetics and general toxicology of iodixanol.
Heglund, F. et al
*Acta Radiologica Supplement, **399**, 69-82 (1995)*

To document the safety of **iodixanol** and to assess its pharmacokinetic properties, extensive tests have been performed. Iodixanol was rapidly excreted, mainly via the kidney, with a plasma half-life in rats and monkeys of 25 and 76 min. respectively. The pharmacokinetic data was consistent with an extracellular distribution of iodixanol. During the 24 hours post-dosing, the urinary excretion was from 72 to 100% in rats and 78% in monkeys. Biliary excretion was 1.5% during the first 4 hours in rats. Fecal excretion was about 7% in rats and 0.8% in monkeys over the first 24 hours after injection. Approximately 0.5 and 1% of the dose was found in the kidneys of rats and monkeys, respectively, 24 hours after dosing. Acute toxicity of iodixanol in rats was low, with an LD_{50} greater than 21gI/kg. In mice the LD_{50} was 21gI/kg and the approximated median lethal dose (ALD_{50}) was found to range from 15 to 21 gI/kg. A single dose of 1 and 3 gI/kg was well tolerated in monkeys. As for other roentgen contrast media, a reversible, dose-related, vacuolation of the proximal tubules in the kidneys was seen in the acute and subacute studies in rats and monkeys. No relationship was seen between the vacuolisation and kidney function. Local tolerance studies demonstrated a low irritation potential for iodixanol when injected by a variety of intravascular and extravascular routes.

The reproductive capacity of male and female rats was unaffected by iodixanol when administered daily at doses up to 2 gI/kg/day. No teratogenic potential in rats and rabbits of iodixanol was observed. Further, no toxic effects on pups were seen when rats were dosed during the lactation period. Each of 4 standard genotoxicity tests was negative. No antigenic potential of iodixanol was observed when assessed by the passive cutaneous anaphylaxis test and the active systemic anaphylaxis test in guinea pigs.

The intravascular tolerability of iodixanol is high and, therefore, iodixanol should be considered as a safe roentgen contrast medium for intravascular use.

1.6 Iodixanol: A nonionic iso-osmotic centrifugation medium for the formation of self generated gradients.
Ford, T., Graham, J. and Rickwood, D.
*Anal. Biochem., **220**, 360-366 (1994)*

The physical and biological properties of **iodixanol**, a new nonionic density gradient medium, are described in this paper. It is effectively a dimer of Nycodenz and it exhibits two significant advantages over previous iodinated density gradient media- its aqueous solutions are iso-osmotic up to a density of 1.32 g/ml and it is capable of forming self-generating gradients in 1 to 3 h. It has a very low toxicity towards biological material and enzyme assays can be carried out in its presence.

1.7 Spectrophotometric determination of iodixanol in subcellular fractions of mammalian cells.
Schroder, M., Schafer, R. and Friedl, P.
*Anal. Biochem., **244**, 174-176 (1997)*

Nonionic iodinated density gradient media are widely used for the separation of cells and organelles. The most suitable of them is **iodixanol**. It displays no cytotoxicity and a number of marker enzymes for cellular organelles can be assayed in the presence of iodixanol. In contrast to other nonionic iodinated density gradient media, such as metrizamide or Nycodenz, iodixanol readily forms self-generated gradients, thus obviating the preparation of gradients using a gradient marker.

A subcellular fractionation of cellular organelles is evaluated by the distribution of marker enzymes in the gradient and the density profile of the gradient. The density profile of a gradient is commonly analyzed by measuring the refractive index of the gradient fractions. Cellular material present in subcellular fractions interferes with the determination of the refractive index, thus necessitating a mock fractionation to determine the density profile of the gradient.

Here we report the direct and specific determination of iodixanol as an example for a nonionic density gradient medium in subcellular fractions. No mock runs are necessary to determine the density profile of the gradient when this method is used. This assay should be more convenient for many laboratories because a refractometer is not required.

1.8 Determination of particle sedimentation rate by ultrasonic interferometry: role of particle size, density and volume fraction

Razavian, S.M., Wenby, R.B., Fisher, T.C. and Meiselman, H.J.
*Biorheology, **34(4/5)**, 349-362 (1997)*

The sedimentation rate (SR) of non-aggregated spherical particles in suspension was determined using an ultrasonic interferometry technique (Echo-Cell); this method is based on A-mode echography and measures the rate of formation of a sediment on a solid plate during settling. The particle accumulation rate, which is related to SR, is obtained from the interference of two waves reflected by two interfaces: one between the plate and the sediment and the other between the sediment and the suspension. Studies were carried out at 25°C using latex spheres of different diameters (7 to 20 μm) and densities (1.062 to 1.190 g/cm^3) suspended in distilled water at various volume fractions (1% to 5%). As anticipated by the Stokes model, linear relations were found between SR and both particle density and the square of particle radius. Experimental SR values decreased with increasing suspension particle concentration; these concentration effects were in good agreement with those predicted by the Steinour model. Our results thus serve to validate the theoretical aspects of the Echo-Cell method and suggest its usefulness as a tool for studies of RBC interaction and RBC aggregation.

1.9 Iodixanol is readily eliminated by hemodialysis

Berg, K.J., Rolfsen, B. and Stake, G.
*Acta Radiologica, **39**, 372-374 (1998)*

The dialyzability of the high-molecular X-ray contrast medium **iodixanol** was examined in an *in vitro* hemodialysis model using two different hollow fiber membranes: one high flux (polysulfone) membrane and one intermediate-flux (cellulose triacetate) membrane. Blood flow was 200 ml/min and membrane area 1.3 m^2. The dialyzer clearance of iodixanol dissolved in a mixture of leucocyte-filtered SAG-M blood and compatible citrate plasma was 143.2 ± 3.6 ml/min for the polysulfone membrane and 113.0 ± 3.6 ml/min for the triacetate membrane. Iodixanol is readily dialyzed through commercial high-flux membranes.

1.10 Stability of the X-ray contrast agent iodixanol - 3,3'5,5'-tetrakis(2,3dihydroxy-propylcarbamoyl)-2,2',4,4',6,6'-hexaiodo-N,N'-(2-hydroxypropane-1,3-diyl)-diacetanilide towards acid, base, oxygen, heat and light.

Priebe, H. et al.
*J. Clin. Pharm. and Therapeut., **24**, 227-235 (1999)*

Background: During the production of the X-ray contrast agent iodixanol the drug substance may be exposed to acid, base, air, heat and daylight, conditions that may cause decomposition products.

Objectives: To investigate the chemical stability of **iodixanol** under accelerating conditions.

Method: Chemometrical stability studies were undertaken to investigate the effect of acid and base on the contrast agent's stability.

Results: Cleavage of the central bridge in iodixanol occurred under ultraviolet irradiation via a Norrish Type-II reaction. Basic conditions (pH 14) combined with heat (60°C) initiated a cyclization reaction. Less than 1% iodixanol decomposed in solution heated to 140°C for 2 days or under both basic conditions (pH 11, 20°C, 5 days) and acidic conditions (pH 0-4, 80°C, 5 days) or under oxygen atmosphere (100°C, 3 days).

Conclusion: Even under highly acidic and basic conditions, iodixanol is stable.