REVIEW ARTICLE

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Biovectors for Tumour Therapy : Lipoprotein Mimicking Biovectorized Systems

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The complicated physiology of tumors has always attracted pharmaceutical scientists to design a delivery system that can shoot to kill it specifically. A number of supramolecular systems, each with their pros and cons have been developed to treat the cancerous growth. The success rate of site-specific supramolecular carriers have urged scientists to direct their focus towards biovectors for drug delivery. Biovectors are intrinsic nutrient carrier systems of the body such as lipoproteins. Their uniqueness lies in the fact that they circulate in the blood for extremely long period and rapidly dividing cancer cells take them specifically in the need for cholesterol. Scientists have successfully modified lipoproteins as neo-lipoproteins for the delivery of anticancer agents. Recently, they have developed lipoprotein mimicking artificial carrier systems for the same purpose. This article reviews natural and various other types of semi-synthetic or synthetic lipoprotein mimicking biovectorized systems developed in recent years.

The twentieth century has witnessed epoch making progress in every branch of science and technology. Even today, despite increasing health consciousness, prevention strategies and developing technology, the word 'cancer' inspires fear. Recent studies reveal that about 6 to 7 lakh new cases of cancer occur every year in our country alone and cancer is the second lethal cause. All over the world, research pursuits in this field aim at achieving a better understanding of cancer-its cause, symptoms, mode of spreading, diagnosis, treatment and possible cure.

Killing a tumor cell is not difficult, the problem is how to avoid killing normal cells. Although intensive efforts have been made to develop new therapeutic moieties and to improve upon existing ones, chemotherapy is still a major therapeutic approach for the treatment of this cell growth disorder. In practice, a number of hurdles complicate cancer chemotherapy such as low therapeutic index, limited efficacy, lack of response, early resistance, unacceptable toxicity and poor specificity of an antineoplastic agent. In addition to these abnormal neovasculature architecture, pattern of perfusion, compromised endothelial integrity, immunogenicity, pattern of perfusion, capacity to metastasize and heterogeneity of distribution within tumor mitigate against effective delivery to tumour cells. Finally, huge disappointment proven by chemotherapy in clinic have led to the search for techniques which considerably alleviate the problems of conventional therapies.

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Supramolecular Drug Carriers:

A number of exiting and technologically challenging approaches like use of polymeric microparticles[1] and nonoparticles[2] have been proposed to allow the use of more toxic agents with simultaneous sparing of the normal tissue. Many literature reports make it clear that the approach based on targeting via supramolecular drug carriers is more attractive. The supramolecular carriers are the structures formed by non-covalent interactions of the molecules with unique physicochemical properties and are capable of carrying drugs in them by

encapsulation, intercalation or adsorption. The common ones are micelles, liposomes[3] and niosomes[4].

Although tremendous efforts have been made for designing these carriers, their applicability is limited by a number of drawbacks. The major hindrance to their clinical acceptance has been the human body's ability to recognize them as foreign. This early recognition and uptake by reticuloendothelial system (RES) is a major hurdle of target oriented drug delivery system. A prerequisite for optimal tumour targeting is prolonged circulation time of supramolecular carrier which in turn depends on reduction of RES mediated clearance.

Circulation Time of Supramolecular Carriers and Tumour Targeting: Implications for Cancer Chemotherapy:

Longer circulation drug carriers have been demonstrated to localise in tumours, regardless of their type or anatomical location[5]. Since tumour targeting appears to require a longer circulation time, plasma stability of drug-carrier association needs improvement over conventional, rapidly cleared supramolecules, to match prolonged exposure to the blood components. Improved plasma stability has been shown to correlate with increased localization of drug bearing supramolecules in the tumour area. Accumulation of the drug carrier in tumour is also controlled by a interplay of particle size that results in increased tumour localization in various animal models due to prolonged circulation time, decreased RES uptake and concomitant increase of carrier uptake by normal tissue[6]. The effect of lymphatic clearance of supramolecules on tumour targeting cannot be overlooked. Two main clearance mechanisms for removal of extravasated particulate materials from tissues are lymphatic pathway and phagocytosis by scavenger cells. Unlike normal tissues, tumours have increased vascular perfusion and permeability but no functional drainage and therefore the retention of particles and macromolecules is greater in tumour tissues than in normal tissues. This phenomenon is known as enhanced permeability and retention effect (EPR) that amplifies the effect of enhanced permeability, resulting in further tumour accumulation of a carrier associated drug or polymer-drug conjugate[7]. Thus, the supramolecular carrier with altered distribution, prolonged circulation, steric stabilization, stealth (terms having overlapping significance) would lead to tumour targeting.

Stealth® systems are less prone to recognition by opsonins and macrophage uptake. Concomitantly, they are cleared slowly and have long circulation half-life in the biological system. They are obtained by surface-modification of carrier particles by hydrophilic polymer grafting like polyethylene glycol so as to form a non-recognisable sheath or halo around them. The collective knowledge from different research fields will be required for the design and optimization of such carriers.

Design of Supramolecular Carriers with Reduced Recognition and uptake by Reticulo-Endothelial System:

Published data indicate a reduction in recognition and uptake by RES by modifying net surface charge[8], composition and membrane rigidity[9], surface hydrophilicity[10], size[11] and surface mobility[12]. As early in 1971, Napper and Netchey[13] published their classic study on the principal of steric stabilization of colloid particles by an adsorbed macromolecular or polymer layer on the surface. Later in 1978, VanOss[14] showed that pathogenic bacteria possess a surface that consists of highly hydrophilic hydrated layer of protein, polysaccharide and glycoproteins, which reduces, their opsonization and makes them less palatable to phagocytes. Following this lead various research groups modified the surface properties of colloidal carriers to render them sterically stabilized and more hydrophilic by incorporating sialic acid-rich gangliosides[15], or biosurface polymers such as polyethylene glycol (PEG)[16,17], or lipid derivatives of hydrophilic polymer into liposome or by adsorption of hydrophilic copolymers or sialic acid rich glycoprotein on the surface of nanoparticles[20]. As a consequence of these modifications, stealth liposomes as well as biodegradable polymeric nanoparticles are generally less recognised by RES and survive for prolonged time in blood flow which is a prerequisite for optimal tumour targeting.

This strategy no doubt restored their potential as widely useful delivery system, but stability of copolymer adsorption layer, stability and integrity of phospholipid vesicles is still uncertain. In light of these challenges attempts have been made to obtain 'stealth' colloidal particles, directly prepared from a hydrophilic polymer such as chitosan, alginate[21,22]. This approach of targeting is in infancy, further experiments are needed to prove their ability.

Initial promises offered by the systems coded with properties of stealthiness e.g. stealth-microspheres,

nanoparticles, liposomes as well as polymeric micelles and macromolecule complexes were not fulfilled enough. Thus, majority of research community turned to the other means of tumour targeting. A great deal of interest focuses on biovectors.

BIOVECTORS:

On the basis of their origin, carriers could broadly be grouped as exogenous e.g. nanoparticles, liposomes, microemulsions and endogenous e.g. reconstituted viruses, erythrocytes, steroids and lipoproteins. Both the classes have their own advantages and shortcomings. The carriers of endogenous origin, biovector, are thought to be more acceptable and versatile. These biovectors are nanoscopic, thermodynamically-stabilized supramolecular vehicles, formed through self assemblage for site specific delivery, do not undergo any enzymatic cleavage, have prolonged circulation life and are loaded with determinants which avoid their RES uptake and give specific receptor-mediated recovery. The representative of these natural stealth vehicles are viruses, a carrier of nucleic acids and lipoproteins, a cholesterol carrier.

Lipoproteins:

Lipoproteins are the physiological carriers of water-insoluble lipid in the vascular system. Since lipoproteins exhibit a considerably longer circulation time in the blood and are not taken up by the RES, they have been considered for drug delivery. Low density lipoproteins (LDL) have attracted particular interest in this respect, because they are able to leave the vascular system by specific receptor-mediated uptake into cells. Tumour cells exhibit an increased uptake of LDL owing to their own cholesterol need, which forms the basis of the LDL drug targeting concepts in cancer chemotherapy[23].

Structurally, lipoprotein consists of an apolar core, composed of cholesterol esters and triglycerides, surrounded by a monolayer of phospholipid in which cholesterol and one or more specific apolipoprotein are embeded[24]. Based on their origin, lipoproteins could broadly be classified as natural, semi-synthetic or synthetic. Plasma lipoproteins comprise four principal classes with different physiological roles : chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). They differ in size and density and show variations in lipid and apoprotein composition.

The relevance of LDL in context of targeting cytotoxic drugs to tumour cells is two fold : firstly, the cholesteryl esters which form the core of LDL particles can accommodate a large number of lipophilic substances[25,26]. Secondly, large requirement of cholesterol by rapidly dividing malignant cells as well as greater LDL receptor activity of the same[27,28].

The fate of a drug in these lipoproteins is dependent upon the metabolism of the carrier which may be modulated by individual apoprotein or modification of surface characteristics on hydrophobic core composition. The ability of chylomicrons[29] and LDL[30,31] to be introduced rapidly into parenchymal cells, acylated LDL into endothelial cells[32,33] lysosylated LDL into kupffer cells[34], lactosylated HDL into parenchymal cells[24], clearly indicate that in complex organs such as liver, successful targeting of lipoproteins to the cell of choice can be achieved.

Other modifications such as incorporation of various cholesterol derivatives into hydrophobic core of lipoproteins[32,33], alteration of phospholipid composition and/or association of negative by charged phospholipid[37,38], treatment with sphingomyelinase[39] and proteoglycans[40] result in an increased macrophage uptake of these altered lipoproteins.

Practical utility of lipoproteins as drug carriers is hampered by several factors. The major drawbacks to use this carrier system are : ability to entrap only lipophilic drugs, handling problems, low loading efficiency, altered biological fate, limited availability of these vectors and ensuring infection risks such as hepatitis and AIDS. However, these problems can be circumvented effectively by utilizing another similar systems of synthetic origin, that can imitate the biological functions of natural lipoproteins. Artificial "Lipoprotein-Mimicking Systems" could be promising in this respect. They can be classified into three types:

1. Emulsion based Lipoprotein Mimicks.
2. Lipopearls/Lipospheres/Solid Lipid Nanoparticles.
3. Supramolecular Biovectors.

Emulsion Based Lipoprotein Mimicks:

Submicron-sized vegetable oil-in-water emulsions have been used as a caloric source in parenteral nutrition for decades[41,42]. Fat microemulsion-based lipoprotein mimicks are protein free analogues of LDL, consisting mainly of phospholipid, cholesterol, cholesterol ester and

triglycerides ratios that correspond to physiological LDL to resemble as closely as possible. These lipoprotein mimicks have mean droplet size ranging from 12-250 nm, which mainly depends upon preparation method.

Fat emulsions are known and used in parenteral nutrition and have proved to be non-toxic. These systems are manufactured in large scale and display an acceptable long term stability. Some commercially available lipid emulsions are Intralipid, Lipofundin-S, Liposyn and Travemulsion. The presence of bulk of drug in non-aqueous environment may lead to an increased stability of drug (e.g. reduced hydrolysis) as well as to a possible controlled release system.

Several reports are available in connection with development and characterisation of synthetic emulsion model of LDL[43,45], VLDL[46], chylomicron[47-50] and HDL[51].

Many more reports are available in literature on drug delivery by i.v. administration of lipid emulsion containing drugs. Examples are the administration of lignocaine, hexobarbital, phenyl butazone[52,53], diazepam[54], narcotic antagonists[55,56], corticosteroids[57], valinomycin[48] and other anticancer drugs[58,48].

Most drawbacks associated with lipid emulsions are caused by susceptibility of carrier toward incorporation of drug. This can be attributed to drug crystallisation within oil droplets and perturbation of the stabilising emulsifier film by drug crystals on diffusing drug molecules which have a high mobility in liquid oil phase. These perturbations may induce instabilities of either mechanical or electrochemical nature causing coalescence, particle growth and drug leakage. The high mobility of incorporated drug molecules also causes a fast release of drug from carrier in biological fluids, so that it is hardly possible to achieve sustained release by an emulsion formulation.

Lipopearls®/Lipospheres/Solid Lipid Nanoparticles (SLNs):

Lipospheres represent a new type of fat-based encapsulation system developed for parenteral and topical drug delivery of bioactive compounds[59-61]. Lipospheres consist of water-dispersible solid microparticles of particle size between 0.2 to 100 μm in diameter composed of a solid hydrophobic fat core (mainly, triglycerides) stabilized by monolayer of phospholipid molecules embedded on their surface. The internal core contains the bioactive compound dissolved or dispersed in the solid-fat matrix.

Lipospheres combine the superiority of colloidal carriers, such as biodegradability and biocompatibility of the carrier material and ease of manufacture. They bear merits of the solid physical state polymeric nanoparticles with respect to size stability, drug leakage and sustained drug release. Possible advantages of solid physical state of lipid carriers systems are :-

1. Avoidance of coalescence -
   - enhanced physical stability
2. Reduced mobility to incorporate drug molecules
   - reduction of drug leakage
   - circumvention of instabilities due to interactions between diffusing drug molecules and emulsifier film.
   - sustained drug release
3. Static solid/liquid interface
   - facilitate surface modification.

Lipid nanospheres (LNs) have been effective in delivering various drugs and biological agents, including vaccines[62,63], local anesthetic[64], insect repellents[65], β-blocking agents[66], peptide[67], antibiotic and antiinflammatory agents[68] and anticancer agents[69]. The presence of static interface, facilitate a surface modification of the carrier particles often to impart stealth behaviour[70]. Stealth lipospheres bearing methotrexate[71] and 6-mercatopurine[72] have been successfully prepared and evaluated by anchoring lipid derivative of PEG on to their surface.

Colloidal solid lipid dispersion represent complex colloidal system which may contain different types of coexisting colloidal structures such as solid lipid particles, non-solidified particles of super-cooled melt, micelles, mixed micelles, and/or vesicles[46]. Despite their complexity, SLNs represent an interesting alternative to conventional lipid based carrier system. A general limitation of solid lipid nanoparticles is, however, represented by limited inclusion capacity for hydrophobic drugs. It has been demonstrated that stability is another problem associated with SLNs, expressed by their recrystallization behaviour and their tendency to form semisolid gels. The high fluidity of core-lipid observed for all LDL and LDL-model preparations at body temperature may, in general, question the usefulness of these carrier systems for drug targeting.

The strategy proposed to face these problems is to use another system of synthetic origin, designed to mimic LDL. This novel system is named as Supramolecular Biovector[73].

Supramolecular Biovector (SMBVs):

The supramolecular biovectors are very small, multilayered vesicles (10 nm to few microns) formed by gelified polysaccharide hydrophilic core capable of capturing the active substances in the links of a network[73]. This central core is surrounded by a crown of fatty acids attached to the core by covalent bonds. The whole is covered by an external sheet of phospholipids attached to the lipid crown by hydrophobic interactions, with their polar heads facing the periphery. The structure of SMBVs allows the entrapment of various drugs especially amphiphilic and hydrophilic substances. The hydrophilic active substances are attached with more or less stability to heart of core and the active lipophilic substances penetrate throughout the double-lipid membrane.

SMBVs are stable and completely synthetic allowing industrial scale processes. Drug entrapment can be achieved with a high loading ratio[74,75]. Their synthesis can be modulated and controlled to allow an efficient drug cellular uptake and intracellular trafficking[75-78]. Chain length of fatty acids decides peripheral organisation of fatty acids and phospholipid around the polysaccharide core of SMBVs[75]. Design and characterization of two types of SMBVs have been reported.

Type I : Neutral SMBVs

Type II : SMBVs whose internal lipidic layer is grafted with both fatty acid and succinic acid as ionic ligands.

Synthesis and characterization of anionic SMBVs for entrapment of cationic molecules gentamicin and doxorubicin[78], interleukin-2[79] and cationic core for incorporation of anionic antisense oligodeoxynucleotides[80] have been reported. We have prepared and characterized SMBVs and their stealth counterparts with egg-box complex core entrapping 5-fluorouracil[81]. The system showed promising for prolonged delivery and lymphatic targeting of the drug. Modular structure characterization e.g. polysaccharide core mesh size, ionic properties, lipid density, increased stability, biocompatibility, biodegradability, non-immunogenicity, ability to entraps hydrophilic as well as lipidic drugs, possibility of surface modifications, can be exploited favourably for pharmaceutical application of SMBVs[82].

CONCLUSIONS

Another novel approach, coexistence of LDL model particle with other colloidal structures such as lipid vesicles (liposomes), has restored their potential as widely useful delivery system. Conceptual representatives 'Emulsomes' and 'Spherosomes' have to be investigated in more details. They are successfully utilized for lymphatic targeting due to their intrinsic nature hence have ability to control tumour metastasis. The overview on the information required from the reports above indicates that this novel system represents potential and promising tool for targeting and prolonged delivery of drugs.

REFERENCES

1. Wang, Y.M., Sato, H., Adachi, I. and Horikoshi, I. **J. Pharm. Sci.,** 1996, 85, 11.
2. Brannonpeppas, L. **Int. J. Pharm.,** 1995, 116, 1.
3. Rahman, A. and Schein, P.S. In; Gregoriadis, G. Eds, Liposomes as drug carriers, John Wiley, New York, 1988, 381.
4. Yoshioka, T. and Florence, A.T. **Int. J. Fharm.,** 1994, 108, 117.
5. Gabizon, A., Price, D.C., Huberty, J., Bresalier, R.S. and Papahadjopoulos, D. **Cancer Res.,** 1990, 50, 6371.
6. Gabizon, A.A. **Adv. Drug. Del. Rev.,** 1995, 16, 285.
7. Huwang, K.J., Padki, M.M. Chow, D.D. Essien, H.E. and Beaurier, P.L. **Biochim. Biophys. Acta,** 1987, 900, 88.
8. Nishida, K., Tonegav a, C., Nakane, S., Takakura, Y., Hashida, M. and Sezaki, H. **Int. J. Pharm.** 1990, 65, 7.
9. Namba, Y., Sakakibara, T., Masada, M.M. Ito, F. and Oku, N. **Chem. Pharm. Bull.,** 1990, 38, 1663.
10. Illum, L., Hunneyball, I.M. and Davis, S.S. **Int. J. Pharm.,** 1998, 29, 53.
11. Allen, T.M., Hansen, C. and Rutledge, J. **Biochim. Biophys. Acta,** 1989, 981, 27.
12. Blume, G. and Cevc, G. **Biochim. Biophys. Acta.** 1993, 1146, 157.
13. Napper, D.H. and Netchey, A. **J. Colloid Interface. Sci.** 1971, 37, 528.
14. VanOss, C.J. **Annu. Rev. Microbiology,** 1978, 32, 19.
15. Gabizon, A. and Papahadjopoulos, D. **Biochim. Biophys. Acta,** 1992, 1103, 94.
16. Senior, J., Delgado, C., Fisher, D., Tilcoch, C. and Gregoriadis, G. **Biochim. Biophys. Acta,** 1991, 1062, 77.
17. Parr, M.J., Ansell, S.M., Choi, L.S. and Cuttis, P.R. **Biochim. Biophys. Acta,** 1994, 195, 21.
18. Zalipsky, S., Brandeis, E., Newman, M. and Woodle, M.C. **FEBS Lett.,** 1994, 353, 71.
19. Harasym, T.O., Tardi, P., Longman, S.A. Ansell, S.M.,

Bally, M.B., Lullis, P.R. and Choi, L.S. **Bioconjugate Chem.,** 1995, 6, 187.
20. Olivier, J.C., Vauthier, C., Taverna, M., Ferrier-Baylocq, D., Puisieux, F. and Couvreur, P. **Proc. Intern. Symp. Control. Release Bioact. Mater.,** 1994, 21, 144.
21. Rajaonarivony, M., Vauthier, C., Couarıaze, G., Puisieum, F. and Couvreur, P. **J. Pharm. Sci.** 1993, 82, 912.
22. Calvo, P. Remuna Lopez, C., Vita-Jato, J.L. and Alonso, M.L. **J. Applied Polymer Sci.,** 1997, 63, 125.
23. De Smidt, P.C. and Van Barkel, T.J.C. **Crit. Rev. Ther. Drug Carrier Sys.,** 1990, 7, 99.
24. Bijsterbosh, M.K. and Van Barkel, T.J.C. **Mol. Pharmacol.,** 1992, 41, 404.
25. Lestavel-Delattre, S., Martin-Nizard, F., Clavery, V., Testard, P., Favre, G., Doualin, G., Sqallihoussaini, H., Bard, J.M., Duriez, P., Delbart, C., Soula, G., Lesieur, D., Lesisur, I., Cazin, J.C., Cazin, M. and Fruchart, J.C. **Cancer Res.,** 1992, 52, 3629.
26. Sumadi-Baboli, M., Favre, G., Canal, P. and Soula, G. **Br. J. Cancer,** 1993, 68, 319.
27. Hynds, S.A., Welsh, J., Stewart, M.J., Jack, A., Soukop, M., Macardle, C.S., Calman, K.C., Packard, C.J. and Shepherd, J. **Biochim, Biophys. Acta,** 1994, 795, 589.
28. Rudling, M.J., Reihner, E., Einarson, K., Ewerth, S. and Angelin, B. **Proc. Natl. Acad. Sci. USA,** 1990, 87, 3464.
29. De Groot, P.H.E., Van Berkel, T.J.C. and Van Tol, A. **Metabolism,** 1981, 30, 792.
30. Harkes, L. and Van Berkel, T.J.C. **Biochem. J.,** 1994, 224, 21.
31. De Water, R., Hessels, E.M.A.J., Barkeren, H.F and Van Barkel, T.J.C. **Eur. J. Biochem,** 1990, 192, 419.
32. Nagelkerke, J.F., Barto, K.P. and Van Barkel, T.J.C. **J. Biol. Chem.** 1983, 258, 12221.
33. Brown, M.S. and Goldstein, J.I. **Annu. Rev. Biochem.,** 1983, 52, 223.
34. Van Berkel, T.J.C., Kruijit, J.K., Spanjer, H.H. Nagelkerker, J.F., Harkes, L. and Kempen, H. J.M. **J. Biol. Chem.,** 1993, 24, 145.
35. Van Berkel, T.J.C. **J. Control. Release,** 1993, 24, 145.
36. Seki, J., Okita, A., Watanable, M., Nakagawa, T., Handa, K., Tatewaki, N. and Sugiyana, M. **J. Pharm. Sci.,** 1985, 74, 1258.
37. Aviram, M. and Major, I. **J. Clin. Invest.,** 1993, 91, 1942.
38. Greenspan, P., Ryu, B.H., Mao, F. and Futman, R.L. **Biochim. Biophys. Acta,** 1995, 1257, 257.
39. Xu, X.X. and Tabas, I. **J. Biol. Chem.,** 1991, 266, 24849.
40. Vijayagopal, P., Srinivasan, S.R., Radhakrishnamurthy, B. and Berenson, G.S. **Arterioscler. Thromb.,** 1992, 12, 237.
41. Schuberth, O. and Wretlind, A. **Acta Chir. Scand. (Suppl.),** 1961, 278, 1.
42. Wretlind, A. **J. Parent. Enternal Nutr.,** 1981, 5, 230.
43. Ginsburg, G.S., Small, D.M. and Atkinson, D. **J. Bio. Chem.,** 1982, 257, 8216.
44. Via, D.P., Craig, I.F., Jacobs, G.W., Van Winkle, W.B., Charlton, S.C. Gotto, A.M. and Smith, L.C. **J. Lip. Res.,** 1982, 23, 570.
45. Halbert, G.W., Stuart, J.F.B. and Florence, A.T. **Int. J. Pharm.,** 1984, 21, 219.
46. Wheeler, J., Wong, K.M., Ansell, S.M., Masin, D. and Baly, M.B. **J. Pharm. Sci.,** 1994, 83, 1558.
47. Jeppsson, R. and Ljunberg, S. **Acta Phar. Suec.,** 1973, 10, 129.
48. Repta, A.J. In; Breimer, D.D. and Speiscr, P. Eds, Topics in Pharmaceutical Sciences, Elsevier, New York, 1981, 181.
49. Redgrave, T.G. and Maranhao, R.C. **Biophys. Acta,** 1985, 835, 104.
50. Van Barkel, T.J.C. Van Dijk, M.C.M. Bijsterbosch, M.K. and Rensen, P.C.N. **J. Control. Release,** 1996, 41, 85.
51. Pittman, R.C. Glass, C.K., Atkinson, D. and Small, D.M. **J. Biol. Chem.,** 1987, 262, 2435.
52. Jeppsson, R. **Acta Pharma. Toxicol.,** 1975, 37, 56.
53. Jesmik, G.J., Walsh, G.M., Ditzer, W. and Woods, E.F. **Proc. Soc. Exp. Bio.,** 1979, 161, 458.
54. Levy, M.Y. and Benita, S. **Int. J. Pharm.,** 1989, 54, 103.
55. Collier, H.O.J., Francis, D.L. and Schneider, C. **Nature,** 1972, 237, 230.
56. Fortner, C.L. Grove, W.R. Bowie, D. and Walker, M.D. **Am. J. Hosp. Pharm.,** 1975, 32, 582.
57. Mizushima, Y., Hamano, T. and Yokoyama, J. **J. Pharm. Pharmacol.** 1982, 34, 49.
58. Litterst, C.L., Mimnaugh, E.G. and Cowles, A.C. **H. Pharm. Sci.,** 1974, 63, 1718.
59. Domb, A.J. **U.S. Patent** 1993, 5188837.
60. Domb, A.J. and Maniar, M. **U.S. Patent** 1993, 5227165.
61. Amselem, S., Alving, C. and Domb, A. In; Cohen, E.S. and Bernstein, H., Eds, Microparticulates Systems for Drug Delivery, Marcel Dekker, New York, 1996, (in press).
62. Domb, A.J. **U.S. Patent Application,** 1992.
63. Amselem, S., Domb, A. and Alving, C.R. **Vaccine Res.,** 1992, 1, 383.
64. Masters, D. and Berde, C. In; Domb, A.J., Ed, Polymer Site Specific Pharmacotherapy, Wilae Chickester, 1994, 443.
65. Domb, A.J., Marlinsky, A., Maniar, M. and Theomim, L. **J. Am. Mosq. Control Assoc.,** 1995, 11, 29.
66. Cavalli, R., Morel, S., Gasco, M.R. Chetoni, P. and Saettone, M.F. **Int. J. Pharm.** 1995, 117, 243.
67. Morel, S., Gasco, M.R. and Cavalli, R. **Int. J. Pharm.** 1994, 105, R1.
68. Domb, A.J. **Int. J. Pharm.** 1995, 124, 271.
69. Cavalli, R., Caputo, O. and Gasco, M.R. **Int. J. Pharm.** 1993, 89, R9.
70. Schwarz, C., Mehnert, W., Lucks, J.S. and Muller, R.H. **J. Control. Release,** 1994, 30, 83.
71. Shelly, U., Khopade, A.J. and Jain, N.K. **Pharm. Acta Helv.** 1999, 73, 275.
72. Khopade, A.J. and Jain, N.K. Die Pharmazie 2000 (Submitted).

73. Samain, D., Bec, J.C., Cohen, E., Nguyen, F. and Peyrot, M. **PCT/FR 89,** 1989, 00229.
74. Peyrot, M. **STP Pharma. Prat.,** 1994, 415, 344.
75. Peyrot, M., Sautereau, A.M., Rabanel, J.M., Nguyen, F., Tocanne, J.F. and Samain, D. **Int. J. Pharm.** 1994, 102, 25.
76. Samain, D., Bec, J.C., Cohen, E., Nguyen, F. and Peyrot, M. **U.S. Patent** 5151264.
77. Samain, D., De-Miguel, I., Meniali, J. and Ioualalen, K. **European Patent** 92911799.
78. De-Miguel, I., Ioualalen, K., Bonnefous, M., Peyrot, M., Nguyen, F., Cervilla, M., Soulet, N., Dirson, R., Rieumajou, V. and Imbertie, L. **Biochim. Biophys. Acta,** 1995, 1237, 49.
79. Castignolles, N., Betbeder, D., Ioualalen, K., Merten, O., Lender, C., Samain, D. and Perrin, P. **Vaccine,** 1994, 12, 1413.
80. Berton, M., Siyou, S., Karvtzoff, R., Dartiques, L., Imbertic, L., Allal, C. and Favre, G. **Biochim. Biophys. Acta,** 1997, 1355, 7.
81. Nagaich, S. M. Pharm Thesis, Department of Pharmaceutical sciences, Dr. Harisingh Gour University, Sagar, (M.P.) 1997, 50.
82. Surekha Nagaich, Khopade, A.J. and Jain, N.K. **Pharm. Acta Helv.,** 1999, 73, 227.