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SYNTHESIS AND CHARACTERIZATION OF FISH GELATIN NANOPARTICLE AND ITS ROLE AS THE DRUG DELIVERY VEHICLE FOR TUBERCULOSIS

L.Kalaiselvi*

Department of Biotechnology, Udaya School of Engineering, Nagarkoil-629 252-India.

* Corresponding Author E-mail:klkselvi@gmail.com

ABSTRACT

Nanoparticle based systems have significant prospective for diagnosis, treatment and prevention of tuberculosis (TB) Gelatin nanoparticle derived from marine sources (fish skin, bone and fins) has been looked upon as a possible alternative to bovine and porcine. Fish gelatin nanoparticle synthesis by two step desolvation method, it was stable nanoparticles and confirm through Scanning electron microscopy (SEM).These nanoparticle were used as carrier for rifampicin. Our aim to develop a Nano particulate carrier of rifampicin for controlled delivery as well as reduced toxicity. In this study, rifampicin loaded fish gelatin nanoparticle was fabricated by an absorption/adsorption method. The effect of several variables on the Nanoparticle's characteristics was calculated.

Key words: Drug delivery, Nanoparticle, Gelatin, Tuberculosis, SEM, Fish gelatin

1.1INTRODUCTION

Nanotechnology can be defined as the science and engineering involved in the design, Synthesis, characterization, application materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale (One billionth a meter greatest values nanotech will be in the development of new and effective medical treatments. Research into the delivery and targeting if therapeutic and diagnostic agents with nanoparticles is at the forefront, if Nano medicine for several reasons. First traditional oral or inject able drugs are not necessarily the most efficient formulations for a given product. This is particularly true for new biologics such as proteins and nucleic acids that require novel delivery

technologies to optimize efficacy, minimize side effects and lead to better patient compliance. These reformulation drugs have the potential advantages of drug side effects, increasing patient compliance, and reducing health care costs. (Dwaine, *et al*., 2006). Several reports are available regarding the use of carrier systems like liposomes, dendrimers, micro spheres, and solid, lipid nano particles for delivery of bio actives.

Nano materials derived from proteins, especially protein nanoparticles are biodegradable, non- antigenic, metabolizable and can also be easily amenable for surface modification and covalent attachment of drugs and ligands. Because of the defined primary structure of proteins the protein based nanoparticles may suggest various possibilities for surface alteration and covalent drug attachment. Now a day's active research is focused on the preparation of nanoparticles using proteins of nanoparticles using proteins like albumin gelatin, gliadin and legumin (Mohsen,*et al*., 2008) Gelatin is one of the protein materials that can be used for the production of nano particles. It's derived by hydrolytic degradation of collagen, the principle component of animal connective tissue. Raw materials from fish and poultry have received considerable attention in recent years. As for as fish gelatin is concerned the huge number of species having very different intrinsic charareristics.This range from natural resources like fish to polymers, the fish gelatin has been obtained from fish skin products and formulated as natural hollow capsules of fish oil, spirulina and cellulose. (HabibaiBenta,*et al*., 2011)

Gelatin from marine sources (warmand coldwater fish skins, bones, and fins) is a possible alternative to bovine gelatin. One major advantage of gelatin sources is that they are not associated with the risk of out breaks of bovine spongiform Encephalopathy Furthermore, Fish Skin, which is a major byproduct of the Fish processing industry, causing waste and pollution, could provide a valuable source of gelatin. Gelatin has been extracted from skins and bones of various cold water (e.g., cod hate, Alaska Pollock, and salmon) and warm water (e.g. Tuna, Catfish, Tilapia, Nile perchandShark) fish.

NANOTECHNOLOGY IN TREATMENT OF TUBERCULOSIS

Treatments with improved sustained release profiles and bioavailability can increase compliance through reduced drug requirements and there in minimize chemotherapy of TB is complex due to the requirement of multidrug regimens that need to be administered over long periods. The poor patient compliance is the single most common reason for chemotherapy failure in TB.Carrier or delivery systems such as liposome's and micro spheres have been developed for the sustained delivery of antiTB drugs and have found better chemotherapeutic efficacy .The advantages of nanoparticles as drug carriers, high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances; and feasibility of variable routes of administration, including oral

administration and inhalation.Jithendra Prasad mathuria, 2009 summarizes on nanoparticulate formulations of the antiTB drugs.

2. MATERIALS AND METHODS

2.1 MATERIALS

Cat fish (*Ictaluruspunctatus*) 400-500g bought from a local market, filleted, washed and the skins were collected. The skins were weighed, packed in polyethylene bags and freezestored at-20ºC if not used immediately. All reagents used in the studies were of analytical grade. Rifampicin purchased from drug stores. *Mycobacterium tuberculosis* obtained from Asaripallam Government Medical College.

2.2 EXTRACTION OF FISH GELATIN

The procedure was essentially based on a mild acid pretreatment for collagen swelling, followed by extraction in water at moderate Temperature (45ºC). The entire process takes about 24hrs. Because of the acid liability of the cross links found in fish skins collagen. Skins were washed under running tap water to remove superfluous materials. The wash skins were drip dried and soaked in the saturated lime solution [Ca (OH) $_2$], at 20ºC for 14days for each kilogram of wet skins.Ca $(OH)_2$solution was used at the soaking medium. After soaking, the skins were then removed and washed with abundant tap water (1:10) to remove excessive Ca (OH) $_2$while maintaining the skins at pH10. This was followed by the soaking in distilled water at 48ºC overnight to solubilize the gelatin. The solution was then filtered through whatman No 541 filter papers before passing through a strong acid cationic exchange resin, which reduced the pH of gelatin to approximately 5.The filtrates were then freezing, dried and analyzed for physio chemical properties (Jamilah,*etal.,*2011).

The better recovery of gelatin is based on the wet and dry conditions of the skin and the use of $Ca(OH)_2$ prior to gelatin extraction. The yield of gelatin was calculated using the following expression

$$\% \text{ of yield (wet weight basis)} = \frac{\text{Dry weight of gelatin}}{\text{Wet weight of skin}} \times 100$$

2.3. FABRICATION METHODS OF GELATIN NANO PARTICLES

Two fundamental methods of cross linking have been described for gelatin Physical and chemical. Physical methods include UV irradiation and dehydrothermal treatment, although these are inefficient and make it difficult to control the cross linking density of the gelatin matrix. Chemical cross linking agents have been categorized into two types as non zero length and zero length. Non zero length cross linkers are functional or poly functional and operate by bridging free carboxylic acid resides or amine groups between adjacent protein molecules, e.g., glutaraldehyde (Simon, *et, al.,* 2005).

GNP was prepared by atwo-stepdesolvation method with slight modifications. Briefly 10g gelatin was

dissolved in distilled water (100ml) under constant heating at 40±1ºC. Acetone (50ml) was added to the gelatin solution as a desolvation agent to precipitate the high molecular weight (HMW) gelatin. The supernatant was discarded, and the HMW gelatin was redissolved by adding distilled water (10ml) with stirring at 600rpm under constant heating. The pHof the gelatin solution at the second desolvation step was adjusted (between 2 and 12) drop wise addition of acetone (30ml) to form GNPs. At the end of the process, glutraldehyde solution (25% V/V aqueous solution) was added 2 µl. It actas a cross linking agent, and the solution was stirred for 12 hours at 600rpm. Effect of parameters like pH, temperature, amount of glutaraldehyde was studies (Manoj, *et al.,* 2008).

2.4. PHYSIO CHEMICAL TESTS

After gelatin production by above mentioned methods, quality factors were determined according to national and international standards.

2.4.1. PROTEIN CONFORMATION TEST

Biuret test for gelatinaqueous sample is treated with an equal volume of 1% strong base (sodium or potassium hydroxide most often) followed by a few drops of aqueous copper (II) sulfate. If the solution turns purple color.

2.4.2. COLOR DETERMINATION

Color of gelatin samples were measured by putting them on white background and compared with each other. Gelatin color must be pale yellow to amber (Hamid Tavakolipour, 2011).

2.5. CHARACTERIZATION THE NANOPARTICLES (FGNP)

2.5.1. SHAPE AND SIZE.

Themorphology of FGPs was determined by Scanning Electron Microscopy (ZEISS from CarlZeiss, India). A Scanning Electron Microscope (SEM) is a type of electron microscope that images a sample by scanning it with a beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity. Thepowdered sample was coated with gold and the accelerating voltage of 95KV.

2.6- DRUG LOADING

Drug loading can be done by two methods namely an Incorporation method and an Adsorption/absorption technique. Ideally,a successful Nano particulate system has a high drug loading capacity.

Adsorption /absorption technique followed to drug loading, absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution.Drug loading of nanoparticles was determined by the method proposed by the amount of RIF loaded was determined by incubating the nanoparticle suspension (1.0ml) in 5.0ml phosphate buffer saline (pbs.pH 7.4) for 2hrs at 800rpm at 25±1ºC.

The amount of unloaded and loaded drug was determined UVspectrophotometrically in the

supernatant obtained after separation of nanoparticles bycentrifugation. The instrument used here was P.C based UV 5704SS Spectrometer. It consists of a spectrophotometer unit and a computer unit. The amount of radiant energy absorbed by a sample at a certain wavelength depends on how much of that substance, which absorbs that wavelength of radiation, is present in the sample. In other words, the absorption of radiant energy is proportional to the absorbing materials. Transmittance (T) is defined as the ratio of intensity of transmitted light and intensity of the light that is failing upon the sample when light falls upon a homogenous medium a portion is transmitted. It is the transmitted light that is actually detected by the instrument.

2.6.1DRUG RELEASE STUDIES

Drug release from known amounts of RIF loaded FGnanoparticles was evaluated using the equilibrium dialysis technique at 37 + 1°C and quantification was carried out by spectrophotometric method. Briefly, 2mlof nanoparticle dispersion was put in the dialysis bag (MWCO 3500 Da,Himedia,India)and were dialyzed against 50ml of PBS (pH 7.4) with 200mg /ml ascorbic acid, which was added as an antioxidant to prevent oxidative degradation of RIF at a rotation speed of 50 rpm. At predetermined time intervals, 0.5ml.samples were withdrawn through sampling portion filtered through 0.45 micro meter membrane filter and the drug content was determined spectrophotometrically.

2.7ANTIBACTERIAL ACTIVITYASSAY WITH DISC DIFFUSION METHOD

Mycobacterium tuberculosis was added onto the l.j.medium and spread uniformly using a spreading tool. After that, the gelatin nanoparticles with drug loaded and drug unloaded were placed in the form of disks on the medium. It was kept under observation for seven days. It was evident that the antibacterial efficacy of the drug loaded gelatin nanoparticles was found to be higher than that of the pure gelatin nanoparticles, from the zone of inhibitionWells with 8 mm diameters were prepared by punching a sterile cork borer onto the agar plates.

3. RESULTS AND DISCUSSIONS

3.1. Preparation and Characterization of Fish Gelatin

Fish gelatin manufacturing process consists of three main stages namely Pretreatment of the raw material, extraction of gelatin, purification and drying. Fish gelatin extracted using an acidic treatment is most suitable for the less covalently cross-linked collagen found in the skin of the fish. Theparameters affecting the process include temperature, time and pH. Preparing fish gelatin typically involves a mild chemical pretreatment of the raw material and requires mild temperature conditions during the extraction process.

Raw materials stained were washed under running tap water to remove superfluous material and the steps mentioned in the extraction procedure were performed.On average, the extraction yield of fish gelatin was found to lie approximately between 6%

and 19% (expressed as grams of dry gelatin per 100g clean skin). The extracted fish gelatin was filtered and then freeze dried. The yield of gelatin was found to be 20% for 500gms of fish skin taken.

3.1.1 Protein Confirmation

An aqueous sample is treated with an equal volume of 1% strong base (sodium or potassium hydroxide most often) followed by a few drops of aqueous copper (II) sulfate. If the solution turns purple, protein is present. Theodor of the dried fish gelatin and fish gelatin solution was evaluated byjudging the intensity of the intensity of fishy odor. Color of the gelatin sample was measured by putting them on a white background and comparing with each other. Fish gelatin color was pale yellow to amber.

3.2 PREPARATION OF FISH GELATIN NANOPARTICLES

Fish gelatin nanoparticles prepared using modified double desolvation method with acetone as the desolving agent and glutaraldehyde as the crosslinking agent was found to be highly stable in water. The sediment obtained after first desolvation step mainly possessed the high molecular weight fish gelatin, while the supernatantwhich was removed, was containing portions of gelatin. The purpose of removing gelatin of low molecular weight was to avoid wider distribution of gelatin, which may not only cause formation of irreversible aggregates after crosslinking, but also form unstable particles. Acetone slowly added to protein solution acts as the desolvating agent. With the addition of acetone, a change occurs in the third structure of protein. When a certain level of desolvation was reached, protein clump was formed. In the next stage, nanoparticles were obtained when cross linked with glutaraldehyde. In order to obtain dispersed nanoparticles, we must stop the system before particles start to accumulate. System turbidity will be increased owing to the desolvation factor. Particles started to accumulatewhenthe turbidityof the system were increased.

Fig 1: Preparation of fish gelatin nano particle

3.3. PARTICLE MORPHOLOGY:

The average particle size of $FGNP_S$ was found to be 300 nm. Scanning electron microscopy was used to determine the shape and surface morphology of the nanoparticles produced. Particles were coated with gold under vacuum before SEM analysis. It was found that the particle size and size distribution are the most important characteristics of nanoparticle system.

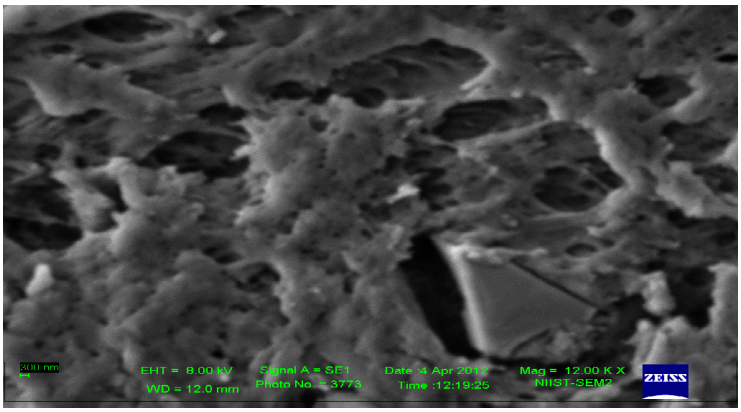


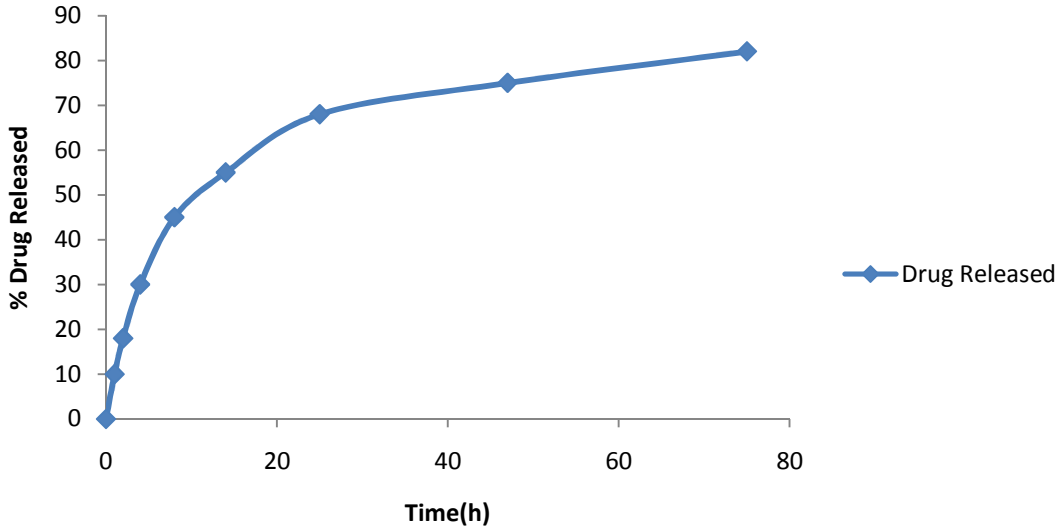
Fig 2: SEM micrograph for fish gelatin nanoparticle.

3.4. DRUG LOADING: Drug loading depends much on the solubility of the solid state drug in a matrix material and the molecular weight. UV-Vis spectra of pure RIF as well as RIF loaded FGNPs were obtained using UV-Vis spectrophotometer. Transmittance (T) is defined as the ratio of intensity of transmitted light and intensity of the light that is falling upon the sample. In drug loaded nanoparticles, the amount of light transmitted is less, because of light absorption by the drug particles, whereas in pure nanoparticles, the amount of light transmitted is high, because of absence ofthe drug particles.

Table 1: Drug Release Study:

3.5DRUG RELEASE: The release occurs by diffusion, where the drug is uniformly distributed. The invitro release of the drug by artificial biological membranes was studied. Drug release from known amounts of RIF's loaded FGPS nanoparticles was evaluated. The in-vitro drug release of RIF- loaded FGPS was studied for 75hours at of pH 7.4 by using a dialysis membrane. In the first 14hrs, 42% of RIF was released from the nanoparticles which increased to 48% in 25 hrs. The release behavior of drug from the fish gelatin matrix when studied was found that the release may have occurred through the matrix of the nanoparticles.

S.no	Time (h)	Drug Released
1	0	0
2	1	10
3	2	18
4	4	30
5	8	45
6	14	55
7	25	68
8	47	75
9	75	82



3.6 ANTIBACTERIAL ACTIVITY STUDIES:

The antimicrobial activity of the nanoparticles was compared with that of the free drug RIF by disc diffusion method.NPs better penetrate the bacterial cell and enables better delivery of RIF to the active site, hence RIF loaded FGNPs possesshigh antibacterial activity against the *Myclobacterium Tuberculosis*. The antibacterial activity of drug loaded NPs weregreater than that of free drug. Nano encapsulation did not increase the antibacterial activity of RIF.Growth inhibition was compared with drug.

CONCLUSION:

A novel approach was followed for the preparation of RIF loaded FGNPs. These nanoparticles possess various therapeutic and biomedical applications. Fish gelatin was extracted successfully from the skin and fin of cat fish by acidic methods.

The fish gelatin nanoparticles were successfully extracted by a two-step

desolvation method, encapsulation and characterization. In vitro release and antibacterial activity of the FGNPs were found out to determine the efficiency of the system. Rifampicin is a bactericidal antibiotic with a wide spectrum of activity and is used to treat Mycobacterium including tuberculosis and leprosy.

It is less active against gram-negative bacteria. RIF inhibits DNA dependent RNA polymerase in bacterial cells by binding to its B subunit. The results demonstrated that the RIF loaded NPs were more effective against gram positive bacteria. The advantage of using gelatin nanoparticles as drug vehicle is that they enable controlled release of drugs.

The dosage of drug required is reduced with gelatin nanoparticles, when compared with that through the oral route; hence side effects are significantly reduced.

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3.3 REFERENCE

1. AhmadZ,Pandey R, Sharma, S,Khuller G.K. (2008) 'Novel chemotherapy for tuberculosis' chemotherapeutic potential of econazole,moxifloxiacinloaded PLG nanoparticles', Journal Antimicrobial Agents 31, pp 142-146.
2. Amesen J.A and Gildberg.A. (2007). 'Preparation and characterization of gelatin from Atlantic salmon skin'.Bioresource Technology, pp 53-57.
3. Ameson J.A. and Gildberg A. (2006).'Extraction of muscle proteins and gelatin from cod head'.ProcessBiochemistry.pp 697-700.
4. AvenaBustillosR.J.OlsenC.W. Chiou.B.YeeE.BechtelJ.&Mc Hugh,T.H.(2006)'Water Vapor Permeability of mammalian and fish gelatin films.' Journal of Food Science, 71, pp202 - 207.
5. BajpaiA.K.ChoubeyJ. (2006). 'Designof gelatin nanoparticles as swelling controlled delivery system for chloroquine phosphate'.Journal.Mater.sci. mater. Med.17pp 345-358.
6. Bala Hariharan.S. KumarM.N. (2004). 'PLGA nanoparticles in drug delivery' Drug carrier syst.21, pp 387-422.
7. BalthasarS.MichaelisK.Dinauer NvonBriesenH.KreuterJ.LangerK. (2005). 'Preparation and characterization of antibody modified gelatin nanoparticles as drug carrier system for

uptake in lymphocytes'. Biomaterials 26, pp 723-2732.

8. BarrowE.W.WinchesterG.A.StaasJ.KQuenelleD.C.Barrow, W.W. (1998). 'Use of micro sphere technology for targeted delivery of rifampintomycobacteriumtuberculosisinfected macrophages'.Antimicrob.Agents Chemother.42, pp2682-2689.
9. CasconeM.G.Lazzeri.Carmignani C. ZhuZ. 2002. 'Gelatin nanoparticles produced by a simple W/O emulsion as delivery system methotrexate'. JournalMater.Sci. Med.13.pp 523-526.
10. ChengLH.Lim.B.L.Chow.K.H. Chong,S.M.&Chang.Y.C.2007 'Using fish gelatin and pectin to make a low fat spread. Journal of Food Hydrocolloids.
11. ChoS.M.KwakK.S.ParkD.CGuY.SjiC.IJang.D.H.(2004).'Processing optimization and functional properties of gelatin from shark cartilage'. Food Hydrocolloids.18, pp573-579.
12. Costas kaparissidei, Sofia alexandridou, katerinakotti and sotirachaitidou.2006,' 'Recent advances in drug delivery systems', Azojono journal of nanotechnology online.
13. Dwaine, Emerich, Christopher G. Thanos 2006 'The pinpoint promise of nanoparticle based drug delivery and molecular diagnosis'LCTBiopharma, pp 171–184.
14. Esther L.W .Barrow, Quenelle and William Barrow (1998) 'Anti-microbialagent's andchemotherapy'2682-2689.
15. FarhanAlhusban, Yvonne perrie, Mohammed (2011) 'Formulation of multi particulate systems as lyophilize dis integrating tablets'. European journal of pharmaceutics and Bio pharmaceutics 79, pp 627 - 634.
16. Farnaz, Mahdi H 'Preparation and antibacterial activity evaluation of rifampicin loaded poly lactase co glycolidenano particles'. 2007,Medical Nanotechnology Research centre, Medical Sciences.
17. GilsenanP.M&Ross Murphy 1999 'Structureandrhelogy of gelatin gels'. In B.T.Stockke, &A.Elgsaeter Polymer networks group review seriesvol.2pp27-30.
18. Gomez, Guillen, GHimenez M.E., LopezCaballero M.P. Montero, (2011). 'Functional and bioactive properties of Collagen and Gelatin from alternative sources' Food hydrocolloids. pp 1813–1827.
19. GuptaU.D.Katoch V.M. (2009). 'Animal models of tuberculosis for vaccine development'.IJMR.(129) pages 11-18.
20. Hamid Tavakolipour (2011) 'Extraction and Evaluation of Gelatin from Silver Carp Waste'. World journal of fish and marine sciences 3(1) pp10-15

21. Ho J. Bae,Hyun J. Park, Seung Hong, Young J. Byun,Darby, Robert, William, (2009). 'Nano composite Effect of clay content homogenization, pH and ultra sonicationon mechanical and barrier properties of fish gelatin composite films'pp 1179 - 1186.

22. HongshumYang, Yiten Wang, Joe M. Regenstein, (2008) 'Effects of alkaline and acid pretreatment on the physical properties and nanostructure of the gelatin from channel catfish skins'FoodHydro colloids (22) pp1541-1550.

23. IngrildJ.HaugI.Draget, 2004 'physical and rheological properties of Fish gelatin compared to mammalian gelatin'. Food Hydro colloids (18) pp. 203 -213.

24. JainS.K.GuptaY.2008.'Manno sylated gelatin nanoparticles bearinganantiHIVdrugdidanosi ne for site specific delivery'.Nanotechnology.Biol ogy Medical. (4)pp41-48.

25. Jain,(2008).'Development, characterization, and toxicity evaluation of amphotericin β loaded gelatin Nano particles. Biology and Medicine pp 252-261

26. M.R.UmihartinaA.Azizah (2011).'Gelatins from three cultured fresh water fish skins obtained by liming processes. Food hydrocolloids (25) pp1256 -1260.

27. Arnesen, (2005)'Extraction of muscle proteins and gelatin from cod head.' Process biochemistry pp 697-700.

28. JessyShaji and Patole V.2008. 'Protein and peptide drug delivery', Oral Approaches, Dept. pharmaceutical sciences pp. 269 -276.

29. Jitendra Prasad Mathuria, (2009). 'Nanoparticles in Tuberculosis Diagnosis, treatment and prevention'; A Hope for Future. Digest journal of Nano materials and Bio structures Vol. 4, No. 2, pp 309 – 312.

30. Karim, A.A, Rajeev Bhat (2009), 'Fish gelatin: properties, Challenges, and prospects as an alternative to mammalian gelatins.' Food hydrocolloids (23) pp563–576.

31. Kaul.G. Amiji M. (2005). 'Tumor Targeted gene delivery using poly (ethyleneglycol) modifiedgelatinnanoparticles'i nvitroandinvivostudies.Pharm. Research. (22). pp 951-961.

32. Klaus Zwiorek, Julia Kloeckner, Ernst Wagner, ConrodCoester. (2005). 'Gelatin Nanoparticles as a new and simple gene delivery system.' Journal Pharm Pharmaceuti. Sciences;pp 28

33. LabanaS .Pandey.R,Sharma.S. Khuller.G.K. (2002)'Chemotherapeutic activity against murine

tuberculosis of once weekly administered drugs (isoniazid and rifampicin)encapsulated in liposomes'.Int.JournalAntimicrob.Agents (20) pp301-304.

34. LeeG.V.P (2006).'Antitumor and antimetastatic effects of gelatin doxorubicin and PEGylatedgelatindoxorubicin nanoparticles in SCC7 Bearing mice'Journal.Drug Target. pp 707-716.
35. Leo E.Came (1999).'Dynamic dialysis for the drug release evaluationfromdoxorubicingelatinnanoparticlesconjugates'.Int.Journal.Pharm.pp 23-30.
36. Sadowska, Wittorkolodziejski, Celina niecikowska (2008). 'Effect of extracting time and temperature on yield of gelatin from different fish offal'. Food chemistry pp 700 – 706.
37. Narendra(2008)'Development Characterization, and toxicity evaluation of amphotericin β loaded gelatin nanoparticles', Nano med: Nanotechnology, and Medicine, Volume 4, Issue 3, Pages 252-261
38. Mohsen Jahanshahi and Zahra Babaei, (2008). 'Protein Nanoparticle: A Unique System as drug delivery vehicles'. African journal of Biotechnology 7(25) pp. 4926–4934.
39. Mohan raj and Chen, Nano particlesReview2006) Tropical Journal of Pharmaceutical Research, vol 5pp 561 – 573.
40. Nader (2010), 'Synthesis and Characterization of gelatin nanoparticles using CDS/NHS as a nontoxic cross linking system'. Journal Sciences: Mater Med 22: pp 63- 69.
41. Nirmaladevi and Tarun Kumar Maji (2009), 'Preparation and evaluation of Gelatin / Sodium Carboxy methyl cellulose poly electrolyte complex micro particle for controlled delivery of isoniazid'; AAPS.
42. Paraskevi, Kallinteri, Sophia Antimisiaris. 2001 .'Solubility of drugs in the presence of gelatin; effect of drug lipophilicity and degree of ionization' International journal of pharmaceutics (221), pp 219 – 226.
43. Paul Szpak, (2011), 'Fish bone chemistry and ultra-structure: implications for taphonomy and stable isotope analysis Journal of Archaeological Science' pp 3358 – 3372.
44. Rajesh pandey and G.K. (2006).'Nanotechnology based drug delivery system for the management of tuberculosis.' Indian Journal of Experimental Biology Vol – 44 pp 357-366.
45. Ricardo (1997). bioactive compounds from marine organisms'Journal of Marine Biotechnology pp 187 – 193.
46. Rustard T. (2003). 'Utilization of Marine by products.' Journal of food chemistry, pp 458 – 461.
47. SahooK, S. Parveen MS, J.J. Panda MS (2007) 'The present and future of nanotechnology in human health care'.

Nanotechnology, Biology and Medicine (3) pp 20–31

48. Simon B Ross Murphy (2004). ‘Structure and rheology of gelatin gels recent progress.’ Polymer physics volume 33 pp 2622 – 2627.

49. Simon Young, Mark Wong, Yasuhiko Tabata, Antonios G. Mikos, 2005 ‘Gelatin as a delivery vehicle for the controlled release if bioactive ’JCR (109) pp- 256-274.

50. Southwick, 2007. “Pulmonary Infections" ‘Infectious Diseases: A Clinical Short Course, 2nd ed. McGraw-Hill Medical Publishing Division’. pp 104.

51. Tobias (2005). ‘Super paramagnetic nanoparticles for biomedical applications’ Journal of Magnetism and Magnetic Materials pp 483–496.