# Air-filled, cross-linked, human serum albumin microcapsules

## Air-filled HSA microcapsules

Kenneth T. Cheng, PhD[1]

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| **Chemical name:** | Air-filled, cross-linked human serum albumin microcapsules |
| **Abbreviated name:** | Air-filled HAS microcapsules |
| **Synonym:** | Quantison™ |
| **Agent Category:** | Albumin |
| **Target:** | Non-targeted contrast agent, blood pool |
| **Target Category:** | Passive nontargeted filling of cardiac chambers |
| **Method of detection:** | Ultrasound (US) |
| **Source of signal/contrast:** | Air-filled microbubble |
| **Activation:** | No |
| **Studies:** | • *In vitro*<br>• Humans |

## Background

[PubMed]

The air-filled, cross-linked, human serum albumin (HSA) microcapsules (air-filled HSA microcapsules) is a stabilized mirobubble formulation that is being developed as an ultrasound (US) contrast agent for use in echocardiography to enhance US images (1)

US contrast agents, or echopharmaceuticals, are designed to change the attenuation (absorption, reflection, and refraction) or impedance (resistance to sound propagation) of sound to enhance the differentiation of the signal (echo) of a target organ from that of the surrounding tissue (2-5). Gas-liquid emulsions (i.e., microbubbles or gaseous particles)

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

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are highly echogenic *in vivo* because of the nonlinear rarefaction and compression effects that lead to volume pulsations of microbubbles (3, 6, 7). HSA, synthetic polymers, and phospholipids have been used to construct the membranes of these bubbles. Microbubble preparations of various formulations have been developed, and their clinical usefulness depends very much on the size and stability of these bubbles *in vivo*. The current clinical application of these agents is in myocardial contrast echocardiography (MCE) (8).

In addition to acoustic parameters, microbubble preparations must meet two other requirements in order to be useful for MCE applications. Microtubble preparations cannot be trapped significantly by the lung capillaries and they must be able to survive the high intracardiac pressure. Air-filled microbubbles stabilized within a galactose matrix were the first commercially available echopharmaceutical (9). However, these microbubbles are not stable enough to pass through the pulmonary capillary bed after a peripheral intravenous injection and can be used only to opacify the right heart chamber. Sonicated human serum microspheres (Sonicated HSM) were the first US contrast agent approved in the United States for cardiac applications. HSM consists of air-filled microbubbles stabilized in a thin shell of HSA with a mean diameter of 3.8 ± 2.5 μm (10). Although these air-filled microbubbles are very sensitive to pressure changes with an *in vivo* half-life ($t_{½}$) of <1 min, they can pass through the pulmonary capillary bed and reach the left heart chamber. The *in vivo* life-times of air-filled microbubbles can be prolonged by increasing the stiffness of the bubble shell (11). Air-filled HSA microcapsules have stabilized shells that can withstand pressures in excess of 300 mm Hg (1). Other microbubble preparations use perfluorocarbons which dissolve poorly in blood to increase the stability of the microbubbles (12).

Serious cardiopulmonary reactions following the administration of ultrasound microbubble contrast agents have been reported (13). In 2007, the US FDA requested that warnings emphasizing the risk for serious cardiopulmonary reactions be added to the labeling of these agents. The uses of these agents are contraindicated in patients with unstable cardiopulmonary status.

## Synthesis

[PubMed]

Air-filled HSA microcapsules are air bubbles encapsulated by a shell of HSA (1, 11). The microbubble shell is formed by spray-drying a solution of HSA. This shell is stabilized by heat fixation and chemical cross-linking. The final preparation is a dry powder that must be resuspended before use. The mean diameter is 3.2 μm, and less than 0.5% of the preparation had a diameter >6 μm. The shell thickness is 200-300 nm and is able to withstand static pressure up to 600 mm Hg without losing the air within the microbubble. The density of the bubbles is approximately 780 $kg/m^3$. The particle density in the preparation is about 1.5 x $10^9$ bubbles per ml.

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Chatterjee and Sarkar (14) suggested an interfacial rheological model with intrinsic surface rheology for studying the US microbubbles. Frinking and De Jong (11) studied the *in vitro* scatter and attenuation properties of air-filled HSA microcapsules based on an adapted version of the Rayleigh-Plesset equation which treats the microbubbles as a viscoelastic solid. The acoustic transmission and scattering were measured in the frequency band from 1-10 MHz. The effective bulk modulus (elasticity) of air-filled HSA microcapsules was 17.4 MPa and independent of the bubble diameter. In comparison, the effective bulk modulus of sonicated HSM was 1.3 MPa and increased for decreasing bubble diameter. The acoustic efficiency of air-filled HSA microcapsules was relatively low for low acoustic pressures but the measured scattering coefficient abruptly increased above an acoustic pressure of 200 kPa.

Moran et al. (15) used an *in vitro* tissue-mimicking phantom to study the scatter phenomenon of air-filled HSA microcapsules. A 3.5-5.0 MHz probe was used. At high concentrations (no dilution), no significant change in mean integrated backscatter was observed during continuous sonication. For medium (1:25 dilution) and low (1:50 dilution) concentrations, mean integrated backscatter values reached their maximum values and then decreased.

In various experiments, Sboros et al. studied the *in vitro* properties of US contrast agents (16-20). Using dilute suspensions of microbubbles, air-filled HSA microcapsules appeared to produce an increasing number of scattering events per unit volume with increasing acoustic pressure. However, the behavior across the acoustic pressure range was not uniform. Free bubbles appeared at the high acoustic pressure of 0.83 MPa peak negative pressure. Postema et al. (21) investigated the phenomenon of sonic cracking. They used high-speed photography to observe the high-mechanical index US-induced air released from air-filled HSA microcapsules For air-filled HSA microcapsules, sonic cracking was observed more often at driving frequency closer to the resonance frequency (1.7 MHz). Most of the released air bubbles had smaller equilibrium diameters (between 1.25 and 1.75 μm).

# Animal Studies

## Rodents

[PubMed]

No publication is currently available.

## Other Non-Primate Mammals

[PubMed]

No publication is currently available.

### Non-Human Primates

[PubMed]

No publication is currently available.

## Human Studies

[PubMed]

Perkins et al. (1) used $^{123}$I-labeled, air-filled HSA microcapsules and gamma imaging to study the biodistribution of air-filled HSA microcapsules in 12 normal healthy male subjects. The chloramines-T method was used to perform radiolabeling radiolabeling. The radiolabeling efficiency was 15%. The preparation was washed four times so that 100% of the radioactivity was bound to the microcapsules. Each subject received a dose of 50 MBq (1.35 mCi) with a total of 50 million microcapsules/kg. All subjects also received 100 mg potassium iodide three times to reduce thyroid uptake of [$^{123}$I]iodide. Gamma planar imaging showed immediate radioactivity uptake in the myocardium. There was evidence of myocardial perfusion at 3 min. The myocardial radioactivity which displayed a doughnut pattern, appeared to be more intense than that in the blood of the left ventricle. The washout of the agent from the myocardium was observed as no radioactivity was detected after 3 h. There was also rapid uptake of radioactivity by the liver and spleen. Posterior images showed some bone marrow uptake. Within the first hour, the liver had the highest radioactivity level of 41.8 ± 10.4% injected dose (ID). Transient lung activity of 4.0 ± 3.4% ID was observed. The maximum heart radioactivity level was 0.7 ± 0.4% ID at 0.06 hr. The spleen and kidneys had maximum radioactivity levels of 11.0 ± 6.2% ID at 1 hr and 1.6 ± 1.0% ID at 5 min, respectively. There was a rapid initial elimination of radioactivity from the blood with 15% ID at 5 min. About 63% ID radioactivity was excreted into the urine over 58 h and <0.5% in the feces over 36 h.

In a dose-finding study, van der Wouw et al. (22) conducted echocardiographic imaging (two-dimensional and color Doppler) in 12 healthy male volunteers. Each volunteer received two i.v. doses. The first dose was 25, 50, 100 or 150 x $10^6$ microcapsules/kg, and the second dose was 300 x $10^6$ microcapsules/kg. Air-filled HSA microcapsules required higher US output for visualization but the US contrast was visible in the left ventricular cavity for up to 30 min in two-dimensional and up to 60 min in color Doppler imaging.

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## References

1. Perkins A.C., Frier M., Hindle A.J., Blackshaw P.E., Bailey S.E., Hebden J.M., Middleton S.M., Wastie M.L. Human biodistribution of an ultrasound contrast agent

(Quantison) by radiolabelling and gamma scintigraphy. Br J Radiol. 1997;**70**(834): 603–11. PubMed PMID: 9227254.

2. Morawski A.M., Lanza G.A., Wickline S.A. Targeted contrast agents for magnetic resonance imaging and ultrasound. Curr Opin Biotechnol. 2005;**16**(1):89–92. PubMed PMID: 15722020.
3. Schutt E.G., Klein D.H., Mattrey R.M., Riess J.G. Injectable microbubbles as contrast agents for diagnostic ultrasound imaging: the key role of perfluorochemicals. Angew Chem Int Ed Engl. 2003;**42**(28):3218–35. PubMed PMID: 12876730.

4. Swanson, D.P., Enhancement agents for ultrasound: Fundamentals, in Pharmaceuticals in Medical Imaging, D.P. Swanson, H.M. Chilton and J.H. Thrall, Editor. 1990, MacMillan Publishing Co., Inc.: New York. p. 682-687.

5. Gobuty, A.H., Perspectives in ultrasound contrast agents, in Contrast media: Biologic effects and clinical application, Z. Parvez, R. Moncada and M. Sovak, Editor. 1987, CRC: Boca Raton, Florida. p. 145-155.

6. Averkiou M., Powers J., Skyba D., Bruce M., Jensen S. Ultrasound contrast imaging research. Ultrasound Q. 2003;**19**(1):27–37. PubMed PMID: 12970614.
7. Miller A.P., Nanda N.C. Contrast echocardiography: new agents. Ultrasound Med Biol. 2004;**30**(4):425–34. PubMed PMID: 15121243.
8. Murthy T.H., Li P., Locvicchio E., Baisch C., Dairywala I., Armstrong W.F., Vannan M. Real-time myocardial blood flow imaging in normal human beings with the use of myocardial contrast echocardiography. J Am Soc Echocardiogr. 2001;**14**(7):698–705. PubMed PMID: 11447415.
9. Correas J.M., Bridal L., Lesavre A., Mejean A., Claudon M., Helenon O. Ultrasound contrast agents: properties, principles of action, tolerance, and artifacts. Eur Radiol. 2001;**11**(8):1316–28. PubMed PMID: 11519538.

10. Summary of safety and effectiveness of Albunex. PMA number P9000059/ Supplement 004, Mallinckrodt, Inc. p. 1-28.

11. Frinking P.J., de Jong N. Acoustic modeling of shell-encapsulated gas bubbles. Ultrasound Med Biol. 1998;**24**(4):523–33. PubMed PMID: 9651962.
12. Mattrey R.F. Sonographic enhancement of Doppler signals and perfused tissues with perfluorooctylbromide. Invest Radiol. 1990;**25Suppl 1**S158–9. PubMed PMID: 2283238.
13. Blomley M., Claudon M., Cosgrove D. WFUMB Safety Symposium on Ultrasound Contrast Agents: clinical applications and safety concerns. Ultrasound Med Biol. 2007;**33**(2):180–6. PubMed PMID: 17254696.
14. Chatterjee D., Sarkar K. A Newtonian rheological model for the interface of microbubble contrast agents. Ultrasound Med Biol. 2003;**29**(12):1749–57. PubMed PMID: 14698342.
15. Moran C.M., Anderson T., Sboros V., Sutherland G.R., Wright R., McDicken W.N. Quantification of the enhanced backscatter phenomenon from an intravenous and an intra-arterial contrast agent. Ultrasound Med Biol. 1998;**24**(6):871–80. PubMed PMID: 9740388.

16. Sboros V., Moran C.M., Anderson T., Gatzoulis L., Criton A., Averkiou M., Pye S.D., McDicken W.N. An in vitro system for the study of ultrasound contrast agents using a commercial imaging system. Phys Med Biol. 2001;**46**(12):3301–21. PubMed PMID: 11768507.
17. Sboros V., Moran C.M., Pye S.D., McDicken W.N. Contrast agent stability: a continuous B-mode imaging approach. Ultrasound Med Biol. 2001;**27**(10):1367–77. PubMed PMID: 11731050.
18. Sboros, V., C.A. MacDonald, S.D. Pye, C.M. Moran, J. Gomatam, and W.N. McDicken, The dependence of ultrasound contrast agents backscatter on acoustic pressure: theory versus experiment. Ultrasonics, 2002. 40(1-8): p. 579-83.
19. Sboros V., Moran C.M., Pye S.D., McDicken W.N. The behaviour of individual contrast agent microbubbles. Ultrasound Med Biol. 2003;**29**(5):687–94. PubMed PMID: 12754068.
20. Sboros V., Ramnarine K.V., Moran C.M., Pye S.D., McDicken W.N. Understanding the limitations of ultrasonic backscatter measurements from microbubble populations. Phys Med Biol. 2002;**47**(23):4287–99. PubMed PMID: 12502050.
21. Postema M., Bouakaz A., Versluis M., de Jong N. Ultrasound-induced gas release from contrast agent microbubbles. IEEE Trans Ultrason Ferroelectr Freq Control. 2005;**52**(6):1035–41. PubMed PMID: 16118985.
22. van der Wouw P.A., Braune A.C., Levi M., Bailey S.E. Quantison, a new long-living ultrasound contrast agent: experience in human volunteers. J Am Coll Cardiol. 1997;**29**Suppl A299A.