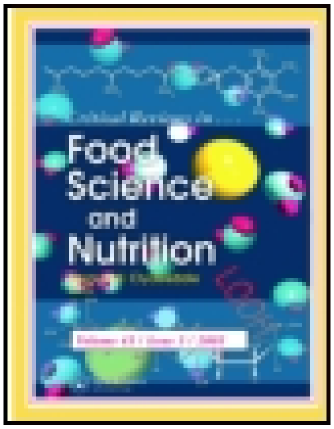

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Nano Delivery: An Emerging Avenue for Nutraceuticals and Drug Delivery

Md Aklakur[a], Mohd Asharf Rather[b] & Neeraj Kumar[b]
[a] Division of Fish Physiology and Biochemistry, Central Institute of Fisheries Education, Mumbai- India
[b] Division of Fish Genetics and Biotechnology, Central Institute of Fisheries Education, Mumbai- India
Accepted author version posted online: 01 Apr 2015.

Nanodelivery for Nutraceuticals and Drugs

Nano delivery: An Emerging avenue for Nutraceuticals and Drug delivery

Md Aklakur*[1], Mohd Asharf Rather[2] and Neeraj Kumar[2]

[1]Division of Fish Physiology and Biochemistry, Central Institute of Fisheries Education, Mumbai- India

[2]Division of Fish Genetics and Biotechnology, Central Institute of Fisheries Education, Mumbai- India

*Corresponding author- Md Aklakur, Central Institute of Fisheries Education, Versova, Mumbai 400 061, India, Fax: +91 22 26361573. Email id- romiakela@gmail.com

Abstract

Aquaculture has been globally recognized as the fastest growing food production sector which plays a major role in meeting the increasing demand for animal protein requirement. A consensus is growing that a dramatic increase in aquaculture is needed to supply future aquatic food needs. However, there are sustained problems with the aquaculture like disease outbreaks, chemical pollution, the environmental destruction and inefficient feed utilization. These altogether raise question mark on sustainability of aquaculture. In spite of the several strategy adopted on national and international level, as improved laboratory facilities, diagnostic expertise, and control and therapeutic strategies in order to handle disease outbreaks more effectively. Aquaculture industry is under uncertainty and the progress has not matched that of the rapidly developing aquaculture sector. In order to control disease prevalence and ensure better health of system and sustainable production, the sector demand more technical innovation for the drug use, disease treatment, water quality management , production of tailored fish for suiting better health, productivity drive by epigenetic and nutrigenomic interaction, better breeding success by efficient delivery of maturation and spawning inducing agent, nutraceutical delivery for rapid growth promotion and culture time reduction, successful use of auto-transgenic and effective vaccine. Nanotechnology has a tremendous potential to revolutionize agriculture and allied fields including aquaculture and fisheries. For these multiple purposes effort, importance of nanotechnology and nanodelivery of drugs, vaccine, nutraceutical, inducing hormones and growth promoting anabolics open tremendous opportunity. The paper has been targeted to delineate the possible future application of nanodelivery for the aquaculture development.

Keywords

Aquaculture, Drugs, Nanodelivery, Nutraceutical, Vaccine

 ACCEPTED MANUSCRIPT

Introduction

Capture fisheries and aquaculture supplied the world with about production of 154 million tonnes, in 2011 of which 131 million tonnes was destined as food. In the last three decades (1980ï 2010), world food fish production of aquaculture has expanded by almost 12 times, at an average annual rate of 8.8 percent. Global aquaculture production has continued to grow. World aquaculture production attained another all-time high in 2010, at 60 million tonnes (excluding aquatic plants and non-food products), with an estimated total value of US$119 billion. When farmed aquatic plants and non-food products are included, world aquaculture production in 2010 was 79 million tonnes, worth US$125 billion. About 600 aquatic species are raised in captivity in about 190 countries for production in farming systems of varying input intensities and technological sophistication (FAO 2012). The rapid growth in this sector has been driven by a variety of factors, including pre-existing aquaculture practices, population and economic growth, relaxed regulatory framework and expanding export opportunities. Single sustained problem the aquaculture growth suffers is the frequent disease outbreak and loss of the production due to disease, especially viral diseases, bacterial diseases and the fungal epizootics like EUS (Epizootic Ulcerative Syndrome). Another major is to be accounted is the current global population is nearly 6 billion with 50% living in Asia. For developing food supply, the drive is to develop drought and pest resistant crops, wild and environmental suitability of species under culture and impact free use of the exotic species as well as the captive and commercial breeding of the high price marine fishes and to maximize yield and overall aquaculture production manifold via horizontal and vertical extension and intensification. These in turn demand involvement of new technology to provide safe and healthier food free of chemical residues. So the biggest challenge of todayôs aquaculture system or other food producing sector is to enhance production through intensification and same time producing the safe and healthier produce without chemical residues.

Among all food producing sectors aquaculture comes to be the fastest growing food production sector with a rate of 8.8 per cent annually (FAO, 2012). But the Single unsolved question mark for aquaculture and its sustainability is ï ñ*disease prevalence and the poor health of system".* Inspite of the several strategy adopted on national and international level, as improved laboratory facilities, diagnostic expertise, and control and therapeutic strategies in order to handle disease outbreaks more effectively. Aquaculture industry is under uncertainty and the progress has not matched that of the rapidly developing aquaculture sector. The sector demand more technical innovation for the drug use, disease treatment, water quality management , production of tailored fish for suiting better health, productivity drive by epigenetic and nutrigenomic interaction, better breeding success by efficient delivery of maturation and spawning inducing agent , nutraceutical delivery for rapid growth promotion and culture time reduction, successful use of auto-transgenics and effective vaccine . For these multiple purposes the effort and importance of the nanotechnology and nano delivery of drugs, vaccine, nutraceuticals, inducing hormones and growth promoting anabolic bears the promise.

The world aquatic health markets and R&D investment are characterized with several unsolved issues as it is least regulated and mostly on thumb rules followed from research in veterinary background. There has been no species and endemic specific approach yet for aquaculture drugs. The various global aquaculture diseases has been causing severe loss to culture such as White Spot Syndrome Virus (WSSV), Early Mortality Syndrome (EMS), Yellow

Head Virus (YHV), Epizootic Ulcerative Syndrome (EUS) etc. and we still lack the solid solution for it . Even several drugs, chemotherapeutics and sanitizing agent used in aquaculture have not been properly studied in term of toxicokinetics, pharmacokinetics and pharmacodynamics in species specific manner. While the most of the drugs used from veterinary background in aquaculture has toxicity to fishes at higher doses and even at double the dose of application fish shows the stress signals. Same time at market front there has been monopoly in business because not much market competition for few products, while a new intruder in market needs to face the challenge of developing the full market channel and product distribution system. There are several pesticides, insecticides and chemotherapeutics, which are used as water bath or immersion to control the pathogenic infestation but they waive a temporary solution with huge impact on the environment and non-target organism lying under trophic and up trophic species in the ecosystem, leading a question mark on sustainability of aquaculture. The products available to control pathogen or cure diseases do not account the protection of host from harsh impact of the drugs, their immunity and health recovery, and protection of vital organ to ensure production performance with no drug residue.

The use of nutraceutical has been much advocated for the production performance boosting immunity and feed utilization enhancement (Sinha *et al* . 2007 ; Tejpal *et al*. 2009; Jha *et al*. 2007). There are various group of nutraceuticals being used in aqua feed industry and on farm such as enzymes, methyl donors, nucleotides, levans and other immunostimulant chitin, chitosan, polycheat and tunicates extract, vitamins , antioxidant minerals , amino acids, anabolic growth promoters, organic acids, caratenoids etc. No doubt that the use of nutraceuticals have the better impact on feed utilization, immunity, flesh quality, stress tolerance, reproductive and productive performance beside better water quality during the culture due to less feed waste and feed utilization efficiency. But aquaculture being a less margin culture system and with longer duration of production cycle compared to poultry has no option to use the cost raising nutraceutical to the feed. The feed cost raised has affect on the final profitability as the feed accounts about 60 % of input cost, therefore the regulated use of the nutraceutical components and efficient delivery systems for nutraceuticals and nutritional supplements becomes essential.

Nanodelivery and nutraceuticals

Nanotechnology provides a mean of manipulating and altering these nutraceutical components more effectively which enables to overcome several technological limitations negatively impacting the development of nutraceuticals and nutritional supplements and their efficient delivery. For instance, the use of nanotechnologies can improve the solubility and utility of nutraceuticals and nutritional supplements. Solubility problems can affect the applicability and performance of a biologically active compound in a variety of ways: First, poor water solubility can limit the range of formulations available for a bioactive compound. For the same reasons, insoluble nutrients are not useful for enhancing feeds utilization (Jani *et al*. 1990). Second, poorly soluble compounds are likely to have limited bioavailability because they do not remain in solution at the site of action, once in the body. This results in lower absorption and, by definition, reduced efficacy. To counter this, administration of higher doses is often necessary. However, higher doses can potentially lead to increased side effects. Third, because of a need for higher doses and/or complex formulations, the cost of drugs and nutrients to both manufacturer and consumer are increased. A number of nano-micelle based carriers for nutraceuticals and nutritional supplements are currently available. Delivery systems for nutraceuticals and supplements utilise a variety of processes, including: nano-emulsions, surfactant micelles, emulsion bilayers, reverse micelles. The advance techniques of nano-based delivery systems have several benefits compared to current tradtional systems of blending as such to feed mix, these are:

- Broader applications for nutraceuticals
- Less chance of leaching to water
- Less amount needed
- Protection against oxidation and other binding to feed ingredients
- Superior efficiency for antioxidants and preservatives
- Superior bioavailability of actives
- Chemically unmodified substances
- Crystal clear, liquid solutions
- Mechanically, thermally, pH stable
- Ready to use for production
- Processing without additives
- Longer storage protection and shelf life

There are several product in market now which already uses the advace delivery system for nutraceutical such as Ubisol-Aqua™ Delivery System Technology from Zymes LLC (limited liability company) (USA), NovaSOL® from AQUANOVA (Germany) and Nano-sized Self-assembled Structured Liquids (NSSL) technology from Nutralease (Israel) etc.(Market report, Agrifood European nanotechnology gateway http://www.observatorynano.eu/project/catalogue/F/). Few of these are discussed here

Ubisol-Aqua™

Ubisol-Aqua™ is a patented family of neutral, non-ionic carriers for water-insoluble compounds (Market report, Agrifood European nanotechnology gateway http://www.observatorynano.eu/project/catalogue/F/-2010). The water insoluble substance to be solubilised forms a non-covalently bound complex with Ubisol-Aqua™, which has both hydrophobic and hydrophilic subsections. This leads to self-assembly into micellar arrays with a hydrophobic interior and a hydrophilic exterior shell when mixed with water. The solubilized complex is both water and lipid soluble and stable through a wide range of temperatures (-80 to 120 °C), phase changes (freezing, melting) and across a broad pH range (2.0-8.0). The Ubisol-Aqua™ technology has successfully solubilised a number of bioactive molecules including for instance: coenzyme Q10, vitamin A acetate, vitamin A palmitate, vitamin B pentapalmitate, squalene, Ǘ-tocopheryl acetate, fish oil (EPA/DHA), ɓ-carotene etc.

NovaSOL®

AQUANOVA offers a key technology, which creates liquid carrier solutions, the so-called "solubilisates", based on its own technology. The solubilisates transport the respective active raw materials and active substances in the smallest capsules, the so-called "product micelles". The product micelle is stable with respect to pH and temperature and has a diameter of approximately 30 nm. Here, where microemulsions and liposomes prove to be problematical and unsuitable, the product micelle represents the optimum solution in the fields of functional food, cosmetics, pharmaceuticals and biotechnology. The 100% water-soluble micelle can be used for aqua feed supplement with better binders. The product micelle is proving to be an optimum carrier system of hydrophobic substances for a higher and faster intestinal and dermal re-sorption and penetration of active ingredients.

The incorporation of micellated fat-soluble or water-insoluble substances into final products occurs directly and without additional intermediate production steps and independently of matrix and final product quality. Due to AQUANOVA product micelles, the physiological mechanism of intestinal micelle formation is bypassed and consequently, a higher bioavailability is achieved. AQUANOVA product micelles are thermally, mechanically and pH-stable as well as being insusceptible microbiologically. Due to higher resorption and better bioavailability or penetration of the micellated active substances, the concentrations of active substances in the final products can be reduced.

Nano-sized Self-assembled Structured Liquids (NSSL)

There are currently some food and beverage products that are in the process of integrating NutraLease NSSL technology into them. The chemical structures that are formed using the technology Nano-sized Self-assembled Structured Liquids (NSSL) are micelles. When adding the targeted compound (the nutraceutical, drug or any other component) to the system, the micelles expand to form the Fortifying Nano-Vehicles (FNVs). When preparing the NSSLs together with the targeted molecule (i.e. the nutraceutical, drug etc.), and if this molecule prefers oil environment, then this molecule finds its way spontaneously into the micelle and only when the structure of the micelle is disturbed this molecule can finds its way out of the micelle. When the targeted molecule prefers an aqueous media then the formulation is such to generate a "reverse" micelle. The improved bioavailability is obtained via the ability of the micelles with the nutraceuticals (FNVs) to release the nutraceuticals into the membrane between the digestive system and the blood.

Bioral® (Nanocochleate Delivery System) from BioDelivery Sciences International (BDSI) (USA)

Bioral® is a novel drug delivery system, based upon cochleate technology. Bioral® encapsulates and protects a drug without chemically bonding to it and may facilitate oral dosing of drugs that typically need to be given by intravenous administration (http://www.wikinvest.com/stock/BioDelivery_Sciences_International_(BDSI)/Bioral_Technology).Alternating layers of lipids spiral around a drug molecule, encapsulating and potentially protecting it from degradation by acid or digestive enzymes in the stomach. The Bioral® technology is being evaluated as a new means of overcoming the poor oral absorption of drugs.In the food and beverage industry, attempts to add micronutrients as a supplement to fish has been hampered by the susceptibility of micronutrients and antioxidants to degradation during manufacturing and storage. The Bioral™ Nanocochleate Delivery System protects micronutrients and antioxidants from degradation during manufacturing and storage. With Bioral™ micronutrients, stability is enhanced and shelf-life is extended. It will serve best for drug delivery to fishes through in feed formulations.

Nanoencapsulation technology has been suggested for carotenoids, trace minerals, vitamins and fatty acids, with increasing bioavailability being the main goal (Acosta, 2009; Bouwmeester *et al*. 2009). For example, nano-micelles made from casein are proposed as a vehicle for hydrophobic ingredients like vitamin D2 (Semo *et al*. 2007). Nanoscale mineral supplements might provide a source of trace metals, without the extensive faecal losses normally associated with mineral salts (*e.g.* Fe salts; Carriquiriborde *et al*. 2004). Nanoforms of sodium selenite are already proposed to improve selenium absorption in ruminants (Zhou *et al.* 2009; Romero-Perez *et al*. 2010). Nanomaterials (NMs) may also offer an alternative to organic forms of food supplements, where antinutritional factors (incidental pesticides, toxic metals, *etc.*) in the ingredient can sometimes be a problem (Berntssen *et al*. 2010). In addition to improving the bioavailability and stability of the food ingredients, NMs may be used to alter the physical properties of fish food. Food wastage and pollution in aquaculture due to poor food stability, texture or inappropriate buoyancy of the pellet is a long-standing problem (Handy and Poxton, 1993). Small additions of NMs can dramatically change the physical properties of food pellets. For example, the additions of single-walled carbon nanotubes (SWCNTs) to trout food can result in a hard pellet that does not fragment easily in water (Handy, unpubl. obs.). Rainbow trout readily eat food containing NMs (up to 100 mg kgī 1 TiO2 NPs, Ramsden *et al*. 2009; 500 mg kgī 1 C60 and 500 mg kgī 1 SWCNT, Fraser *et al*. 2010) without loss of appetite or growth rate. To improve the delivery efficiency of drugs, hormones, vaccine, enzymes and nucleic acids; number of polymeric compounds are being used in medical science (Senel *et al*.2004). Chitosan nanoparticles may act as an excellent delivery system for yeast-RNA because of its versatile properties like biocompatibility, biodegradability, membrane forming ability, reactive surface functional groups for easy surface modification, low toxicity, immunomodulation, membrane adhesiveness, improved stability and enhanced permeability of epithelial membrane (Dodan *et al.*1998). As chitosan carries net positive charge and intestinal mucosa carries negative charge, chitosan nanoparticles enhances the delivery efficiency and also protect the encapsulated RNA from nuclease degradation (Mao *et al* .2001). Chitosan and chitosan derivatized polymers are being extensively used for the preparation of nanoparticles for oral controlled delivery therapeutic agents such as insulin, antibiotics, heparin, anti-oxidants and nucleic acids (Chaudhury *et al* . 2011) . The safety and non-toxicity of chitosan nanoparticles has been shown in animal models and in humans (Staroniewicz *et al*. 1994) including fish (Wang *et al.* 2011). Adding a few mg of an NM to fish feed to modify the physical properties of fish pellets would therefore seem a practical proposition for the aqua feed industry.

Several such delivery mean based on liposome micelle or other encapsulation polymers based nanoparticles can be used for the regulation of solubility of drugs or nutraceutical as needed in fish nutrition. Nutraceuticals are gaining acceptance for their ability to address several diseases. Vitamins, Minerals, enzymes, nucleotides, phospholopids and Nutrients constitute about 85% of the market while antioxidants and preservatives account for 10% other segments such as herbal extracts gaining importance globally in aquaculture feed. The effective treatment of various diseases has now been advised with the combinatorial use of nutraceuticals and pharmaceuticals to activate the immunity of fish as well early health recovery without productivity reduction of the system. Same time the use of nutraceutical for therapeutic nutrition to treat or control the various stress lead anomalies are gaining momentum in aqua feed too. The broader application of nutraceutical in modern fish nutrition can be illustrated as in figure.1.

Nano delivery of drugs in aquaculture

Aquaculture drugs are mostly administered through major three delivery root as bath or immersion, second through in-feed or oral and the third by injection. While first mode of immersion or bath is more applicable, but it require the drug in more amount and the handling to fish cause unavoidable stress. So the most ease method come to be through in-feed formulation where the drugs are applied with normal feeding without stress and extra cost. And lastly the injection seems to be impractical for fishes. The oral administration of drugs essentially needs the study of toxicokinetics, pharmacokinetics and pharmacodynmics of drugs in species and stage specific manner before in-feed administration. In in-feed delivery of drugs especially for the peptides, vaccine and the DNA or RNA components the major problem is the gastric digestion or denaturation before reaching to intestine for absorption (Fluorence *et al*.1995). So the in-feed formulation and delivery of drugs through feed needs an immediate intervention of an innovative step. Here the nano encapsulated or nano coated drug delivery give a solution where the drugs , vaccines , adjuvant, enzymes etc as protected and passed to intestine and made to stay for longer time in intestine for better absorption and assimilation. One of the most promising and productive areas of nanotechnology application to animal research is the nanopharmaceuticals (Tomlinson and Rolland 1996). One of the major classes of drug delivery systems is materials that encapsulate drugs to protect them during transit through the body. When encapsulation materials are produced from nanoparticles in the 1 to100-nanometer size range instead of bigger micro particles, they have a larger surface area for the same volume, smaller pore size, improved solubility, and different structural properties. This can improve both the diffusion and degradation characteristics of the encapsulation material. Another class of drug delivery system is nanomaterials that can carry drugs to their destination sites and also have functional properties. Certain nanostructures can be controlled to link with a drug, a molecule or an imaging agent, then attract specific cells and release their payload when required. The antibacterial properties of nanotubes are being studied. Self-assembled stacking of cyclic peptides having an even number of alternating and L-amino acids forms the nanotubes. The nanotubes insert themselves readily into bacterial cell membranes and act as potent and selective antibacterial agents; both nanomaterials buckyballs and nanotube will undoubtedly become an important part of the total pharmaceutical tool kit over the next few years (Kannaki and Verma 2006). Targeting Ligands and receptor or ligand specific delivery of drug is one key for targeted drug or gene or DNA delivery, which needs various ligands as small molecules galactose glucose and mannose, protein like transferins, antibodies, and low density lipids (LDLS). Drug delivery is an area where nanotechnology has already had a significant impact (La Van *et al*. 2003) and surely for this aquaculture drugs has the major share**,** because the drugs and nutraceuticals in aquaculture and their delivery methods need several concern as

1. Cost of the drugs and nutraceutical used in culture.
2. Same time we need to target Less waste as feed cost is the major cost and aquaculture is the margin sifting industry.
3. Efficacy and cost analysis of such drugs being used in aquaculture.
4. Environmental impact of these additives and drugs used.
5. Monitoring the residues level and its impact in food value.
6. Uncertain fate of the administered feed in the water.
7. Feed palatability and acceptance by fish after use of drugs and nutraceuticals
8. Toxicity of drugs to fishes at higher dose.

The factors mention above strongly suggest need of the effective delivery of the drugs where the efficacy can be achieved at low dose in sustained manner , without any toxicity to fish and residue of drug in the flesh . Nano deliveries -has satisfaction for several of these issues in aquaculture drugs like drug safety, residue level in flesh and sustain release to reduce the frequency of dosing and overall cost of drug or treatment. There are several natural polymeric and other nano carriers which can easily be applied for aquaculture drug without adding much cost. Goal of more sophisticated drug delivery techniques like nanodelivey is to

1. Deploy to a target site to limit side effects
2. Shepard drugs through specific areas of the body without degradation
3. Maintain a therapeutic drug level for prolonged periods of time
4. Predictable controllable release rates
5. Reduce dosing frequent and increase fish compliance without frequent handling and stress.

Therefore the delivery of drugs and nutraceuticals in aquaculture will have to find the way through nano delivery (Rather *et al*. 2011). Nanomedicine is a rapidly growing aspect of nanotechnology and there is an opportunity to use these technological advances to monitor and improve fish health. The poor stability of pharmaceuticals in natural water has inevitably led to many fish medicines being delivered via the food, or accepting that much of any aqueous treatment may be simply washed away. Nano carriers have been exploited to make new drug delivery systems for humans, and these may also be used for veterinary medicines including those for fishes. The approaches include solid core drug delivery systems (SCDDS), which involve coating a solid NP with a fatty acid shell to contain the drug of interest. This methodology works at relatively low temperature and pressure, making it especially useful for heat sensitive or labile pharmaceuticals. Porous NMs can also be used as a drug delivery matrix. For example, mesoporous silica particles can be used for the controlled release of substances. Oral delivery is the most easy and practical through in feed formulations and only option for the nutraceuticals delivery. The various in feed formulation forms like pelleted form, micro encapsulated, microparticulated form and nano particulate or encapsulated forms are the mean of delivery. As the Immersion pose handling stress and more amount of drugs required which raise the treatment cost, impact on environment and may cause possible threat on other organism and resistance building. While the Injection method becomes unrealistic for field application. The oral/ in-feed delivery with add of nano carriers becomes excellent mean of delivery for drugs and nutraceuticals (Rather *et al*. 2011)

Gene or DNA delivery

There is relation between nanoparticles and gene or DNA delivery, Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system (Gurunathan *et al*. 2000). The key ingredient of polynucleotide vaccines, DNA, can be produced cheaply and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines. Hence, polynucleotide vaccines are set to supersede many conventional vaccines particularly for viral diseases. However, there are several issues related to the delivery of polynucleotide which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site (Mohanraj and Chen, 2006). Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo lysosomal compartment to the cytoplasmic compartment (Panyam *et al*. 2002). Hedley *et al*. (1998) reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate the transgenic production, for modulating the expression of a gene, say GH gene in fishes to boost growth, produce the tailored fish product or health food. The DNA vaccines administered as liposomal complexes also improve the antibody response over that seen with free DNA (Gregoriadis *et al.* 1997). These include a reduction in the rapid clearance of cationic liposomes and the production of efficiently targeted liposomes. At the cellular level, the problems may be overcome by improving receptor mediated uptake using appropriate ligands, the endowment of liposomes with endosomal escape mechanisms, and a more efficient translocation of DNA to the nucleus and the efficient dissociation of the liposome complex just before the entry of free DNA into the nucleus (Smith *et al.* 1997). If it is delivered though in feed formulations, then the protection of the chitosan or alginate nano-capsulation in single layer or a layer over other carrier will ensure the gastric protection. Promising results were reported in the formation of complexes between chitosan and DNA (Rajshekhar *et al*. 2009). Although chitosan increases transformation efficiency, the addition of appropriate ligands to the DNAï chitosan complex seems to achieve a more efficient gene delivery via receptor-mediated endocytosis (Eldridge *et al.* 1990). These results suggest that chitosan has comparable efficacy without the associated toxicity of other synthetic vectors and therefore, can be an effective gene-delivery vehicle *in vivo* (Roy *et al.* 1997; Murta *et al*. 1998).

Vaccine-adjuvant

Vaccination is one of the important methods of prevention of disease in advance by developing antibody against the particular pathogen. Most of the vaccines are applied as a fluid form and generally injected in to blood stream. These vaccines require cool temperature to be stored and they also have limited life span within which they are to be utilized. These two limitations have prevented the utility of vaccines particularly in the fishes and shrimp. Therefore, more robust and durable vaccines are the only solution for the successful eradication of particular disease.

Many organisms, particularly microorganisms, have novel and interesting structures that could be exploited, for example, the lattice-type crystalline arrays of bacterial S-layers and bacterial spore coats both of which have protective prosperities. In principle, the spore coat could be used not only as a delivery vehicle for a variety of different molecules but also as a source of new and novel self-assembling proteins. Spore coats are comprised of protein, have ordered arrays of photometric subunits, exhibit self-assembly and have protective prosperities. A spore-based display system provides several advantages with respect to systems based on the use of conventional vaccines, these include the robustness of the bacterial spore allowing storage in the desiccated form, ease of production and safety. And it can be suitably used for aquaculture bacterial vaccines. Similarly the Liposomes as vaccine adjuvants, liposomes have been firmly established as immune-adjuvants (enhancers of the immunological response), potentiating both cell mediated and humoral immunity (Gregoriadis *et al*. 1996). Liposomal vaccines can be made by associating microbes, soluble antigens, cytokines (Gregoriadis *et al.* 1996) or deoxyribonucleic acid (DNA) (Gregoriadis *et al.* 1997) with liposomes, the latter stimulating an immune response on expression of the antigenic protein (Gregoriadis 1997). Liposomes encapsulating antigens which are subsequently encapsulated within alginate lysine microcapsules (Cohen *et al.* 1991), or to chitin or chitosan or other polymeric microcapsules to protect the gastric digestion and to control antigen release for improving the antibody response. Liposomal vaccines may also be stored dried at refrigeration temperatures for up to 12 months and still retain their adjuvanticity (Kim and Jeong 1995). DNA Nanovaccines Using Nanocapsules and Ultrasound Methods, the United States Department of Agriculture (USDA) is completing trials on a system for mass vaccination of fish in fishponds using ultrasound. Nanocapsules containing short strands of DNA are added to a fishpond where they are absorbed into the cells of the fish. Ultrasound is then used to rupture the capsules, releasing the DNA and eliciting an immune response from the fish. This technology has so far been tested on rainbow trout by Clear Springs Foods (Idaho, US)-a major aquaculture company that produces about one-third of all U.S. farmed trout (Mongillo 2007; ETC 2003).

Advances in nanotechnology have also proved to be beneficial in therapeutic fields such as drug discovery, drug delivery and gene/protein delivery. Now a day synthetic siRNA is considered as a highly promising therapeutic agent for viral disease like WSSV. However, clinical use of siRNA has been hampered by instability in the body and inability to deliver sufficient RNA interference compounds to the tissues or cells. To address this challenge, we present here a single siRNA nanocapsule delivery technology, which is achieved by encapsulating a single siRNA molecule within a degradable polymer nanocapsule with a diameter around 20 nm and positive surface charge. Several works provides a potential novel platform for siRNA delivery that can be developed for therapeutic purposes (Yan *et al*. 2012). Recently development of Chitosan based DNA-nano construct against nodavirus in

Macrobranchium rosenbergii. Persistence study showed the presence of the DNA construct up to 30th day post treatment. The oral treatment found to be better than the immersion treatment for delivery of the chitosan conjugated DNA construct (Ramya *et al*. 2013).

Adjuvant properties

Adjuvants are agents added to a vaccine to augment immune responses toward antigens. A number of studies describe the use of nanoparticles as adjuvants. For example, polymethylmethacrylate nanoparticles induced long-lasting antibody titres (the quantity of substance required to produce a reaction) .The antibody response was 100 times higher than when a traditional aluminium sulphate (alum) adjuvant was used (Stieneker *et al*. 1991). Similarly, when immunized with rabies glycoprotein, lipid-coated polysaccharide nanoparticles induced four times more immunoglobulin G than when immunized with alum adjuvant (Castignolles *et al*. 1996). Immunization of animals with both complete antigens and haptens (small molecules that can elicit an immune response only when attached to a large carrier such as a nanoparticle or a protein) conjugated to the surface of colloidal gold particles generated higher levels of specific antibodies than immunization of the same antigens with classical adjuvants (Dykman *et al*. 2004). Furthermore, the amount of antigen required to achieve a high antibody response was an order of magnitude lower than for immunization with Freundôs adjuvant (Dykman, *et al*. 2004). Injection of C60 (Carbon 60 derivatives into mice resulted in an approximately 20-fold increase in anti-ovalbumin antibodies (Andreev *et al.* 2000). Although the mechanisms of nanoparticle adjuvanticity are not completely understood, some studies suggest that nanoparticles can enhance antigen uptake and/or stimulate antigen-presenting cells. In summary, nanoparticles can be engineered to serve as vaccine carriers and adjuvants in aquaculture too. But Comprehensive structure and activity relationship studies are necessary further to understand the critical parameters that determine the antigenic and adjuvant properties of nanoparticles for fish and shellfishes.

Management of animal breeding

Aquaculture today is growing vertically and horizontally, growing rate of mariculture indicates the kind of horizontal expansion in the sea where the immediate need felt is for potentiating the maturation of the fishes for early and effective breeding same time easy artificial spawning to ensure uninterrupted supply of quality seed which is backbone for aquaculture growth. The nano delivery of the hormones, anabolic agents, hormone analogue and spawning inducing agent is very important for it where the effective delivery without much wastage can be targeted through in-feed formulation in marine cages. Same time the oral delivery of the nano carrier based or nanoencapsulated hormones/pheromones will have an option of mass breeding of the fishes in confined water in natural or artificial habitat (Sharma *et al* .2012; Rather *et al*. 2013).

Nano smart delivery system in cell vs. Transgenic

Today the world wide discussion is about the uses and abuse of transgenics. Human need and greed over the luring enhanced production and desirable trait attainment for diseases resistance and environmental tolerance seems necessary but same time the impact on biodiversity and food safety bring a big question mark even on survival and sustainability. Here the big dilemma can be solved by the nano delivery by using smart nano cell delivery, which will ensure only the cell specific or tissue specific modification of expression leading production of particular hormone say growth hormone in specific tissue. The delivery will be on basis of cell surface based receptor interaction and multiple nano coating material, this will be able to release the gene or DNA construct in the nucleus of decided cell. It will also be used for triggering the protein- protein interaction channel to trigger the various synthetic cascade of the cell leading to expression of particular gene by various factors or cofactors activation and triggering binding to genes response element. This particular mean will be effective as it will lead in temporary and even long lasting change in the somatic cell only. The epigenetic or protein -protein based triggering will have a definite life of triggering, while the genetic integration or transgenics will be a lifelong modification of expression in that animal. Beside that it will have the chance of genetic contamination and environmental impact. As the somatic cell alteration will not be carried to the germplasm. Oral delivery if achieved it becomes super simple, over all it will be the least tedious than transgenic gene transfer to embryo where the success rate is less and it require the sophisticated techniques or equipment (The United States Department of Agriculture, Report of National Planning Workshop, 2002). Establishment and Optimization of a Nano Transgenic System for Molecular Breeding of *Jatropha curcas.* The nano transgenic system of QDs labeling chitosan-DNA complex was shown to have good fluorescence properties and uniform particle size (Jienan *et al*. 2013). Long-term Transgene Expression in the Central Nervous System Using DNA Nanoparticles. In this study, Plasmid DNA was compacted with polyethylene glycol substituted lysine 30-mer peptides, forming rod-like nanoparticles with diameters between 8 and 11 nm. An intracerebral injection of compacted DNA can transfect both neurons and glia, and can produce transgene expression in the striatum for up to 8 weeks, which was at least 100-fold greater than intracerebral injections (Yurek *et al*. 2009).

In feed or oral -nanodelivery

Nanodelivery have supremacy over the free oral delivery of Proteins, drugs, epitope vaccine, or the sub-unit vaccine or nutraceutical kinds like enzymes, anabolic hormones, maturation inducing agents etc., which get denatured or degraded in the acidic environment in the stomach and digested by digestive or hydrolytic enzymes in the GI tract. ñSecondarily, the Penetration and absorption of drug across the intestinal mucosa and epithelium must be ensured in efficient manner (Jani *et al*. 1990). And lastly for smart delivery of drugs in tissue and organ specific manner the nano delivery comes as best mode. Overall the use of nano carrier based delivery comes to be very important, the presently several nanodelivery means are applicable for drug delivery such as ñComplexation and pHò Responsive Hydrogels, Polymeric nanoparticles, Nanosuspensions, Solid lipid nanoparticles, Magnetic nano spheres , Nanogels, Nanocapsules, Fullerenes, Metal nanoparticles and Self-assembling Systems Like Vesicles, Liposomes and Niosomes. These carriers perform the various functionaries as

1. Prevention of drug from biological degradation
2. Effective Targeting
3. Fish Compliance and stress reduction
4. Controlled and sustained release
5. Cost effectiveness
6. Product life extension

Therefore the nano delivery can be used in various priority areas of aquaculture such as the

- DNA and siRNA Vaccines for parasitic, bacterial and viral diseases
- Peptide coated vaccine

- Oral routes for systemic delivery of proteins and peptides, enzymes easily accessible form of the Netraceuticals viz minerals vitamins etc
- Maturity inducing agents, harmone and harmone release factors
- Commercial oral spawning agents
- Smart Nanomedicine Systems with Control of Gene/Drug Delivery within Single Cells
- Nano delivery to regulate the gene expression as cell nanofactories for treating the systemic diseases or promoting expression of growth or immune genes
- Nano delivered antigenic ,epigenetic factors and trans genes for bio reactor or tailored animal production
- Specific pathogen control in aquatic envirnment on basis of the specific cell receptors and lysis of entire specicic pathogenic cell mass of the water bodies

Beside above various use in above mention area in aquaculture, no doubt there are risk associated (FOE. 2008) with the unregulated and unjustified use of nano material for aquaculture also. Card *et al*. (2011) evaluated published literature on the safety of oral exposure to food-related nano materials and found that there are currently insufficient reliable data to allow a clear safety assessment. Card *et al*. (2011) also considered that non-food-related engineered nanomaterials require evaluation of oral toxicity in light of possible contamination of the food supply. Morris (2011) concluded that the lack of information on the possible toxicity of nano materials makes it difficult to assess the safe or acceptable daily intake. According to Magnuson, et al (2011) the literature on the safety of oral exposure to nanomaterials inadequately characterizes nano materials with insufficient physicochemical parameters, concluding that ñUnless nano materials are adequately characterized, the results of the toxicology studies cannot be utilized to predict toxicity of other nano materials as changes in any of the characteristics may result in changes in biological activityò. But it is quite essential that nanoparticles for drug delivery must fulfill some characteristics such as

- it must give easy delivery
- The material should be biocompatible
- The material should be biodegradable &/or bioeliminable
- The material should be non-antigenic
- The particle surface should be amenable to chemical modifications
- The particle should be able to encapsulate as much drug or ntraceuticals as possible
- The particle should be able to protect the drug or nutrceuticals in vivo
- The particle should be able to chemically sustain in the entire pathway from the site of administration to targeted site
- The particle should be able to release the drug at the targeted site
- The particle should be eliminated from the body after delivery

Conclusion

Nanoencapsulation techniques needed now to produce nano sized particles and capsules to address the high performance needs of many applications and for it techniques used are , Micelles, Liposomes and polymersomes, phase inversion/precipitation, solvent evaporation, polyelectrolyte complexes, layer-by-layer deposition, controlled precipitation, surfactant-free particle formation, templating, molecular encapsulation . These techniques intern give various application as Protein, DNA and RNA stabilization, Small molecule delivery, Extending circulatory half-life, modifying drug transport etc., which can be suitably used in aquaculture

ACCEPTED MANUSCRIPT

nutrition, fish breeding, aquaculture drug development and fish health management. Another specific area of interest is the use of nanomaterials in wastewater treatment to improve the quality and safety of water used for aquaculture and aquaculture discharge. It might be possible to develop, for example, low-cost nanofilter/nanomembrane materials that could be of interest to developing countries. However, such new materials and uses may pose safety issues not related to food safety, such as their disposal at the end of their life cycle (FAO/WHO, 2012). For animal health, the development of so-called ñnanovaccinesò with improved delivery routes to target animals of small size in aquaculture (e.g. fish larvae, shrimp) could be of benefit from a cost and animal welfare point of view. However, research seems to be in an early conceptual stage; no information is available on already ongoing technical projects (FAO/WHO, 2012). But surely nanodelivery carries tremendous opportunities for aquaculture drugs and nutraceutical delivery, the only need is to explore the green ways according to natural suitability in environmentally sustainable manner.

Declaration of Interest

The authors report no conflict of interest.

Acknowledgements

The authors are grateful to Dr W.S. Lakra, Director, Central Institute of Fisheries Education, Mumbai, India, for providing necessary facilities for carrying out research in this new emerging area in various departments in Central Institute of Fisheries Education, Mumbai, India. Authors are also thankful to Dar Jaffer and Irfan Bhat for a very careful and critical reading of the revised manuscript and making necessary corrections shortly before its submission.

References

Acosta E, (2009) Bioavailability of nanoparticles in nutrien and nutraceutical delivery. *Current Opinion in Colloid Interface Science* **14:** 3ï 15.

Amit KS, Kartik, Dipesh D, Pal AK (2007) Nutrizymes, Ideal nutraceuticals in Aquafeed: Potential and Limitation. *Aquaculture Health International*. **11**: 4-6

Andreev SM, Babakhin AA, Petrukhina AO, Romanova VS, Parnes ZN, Petrov RV (2000) Immunogenic and allergenic properties of fulleren conjugates with aminoacids and proteins. *Doklady Biochemistry and Biophysics*. **370:** 4ï 7

Berntssen MHG, Julshamn K, Lundebye AK (2010) Chemical contaminants in aquafeeds and Atlantic salmon (*Salmo salar*) following the use of traditional- versusalternative feed ingredients. *Chemosphere* .**78:** 637ï 646.

Bouwmeester H, Dekkers S, Noordam MY, Hagens WI, Bulder AS, de Heer C, Sandra ECGT V, Susan WPW, Hans JPM , Adriënne JAM S, (2009) Review of health safety aspects of nanotechnologies in food production.*Regulatory Toxicology and Pharmacology*. **53**(1), 52ï 62.

Card JW, Jonaitis TS, Tafazoli S, Magnuson BA (2011) An appraisal of the published literature on the safety and toxicity of food-related nanomaterials. *Critical Reviews in Toxicology* .**41**(1): 20ï 49.

Carriquiriborde P, Handy RD, Davies SJ (2004) Physiological modulation of iron metabolism in rainbow trout (*Oncorhynchus mykiss*) fed low and high iron diets. *Journal of Experimental Biology* . **207:** 75ï 86.

Castignolles N, Morgeaux S, Gontier JC (1996) A new family of carriers/biovectors enhances the immunogenicity of rabies antigens. *Vaccine* **14:** 1353ï 1360.

Chaudhury A, Das S (2011) Recent advancement of chitosan-based nanoparticles for oral controlled delivery of insulin and other therapeutic agents. AAPS PharmSciTech **12**(1):10-20

Cohen S, Bernstein H, Hewes C, Chow M, Langer R (1991) The pharmacokinetics of, and humoral responses to,antigen delivered by microencapsulated liposomes. *Proceedings of the National Academy of Sciences USA* **88**: 10440-4.

Dodane V, Vilivalam VD (1998) Pharmaceutical applications of chitosan. Pharmaceutical Science & Technology Today. **1**: 246-253.

Dutkiewicz J, Kucharska M (1992) Advances in Chitin and Chitosan (Brine CJ, Sandford PA, Zikakis JP, eds). *Elsevier Science* 54ï 60.

Dykman LA, Sumaroka MV, Staroverov SA (2004) Immunogenic properties of colloidal gold. *Izvestiia Akademii Nauk Seriia Biologicheskaia.* **1:** 86ï 91 .

Eldridge JH, Hammond CJ, Meulbroek JA, Staas JK, Gilley RM, Tice TR (1990) Controlled vaccine release in gut-associated lymphoid tissues. Orally administered biodegradable microspheres target the Peyerôs patches. *Journal of Controlled Release* **11:** 205ï 214.

ETC (Action Group on Erosion, Technology and Concentration) (2003) Down on the Farm: The Impact of NanoscaleTechnologies on Food and Agriculture (http://www.etcgroup.org.)

FAO & WHO (2012) State of the art on the initiatives and activities relevant to risk assessment and risk management of nanotechnologies in the food and agriculture sectors .

FAO (2012) Status and trends, World review of fisheries and aquaculture.

FAO/WHO (2012) Joint FAO/WHO Seminar on Nanotechnologies in Food and Agriculture,. Rome, Food and Agriculture Organization of the United Nations.

Florence AT, Hillery AM, Hussain N, Jani PU (1995) Nanoparticles as carriers for oral peptide absorption: studies on particleuptake and fate. *Journal of Controlled Release* **36:** 39ï 44.

FOE (Friends of the Earth) (2008) Out of the laboratory and onto our plates: Nanotechnology in food and agriculture.[http://www.foe.org/]

Fraser TWK, Reinardy HC, Shaw BJ, Henry TB, Handy RD (2010) Dietary toxicity of single-walled carbon nanotubes and fullerenes (C60) in rainbow trout (*Oncorhynchus mykiss*). *Nanotoxicology* **5:** 98ï 108.

Gregoriadis G (1997) Genetic vaccines: strategies for optimization. *Pharmological Research* .**15:** 661-9.

Gregoriadis GR, Saffie JB, DaSouza (1997) Liposome mediated DNA vaccination. *FEBS Lett.* **402:** 107-10

Gregoriadis *G,* Gursel *I,* GurselM*,* McCormack B (1996) Liposomes as immunological adjuvants and vaccine carriers. *Journal of Controlled Release* .**41**: 49-56.

Gurunathan SC, Freidag WB (2000) DNA vaccines: a key for inducing long-term cellular immunity. *Current Opinion in Immunology.* **12:** 442-447.

Handy RD, Poxton MG (1993) Nitrogen pollution in mariculture ï toxicity and excretion of nitrogenous compounds by marine fish. *Reviews in Fish Biology and Fisheries.* **3 :**205ï 241.

Hedley, Curley MJ, Urban R (1998) Microspheres containing plasmid-encoded antigens elicit cytotoxic T-cellresponses. *Nature Medicine.* **4:** 365-368.

Jani P, Halbert GW, Langridge J, Florence AT (1990) Nanoparticles uptake by rat gastrointestinal mucosa: quantitation andparticles size dependency. *Journal of Pharmacy and Pharmacology.* **42:** 821ï 826.

Jha AK, Pal AK, Sahu NP, Kumar S, Mukherjee SC (2007) Haematoimmunological responses to dietary yeast RNA, ɤ-3 fatty acid and Ɓ-carotene in *Catla catla* juveniles. *Fish and Shellfish Immunology.* **23:** 917-927.

Jienan C, Qiong W, Lin Z, Qianqian K (2013) Establishment and Optimization of a Nano Transgenic System for Molecular Breeding of *Jatropha curcas* . *J of Bionanoscience*. **7**: 251-255

Kannaki TR, Verm PC (2006) The Challenges of 2020 and the Role of Nanotechnology in Poultry Research, Proceedings of National Seminar Central Avian Research Institute, Izatnagar- (India) 273-277.

Kim CKE, Jeong J (1995) Development of dried liposome as effective immuno-adjuvant for hepatitis B surfaceantigen. *International Journal of Pharmacy.* **115:** 193-9.

Kuzma J, Verhage P (2006) Nanotechnology in Agriculture and Food Production: Anticipated Applications. Project on Emerging Nanotechnologie and the Consortium on Law, Values and Health and Life Sciences. Centre for Science, Technology and Public Policy (CSTPP).

LaVan DAT, McGuire R (2003) Langer Small-scale systems for in vivo drug delivery. *Nature Biotechnology.* **21:** 1184ï 1191

Magnuson BA, Jonaitis TS, Card JW (2011) A brief review of the occurrence, use, and safety of food-related nanomaterials. *Journal of Food Science.* **76 (6):** 126ï 133.

ACCEPTED MANUSCRIPT

Market report , 2010 Agrifood European nanotechnology gateway Delivery systems for nutraceuticals and nutritional supplements, Technology Centre ASCR.

Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y (2001) Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. *Journal of Controlled Release.* **70**:399-421

Mohanraj VJ, Chen Y (2006) Nanoparticles ï A Review. *Tropical Journal of Pharmaceutical Research.* **5**(1) **:** 561-573.

Mongillo JF (2007) Nanotechnology 101, Greenwood Press, 88 Post Road West, Westport, CT 06881. Animprint of Greenwood Publishing Group.

Morris VJ (2011) Emerging roles of engineered nanomaterials in the food industry. *Trends in Biotechnology.* **29**(10): 509ï 516.

Murata JY, Ohya T, Ouchi (1998) Design of quaternary chitosan conjugate having antennary galactose residues as a gene delivery tool. *Carbohydrate Polymers.* **32** (2)**:** 105-109.

Panyam, Zhou JWZ, Prabha S, Sahoo SK, Labhasetwar V (2002) Rapid endo-lysosomal escape of poly (DLlactide- co-glycolide) nanoparticles: implications for drug and gene delivery. *Federation of American Society of Experimental Biology Journal* **16:** 1217-26.

Rajeshkumar S, Venkatesan C, Sarathi M, Sarathbabu V, John T, Anver Basha,K, Sahul Hameed AS (2009) Oral delivery of DNA construct using chitosan nanoparticles to protect the shrimp from white spot syndrome virus (WSSV). *Fish & Shellfish Immunology.* **26:** 429-437.

Ramya VL, Sharma R, Gireesh BP, Sreenivas RP, Rather M A, Nandanpawar PC and Suresh E (2013)Development of Chitosan based DNA-nano construct against nodavirus in *Macrobranchium rosenbergii. J of fish diseases.(In Press)*

Ramsden CS, Smith TJ, Shaw BS, Handy RD (2009) Dietary exposure to titanium dioxide nanoparticles in rainbow trout (*Oncorhynchus mykiss*): no effect on growth, but subtle biochemical disturbances in the brain. *Ecotoxicology.* **18:** 939ï 951.

Rather MA, Sharma R, Aklakur M, Ahmad S, Kumar N, Khan M **(**2011) Nanotechnology: A novel tool for aquaculture and fisheries development.A prospective mini-review. *Fisheries and Aquaculture development.* **201:** 1-5.

Rather MA., Rupam S., Subodh G., Ferose K., Ramya VL., and Sanjay B J.(2013) Chitosan-Nanoconjugated Hormone Nanoparticles for Sustained Surge of Gonadotropins and Enhanced Reproductive Output in Female Fish. *PLOS ONE* **8** (2):1-10

Romero P, Garcia-Garcia AE, Zavaleta-Mancera A, Ramirez-Bribiesca JE, Revilla-Vazquez A, Hernandez-Calva LM, Lopez-Arellano R, Cruz-Monterrosa RJ (2010) Designing and evaluation of sodium selenite nanoparticles *in vitro* to improve selenium absorption in ruminants. *Veterinary Research Community.* **34:** 71ï 79.

Roy KH, Mao Q, Leong KW (1997) DNA-chitosan nanospheres: Transfection efficiency and cellular uptake. *Proceedings of the Controlled Release Society.* **24:** 673-674.

Semo E, Kesselman E, Danino D, Livney YD (2007) Casein micelle as a natural nano-capsular vehicle for nutraceuticals. *Food Hydrocolloid.s* **21:** 936ï 942.

Senel S, McClure SJ (2004). Potential applications of chitosan in veterinary medicine. Advanced Drug Delivery Reviews **56**: 1467-80.

Sharma R, Rather MA., Ramya VL., Himadri S, Suresh BPP, Shabir AD, and Sukham M.(2012) Preliminary observations on the effect of nanoconjugated pheromones on *Clarias batrachus*. *Aquaculture Research.* (Inpress)

Smith, Zhang JYL, Niven R (1997) Toward development of a non-viral gene therapeutic. *Ibid* **26:** 135-50

Staroniewicz Z, Ramisz A, Wojtasz-Pajak A, Brzeski MM (1994) Studies on antibacterial and antifungal activity of chitosan. In: Karnicki ZS, Brzeski MM, Bykowski PJ, Wojtasz Pajak A, eds. Chitin World. Wirtschaftverlag, Germany, pp-374-377

Stieneker FJ, Kreuter J (1991) Lower High antibody titres in mice with polymethylmethacrylate nanoparticles as adjuvant for HIV vaccines. *AIDS* **5:** 431ï 435.

Tejpal CS, Pal AK, Sahu NP, Ashish KJ, Muthappa NA, Vidya S, Rajan MG (2009) Dietary supplementation of L-tryptophan mitigates crowding stress and augments the growth in *Cirrhinus mrigala* fingerlings. *Aquaculture.* **293:** *272-277.*

Tomlinson E, Rolland AP (1996) Controllable gene therapy pharmaceutics of non-viral gene delivery systems. *Journal of Controlled Release* **1:** 357-372.

Wang Y, Li J (2011) Effect of chitosan nanoparticles on survival growth and meat quality of Tilapia (*Oreochromis nilotica*). *Nanotoxicology* **5(**3):425-31

Yan, Min ML, Jing W, Yang L, Yunfeng L, Irvin SY **(**2012) Single siRNA Nanocapsules for Enhanced RNAi Delivery. *Journal of American Chemical Society.* **34** (33): 13542ï 13545

Yurek, DM , Fletcher, AM Smith GM, Seroogy KB , Ziady AG , Molter J, Kowalczyk T H , Padegimas L and Cooper MJ (2009) Long-term Transgene Expression in the Central Nervous System Using DNA Nanoparticles *Molecular Therapy* **17** : 641ï 650

Zhou, Wang XY, Gu Q, Li W (2009) Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*). *Aquaculture* **291:** 78-81.

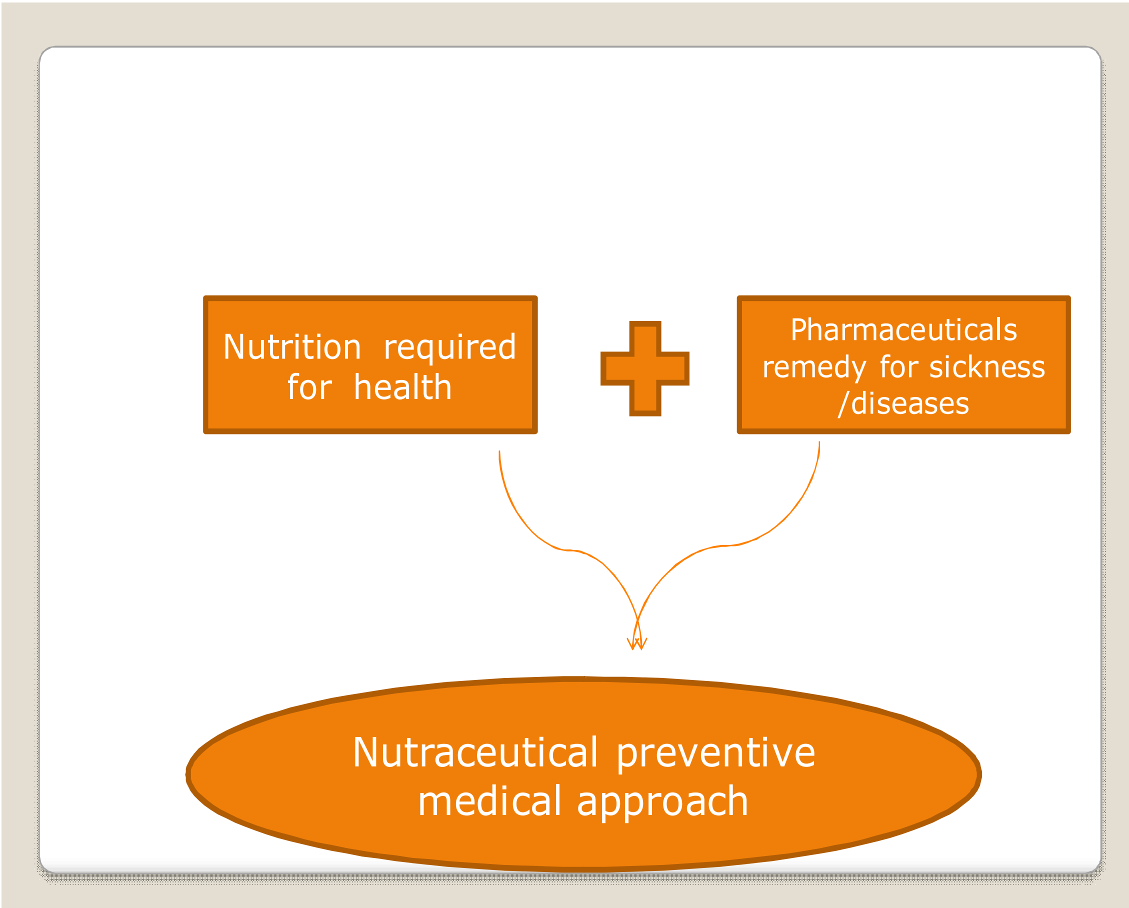

Figure.1 Nutraceutical as preventive medical approach/ therapeutics