

NANOCARRIERS IN ORAL DELIVERY OF MACROMOLECULES: UPDATED INSIGHT

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ABSTRACT

Having handy reference as a source of outstanding knowledge for scientist engaged in developing nanocarrier based oral synergising their effect by amalgamating nanotechnology and presenting them as oral delivery system. Diverse methods and technology devised to achieve goal. All of them abreast increasing efficacy, specificity, tolerability and therapeutic index, and minimising toxicities. Each and every system has its own limitation and superiority over others. Amongst them nanocarrier based systems receiving attentions. These systems provide improved performance, stability, and patient compliance, and have potential for improved marketability. Their design and development progressed to make best formula through method of optimisation. This considered being a state of art involves multidisciplinary activity and creates difficulties as several causes needed to be achievable, associated with many features. Available literatures are unable to provide insight on issues relating their development while some are unhandy. In this regard, information collected from databases and presented as a handy note. This insight features delivery system of macromolecules (NBODSM). Macromolecule based biopharmaceuticals (proteins, peptides, monoclonal antibodies, and vaccines) explored for delivering associated proclaimed benefits, in diverse domain. Interest evoke for on issues relating development of NBODSM and has applicability in product evolution. Presented information will be a helping hand and will offer an outstanding knowledge to developers, while designing them with excellent feature.

Key Words: Delivery, macromolecules, nanocarriers, nanotechnology, oral, strategy.

INTRODUCTION

Nanotechnology possesses enormous potential to produce products, with novel properties, in various domains, whose importance is expected to get enhanced, in near future. Novel materials and devices resulting from said technology have broad introduction potentiality in all domain including biotechnology, food, and pharmaceutical sector. In pharmaceutical field, introduction of nanotechnology is achieving in devising nanotherapeutics and nanodevices. Semi-biological nanodevices are multifunctional offering versatile therapeutic services and demonstrating unitary biochemical activities. While amalgamation of imaging and therapeutic function in a single nanodevice can provide prognostic information concurrent with therapeutic intervention¹.

Presently, pharmaceuticals containing nanotherapeutics and nanodevices are under extensive study for improving performance¹. Ongoing attempt is to present them as disinfectants; biosensor or bio-tracer based diagnostic agent for detecting toxins, pathogens, volatile compounds, and organic components of body fluids; and for monitoring diseases¹. Besides these they will revolutionise offering of device for site-specific controlled delivery of drug or drug targeting, and presenting of differential device-activity in dissimilar physiological environments, under direction of an external operator / physician²⁻⁶.

Several nano sized pharmaceuticals were designed for oral delivery of nanotherapeutic macromolecules but polymeric nanocarriers gaining popularity⁷. They allow paracellular and transcellular transcytosis of macromolecules and site specific delivery or targeting, and provide desirable biopharmaceutical and pharmacokinetic profile⁸⁻¹¹. Their

consensus on increasing efficacy, specificity, tolerability and therapeutic index and prognostic on minimising toxicities are available^{3, 4, 12-17}.

Polymeric nanocarriers with diverse functionality were designed using several materials and following various process or technique. They be either a vesicular system (i.e., nanovesicles, encapsulates drug in a cavity of polymeric membrane) or a matrix system (i.e., nanoparticles (NPs), in which drug is physically and uniformly dispersed)^{7, 12, 18, 19}. All of these systems have limitations and superiority over others. Choice of system afoot on desired physicochemical properties and targeted therapeutic objective^{3, 6, 7}. Biodegradable polymers of natural or synthetic origin used to wider extent while non-biodegradable polymers are in rare use^{1, 20}. Table 1 depicts glimpse on IUPAC name and structure of polymers used for developing nanocarriers, while Table 2 presents glimpse on specialism of nanocarriers.

Adsorbing or grafting of molecules on surface of nanocarriers modifies its surface property that in turn modifies interaction with intestinal mucosa. Ligand molecule like antibodies, glycoproteins or peptides confers targeting^{9, 22-25} while that of hydrophilic one, polyethylene glycol (PEG), improves transcytosis²¹. Adsorbing or coating of them with mucoadhesives (viz. chitosan) imparts mucoadhesive property^{1, 5}.

Present work insights design and manufacturing of NBODSM and underline balancing their physicochemical properties. Presented information has applicability in product evolution and will be a helping hand for developers, while designing them with excellent feature.

Table 1: Depicts IUPAC name and structure of polymers used for developing nanocarriers

POLYMER	IUPAC NAME	STRUCTURE
Polyethylenimine	Poly(iminoethylene)	
Poly(ε-caprolactone)	(1,7)-polyoxepan-2-one	
Poly(glycolide)	Poly[oxy(1-oxo-1,2-ethanediyl)]	
Poly(lactide)	Poly[3, 6-dimethyl-1, 4-dione]	
Poly(lactide-co-glycolide)	----	
Poly(cyanoacrylates)	----	
Pluronic block copolymers	----	
Lecithin	1,2-diacyl-sn-glycero-3-phosphatidylcholine	
Tween	Polyoxyethylenesorbitanmonooleate	
Span	Sorbitan mono-oleate	
Cetylpalmitate	Hexadecylhexadecanoate	
Sodium glycocholate	Sodium 2-[4-(3,7,12-trihydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoylamino]acetate	

NANOPARTICLES

Physicochemical properties of NPs modulated with change in polymers and processing conditions. Modulation in hydrophobicity or surface charges, extent of drug loading, drug release profile, and biological behaviour make them versatility thereby amenable in orally delivering wide variety of drug with or without target specificity^{5, 16, 26}.

Diverse methods followed for preparing them, depending on nature of polymer and drug. Use of process involving electrostatic interaction wise option, employ polyelectrolyte complexes and eliminates application of detrimental or aggressive in-process conditions^{27, 28}. While method requiring heat, organic solvent, sonication or vigorous agitation is unsuitable for biopharmaceuticals^{1, 20}.

Table 2: Nanocarriers exploited for developing oral delivery system of macromolecules

Type of Nanocarriers	Composition	Size Range	Specialism
Nanoparticles	Natural or synthetic, biodegradable or non-biodegradable polymers	1-100 nm	High kinetic stability, static structure
Liposomes	Natural or synthetic phospholipids	20 nm-10 μ m	Have dynamic structure and are biocompatible, safe, and well-tolerated.
Solid-lipid nanoparticles	High melting point natural or synthetic fats	50-1000 nm	
Micelles	Ionic or non-ionic surfactants	2-20 nm	
Lipospheres	High melting lipid, phospholipids	0.2-100 nm	
Submicron lipid emulsions	Lipids, hydrophilic liquid, surfactants	1-100 nm	

Natural, biocompatible polymers are alginate, agar, chitosan and its derivatives, dextran, gelatine, and so on. Synthetic, biocompatible and/or biodegradable are poly(cyanoacrylates) (PCA), poly(glycolide) (PGA), poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA), polyethylenimine or poly(ϵ -caprolactone) (PCL)^{4-7, 20, 29, 30}. Chitosan and its derivatives used popularly linked with biocompatible, non-toxic, mucoadhesive, targeting, and tight junctions (TJs) opening property²⁹⁻³³. Its limited solubility at pH > 6.5 being overcome by introducing alkyl groups to amine groups³⁴. Polyelectrolyte complexes of 200-400 nm size formed with these²⁷. PLA, PLGA, PCL biodegrade through hydrolysis while PCA with esterase. PCL degrade slowly than PLGA, thus suitable for extending delivery^{20, 35}. Chitosan used to greater extent while dextran, gelatine and alginate used to lesser extent¹.

NANOVESICLES

Surfactants and amphiphilic polymers / copolymers form colloidal dispersions of molecular aggregates or vesicles^{13, 19, 36}. Lipid-based vesicles are solid-lipid nanocarriers (SLN), micelles, lipid microspheres (lipospheres) and liposomes (LIP) while surfactant based is niosomes^{13, 14, 19, 37, 38}. In contrast to NPs, which display a static and stable structure, they form a dynamic structure. These can carry drug in core or on corona and can solubilise poorly soluble drugs and partly protect drug from aqueous environment, thus preferred for poorly soluble drugs^{15, 18, 19}. SLN and niosomes are more stable and advantageous comparing LIP, can incorporate hydrophilic and/or hydrophobic drug. They protect incorporated drug from degradation; are biocompatible, safe, and well-tolerated; offer possibility for controlled release and drug targeting; and so on^{14, 15, 18, 38}. SLN exhibit beneficial solid state behaviour (crystallinity, polymorphism and thermal behaviour) as

consequences of non-spherical particle shape, usually. Crystalline natured lipid core of which contribute additional beneficent features, including altering pharmacokinetics profiles, comparing others^{12, 37}.

Copolymer of PLA-PEG, PCL-PEG, or low molecular weight polyester-PEG is cheaper and safe but their use limited by involvement of sophisticated technology and machine, and organic solvents. Lecithin, tween 80, poloxamer 188, span 85, cetyl palmitate, and sodium glycocholate are safe³⁸. Amphiphilic copolymers provide enhanced kinetic and thermodynamic stability comparing surfactants^{14, 15, 18}. Copolymer of pluronic-polyacrylic acid and poloxamer results pH-sensitive micelles^{39, 40}. Derivatives of PEGylated α -tocopherol and tocopherol polyethylene glycol succinate (TPGS), is biocompatible and suitable for delivering both soluble and poorly water soluble drugs^{14, 15, 19, 38}. Poloxamer solubilise drugs and enhance drug transport across the intestinal barriers^{11, 41}.

SLN formulated by solvent evaporation method or melt dispersion method but latter is advantageous as eliminates use of organic solvents⁴²⁻⁴⁴. LIP, lipospheres and niosomes are formed either by simple direct dissolution in water or by dissolving drug and polymer in organic solvents before solvent evaporation or dialysis^{19, 36, 45, 46}.

The polymers or copolymers use for micelles should (i) spontaneously self-assemble in water, (ii) enhance drug solubility by several fold (iii) provide high loading efficiency (iv) remain stable in the gastrointestinal (GI) tract, (v) be biocompatible and non toxic and (vi) easy to synthesise in commercial scale (vii) cheap and easily assessable.

STABILITY OF NANOCARRIERS IN GI TRACT

After oral administration, nanocarriers degrade with variation of pH levels and presence of enzymes or bile salts. Their composition strongly influences stability in GI tract. Nanocarriers prepared with insoluble polymers neither immediately degrades nor rapidly releases drug. While that with water-soluble polymers, forming polyelectrolyte one, are more likely to destabilise by pH or ionic strength. Kinetic stability of NPs is better than nanovesicles^{7, 13}.

BIODISTRIBUTION OF NANOCARRIERS

ATP-binding cassette (ABC) transporters (like P-glycoprotein (P-gp) and multi-drug resistance-associated proteins expressed by intestinal epithelial membrane) and various solute transporters facilitate absorption of nanocarriers and or drugs. The ABC transporters are ATP dependent thus limits absorption in a dose-dependent, inhibitable and saturable manner and can pump against steep of concentration^{41, 47-50}.

Upon crossing mucus the nanocarriers are traversing intestinal epithelium via paracellular pathway and/or transcytosis. Receptor-mediated transcytosis (internalization through endocytic pathways) is specific to intracellular locations and processes and mediated by enterocytes or M cells⁵⁰. Endocytosis mechanism involves phagocytosis and pinocytosis. Phagocytosis is restricted to M cells and phagocytic immune cells^{47, 51}. Pinocytosis occurs by macropinocytosis (a transient process) and micropinocytosis (a constitutive pathway)^{5, 41}.

Micropinocytosis involves clathrin-mediated endocytosis (CIME), caveolae-mediated endocytosis (CaME) and clathrin- and caveolae-independent endocytosis. CIME

involves either specific receptor–ligand interaction or non-specific endocytosis. Macropinocytosis and CIME involves fusion with endolysosomes whereas CaME involve direct exocytosis by escaping endolysosomes. CaME involves formation of flask-shaped invaginations, a static structures with a size of 50 to 100 nm, of the plasma membrane in cholesterol and sphingo lipid-rich microdomains^{47, 50, 51}.

After absorption, drug and/or drug loaded nanocarriers can be included in cytoplasmic vesicles or diffuse in cytoplasm and be discharged in serosal spaces for gaining access to mesenteric lymph or blood^{5, 26, 41, 48}. The size, composition, surface characteristics and architecture of them along with physicochemical properties of polymer (viz. molecular weight, conformation, hydrophobicity, and so on) will monitor their absorption^{9, 10, 47}. Water-insoluble polymers forms stable nanocarriers and are more likely absorbed as particles⁵. Polymers forming less stable particles, polyelectrolyte complexes, vesicles or micelles will partly get dissociate and will not completely get absorbed as particle^{10, 14, 15, 38}. Nanocarriers taken up by absorptive enterocytes mainly delivered in blood. While those taken up by M cells will transcytosis close to immune cells and are likely be delivered to the gut-associated lymphoid tissue and lymphoid cells^{5, 9, 10}. Drugs encapsulated in NPs are not likely to be substrate of efflux pump while hydrophobic drugs forming core of micelles are more likely to be transported by efflux pumps^{5, 10, 11}. Delivery mechanism of macromolecule through oral route is presented with Figure-1.

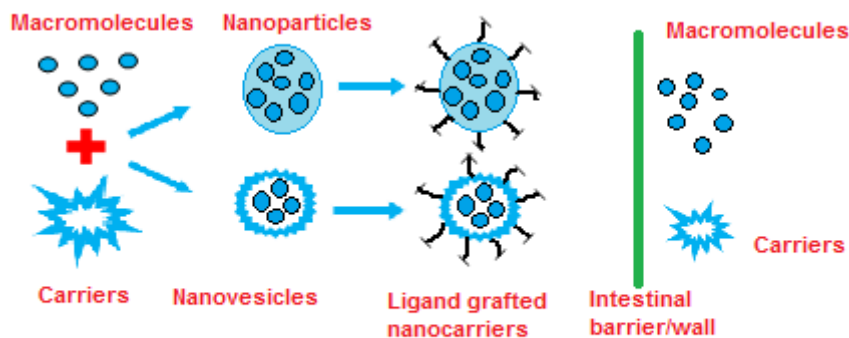


Figure-1: Pictorial representation of delivery mechanism of macromolecules, via oral route

BIOAVAILABILITY TECHNIQUES

In paracellular route junction-proteins present at tight or adherens junction restrains passage of macromolecules or aggregates with size more than one nm. Consensus on nanocarriers is generally they do not transverse intestinal barrier by paracellular route^{22, 26, 48}. While in transcellular route major limiting factor is intestinal mucosa which requires redresses for improving bioavailability of orally delivered macromolecules^{5, 8, 16, 22, 41, 47, 50, 52}.

IMPROVEMENT

Improvement in delivery of drug, by paracellular way, can be achievable by grafting modulators of junctional proteins on the surface of nanocarriers^{5, 22}. These modulators act directly or indirectly on TJ components, proteins or surface receptor resulting reversible opening of membranous barriers^{32, 33}. Some modulators disrupt the TJs via activation of protein kinase C and the adherens junctions by chelating calcium^{22, 47, 50}.

Transcellular translocation of nanocarriers seems influencing by several physicochemical parameters including surface hydrophobicity, polymer nature and

particle size. These factors influence their fate within enterocytes or M cells while efflux pumps may strongly reduce extent of net drug uptake^{8, 16, 41, 47, 50}.

Bioavailability or performance of nanocarriers ought to be improving through protecting drug from detrimental GI tract environment, prolonging their GI-residence time by mucoadhesion, their endocytosis, and permeabilising effect of polymer^{1, 5, 8, 39, 47}.

ENDOCYTOSIS

Internalization of nanocarriers through endocytosis pathway depends on physicochemical characteristics and size of the particle and the cell type. Their transport can be enhanced by specifically targeting receptors (mediating endocytosis) through grafting or coating them with ligand, having affinity for receptors^{8, 22, 41, 47, 49, 50}. Several non-exhaustive receptors are there whose targeting can be done to increase their transport mediated by intestinal cells, goblet cells or M cells^{9, 24, 47, 49, 51, 53, 54}. Pattern recognition receptors (PRRs) such as toll-like receptor-4, platelet-activating factor receptor and $\alpha 5\beta 1$ integrin present on the surface of M cells considered as important in antigen transcytosis⁵⁵. A few specific ligands of human M cells have been identified^{3, 9, 23}. Grafting of these M cell homing peptides (CKSTHPLSC (CKS9), CSKSSDYQC (CSC)) results in targeting of M cells and enhances transport^{23, 56, 57}. Nanocarriers mimicking structure of pathogen or having affinity for M cells is suitable for oral immunisation⁵⁵. Integrins can be targeted with Arg-Gly-Asp (RGD) and aspartic-acid-based ligands. Among these RGD peptides cyclic peptides c(RGDfK) and c(RGDyK), RGD4C, and RGD10 showed high binding affinity and selectivity for integrin⁵⁴.

TARGETING

Many emerging nanocarriers are essentially delivery devices strongly emphasising on control of therapeutic action in time and space¹. Targeted delivery devices depending on biologic affinity interactions (using vascular address system) and physical/chemical properties (enhanced permeability and retention effect), and pro-drug strategies (devices that are active only at preselected sites of disease) have emerged recent years^{3, 6, 9, 16}. Other related modalities are controlling the assembly or activity of devices at particular sites or at particular times in response to a signal provided by an external operator. Such signals are physical (a magnetic field) or biochemical (an enzyme transforming therapeutic to an active form) in nature^{32, 49}. Up-regulating expression of some receptors using an appropriate stimulant is another strategy of targeting. Stimulation of these receptors with ligands, lipopolysaccharide or cytokines increases particle uptake by human follicle-associated epithelium cells^{5, 8, 9, 56, 57}.

Some lectins can result targeting of adhesion molecules present on enterocytes and M cell membranes. Grafting of ligand is particularly attractive for oral vaccine delivery^{9, 23, 57}. Adhesive factors (flagellin and invasins) derived from microorganisms, vitamins, and carbohydrates are used as ligands^{1, 5, 58, 59, 60}.

Conjugating lectins to nanocarriers increases transport across the intestinal mucosa while grafting them with

vitamin B₁₂ allows targeting to intrinsic factor specific receptor^{10, 50, 60, 61}. Targeting of NPs and internalisation of micelles takes place via CIME. Endocytic translocation of small particles (with size 50-100 nm) mediated by enterocytes whilst larger particles mediated by M cells^{10, 41, 47, 50, 51}. Chitosan and PLGA nanocarriers enhance transport via CIME³¹. Nanocarriers coated or grafted with ligands like folic acid, albumin and cholesterol, gets internalized by CaME⁵¹. Targeting of goblet cell⁵³, peyer's patches (PPs)^{45, 56}, dendritic cells (DCs) of PPs⁶² done by grafting ligands, having homing property or affinity for them. Pluronic 85 unimers enter epithelial cells through caveolae-dependent and caveolae-independent pathways⁶³. Thiamine-coated NPs captured by PPs^{58, 56}.

MUCOADHESION

Prolongation in GI-residence time of nanocarriers will improve efficiency in transversing intestinal epithelium, thereby improving bioavailability^{26, 39, 58}. This ought to get improved by decreasing their size below 200 nm, which in turn will diffuse through mucus and circumvent elimination by mucilliary clearance. Mucoadhesion translates to cumulative protein release and absorption^{39, 59, 64}.

DISCUSSION AND STRATEGY FOR DEVELOPMENT

Nowadays NBODSM considered as promising alternative to parenteral one. pH sensitive, untargeted, or targeted (with or without grafting of ligand) NBODSM had been exploited in most cases^{1, 5, 16, 30, 65}. Future of their marketing remains uncertain due to following facts.

- (i) High cost of synthesising polymer, manufacturing nanocarriers and scaling-up,
- (ii) Improvement of bioavailability to stable and efficient therapeutic level,
- (iii) High inter- and intra-individual variations in pharmacokinetics of macromolecules, and
- (iv) Optimizing polymeric composition of them for specific macromolecules.

Selection of nanocarrier remains controversial for delivering macromolecules. Amenability of NPs for modulation in physicochemical and biological behaviour makes them versatile, thus suitable for delivering wide variety of drug with or without target specificity⁵. Micelles considered viable approach for delivering poorly soluble macromolecules comparing cyclodextrins based solid dispersions or lipid-based systems^{13, 19, 36, 38}. pH-sensitive micelles minimises initial burst release of macromolecules in stomach but releases them at pH 5, in a molecularly dispersed form⁴⁰. LIP and lipospheres have lower toxicity and higher tolerability, and suitable for poorly soluble macromolecules^{45, 46}. SLN, comprises physiological and well-tolerated lipids, offer controlled release and targeting, provide protection against degradation, and is advantageous over other colloidal carriers^{12, 42, 44, 66, 67}.

Size of nanocarriers should be essentially within 10-200 nm, to promote diffusion in mucus and uptake by intestinal cells, and to decrease uptake by reticulo-endothelial system (RES)¹⁶. Surface modification of them with PEG will prevent their uptake by RES and prolong circulation

half-life^{21, 25}. Imparting mucoadhesion property will prolong their residence time without impeding diffusion in mucus^{39, 58, 59}. Their surface charge has to be positive favouring interaction with mucus and cell membrane but be neutral to decrease RES clearance^{26, 39, 64}.

The mechanisms of drug absorption from micelles differ from NPs uptake. Nanocarriers should be stable in GI tract while releasing drug at desired site with predetermined rate. They may have targeting potentiality for M cells or immunocompetent cells of GI tract^{9, 38, 47, 50}.

Chitosan have ability to cause reversible opening of TJs and have mucoadhesive property^{29, 32, 33, 68}. Improvement in paracellular permeability did not increase permeability of endotoxins and lipopolysaccharide thus is safe⁶⁹. Polyelectrolyte complexation of drug with chitosan prevents enzymatic degradation in GI environment²⁸. Hydrogel system of alginate, dextran sulphate and poloxamer 188 consisting multilayered NPs of chitosan improves entrapment and gastric retention. Poloxamers improves solubility and transport of drug^{8, 41, 48}. Decreasing concentration of Ca²⁺ and increasing concentration of poloxamer and albumin results smaller NPs. Reduction in albumin content improves entrapment and gastric retention^{10, 11}.

Loss in mucoadhesive and TJ opening properties of chitosan at pH 7.4 is improved by derivatisation^{29, 33, 34}. N-trimethylated chitosan (NTC), thiolated chitosan (TC)⁶⁸, N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan (NTAC)⁷⁰, are important derivatives²⁹. These categorised as pH-sensitive polymers used for improving bioavailability alone or in combination with other polymer^{4, 71}. PEGylated chitosan have enhanced permeability and lower toxicity²⁵. Gama-polyglutamic acid (γ PGA)-NTC based NPs allow absorption of macromolecules throughout the intestinal tract^{71, 72}.

NTC based NPs involve active transport and electrostatic interaction, which upon conjugation with ligand CKS9, CSC enhances absorption, further, via clathrin- and caveolae-mediated endocytosis^{23, 53, 56, 57}. From pH-responsive NPs macromolecules gets absorbed into systemic circulation while carrier retained mainly in oral tract^{65, 73}. Some pH-responsive nanocarriers are biodegradable⁴⁰. TC improves mucoadhesion and absorption eliminating toxicity and biocompatibility issue⁶⁸. NTAC exhibit higher cationic charge density, increased mucoadhesion, TJ opening ability and were non-cytotoxic⁷⁰.

NPs of chitosan- γ PGA increase their absorption in intestine and sustain release. Their conjugation with CSC results targeting of goblet cells, but with diethylenetriaminepentaacetic acid disrupts TJs⁷¹. Blending chitosan and γ PGA with tripolyphosphate and MgSO₄ result multi-ion-crosslinked NPs, possessing stability over broader pH ranges and improved transporting capability, and more compact one^{8, 29}.

Acrylic-based polymers and copolymers like poly(isobutylcyanoacrylate) (PIBCA), poly(acrylic acid) (PAA), poly(alkylcyanoacrylate), PEG grafted methacrylic acid (MAA) or acrylic acid used to get pH sensitive NPs. Copolymerising MAA with PEG dimethacrylate (PG-

DMAA) having molecular weights of 400-4000 g/mol results hydrophilic and pH-sensitive poly(PG-DMAA-MAA) NPs^{5, 7, 25, 30}. Modification of dextran based NPs through conjugation of its surface with vitamin B₁₂ results targeting with better transport and higher bioavailability⁶⁰. Thiolated poly(acrylic acid) or its blend with chitosan improves mucoadhesive properties of NPs^{68, 74}.

PLGA NPs gets distributed in liver, spleen, lungs, brain and kidneys, while majority were located in liver but these upon PEG-grafting rapidly taken up by peritoneal macrophages³¹. PLGA or its blend with β -cyclodextrin (PLGA- β CD), hydroxypropyl methylcellulose phthalate (PLGA-HP55) and Eudragit® RS (PLGA-ERS) are also exploited⁷⁵. PLGA- β CD, PLGA-HP55 and star-branched form of PLGA exhibit burst release effect. While PLGA-ERS based NPs remains attached to intestinal epithelium and able to open TJs⁷⁵. γ PGA grafted mesoporous silica NPs are pH-sensitive and possess much higher release rate at pH 5.5 comparing pH 7.4⁷². PLGA based NPs upon coating with chitosan exhibit improved mucoadhesion⁵⁹. Co-encapsulating antacids (viz. magnesium hydroxide, magnesium carbonate or zinc carbonate) in PLGA NPs preserve the structure of macromolecules and protect them from GI tract degradation⁷⁶. PLGA-based NPs targeting M cells might be more efficient for vaccine delivery that sustain release and deliver antigen directly to the immune cells and might achieve and have intrinsic adjuvant effect on DCs^{31, 57, 77}. PCL-ERS, and poly(allylamine) are alternatives for getting mucoadhesive NPs.

Dipalmitoyl-phosphatidylcholine (DPPC), egg, phosphatidylcholine, or phosphatidylinositol-cholesterol based LIP are used while DPPC offers protection of drug against degradation in GI tract and their entry in bloodstream, intact^{45, 46}. Surface conjugation or coatings of LIP with mucin, PEG, wheat germ agglutinin (WGA), tomato lectin (TL) and Ulex europaeus agglutinin 1 (UA1) results targeting. Mucin-LIP and PEG-LIP results sustained release and provides protection against bile salts^{1, 45, 46}. LIP-WGA is taken up by endocytosis, LIP-TL exhibits resistance to enzymatic action in the intestine and mucoadhesion, LIP-UA1 have targeting specificity to M cells of PPs^{9, 45, 56, 57}. Co-encapsulation of permeation enhancers (e.g. sodium taurocholate, dimethyl palmitoylammoniopropanesulfonate) enhances bioavailability and paracellular translocation, through disrupting TJ⁷⁸.

Poly(allylamine) are amphiphilic and protects macromolecules from degradation by pepsin, trypsin and chymotrypsin along with improvement of transcellular and paracellular transport while latter being via reversible disruption of TJs^{28, 79}. PLA-Pluronic block copolymer based vesicles have ability to pass through cell membranes and attracted to small intestine⁶³. Copolymer of pluronic-PAA and poloxamer minimise initial burst release of drug, in the acidic stomach, but releases drug, in a molecularly dispersed form, at pH five. Targeting and mucoadhesion property of these synergises efficiency and efficacy³⁹. SLN of cetyl palmitate sustain release while that of WGA-N-glutarylphosphatidylethanolamine exhibits protection against enzymatic degradation^{11, 12, 43, 67, 80}. Micelles of

monomethyl PEG750-poly(caprolactone-co-trimethylcarbonate), copolymer, confers 1 to 3 fold increase in solubility of poorly soluble drugs and bioavailability^{14, 18}.

To avert drug release in GI tract, concentration of micelle to be above critical micelle concentration (CMC) and it shall expose to an ionic strength below their flocculation point^{27, 81}. The inhibition of P-gp by pluronics is highest just below CMC whereas that of PCL-PEG is above CMC^{14, 18}. Micelles of poorly soluble drugs could be passively target by enhanced permeabilisation-retention effect¹⁴. Poly-ion complex micelle enhances transport across intestinal epithelial barrier^{27, 28}.

Pluronics, poloxamers, TPGS and PCL-PEG inhibit P-gp and enhance net drug transport through intestinal barrier^{14, 18, 41, 63}. Some NPs enhances transcytosis through proteaseinhibition⁸². Multilayered NPs of alginate co-localises in the small intestinal mucosa. Amine-modified graft polyesters-chitosan NPs or thiolatedtrimethylchitosan NPs conserving degradation of macromolecules and facilitate their uptake, while latter had intrinsic adjuvant effect on DCs^{48, 77}.

Lipid-based systems (lipospheres, micelles, and LIP), prepared with several lipids including orexcluding emulsifying agents, well tolerated in living systems did not exhibit any cytotoxic effects, up to total of 2.5% lipid content. LIP is advantageous in terms of amphiphilic character, biocompatibility, and ease of surface modification. SLN is innovative system having expectation for more contributions, particularly, poor aqueous soluble macromolecules. Considering tolerability, surfactant of GRAS status used with preference, comparing lipids. Acylglycerols composed of fatty acids and lecithins mostly accepted as safe^{19, 38}.

Surface charges or chemistry of nanocarriers seem to monitor their fate. Negatively charged one display higher transport rates referring near neutral, or positively charged^{5, 64}. Fast-diffusing nanocarriers are achievable by coating their surface with PEG. This surface engineering modulates their mucoadhesive properties. Dense coating effectively minimises adhesive interactions of them with mucins, allowing penetration²¹. GI-residence time can be improved with cationic polymers or coating them with cationic groups or with thiol groups (binds to mucin)^{21, 35}. However, a balance between mucoadhesion and mucus penetration is important⁵.

Nanocarriers must be small enough to avoid significant steric inhibition by the fibre mesh and should avoid adhesion to mucin fibres; and to allow diffusion and uptake, but large enough to carry a favourable amount of macromolecules⁴⁸. They being stable, non-toxic, bioabsorbable, non-thrombogenic, non-immunogenic, non-

inflammatory, avoid uptake by RES and have versatile applicability^{1, 17, 20}. Cytotoxicity of nanovesicles is an essential product parameter of in vivo tolerance evaluation in humans and/or animals^{17, 19, 38}. Their stability assessed involving approved method and accordingly shelf-life be assigned³⁹.

Targeting of nanocarriers to globet cell^{53, 54} PPs^{9, 45, 56} DCs of PPs^{57, 62} achieved with grafting of ligands. Optimisation of ligand density on their surface must allow tissue penetration and cellular uptake resulting in optimal therapeutic efficacy. Redressing issue of variation in M cells populations and receptors needed; those vary with species, anatomical location, age, sex and exogenous factors^{1, 9, 10}.

In vitro models used for understanding fate of nanocarriers within intestinal epithelium is critical for their development. Combining both quantitative analysis method studying their transport and confocal microscopy, locating them, improve efficiency of study^{39, 83}. Cellular tracking of them can be done with quantum dots technique that improves their visualization and dynamic colocalization microscopy^{61, 83}. Quantitative and qualitative information such as diffusivity, viscoelasticity, pore size, velocity, directionality and transport mode of drug vectors can be determined from particle trajectories that reflect transport in mucus. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays provide cytotoxic report of the nanocarriers⁷⁰. Association of nanocarriers with DCs and their effect on their maturation determined flow-cytometrically⁷⁷.

Novel NBODSM can be developed through¹.

- (i) Development of biocompatible polymers with tailored attributes for orally administered nanocarriers,
- (ii) Understanding the mechanisms of their cellular uptake,
- (iii) Devising novel techniques for studying their fate including its components, and
- (iv) Identifying ligands for their targeted delivery.

CONCLUSION

NBODSM will be a promising platform for orally delivering macromolecules. In vivo experimental results of animal models need to be interpreted and correlated with care, associated with existence of differences in anatomy and physiology of laboratory animals and humans. In order have successful NBODSM bioavailability of them to reach therapeutic level, with minimal inter and intra individual variation and toxicity had to be considered. Greater stability along with a financially feasible manufacturing process for mass production is prerequisite. Future will evidence utility of NBODSM in devising projected benefit in a cost-effective and sustainable way. Step to set up to arouse professional in delivering worthwhile effects to society.

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