Review Article

Medical Applications of Siderophores

Basavraj Nagoba, Deepak Vedpathak

[1]*Maharashtra Institute of Medical Science and Research Medical College, Latur*

[2]*Rajarshi Shahu College, Latur*

Eur J Gen Med 2011;8(3):229-235

Received: 13.11.2010

Accepted: 01.01.2011

***Correspondence**: Dr. B. S. NAGOBA*
Assistant Dean, Research & Development,
MIMSR Medical College, Latur - 413 531 (M.S.) India
GSM: 9109423075786, Fax: 9102382227246
E-mail:dr_bsnagoba@yahoo.com,
bsnagoba@indiatimes.com

ABSTRACT

The literature survey presented in this review shows that the knowledge regarding the structure of various siderophores and the presence of microbial membrane receptors that operates in iron uptake from these iron - siderophore complexes have opened newer avenues for research. Siderophores and their substituted derivatives have a large number of applications in medical sciences. The most important application is selective drug delivery, a Trojan horse strategy, to defeat drug resistant bacteria. The Trojan horse strategy is most promising way to specifically attack multiple antibiotic resistant strains of bacteria associated with life threatening infections. Other major clinical applications include treatment of diseases like haemochromatosis, thalassemia, dialysis encephalopathy, removal of transuranic elements such as aluminium and vanadium and anti-malarial activity.

***Key words:** Siderophores, trojan horse strategy, medical applications*

Siderophor'ların Tıbbi Uygulamaları

ÖZET

Bu derlemede sunulan literatür taraması, çeşitli siderophores yapıları ve yeni araştırmalara yol açan, demir siderofor komplekslerinden kaynaklanan demirin alımında faaliyet gösteren mikrobiyal membran reseptörlerinin varlığı ile ilgili bilgiler vermektedir. Siderophoresler ve onların türevlerinin tıp bilimlerinde çok sayıda uygulamaları vardır. En önemli uygulama, ilaca dirençli bakterileri yenmek için, Truva atı starejisi gibi selektif ilaç teslimidir. Truva atı stratejisi, hayatı tehdit eden enfeksiyonlar ile ilişkili bakterilerin, özellikle birden fazla antibiyotiğe dirençli suşlarına saldırmak için çok gelecek vaat eden yoldur. Diğer önemli klinik uygulamaları, hemokromatoz, talasemi, diyaliz ensefalopatisi, alüminyum ve vanadyum gibi uranyum ötesi elementleri kaldırılması ve sıtma gibi hastalıkların tedavisini içerir.

Anahtar kelimeler: *Siderophor, truva atı starejisi, tıbbi uygulamalar*

INTRODUCTION

The concern over increasing antibiotic resistance in pathogenic bacteria, due to their capacity to adapt by natural selection, is wobbling the medical field all over the world. It has forced the pharmaceutical industries to search for newer effective antibiotics. To overcome the drug resistance problem, the sensible use of antibio tics available with us must be respected. One newer attitude that has been explored to resolve the problem is to smuggle the antibiotic molecule into the bacterial cell by binding it to a siderophore molecule (the Trojan Horse strategy).

Most organisms require iron as an essential element in a variety of metabolic and informational cellular pathways. More than 100 enzymes acting in primary and secondary metabolism possess iron containing cofactors (1). Although iron is abundantly present in the environment, ionic forms of iron are insoluble under physiological conditions and hence are difficult to assimilate for microorganisms (2). Three to four gram of iron is generally present in Human body, out of which haemoglobin and myoglobin acquire the major share of 70% (3). Mammalian iron-binding proteins, such as transferrin and lactoferrin, reduce the amount of iron available to the microbes. The iron acquiring skill of pathogens is essential for their ability to cause infection in animal hosts and to establish the host-parasite relationship (4 -7). Under such iron-depriving conditions, the aerobic and facultative anaerobic bacteria and other microorganisms frequently produce organic chelates that combine with inorganic iron and greatly enhance its solubility. These microorganisms also produce the unique outer membrane proteins (OMPs) which serve to recognize process and transport the siderophore- iron complex (es) into cell. These chelates, which were designated earlier as siderochromes, sideramines, and sideromycins, are now conveniently termed as Siderophores (meaning in Greek: sideros = iron and phores = bearer). Siderophores are technically defined as the ferric iron specific, low molecular weight (<1500) compounds, which solubilize and transport iron in to the cell (8-11).

Classes of Microbial Siderophores

A large number of Siderophores produced by different microorganisms have been documented. The most common Siderophores can be classified traditionally as phenol-catecholates and hydroxamates, depending upon the chemical moieties that are involved in coordination of the ferric iron (12), while siderophores that contain neither of these ligand systems have extended the classification to a third main class i.e. carboxylate siderophores. Particularly the first two classes exhibited a high structural diversity and were found in a vast number of microorganisms. Fungi predominantly produce hydroxamate type of Siderophores whereas bacteria and related prokaryotes produce hydroxamate as well as catecholate types (13, 14). The siderophores of fluorescent *Pseudomonas spp.* and *Escherichia coli* are most extensively studied. The succinate medium is widely used for production of siderophores by *Pseudomonas spp.* by a process of fermentation. The appearance of parrot green colour in the medium is an indication of production of siderophores (Figure 1). The siderophore produced can be extracted and purified for further studies by using phenol-chloroform solvent extraction method (Figure 2).

Some Medically Important Bacterial Siderophores

Medically important bacterial species producing siderophores are summerized in Table 1.

Medical Applications of Siderophores

The obligate nutritional requirement for iron by multiple drug resistant pathogens can be exploited to control its infection. The three fundamental perceptions of iron dependant pathogen control include the Trojan horse concept to facilitate the cellular uptake of antibiotics, artificial iron starvation by using siderophores or antagonists that cannot be utilized as an iron source by the pathogen and inhibition of iron metabolism pathways (23). Siderophores and their substituted derivatives have a large number of applications in medical sciences. Siderophores have potential applications in the treatment of some human diseases and infections. These are as follows:

1. Selective Drug Delivery - Trojan horse strategy (Siderophore-antibiotic conjugates - Sideromycins)

Siderophores can be used for selective delivery of antibiotics in antibiotic resistant bacteria. It is a newer antimicrobial approach to defeat drug resistant bacteria. It is the potentially powerful application that uses the iron transport abilities of siderophores to carry drugs into cells by preparation of conjugates between siderophores and antimicrobial agents. It uses siderophores as mediators to facilitate the cellular uptake of antibiotics. This interaction of antibiotic with siderophores results in a

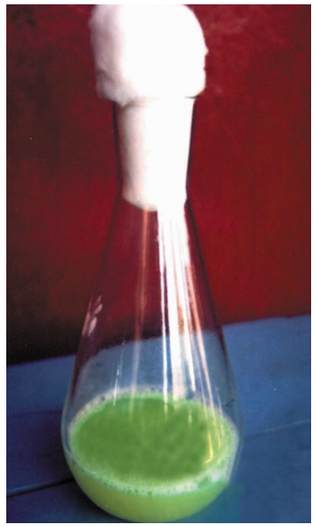

***Figure 1**. The fermented succinate broth showing siderophore production*

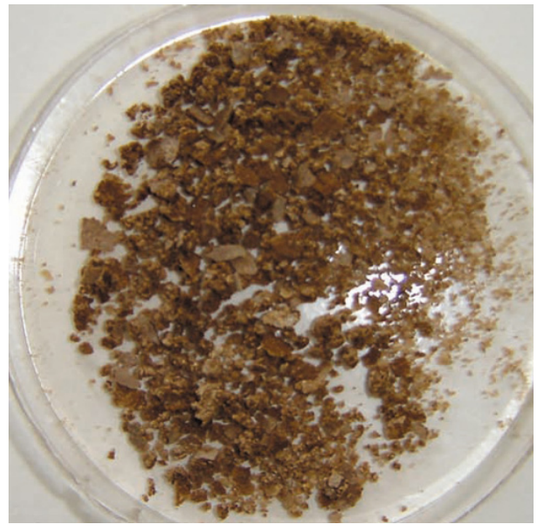

***Figure 2**. Crude siderophore crystals obtained by solvent extraction method*

formation of siderophore – antibiotic conjugates known as Sideromycins. These are the microbe selective compounds that put together two utilitarian molecules, a si derophore and an antibiotic, into one structural scaffold. The siderophore part of this conjugate is able to scavenge iron and is recognized by cellular Fe-Siderophore uptake systems, while the other part of conjugate bears an antibiotic activity that uses the siderophore as a Trojan horse and mediates iron transport mediated drug delivery.

The antibiotic molecule recruits the siderophore as a "Trojan horse" vehicle, taking advantage of the Fe- siderophore uptake system, to enter the microbial cell. The drug conjugate usually also consists of a linker for attac hing the drug to the siderophore. The linker allows controlled, chemical or enzymatic, release of drugs in the target bacterial cell. On reaching into the cell, the side rophore–drug conjugate kills the cell by releasing the drug or by acting as an intact antibacterial agent or by blocking further iron acquisition. The siderophore – antibiotic conjugates are of two types - natural and synthetic:

a. Natural siderophore–antibiotic conjugates are: Albomycins, ferrimycins, danomycins, salmycins (isolated from Streptomyces and Actinomyces), microcins (isolated from enteric bacteria)

- Albomycins–blocks protein synthesis by inhibiting t- RNA synthetase in *E. coli*

- Danomycins and salmycins–inhibit protein synthesis in Gram positive bacteria

- Ferrimycins–inhibit Gram positive bacteria by altering protein biosynthesis

- Microcins–inhibit *E. coli* and *Klebsiella spp.*

These are siderophore transport system to gain access to the cell after gaining entry inside the cell – exert antibacterial activity by inhibiting protein synthesis.

b. Synthetic Siderophore–antibiotic conjugates-These can be prepared – using antibiotics with improved cell permeability and reduced susceptibility to resistance mechanisms. Siderophores can be bound to potent antibiotics such as beta lactam (Carbacephalosporins), erythromycin, sulphonamides, spiramycin, vancomycin, nalidixic acid, norfloxacin, etc. Antifungal agents – such as 5- flourocytosine and neoenactins can also be conjugated and used against fungi. The results indicate that these conjugates give better results and this siderophores mediated drug delivery is remarkably effective. Microbes recognize the siderophore component as an iron delivery agent and assimilate the conjugate and commit suicide as the attached drug is lethal to them. The siderophore-antibiotic conjugates can also be used against pathogenic

***Table 1**. Some medically important bacterial siderophores*

Organism	*Siderophore*	*Reference*
Escherichia coli /Salmonella spp.	*Enterobactin*	*(15)*
Pseudomonas aeruginosa	*Pyoverdin and pyochelin*	*(16)*
Aerobacter aerogenes/ Klebsiella pneumoniae	*Aerobactin*	*(17)*
Vibrio cholerae	*Vibriobactin*	*(18)*
Mycobacterium tuberculosis	*Mycobactin*	*(19)*
Yersinia pestis	*Yersiniabactin*	*(20)*
Staphylococcus aureus	*Aureochelin/ Staphylobactin*	*(21,22)*

bacteria, which produce and use siderophores to acquire iron (24-29).

2. Use of Gallium as Trojan Horse to Iron Seeking Bacteria

It is well known fact that iron is critical for the growth of bacteria and for their ability to form biofilms in slime encased bacteria that cause many chronic infections. In a study carried out at university of Washington, gallium, a metal very similar to iron (looks like iron) was used instead of iron. Because of structural similarity to iron, gallium is also chelated like iron by siderophores and siderophores are recognized by the cellular receptors on bacterial cells that allows the molecule (Siderophore-gallium complex) to enter inside the bacterial cells. Gallium was found to act Trojan horse to iron -seeking bacteria and killed them and inhibited biofilm formation. Gallium was also found effective against multiple antibiotic resistant strains of *Pseudomonas aeruginosa*. In mice, gallium treatment blocked both chronic and acute infections caused by *Pseudomonas aeruginosa*. This approach is under trial and results are promising (30).

3. Treatment of Iron Overload Diseases

Some siderophores are used in the treatment of acute iron intoxication and chronic iron overload diseases associated with excessive blood transfusions. In acute iron intoxication and chronic iron overload diseases, siderophores can be used as chelating agents, which are able to bind with iron to produce complexes that lead to formation of ferrioxamine. The ferrioxamine is soluble in water and readily excreted through the kidneys. It binds with iron in the blood and enhances its elimination via urine and faeces. Hence, it is of value in the treatment of acute iron intoxication and chronic iron overload diseases. Thus, it can be used to decrease the iron overload in the body.

These acute iron intoxication and chronic iron overload diseases include:

a. Haemochromatosis

Haemochromatosis is a disorder in which there is a prog ressive increase in the iron content of the body causing deposition of iron in the liver, heart or pancreas. It is the most common form of iron overload disease that occurs as a primary haemochromatosis (hereditary due to genetic abnormalities) or as a metabolic disorder. It causes body to absorb and store too much of iron from gastrointestinal tract. Haemosiderosis is a secondary haemochromatosis caused by excessive blood transfusions. Secondary haemochromatosis occurs as a result of severe chronic haemolysis, multiple frequent transfusions, excessive parenteral iron supplements and excess dietary iron intake. Clinically, haemochromatosis is manifested as joint pain – most common (Arthritis), fatigue, lack of energy, abdominal pain, loss of sex drive, heart problems, liver disease, such as cirrhosis, damage to pancreas, abnormal pigmentation of skin and damage to adrenal glands.

Treatment of haemochromatosis; Desferal (Deferoxamine mesylate) is a chelating agent which binds with trivalent iron and form complexes that leads to formation of ferrioxamine. The complex ferrioxamine is soluble in water and readily excreted through the kidneys. It binds with iron in the blood and enhances its elimination via urine and faeces. Hence, it is of value in the treatment of haemochromatosis.

Doses and Administration; 6.0 gm in 24 hrs – not more than this is to be given intramuscularly or by slow subcutaneous injection or intravenous (IV) infusion. By intramuscularly or subcutaneous route it must be taken over 8-24 hrs due to its short duration of effect within body. Once its infusion stops, iron removal stops shortly thereafter, hence intermittent infusion has little effect on reducing iron overload. In chronic iron overload for infusion

treatment the average daily dose of 1-4 gm (20-60 mg/Kg) depending up on iron overload is given by subcutaneous or intravenous route over a period of 12 hrs.

b. Sickle cell disease

It is a lifelong genetic disorder characterized by abnormal, rigid and sickle shaped red blood cells (RBCs) that needs repeated blood transfusions. Iron overload in these cases is likely to be detected after 20 transfusions. Iron overload can be detected by monitoring serum ferritin level but study of liver biopsy is more accurate test for the diagnosis. Desferal is the treatment for sickle cell di sease.

c. Thalassemia major

It is a genetically transmitted haemoglobin abnormality that leads to anemia. It needs multiple transfusions because of which iron overload is likely to occur after the first 10-20 transfusions (near the age of three years). Iron overload is monitored by serum ferritin level (serum ferritin level > 1000 microgram/L is an indicator) but this test is not reliable, hence for confirmation liver biopsy and MRI are used. Desferal is the drug used for the treatment of thalassemia major (31 -35).

4. Siderophores in the Treatment of Malaria - Antimalarial Activity

Some siderophores have been found to be useful in the treatment of malaria caused by *Plasmodium falciparum*. Desferrioxamine B produced by *Streptomyces pilosus* (Now produced by chemical synthesis also) is active against *P. falciparum in vitro* as well as *in vivo*. This agent enters the parasite and causes intracellular iron depletion. This agent conjugated with methylanthranilic acid shows 10- fold higher *in vitro* activity against *P. falciparum*, which could be increased further by using nalidixic acid as a conjugate against multidrug resistant *P. falciparum*. The mode of action of this conjugate is metal-catalyzed oxidative DNA damage (36 -38).

5. Siderophres in the Removal of Transuranic Elements

The development of electricity generation by nuclear energy has led to increased human exposure to transuranic elements such as aluminium. Siderophores can be used to remove such elements from the body.

a. Removal of Aluminium

Aluminium overload occurs in dialysis encephalopathy (is a major complication of long - term dialysis, which is caused by the accumulation of aluminium in the brain) and dialysis patients with end stage renal failure (ESRF). In the treatment of chronic aluminium overload - Desferol can mobilize and chelate tissue bound aluminium forming an aluminoxamine complex, which are freely soluble in water and are readily excreted through the kidneys (urine) and may also be excreted in faeces. Desferol forms complex with aluminium ions, hence can be used in the treatment of aluminium overload. It is given as a single dose, slow intravenous infusion - 50 mg/kg (15 mg/kg/h). In children with ESRF- 15-20mg/kg is given. In chronic aluminium overload with ESRF -5.0 mg/kg IV infusion (15 mg/kg/h) once weekly for three months. Monitoring Criteria –Aluminum level is to be monitored properly. The aluminium level of 60 ng/ml and aluminium related bone diseases (diagnosed by bone biopsy) are the criteria for monitoring the same.

b. Removal of Vanadium

Desferal can also be used for removal of vanadium from the body. It has been found that in rats, desferal reduced the vanadium content in kidney by 20%, in lungs by 25% and in liver by 26%. It has been found that desferal increases the urinary and faecal excretion of vanadium. It is poorly absorbed through gastrointestinal tract (GIT). Inhalation causes adverse effects on the respiratory tract. Oral / inhalation exposures – affect blood parameters and create liver problems, neurological problems and problems in other organs. These effects have been studied in rats (39 -42).

6. Other Uses of Siderophores

a. Deoderant - Siderophore from *Klebsiella pneumoniae* has been used in cosmetics as deodorant (43).

b. Cancer Therapy - Some siderophores, e.g, dexrazoxane, O- trensox, desferriexochelins, desferrithiocin, tachpyridine, have been found useful in cancer therapy (24).

REFERENCES

1. *Chincholkar SB, Chaudhari BL, Talegaonkar SK, Kothari RM. Microbial iron chelators: A sustainable tool for the biocontrol of plant diseases. In: Upadhaya RK, Mukerji KG, and Chamola BP (eds.), Biocontrol potential and its exploitation in sustainable agriculture, New York; Kluwer Academic/Plenum Publishers, 2000; 49-70.*
2. *Barber SA. Iron; soil nutrient bioavailability- a mechanistic approach. John Wily and Sons Inc. 1995;330-7.*

3. Messenger A J M, and Ratledge C. Siderophores. In: Moo Young M. (eds.), Comprehensive biotechnology. Vol.3. Pergamon press, 1985; 275-295.

4. Carmen VS and Richard AF. Vibrio cholerae Expresses Iron-Regulated Outer Membrane Proteins In- Vivo. Infect Immun 1983;42(3);990-6.

5. Bulien JJ. The significance of iron in infection. Rev Infect Dis 1981;3:1127-38.

6. Finkelstein RA, Sciortino CV and McIntosh MA. Role of iron in microbe-host interactions. Rev Infect Dis 5(Suppl. 4):1983;759-77.

7. Weinberg ED. Iron withholding: a defense against infection and neoplasia. Physiol Rev 1984;64:65-102.

8. Emery T. The storage and transport of iron, in: Metal ions in biological systems, Iron in model and natural compounds, H. Sigel (eds.), New York, Marcal Dekker Inc., 1977;7:77-125.

9. Neilands JB. Microbial iron compounds. Annu Rev Biochem 1981;50:715-31.

10. Neilands JB. Microbial envelope proteins related to iron. Annu Rev Microbiol 1982;36:285-309.

11. Lankford CE. Bacterial assimilation of iron. Crit Rev Microbiol 1973;2:273-331.

12. Neilands JB. Siderophores of bacteria and fungi. Microbiol Sci 1984;1:9-14.

13. Hofte M. Classes of microbial siderophores. In : Iron chelation in plants and soil microorganisms. Academic Press Inc. 1993;3-26.

14. Winkelman G. and Drechsel H. Microbial siderophores. In: products of secondary metabolism. Vol.7. Kleinkauf, H. and von Dohren, H.(eds.). Germany, Wiley -VCH. Weinheim, 1997;200-46.

15. Dertz EA, Jide Xu, Stintzi A, and Raymond K N. "Bacillibactin-Mediated Iron Transport in Bacillus Subtilis". J Am Chem Soc 2006;128(1):22-3.

16. Budzikiewicz H. Siderphores of the human pathogenic fluorescent pseudomonads. Curr Topics in Med Chem 2000; 11(1):1-6.

17. Gibson F and Margrath DI. The isolation and characterization of a hydroxamic acid (aerobactin) formed by Aerobacter aerogenes 62-I. Biochem Biophysics Acta 1969; 92:175-84.

18. Griffiths Gl, Sigel SP, Payne SM and Neilands JB. Vibriobactin, a siderophore from Vibrio cholerae. J Biol Chem 1984;259:383-5.

19. Snow GA. Mycobactins: iron-chelating growth factors from Mycobactaria. Bacteriol Rev 1970;34:99-125.

20. Perry RD, Balbo PB, Jones HA, Fetherston JD and Demoll E. Yersiniabactin from Yersinia pestis: biochemical characterization of the siderophore and its role in iron transport and regulation. Microbiology 1999;145:1171-90.

21. Courcol RJ, Trivier, D, Bissinger MC, Martin, GR and Brown, MRW. Siderophore production by Staphylococcus aureus and identification of iron-regulated proteins. Infect Immun 1997;65:1944-8.

22. Dale SE, Doherty-Kirby A, Lajoie G and Heinrichs DE. Role of siderophore biosynthesis in virulence of Staphylococcus aureus: identification and characterization of genes involved in production of a siderophore. Infect Immun 2004;72:29-37.

23. Ballouche M, Cornelis P and Baysse C. Iron Metabolism: A Promising Target for Antibacterial Strategies. Recent Pat Antiinfect Drug Discov 2009;4:190-205.

24. Miethke M and Marahiel M A. Siderophore-Based Iron Acquisition and Pathogen Control. Microbiol Mol Biol Rev 2007;71(3):413-51.

25. Ding P, Schous C E, Miller M J. Design and synthesis of a novel protected mixed ligand siderophore. Tetrahedron Lett 2008;49:2306-10.

26. Budzikiewicz H. Siderophore-Antibiotic Conjugates Used as Trojan Horses Against Pseudomonas aeruginosa 1. Curr Top Med Chem 2001;1:73-82.

27. Kinzel O, Tappe R, Gerus I.; Budzikiewicz H. The synthesis and antibacterial activity of two pyoverdin ampicillin conjugates, entering Pseudomonas aeruginosa via the pyoverdin-mediated iron uptake pathway. J Antibiotics. 1998;51:499-507.

28. Diarra MS, Lavoie MC, Jacques M, Darwish I, Dolence EK, Ghosh AJA, Miller MJ and Malouin F. Species selectivity of new siderophore - drug conjugates that use specific iron uptake for entry into bacteria. Antimicrob Agents Chemother 1996;40:2610-7.

29. Heevwer Budzikiewicz. Siderophore - Antibiotic conjugates as Trojan horses against Pseudomonas aeruginosa. Curr Top Biochem 2001;1:73-82.

30. Reedy J. Trojan horse strategy defeats drug resistant bacteria (online). 2007 June 9; Available from: File://D:\siderophore\Trojan horse strategy defeats drug-resistant bacteria.htm

31. Propper RD, Cooper B, Rufo RR, Nienhuis AW, Anderson WF, Bunn F, Rosenthal A and Nathan DG. Continuous subcutaneous administration of deferoxamine in patients with iron overload. N Engl J Med 1977; 297: 418 - 423.

32. Robotham JL and Lietman PS. Acute iron poisoning - a review. Am J Dis Child 1980; 134: 875 - 897.

33. Summers MR, Jacobs A, Tudway D, Perera P and Rickets C. Studies in desfferoxamine and ferrioxamine metabolism in normal and iron loaded subjects. Br J Haematol 1979; 42: 547- 555.

34. Cao A, Gabutti V, Masera G, Modell B, Sarchia G and Vullo C. Management protocol for the treatment of thalessima patients. Newyork Cooly's Anaemia Foundation.

35. Möllmann U, Heinisch L, Bauernfeind A, Köhler T, Ankel-Fuchs D. Siderophores as drug delivery agents: application of the "Trojan Horse" strategy. Biometals 2009;22(4):615-24.

36. Loyevsky M, John C, Dickens B, Hu V, Miller JH and Gordeuk VR. Chelation of iron within the erythrocytic Plasmodium falciparum parasite by iron chelators. Mol Biochem Parasitol 1999;101:43- 59

37. Loyevsky M, Lytton SD, Mester B, Libman J, Shanzer A and Cabantchik ZI. The antimalareial action of desferal involves a direct access route to erythrocytic (Plasmodium falciparum) parasites. J Clin Investig 1993; 91:218-24.

38. Gysin Jurg, Crenn, Yvwa, Preira da silva, Luiz, Breton, Catherine. Siderophores as anti parasitic agents. US patent 5, 1991:192, 807.

39. Ackrill P, Raiston AJ, Day and Hoodge KC. Successful removal of aluminum from patients with encephalopathy. Lancet 1980;2:692-3.

40. Arze RS, Parkinson IS, Cartilidge NEP, Britton P and Ward MK. Reversal of aluminum dialysis encephalopathy after desferrrioxamine treatment. Lancet 1981;2:1116.

41. Pogglitsch H, Petek W, Wawschinck O and Holzer. Treatment of early stages of dialysis encephalopathy by aluminium. Lancet 1981;2:1344-5.

42. Hansen TV, Aaxeth J and Alexander J. The effect of chelating agents on vanadium distribution in the rat body and on uptake by human erythrocytes. Arch Toxicology 1982; 50:195-202.

43. Johnson PA, Bebington GB, Landa, Andrew Sjakk, Markin, Stephan Anthony, Mckay Victroria Anne, 2003. Deodorant produts, US patent no. 6, 503,490.