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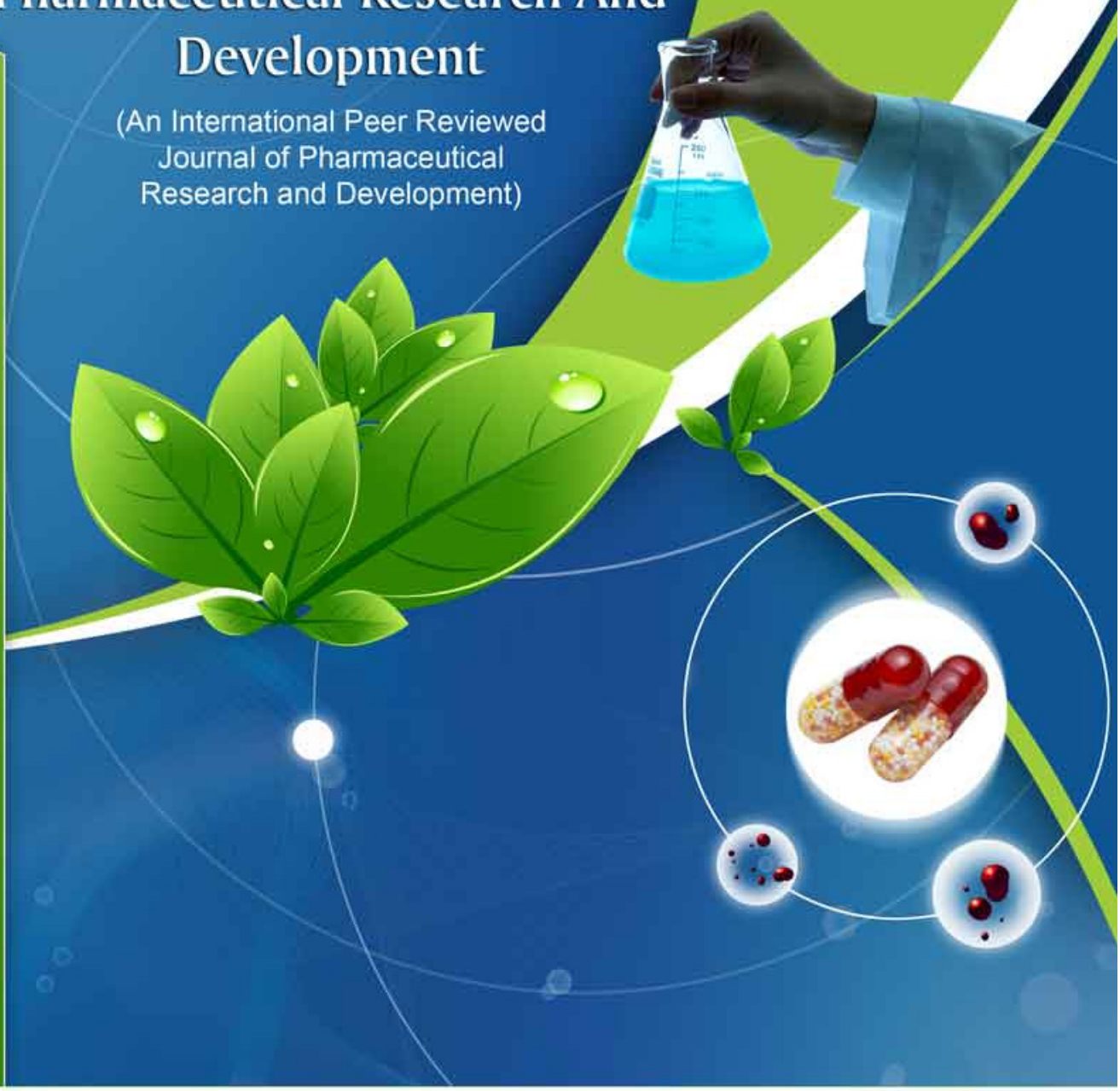

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Review Article

A REVIEW NANOERYTHROSOMES: MILESTONE IN NOVEL DRUG DELIVERY SYSTEM

Sagar R. Paygude *

Department of Pharmaceutics, Sinhgad College of Pharmacy, **Vadgaon (Bk.), Pune-41**

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ABSTRACT

Drug delivery is now entering quite exciting and challenging era. The search for an innovative drug delivery system which is cost effective, biocompatible, targeted and pharmacologically effective resulted into usage of cellular carriers like leukocytes, fibroblasts, erythrocytes, etc. Among them erythrocytes are most abundant cells circulating throughout the body. These are biocompatible, biodegradable, having very long circulation half lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods. Erythrosomes are specially engineered vesicular systems that are chemically cross-linked to human erythrocytes upon which a lipid bilayer is coated. These vesicles have been proposed as useful encapsulation systems for macromolecular drugs. Nanoerythrosomes are erythrocytes prepared by extrusion of erythrocyte ghost to produce small vesicles suspension using polycarbonate filter having a diameter 100 nm on which desired drug is incorporated using glyceraldehydes as cross linker. Nanoerythrosomes have added benefits like greater retention time, bypasses macrophage uptake and systemic clearance. The use of nanoerythrosomes looks promising for a safe and sure delivery of various drugs for treating diabetes mellitus, rheumatoid arthritis, HIV infection, drug addiction, cancer, etc. However the concept needs further optimization to become a routine drug delivery system.

KEY WORDS: *Erythrocytes, Erythrosomes, Nanoerythrosomes, Extrusion, Erythrocyte ghost.*

INTRODUCTION

***Nanotechnology*:**

The study, manipulation and engineering of devices and structures less than 100 nanometers have become known as nanotechnology. As particles become nano-sized, they exhibit unique chemical, biological, electrical and mechanical properties unlike their normal macroscopic state. Recently, much attention has been devoted toward using nanotechnology to improve health care, and the medical application of nanotechnology has become known as nanomedicine.

*Corresponding Author
Sagar R. Paygude
Department of Pharmaceutics,
Sinhgad College of Pharmacy,
Vadgaon (Bk.), Pune-41
Email: sagar7pharma@gmail.com

Three important aspects of nanomedicine viz. drug delivery, tissue engineering and nanosurgery are being studied with intense determination. This paper reviews current nanomedicine advances in these three areas along with citing potential applications for the future. [1, 2, 3]

***Drug Delivery*:**

The limitations of current drug delivering systems include suboptimal bioavailability, limited effective targeting, potential cytotoxicity and long, frequent treatments are often required. Nanoscale drug delivery devices called nanocarriers overcome these limitations. Nanocarriers are also able to maximize therapeutic activity while minimizing toxic side effects and target specific cells rather than tissues because their unique properties allow for easy surface

functionalization. Functional groups may be placed on the nanocarrier to increase or decrease solubility, increase immunocompatibility, encourage cellular uptake and determine the drug's final destination. The nano-size character of the nanocarrier also allows for easy penetration of cellular membranes, including the blood brain barrier of the central nervous system. There are several different types of nanocarriers being used as drug-delivery devices: polymeric micelles, liposomes, nanoerythrosomes and dendrimers. [2]

Erythrocytes:

Erythrocytes or red blood cells are produced in bone marrow & they constitute the largest population of blood cells in the body. The erythrocytes are mainly responsible for carrying haemoglobin and are for exchange of oxygen and carbon dioxide. Red blood cells offer a number of possibilities as drug carriers in controlled drug delivery systems and they can be used as carriers in two ways – i.e. Targeting particular organ or tissue, for continuous or prolonged release of drug. Erythrocytes are biocompatible, biodegradable, possess long circulation half lives. Surface modification with gluteraldehyde antibodies, carbohydrates like sialic acid and biotinylation of loaded erythrocytes to improve their target specificity and circulation half life has been explored. Upon reinjection, the drug loaded erythrocytes serves as a slow circulation depots, targets the drug to the RES and decrease the side effects. [4-9]

***Erythrosomes*:**

Erythrosomes are specially engineered vesicular systems that are chemically cross linked to human erythrocytes upon which a lipid bilayer is coated. These vesicles have been proposed as useful encapsulation systems for macromolecular drugs.

Nanoerythrosomes:

Nanoerythrosomes are small vesicular structures formed by the consecutive extrusion of erythrocyte ghost suspension through a polycarbonate filter membrane under nitrogen pressure and they have an average diameter of 100 nm.

The source of erythrocytes mainly include erythrocytes of mice, cattle, pigs, dogs, sheep, goats,
monkeys, chicken, rats and rabbit.

The release rate of a drug from erythrosomes depends on the following characteristics-

- Considerable degree of water solubility.
- Resistance against degradation within erythrocytes,
- There should not be any physical or chemical interaction with the erythrocyte membrane.
- Should have well defined pharmacokinetic and pharmacodynamic properties.

Isolation, Separation and washing of erythrocytes:

Blood samples can be collected from animals by puncturing and it is centrifuged and the sedimented erythrocytes are washed and refrigerated. Erythrocyte ghosts are formed when erythrocytes are subjected to a reversible process of osmotic lysis, a cell like structure is formed known as "erythrocyte ghost". They contain 5-10% of their original haemoglobin as a major cytosolic component. On resealing, the cells lose some of the properties of normal erythrocytes usually referred as resealed erythrocytes. Such erythrocytes which contain no or little haemoglobin are called ghosts. Pink ghosts are superior to white ghosts as slow release carrier. The erythrocyte ghosts obtained do not circulate in blood for prolonged periods of time as they are rapidly phagocytised by the liver and spleen. These ghost cells are useful for the delivery of substances to lysosomes of erythrophagocytic cells though they cannot survive in vivo. Recently they are used as vehicle for drug delivery and enzyme therapy. They reduce the need for taking of high doses because

instantaneously administration of large doses may lead to toxicity. Side effects of certain drugs like aspirin, steroid and anticancer drugs can be reduced by erythrocyte ghosts. The disadvantage of using ghosts is that they can be targeted to the tissue which contain phagocytic cells (liver and spleen) and not to all other tissues in the body. There are different methods for encapsulation of drug in erythrocytes such that they remain in circulation for prolonged periods of time and release the drug at a slow and steady state. [1, 10]

The erythrocyte ghosts are for encapsulation of:

- Metabolic enzymes to replace deficient activities (eg. Phenylketonuria)
- Enzymes (NZ) to remove nutrients (eg. Asparginase to remove Asparagine required for tumour growth)
- Polar drugs (eg. Methotrexate or antivirals)
- DNA for gene therapy
- Erythropoetin
- Anti-inflammatory drugs or steroids

Preparation of erythrocyte ghosts:

Hypotonic osmotic lysis method for ghost suspension preparation [1]:
The cells are lysed and washed with hypotonic saline solution (0.7%), centrifuged at 1000 rpm for 10 min at 4°C in a refrigerated centrifuge, the supernatant is discarded and the ghost cell suspension is diluted with 0.9% saline to obtain 50% haematocrit and stored at 4°C.

Preparation of Nanoerythrosomes and drug loading :
There are three methods for loading of drugs in nanoerythrocytes.

Extrusion:

The erythrocyte ghost suspension (50% haematocrit) is extruded through the 25 mm polycarbonate membrane filter pore obtained by 8-10 consecutive extrusions under nitrogen pressure. The ghosts obtained are stained with uranyl acetate and they are observed under microscope. The extrusions are performed in a thermostatically controlled extrusion device at 37°C and the final preparation is stored in a refrigerator at 4°C. The extrusions are performed at 37.8°C in a thermostatically controlled extrusion device.

Sonication:
Erythrocyte ghosts are converted into small vesicles using a dismembrator.

Electrical breakdown method:

It is used to convert ghosts into small vesicles under the influence of electric potential.
The nanoerythrosomes can also be considered as lipoproteosomes (vesicles constituted of both lipids and proteins) by analogy with liposomes. Because of their high surface to volume ratio (approximately 80 fold higher than the parent red blood cell) and they remain in suspension for prolonged periods of time are called buoyant vesicles. The nanoerythrosomes can carry various types of drugs or peptides that are transported through the blood stream to various organs. They can be coupled to antibodies or peptide ligands for selective delivery to cells. For example a biologically active molecule can be coupled with Nanoerythrosomes or Nanoerythrosomes themselves can encapsulate a biologically active molecule. Thus nanoerythrosomes are very versatile bioactive drug carriers or drug delivery system. These nanoerythrosomes are stable and maintain both the cytotoxic & antineoplastics activity of daunorubicin against mice leukaemia p338D cells. The Nanoerythrosomes compositions further leads to the formation of bioassays. In case of Gauchers disease glucocortisone was encapsulated in erythrocytes and heparin was encapsulated in erythrocytes to prevent thromboembolism.

Nanoerythrosome- drug conjugation:

Nanoerythrosomes were designed in such a way such that with low doses, they maintain the optimum concentration of the drug for prolonged periods of time & they provide sustained action. They are used for drug delivery in case of antineoplastics, phototherapeutic agents and peptides. Various

groups can be used to couple the bio active agent to the Nanoerythrosomes. Such groups mostly found are NH_2, COOH, SH & OH groups. The coupling agent should not interfere with bioactive agent. The bioactive agent may be photosensitive compounds, drugs, antibiotics, antineoplastics, antiinflammatoryagents, proteins, enzymes, nucleotide sequences, nucleic acids or parts thereof, oligonucleotides, antisense genes vectors, expression vectors, radioactive isotopes. Coupling of active drug molecule to ligand coupled nanoerythrocytes. In this we target the biologically relevant substance to the receptor which is recognized by the ligand coupled nanoerythrocytes.

Route of administration

In men and animals nanoerythrosomes are administered through various routes like intravenous, intraperitoneal, intramuscular and subcutaneous intraauricular.

Advantages of Nanoerythrosomes:

- They are natural products of the body.
- Bio degradable in nature
- Isolation is easy and large amount of drug can be loaded in small volume of cells.
- Non immunogenic in action and can be targeted to disease tissue /organ.
- Prolong the systemic activity of drug while residing for a longer time in the body.
- Protect the premature degradation, inactivation and excretion of proteins and enzymes.
- Act as a carrier for a number of drugs.
- Target the drugs within RES as well as non RES organs/sites
- Biocompatible
- No possibility of triggered immunological response
- Drug is chemically bonded with the protein of the erythrocyte membrane
- Less prone to aggregation and fusion
- Flexibility of membrane allows them to escape RES for longer periods.

The currently available albumin, albumin microspheres, acrylic microspheres, liposomes, magnetic polymers, lectins and mAbs are limited to a group of antineoplastics as these are recognized as foreign materials by human body and cause immunological reactions and also they get accumulated in liver and spleen leading to toxic effects.

Limitation of Nanoerythrosomes:

Nanoerythrosomes cause immune reactions. They are destroyed by gastric juices and hence could not be used in unmodified form. By nature nanoerythrosomes comprises of both lipids and proteins. If one mammal is receiving nanoerythrosomes from another incompatible mammal it leads to undesirable protein-protein interaction which limits the potential for diagnostic and commercial applications of nanoerythrosomes. So there is a need to provide Nanoerythrosomes composition which is more stable at low pH conditions such as that of stomach.

CHARACTERIZATION [11, 12, 13]**:**

Drug content:

Packed loaded erythrosome are first deproteinized with acetonitrile and subjected to centrifugation at 2500 rpm for 10 min. The clear supernatant is analyzed for the drug content.

In vitro drug and hemoglobin release:

Normal and loaded Erythrosome are incubated at 37± 2°C in phosphate buffer saline (pH 7.4) at 50% haematocrit in a metabolic rotating wheel incubator bath. Periodically, the samples are withdrawn with the help of a hypodermic syringe fitted with a 0.8 µ spectropore membrane filter. Percent haemoglobin can similarly be calculated at various time intervals at 540 nm spectrophotometrically. Laser light scattering may also be used to evaluate haemoglobin content of individual resealed erythrocytes. Mean corpuscular [hemoglobin (g/100ml) x 10 hemoglobin = erythrocyte count (per mm^3).

Osmotic fragility:

It is reliable parameter for in vitro evaluation of carrier erythrocytes with respect to shelf life, in vivo survival & effect of encapsulated substances. When RBCs are exposed to solution of varying tonicities their shape changes due to osmotic balance. To evaluate the effects of varying tonicities, drug loaded erythrocytes are incubated with saline solutions of different tonicities' at 37±2°C for 10 min. The suspension after centrifugation for 15 min, 2000 rpm is assayed for drug or hemoglobin release.

Osmotic shock:

Osmotic shock describes a sudden exposure of drug loaded erythrocytes to an environment, which is far from isotonic to evaluate the ability of resealed erythrocytes to withstand the stress and maintain their integrity as well as appearance. Incubating the resealed erythrocytes with distilled water for 15 min followed by centrifugation at 3000 rpm for 15 min, may cause the release of haemoglobin to varying degrees, which could be estimated spectrophotometrically.

Turbulence shock:

This parameter indicates the effects of shear force and pressure by which resealed erythrocyte formulations are injected, on the integrity of the loaded cell. Loaded erythrocytes are passed through a 23-guage hypodermic needle at a flow rate of 10 ml/min. After every pass, aliquot of the suspension is withdrawn and centrifuged at 300 g for 15 min and hemoglobin content, leached out is estimated spectrophotometrically.

Morphology and percent cellular recovery:

Phase contrast optical microscopy, transmission electron microscopy and scanning electron microscopy are the microscopic methods used to evaluate the shape, size and surface features of loaded erythrocytes. Percent cell recovery can be determined by assessing the number of intact erythrocytes remaining per cubic mm with the help of hemocytometer.

Percentage cell recovery:

May be determined by counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug.

Morphology:

Phase contrast or electron microscope may be used for normal and erythrocytes.

Determination of entrapped magnetite:

Atomic absorption spectroscopic method is reported for determination of the concentration of a particular metal element in a sample. The HCl is added to a fixed amount of magnetite bearing erythrocytes and content are heated at 60°C for 2 hours. Then 20%w/v trichloroacetic acid is added and supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy.

Erythrocyte sedimentation rate (ESR):

It is an estimate of the suspension stability of RBC in plasma and is related to the number and size of the red cells and to relative concentration of plasma protein especially fibrinogen and α, β globulins. This test is performed by determining the rate of sedimentation of blood cells in a standard tube. Normal blood ESR is 0 to 15 mm/hr. High rate is indication of active but obscure disease processes.

Miscellaneous:

Nanoerythrosomes can also be characterized by cell sizes, lipid composition, membrane fluidity, rheological properties and density gradient separation.

Deformability:

It evaluates the ease of passage of erythrocytes through narrow capillaries and the RES there by evaluates the life span of the cells. The deformability is measured by passage time of

definite volume of cells through capillary of 4 mm diameter or polycarbonate filter with average pore size of 45 mm. This is done to determine the rheological behaviour of the cells and is dependent on the viscoelasticity of the cell membrane, viscosity of the cell contents and the cellular surface-to-volume ratio.

Percent drug conjugation:

Drug loaded Nanoerythrosomes (0.2 ml) are deproteinized using acetonitrile (0.2 ml) after centrifugation at 20,000 g for 15 min. The clear supernatant is withdrawn and analyzed by HPLC using C-18 column and the mobile phase consisted of phosphate buffer (0.05 M, pH 5): acetonitrile: concentrated perchloric acid (750:300:2.5, v/v/v) at a flow rate of 1 ml/min. The percent drug loading is determined.

Viscosity & sedimentation volume:

Viscosity is determined by using a rotator Brookfield viscometer.
Sedimentation volume of formulation was obtained by measuring the height of sediment in a graduated measuring cylinder.
$F= v_u/ v_0$
F=sedimentation volume
V_u=ultimate volume of sediment
V_0=original volume of formulation.
Centrifugal stress:
For this, Nanoerythrosomes drug conjugates are centrifuged at variable rpm in a refrigerated centrifuge at 4°C for 15 min. Drug leakage in supernatant solution was estimated.

Hematological tests:
Routine clinical hematological tests also can be carried out for drug loaded cells including mean corpuscular volume, mean corpuscular haemoglobin content, ESR, etc.

Stability:

Stability studies of the prepared nanoerythrosomes are carried out by storing all the formulations at 5±3°C, 30±2°C/ 65%±5% RH and at room temperature for 2 weeks and 1 month period. Parameters like percentage drug content and in vitro release studies of the formulation are carried out. [14, 15]

Applications:

- Slow drug release
- For sustained delivery of antineoplastics, antiparasitics, veterinary antiamoebics, vitamins, steroids, antibiotics and cardiovascular drugs.
- Drug targeting
- Surface-modified erythrocytes are used to target organs of mononuclear phagocytic system/ reticulo endothelial system
- Targeting RES organs :
- Resealed erythrocytes, by modifying their surface characteristics with antibodies, gluteraldehyde, sialic acid, sulphydryl and surface chemical cross-linking e.g. delivery of 125 I-labelled carbonicanhydrase loaded in erythrocytes cross-linked with sulfosuccinamidyl propionate.
- Targeting the liver- enzyme deficiency/replacement therapy
- Treatment of hepatic tumours.
- Because of selective accumulation of resealed erythrocytes within RES organs like liver and spleen make them useful tool for delivery of antimalarial, antileishmanial, antiamoebics drugs.
- Removal of RES iron overload
- Desferrioxamine-loaded erythrocytes have been used to treat excess iron accumulated because of multiple transfusions to thalassemic patients.[4, 16-20]

Targeting organs other than those of RES:

The various approaches include entrapment of paramagnetic particles, photosensitive material along with the drug and antibody attachment to erythrocyte membrane to get specificity of

RELEASE OF DRUGS FROM THE ERYTHROCYTES:

There are mainly three ways for a drug to efflux out from the erythrocyte carriers [21]

- Phagocytosis;
- Diffusion through the semipermeable membrane of the cells;

- Using a specific transport system. RBCs are normally removed from circulation by the process of phagocytosis.

The rate of diffusion depends upon the rate at which a particular molecule penetrates through a lipid bilayer. It is greatest for a molecule with high lipid solubility. Considerable control over the release rate is possible by introducing or eliminating polar or charged substituent. Many substances enter cells by a specific membrane protein system because the carries are proteins with many properties analogous to that of enzymes, including specificity. Moderate modifications of the compound can often dramatically alter the rate of exit. Nucleotides and nucleosides provide a simple example of this. Nucleosides are transported rapidly the facilitated diffusion but nucleotides are not transported across the membrane and hence can be entrapped in the erythrocytes. However, erythrocyte carriers have the potential of releasing encapsulated substance following zero order kinetics. By incorporating polymers the release pattern can be modified. Eichler et.al, demonstrated that carrier erythrocytes are not suitable for non diffusible drugs and for diffusible drugs; efflux rate must be controlled before constant release rates over prolonged periods can be achieved.

Future Perspectives:

Nowadays Nanoerythrosomes composition has significantly reduced immunogenic potential, more particularly Nanoerythrosomes -PEG composition. In order to reduce the immunogenicity of proteins the polyethylene glycol coat is preferred. Research is going on whether these Nanoerythrosomes could withstand PEG coupling without collapsing or retain biological activity. However there is a need to develop Nanoerythrosomes with less immunogenic potential yet retaining its biological activity as drug delivery system. In future Nanoerythrosomes technology will remain an active arena for research. Nanoerythrosomes coupled with antibodies can increase test sensitivities. In near future, Nanoerythrosomes based delivery system with an ability to provide controlled and site specific drug delivery may be developed for disease management

CONCLUSION

During the past decade, numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. The use of resealed erythrocytes looks promising for a safe and sure delivery of various drugs for passive and active targeting. However, the concept needs further optimization to become a routine drug delivery system. The same concept also can be extended to the delivery of biopharmaceuticals and much remains to be explored regarding the potential of resealed erythrocytes. Resealed erythrocytes technology will remain an active arena for the further research. Most of the studies in this area are in the in vitro phase and the ongoing projects worldwide remain to step into preclinical and, then, clinical studies to prove the capabilities of this promising delivery system.

REFERENCES

1. *Jain NK. Controlled and novel drug delivery. CBS publishers and distributors; 2010; 1: 256.*
2. *Bajaj A., Desai M., Challenges and strategies in novel drug delivery technologies: Pharma Times 2006; 38: 12-16.*
3. *Torchilin VP. Cells and cell ghosts as drug carriers. In: Nanoparticulates as drug carriers, illustrated (eds.), London:Imperial College Press; 2006; 330.*
4. *Arstensen ST. Drug stability, principles and practices. 2nd Revised and Expanded edition, New York: Marcel Dekker; 1998; 538-559*
5. *Vyas SP., Khar RK., Resealed Erythrocytes in Targeted and Controlled Drug Delivery: Novel Carrier Systems, India: CBS Publishers and Distributors; 2002; 87–416.*
6. *Jain S., Jain K., "Resealed Erythrocytes as drug Carriers in Controlled and Novel Drug Delivery, Indian Journal of Pharmaceutical Science 1997; 256–291.*
7. *Patel R., Patel M., Patel N., An Overview of Resealed Erythrocyte Drug Delivery. Journal of Pharmacy Research 2009; 2(6): 1008-1012.*
8. *Mangal P., Kaur A., Electroporation of Red Blood Cell Membrane and its Use as a Drug Carrier System. Indian Journal of Biochemistry Biophysiology 1991; 28: 219-221.*
9. *Gothoskar A., Resealed erythrocytes: A review. Pharmaceutical Technology, 2004; 1-6.*
10. *Gopal V., Kumar A., Usha A., Karthik A., Udupa N., Effective drug targeting by Erythrocytes as Carrier Systems. Current Trends in Biotechnology and Pharmacy 2008; 1: 18-33.*
11. *Hamidi M., Tajerzadeh H., Evaluation of hypotonic preswelling method for encapulation of enalaprilat*

in intact human erythrocytes. Drug Development and Industrial Pharmacy 2000; 26(12): 1247-1257.

12. *Richieri G., Mel C., Temperature effects on osmotic fragility, and the erythrocyte membrane. Biochemicact Biophysica Acta (BBA) Biomembranes 1985; 813: 41-50.*
13. *Tajerzadeh H., Hamidi M., Dehpour A., Rouini M., Mehr S., In vitro characterization of human intact erythrocytes loaded by Enalaprilat. Drug Delivery 2001; 8: 223-230.*
14. *Pinilla M., Jordan J., Danz J., Luque L., In vitro stability properties of cross-linked red blood cells. Advanced Bioscience 1994; 92: 7-16.*
15. *Tesconi S., Sepassi K., Freeze drying above room temperature. Journal of Pharmaceutical Science 1999; 88(5): 501-505*
16. *Zimmermann U., Cellular Drug-Carrier Systems and Their Possible Targeting In Targeted Drugs, EP Goldberg, Ed. New York: John Wiley & Sons; 1983; 153–200.*
17. *Lewis D., Alpar HO., Therapeutic Possibilities of Drugs Encapsulated in Erythrocytes. International Journal of Pharmaceutics 1984; 22: 137–146.*
18. *Venkata B., Varun D., Gopal P., Babu Rao C., Nanoerythrosomes - a novel drug delivery systems. International Journal of Advances in Pharmaceutical Sciences 2011; 2 :2-3.*
19. *Jain S., Jain K., Engineered erythrocytes as drug delivery system. Indian Journal of Pharmaceutical Sciences, 1997; 59(6): 275- 281.*
20. *Khar R., Diwan M., Targeted delivery of drugs. In: Jain NK (editor). Advances in controlled and novel drug delivery. New delhi: CBS Publishers and Distributors; 2001.p. 420-456.*
21. *Eichler HC., Gasic S., Daum B., Bacher S., Sreger G., In Vitro Drug Release from Human Carrier Erythrocytes. Advanced Bioscience 1987; 67: 11-15.*