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Techniques for Preparation of Pharmaceutical Coated Nanoparticles: A Comprehensive Review

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ABSTRACT

The traditional formulations like solution, suspension or emulsion suffer from certain limitations like high dose and low bioavailability, first pass metabolism, intolerance, instability and also exhibit fluctuations in plasma drug levels and do not provide sustained effect, therefore, there is a need for some novel carriers which could attain ideal requirements of a drug delivery system. Nanoparticles delivery system has been proved nearly ideal one. Nanoparticles are, a type of colloidal drug carrier system comprising particles with diameter of nano-metric range. The rapid development of nanotechnology and nano-materials has led to a need for nanoparticle surface modification for a variety of applications. The surface can be tailored to specific physical, optical, electronic, chemical, and biomedical properties by coating a thin film of material on the surface of the nanoparticles. The coating or encapsulation of nanoparticles has been found to be of particular interest for the controlled release of drugs, genes and other bioactive agents. Controlled release systems provide the benefits of protection from rapid degradation, targeting delivery, control of the release rate and prolonged duration of bioactive agents. This article have a keen emphasize on the techniques used for the formulation of nanoparticles and their coating too along with associated cautions and significance related to special applications which reveals a better way to choose the suitable and efficacious technique to obtain a nano-sized formulation. It also reveals the need of regulatory framework for handling of nanoparticles.

Keywords: Nanoparticles, coated nanoparticles, coating techniques, targeted drug delivery.

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INTRODUCTION:

The solid particles or particulate dispersions having a size range of 10-1000 nm are termed as nanoparticles (NPs). Although NPs have been used as potential carriers with unique features including protecting drugs from degradation and reduction in toxicity or side effects but their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability while the coated or polymeric NPs enhance the stability of drugs/proteins, provide site-specific drug delivery and controlled release patterns which ultimately lead to the increase in drug therapeutic efficacy and reduced side effects [1-3]. Most polymeric nanoparticles are biodegradable and biocompatible i.e. why used for nano-material drug delivery. Also their surface can be modified via chemical transformations which provide excellent pharmacokinetic control and are suitable for the entrapment and delivery of various therapeutics agents [4]. The biodegradable polymeric NPs, particularly those coated with hydrophilic polymer like poly (ethylene glycol) (PEG) known as long-circulating particles or potential drug delivery devices because of their ability to circulate for a prolonged period time in body to target a particular organ, especially as carriers of DNA in gene therapy and their ability to deliver proteins, peptides and genes [5-7]. The techniques used to formulate and coat NPs are shown below in pyramidal representation (figure 1).

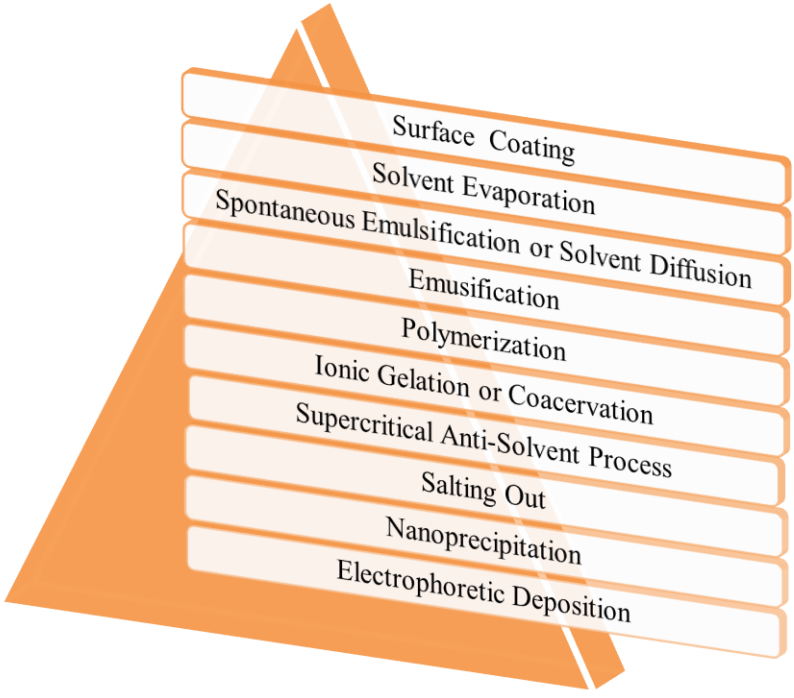


Figure 1: List of Coating Techniques.

Surface Coating Technique

The surface coating of NPs is crucial to determine their properties. A coating that is multivalent or polymeric confers high stability. For biological applications, the surface coating should be polar to give high aqueous solubility and prevent nanoparticle aggregation. In serum or on the cell surface, highly charged coatings promote non-specific binding, while polyethylene glycol linked to terminal hydroxyl or methoxy groups repel non-specific interactions.

NPs can be linked to biological molecules which can act as address tags, to direct the NPs to specific sites within the body, specific organelles within the cell, or to follow specifically the movement of individual protein or RNA molecules in living cells. Common address tags are monoclonal antibodies, aptamers, streptavidin or peptides. These targeting agents should ideally be covalently linked to the NPs and should be present in a controlled number per NP. Multivalent NPs, bearing multiple targeting groups, can cluster receptors, which can activate cellular signaling pathways, and give stronger anchoring. Monovalent NPs, bearing a single binding site, avoid clustering and so are preferable for tracking the behavior of individual proteins [8, 9]. Monolayer coated gold NPs also provide attractive vehicles for delivery of drugs, genetic materials, proteins, and small molecules [10].

Solvent Evaporation Technique

This is one of the popular techniques used for the encapsulation of drug within water insoluble polymer [11].The preparation of NPs using solvent evaporation technique can be easily understood from the Figure 2.

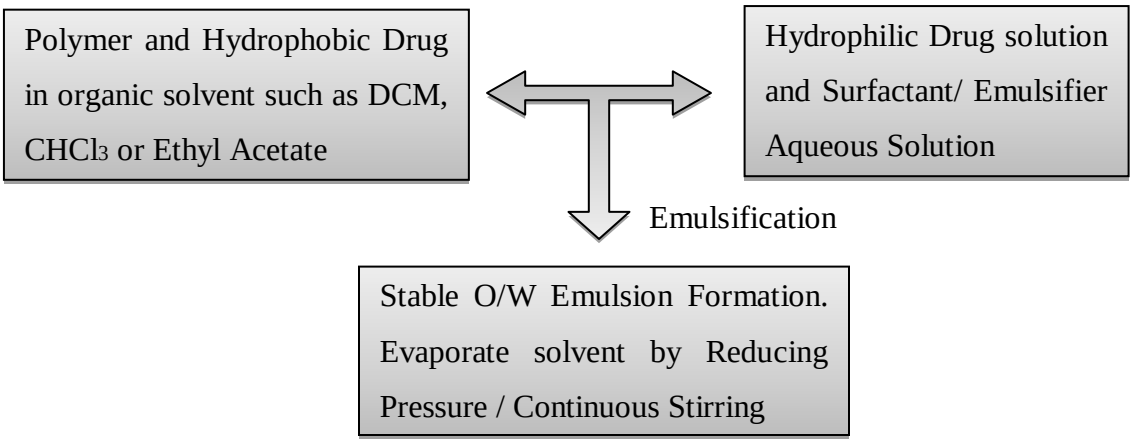


Figure 2: Flow diagram for Solvent Evaporation Technique.

Cautions: Particle size can be influenced by the type and concentrations of stabilizer, homogenization speed and polymer concentration. Small particle size can be achieved by a high-speed homogenization or ultra-sonication [12].

Spontaneous Emulsification or Solvent Diffusion Technique

It's a modified version of solvent evaporation technique in which the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. An interfacial turbulence is created between the two phases leading to the formation of small particles due to the spontaneous diffusion of solvents to obtain the NPs.

Cautions: A decrease in the size of particle can be obtained by increasing the concentration of water miscible solvent. The multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase in case of hydrophilic drugs [13].

Emulsification Technique

This technique is basically associated with the four steps to prepare the NPs (Figure 3). During the emulsion preparation, the organic solvent and the strong shearing force, temperature, pH, and the interface between the oil and water phases may affect and/or alter the structure of the bioactive agents.

Moreover, some severe drawbacks such as residual organic solvent in the final product, volatile organic compounds emission and heavy downstream processing are involved in emulsion processes [14, 15].

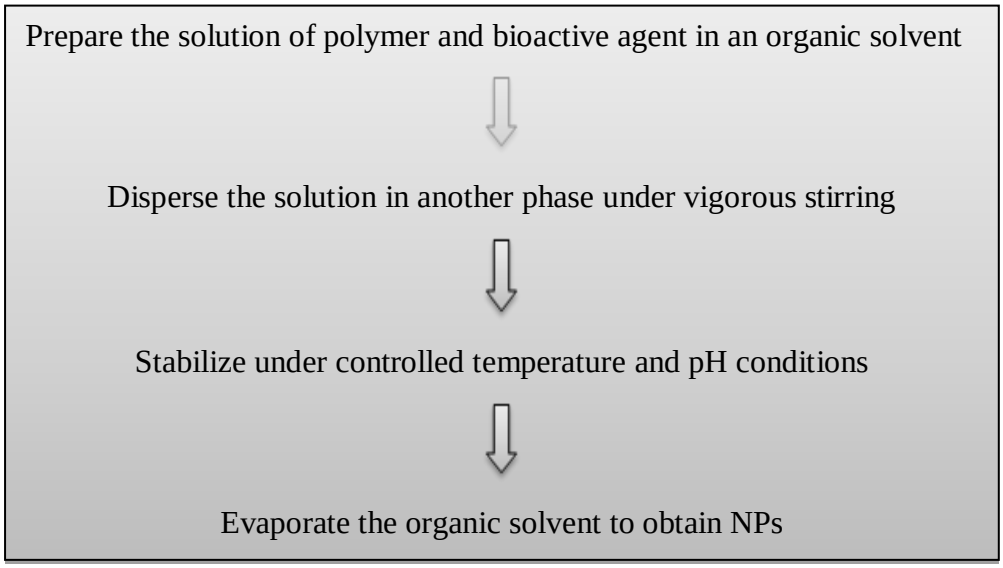


Figure 3: Flow diagram for Emulsification Technique.

Polymerization Technique

The monomers are polymerized to form NPs in an aqueous solution. Drug is incorporated either by dissolving in the polymerization medium or by adsorption onto the NPs after completion of polymerization. The purification of NPs suspension is carried out to remove various stabilizers and surfactants employed for polymerization by ultra-centrifugation and re-suspending the

particles in an isotonic surfactant free medium. It is mainly employed to prepare polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles.

Caution: The concentration of the surfactants and stabilizers used affect the NPs formation and their particle size [16, 17].

Ionic Gelation Technique or Coacervation

Calvo and co-workers developed the ionic gelation technique for the preparation of hydrophilic chitosan NPs. A wide research work has been carried out on the preparation of NPs using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate [18, 19]. This method consists a mixture of two aqueous phases, of which one is the chitosan polymer, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a poly-anion sodium tri-polyphosphate. In this technique, positively charged amino group of chitosan interacts with negative charged tri-polyphosphate to form coacervates with a size in the range of nanometers. Ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature, whereas, coacervates are formed as a result of electrostatic interaction between two aqueous phases. Chitosan is dissolved in a solution of acetic acid and tween 80. The formation of the particles can be achieved after the addition of sodium sulfate solution to the chitosan solution. The addition is to be done under mild agitation (<50 rpm) and continuous sonication to obtain a desired size range.

Caution: The rate of addition of ionic solution and stirring conditions are the main key factors which affect the size of nano-formulation [1, 2, 20].

Supercritical Anti-Solvent (SAS) Technique

The supercritical Anti-solvent technique has been investigated as an alternative to prepare biodegradable NPs because of environmentally safe nature of supercritical fluids. This process is also termed as Supercritical Fluid Technology. A supercritical fluid is defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO_2 (SC CO_2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1 °C, Pc = 73.8 bars), non-toxicity, non-flammability and low cost.

The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, e.g. methanol, which is completely miscible with the supercritical fluid (SC CO_2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the

instantaneous precipitation of the solute, resulting the formation of NPs (figure 4). This technique is clean because the precipitate is basically solvent free.

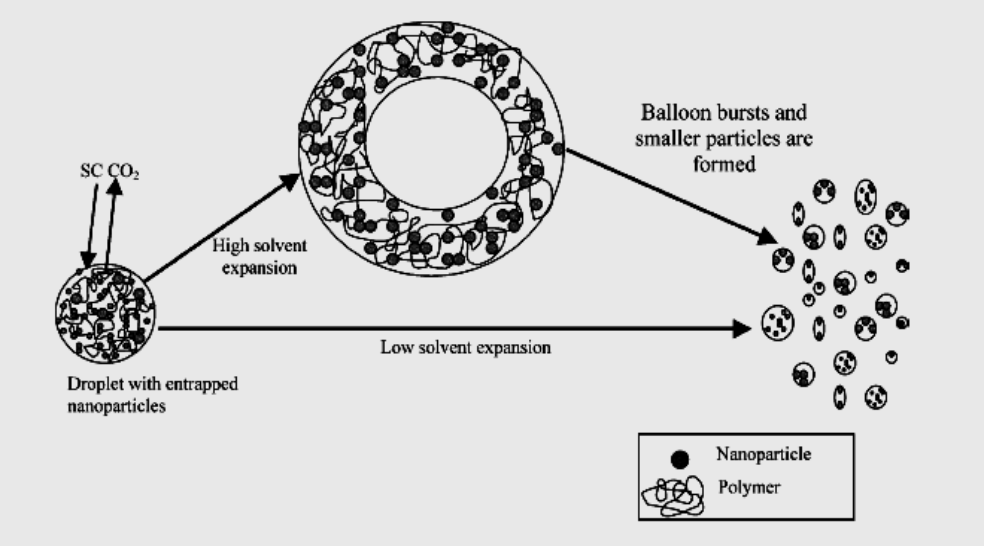
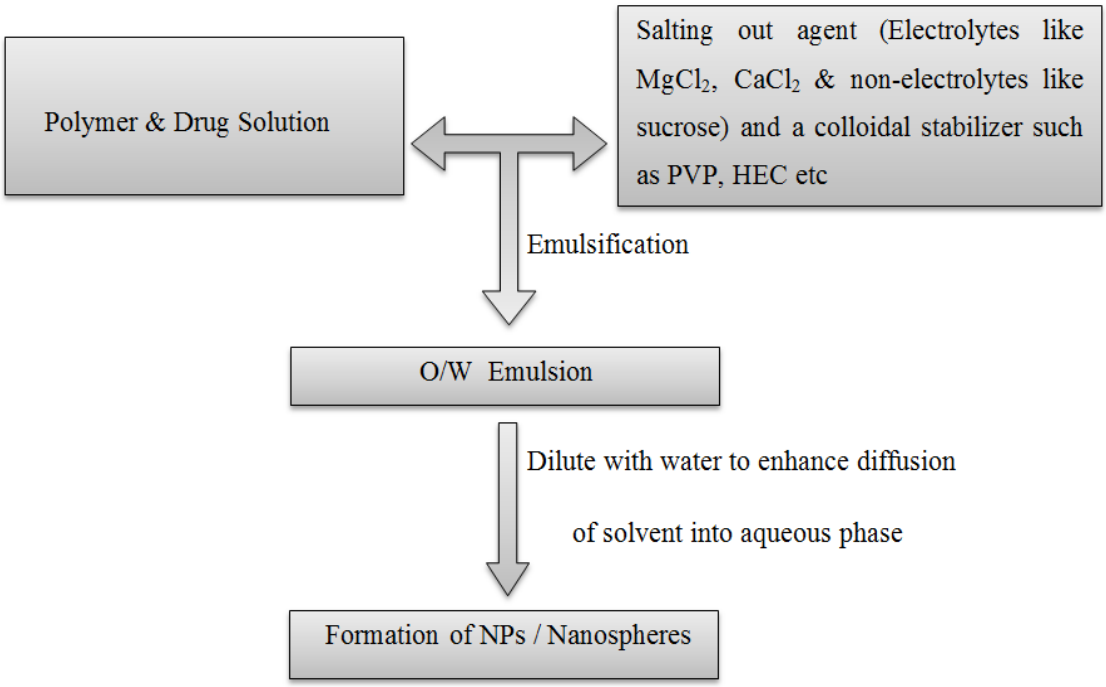


Figure 4 : Schematic mechanism of fine particle encapsulation using the SAS process.



Cautions: RESS differs from the SAS process because in that solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region of lower pressure. Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is more expensive because it requires specially designed equipment [1, 21].

Salting Out Technique

The NPs can also be obtained by salting out technique in which the salting out is achieved by the separation of a water miscible solvent from aqueous solution. The procedure for this technique can be easily understood by figure 5.

Cautions: The polymer concentration in organic phase, internal or external phase ratio, type of electrolyte, type and concentration of stabilizer in aqueous phase and stirring rate affect the formation of NPs [22-24].

Nano-precipitation Technique

This technique is developed and patented by Fessi and co-workers [25]. It is also known as solvent displacement technique and proved to be more versatile and flexible method for encapsulation of hydrophilic drugs (e.g. proteins) with slight modifications in solvent/ non-solvent volume ratio and polymer concentration [26]. In this technique, the precipitation of a preformed polymer from an organic solution occurs and the organic solvent diffusion is achieved in the aqueous medium either in presence or absence of surfactant (figure 6).

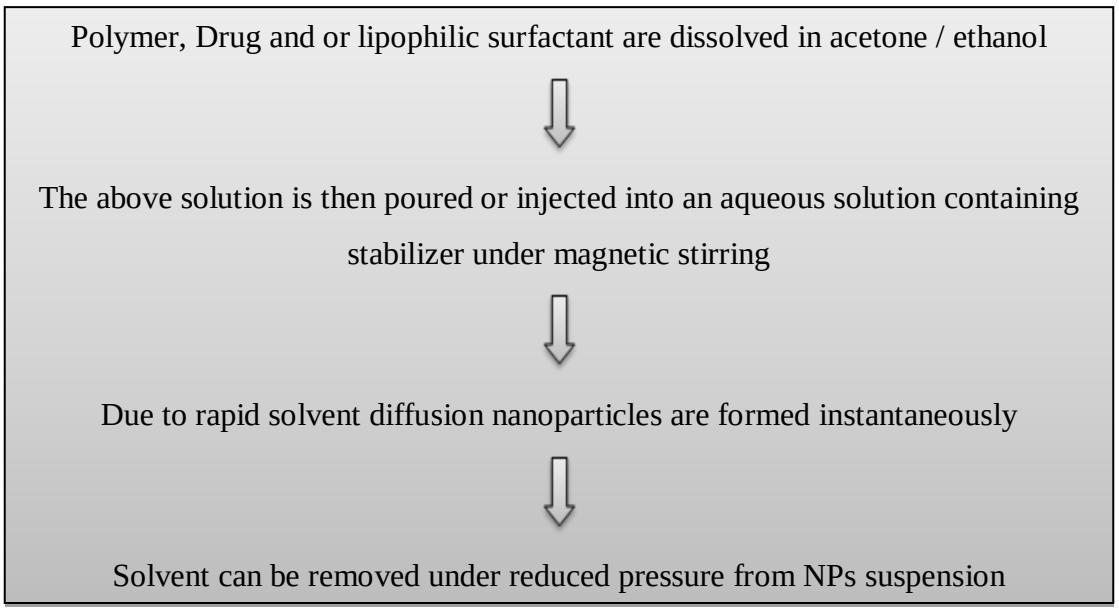


Figure 6: Flow diagram for Nano-precipitation Technique.

Cautions: The particle size is affected by the rate of addition of organic phase into the aqueous phase. It was found that a decrease in particle size as well as drug entrapment occurs when the mixing rate of two phases increases [24].

Electrophoretic Deposition Technique

Electrophoretic deposition (EPD) is achieved via the forced motion of charged particles under an applied voltage towards an electrode of the opposite charge, followed by the coagulation of the particles to form a deposit. The electrodes used may be of stainless steel (316L) plate and a

stainless steel (316L) stent. They are arranged in a 5 mm apart parallel configuration. Both electrodes are immersed in a container holding a liquid medium containing NPs as shown in figure 7.

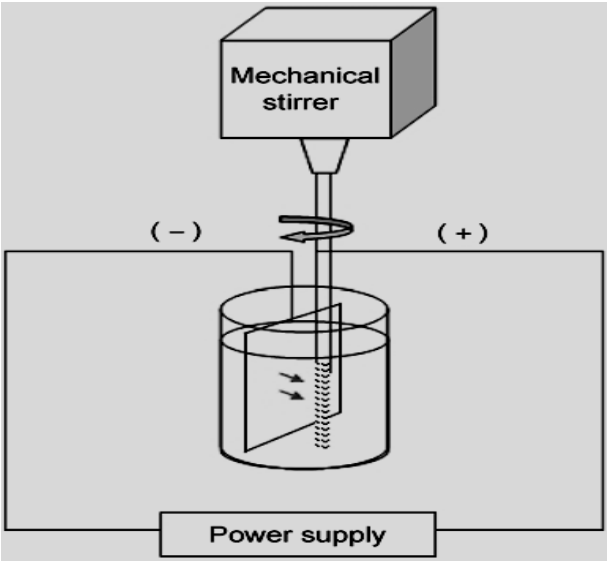


Figure 7: Apparatus for Electrophoretic Deposition.

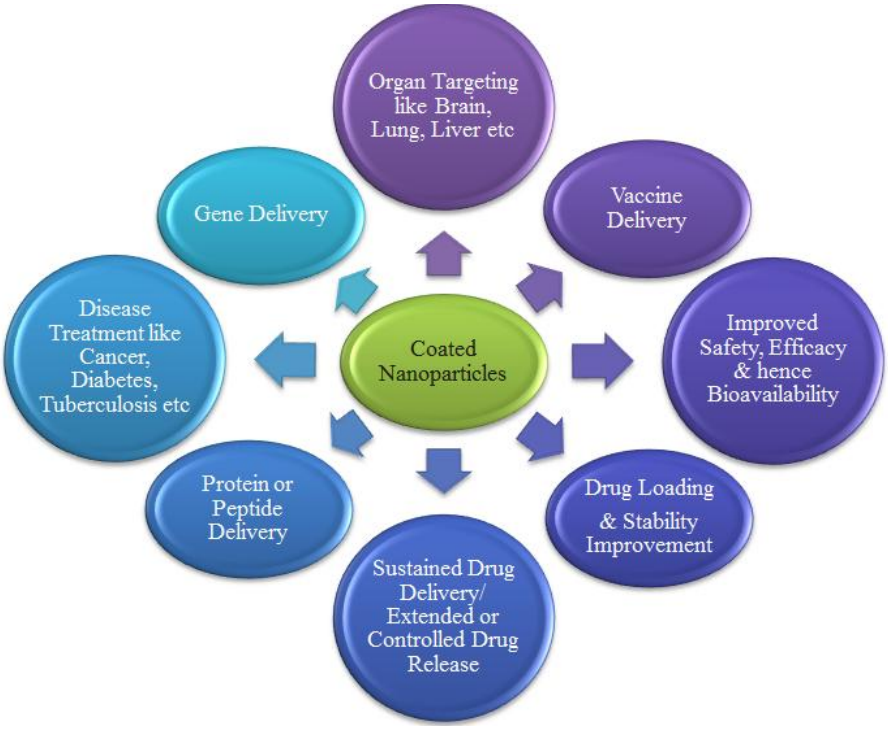


Figure 8: Significance of Coated NPs.

EPD is carried out under various conditions of voltage and time. Then applied voltages of 5 V, 9 V and 13 V and set EPD times to 10 min, 20 min, 30 min, 40 min and 60 min. The concentration of NPs means weight percent value of total weight sum of polymer and drugs used for NPs preparation in media. The stainless steel plate, as the anode, is connected to the negatively

charged terminal of a power supply. The stent, as the cathode, is connected to the positively charged terminal of a power supply. Since the stent has cylindrical structure, it is constantly rotated to give a uniform NPs coating. The EPD is carried out in an ice bath in order to diminish the heat generated during the process [27].

Significance

The utility of above discussed techniques used to obtain coated NPs can be summarized as shown below in the cycle (figure 8). The potential outcomes of various techniques to form and coat NPs along with the polymers used and incorporated drugs are listed in table 1 from the published literature.

Table 1: Some instances from published literature

Sr. No.	Drug	Delivery/ Targeting	Coating Polymer	Potential Outcomes	References
1.	Ovalbumin	Mucosal Vaccine Delivery	Sodium alginate	Burst release of ovalbumin was prevented and enhanced stability at physiological temperature in GIF and SIF	20
2.	Savoxepin	Injectable Extended Release Dosage Form	Poly lactic acid	Improved drug loading and entrapment efficiency	23
3.	Curcumin	Model for local drug delivery	Poly acrylic acid	An efficient coating method for creating uniform surface morphology	27
4.	Dil	Brain	Tween 20 Tranferrin/ BSA	Improved endocytosis	28
5.	Rivastigmine	Brain	Polysorbate 80	Enhanced brain concentration of IV injected rivastigmine	29, 30
6.	Doxorubicin, Loperamide	Brain Tumor	Pluronic F-68 or Tween 80	Efficient delivery to site of action after IV injection	31
7.	Paclitaxel	Cancerous Cells	Hyaluronic acid	Increased cellular uptake using S-180 cells	32
8.	Coumarin 6	Cancerous Cells	PVA/ Vitamin E TPGS	Improved cellular uptake using caco-2 cells	33
9.	Oxaliplatin	Colorectal Cancerous Cells	Hyaluronic acid	Effective delivery to treat colon tumors	34
10.	Insulin	Peroral Delivery	Dextran	Enhanced uptake capacity of NPs and Vitamin B12	35

				transport to deliver orally effective insulin	
11.	Insulin Phenol Red	Trans-mucosal Delivery	Chitosan PMMA PAA Carbopol	Improved bioavailability, prolonged residence time, protection of proteins/peptides from enzymatic degradation	36
12.	Didanosine	Macrophage Targeting	Mannan	Significantly higher concentration of didanosine in spleen, lymph nodes and brain	37
13.	Hydrophilic Drug	Protein/ Peptide Delivery	PLA-PLGA copolymer	DMSO was found to be most useful solvent especially for protein drugs and promising for higher protein encapsulation	38, 39
14.	Hepatitis B surface antigen & CpG ODN	Vaccinated nasal delivery	Sodium Alginate	Higher production of interferon-Ɣ and also rise in humoral mucosal immune responses	40
15.	PEI condensed plasmid DNA & Transferrin	Gene Delivery	DDAB, Soya PC	Very rapid and immediate gene transfer	41
16.	$FeCl_2$ and $FeCl_3$	Assisted drug delivery systems	Chitosan	Immobilization of enzymes or other biomolecules and the preparation of immunological assays	42
17.	$FeCl_2$ and $FeCl_3$	Drug delivery, Cell tracking	Sodium oleate & PEG-6000	Improved intracellular uptake of NPs after coating with PEG	43
18.	INH, RIF, PYZ & ETB	Lung Targeting	Chitosan	Huge potential for intermittent therapy	44
19.	Thymopentin	Oral Delivery	Chitosan glutathione conjugate	Improved oral bioavailability	45
20.	Dexamethaso-ne phosphate	Sustained Drug Delivery	PLGA	Provide sustained drug release without initial burst release	46

NEED OF REGULATORY GUIDELINES

The US Food and Drug Administration (FDA) have approved biodegradable polymeric nanoparticles, such as PLA and PLGA for human use. They may be formulated to encapsulate several classes of therapeutic agents [4] although there is neither an international regulation of

nano-products or the underlying nanotechnology nor are there any internationally agreed definitions or terminology for nanotechnology, no internationally agreed protocols for toxicity testing of nanoparticles and no standardized protocols for evaluating the environmental impacts of NPs. Computer modeling and simulation techniques needed to be developed for further understanding of drug nanoparticles. Addition to this, clinical trials also needed for well characterization of NPs because till date there is an insufficient data available to identify any generic rules governing the likely toxicology and ecotoxicology of NPs in general. To maintain a high level of public health, occupational health and environmental protection, it is essential that a specific risk assessment is conducted if there is any potential for humans and the environment to be exposed to particular forms of NPs. A regulatory framework is the need of hour that enables both human and environmental risk assessment. Thus a number of the conventional toxicity tests may require some modification for the assessment of NPs in order to ensure that the exposure conditions simulate realistic exposure scenarios and the end points are directly associated with the NPs to be assessed [47-49].

CONCLUSION AND VITAL ASPECTS

The above review has a potential aspect to help in resolving the ambiguities in selection of not only an appropriate technique for the preparation of NPs and but also choice of coating polymer for targeting as in present scenario nano-particulate system has vital role in drug targeting, delivery and play a massive role in combined diagnosis and therapy.

Nano-medicines are just beginning to enter drug regulatory processes but within a few decades it could comprise a dominant group within the class of innovative pharmaceuticals. The current government safety and cost-effectiveness regulators appearing to be that these products give rise to few if any nano-specific issues.

REFERENCES

1. Mohanraj VJ, Chen Y. Nanoparticles - A Review. Trop J Pharm Res 2006; 5: 561-73.
2. Manmode AS, Sakarkar DM, Mahajan NM. Nanoparticles-Tremendous Therapeutic Potential: A Review. Int J PharmTech Res 2009; 1: 1020-7.
3. Mudshinge SR, Deore AB, Patil S, Bhalgat CM. Nanoparticles: Emerging carriers for drug delivery. Saudi Pharmaceutical Journal 2011; 24: 129-41.
4. Faraji AH, Wipf P. Nanoparticles in cellular drug delivery. Bioorg Med Chem 2009; 17: 2950-62.

5. Bhadra D, Bhadra S, Jain P, Jain NK. Pegnology: a review of PEG-ylated systems. Pharmazie 2002; 57: 5-29.
6. Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm Res 2005; 22: 1-10.
7. Kommareddy S, Tiwari SB, Amiji MM. Long-circulating polymeric nanovectors for tumor-selective gene delivery. Technol Cancer Res Treat 2005; 4: 615-25.
8. Sanvicens N, Marco MP. Multifunctional nanoparticles--properties and prospects for their use in human medicine. Trends Biotechnol 2008; 26: 425-33.
9. Torchilin VP. Multifunctional nanocarriers. Adv Drug Deliv Rev 2006; 58: 1532-55.
10. Rana S, Bajaj A, Mout R, Rotello VM. Monolayer coated gold nanoparticles for delivery applications. Adv Drug Deliv Rev 2012; 64: 200-16.
11. Hoa LTM, Chi NT, Triet NM, Nhan LNT, Chien DM. Preparation of drug nanoparticles by emulsion evaporation method. J Physics: Conference Series 2009; 187: 012047.
12. Jaiswal J, Gupta SK, Kreuter J. Preparation of biodegradable cyclosporine nanoparticles by high-pressure emulsification-solvent evaporation process. J Control Release 2004; 96: 169-78.
13. Ubrich N, Bouillot P, Pellerin C, Hoffman M, Maincent P. Preparation and characterization of propranolol hydrochloride nanoparticles: a comparative study. J Control Release 2004; 97: 291-300.
14. Zambaux MF, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso MJ et al. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. J Control Release 1998; 50: 31-40.
15. Bilati U, Allemann E, Doelker E. Nanoprecipitation versus emulsion-based techniques for the encapsulation of proteins into biodegradable nanoparticles and process-related stability issues. AAPS PharmSciTech 2005; 6: E594-604.
16. Zhang Q, Shen Z, Nagai T. Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats. Int J Pharm 2001; 218: 75-80.
17. Boudad H, Legrand P, Lebas G, Cheron M, Duchene D, Ponchel G. Combined hydroxypropyl-beta-cyclodextrin and poly(alkylcyanoacrylate) nanoparticles intended for oral administration of saquinavir. Int J Pharm 2001; 218: 113-24.

18. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res 1997; 14: 1431-6.
19. Grenha A, Seijo B, Remunan-Lopez C. Microencapsulated chitosan nanoparticles for lung protein delivery. Eur J Pharm Sci 2005; 25: 427-37.
20. Borges O, Borchard G, Verhoef JC, de Sousa A, Junginger HE. Preparation of coated nanoparticles for a new mucosal vaccine delivery system. Int J Pharm 2005; 299: 155-66.
21. Wang Y, Dave RN, Pfeffer R. Polymer coating/encapsulayion of nanoparticles using a supercritical anti-solvent process. J Supercrit Fluids 2004; 28: 85-99.
22. Catarina PR, Ronald JN, Antonio JR. Nano capsulation 1. Method of preparation of drug-loaded polymeric nanoparticles. Nanomed Nanotech Biol Med 2006; 2: 8-21.
23. Allemann E, Leroux JC, Gurny R, Doelker E. In vitro extended-release properties of drug-loaded poly(DL-lactic acid) nanoparticles produced by a salting-out procedure. Pharm Res 1993; 10: 1732-7.
24. Pal SL, Jana U, Manna PK, Mohanta GP, Manavalan R. Nanoparticle: An overview of preparation and characterization. J Appl Pharm Sci 2011; 1: 228-34.
25. Fessi H, Puiseiux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial deposition following solvent displacement. Int J Pharm 1989; 55: R1-R4.
26. Bilati U, Allemann E, Doelker E. Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles. Eur J Pharm Sci 2005; 24: 67-75.
27. Nam SH, Nam HY, Joo JR, Baek IS, Park JS. Curcumin-Loaded PLGA Nanoparticles Coating onto Metal Stent by Elecrophoretic Deposition Techniques. Bull Korean Chem Soc 2007; 28: 397.
28. Chang J, Jallouli Y, Kroubi M, Yuan XB, Feng W, Kang CS et al. Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood-brain barrier. Int J Pharm 2009; 379: 285-92.
29. Wilson B, Samanta MK, Santhi K, Kumar KP, Paramakrishnan N, Suresh B. Poly(n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease. Brain Res 2008; 1200: 159-68.
30. Kreuter J, Ramge P, Petrov V, Hamm S, Gelperina SE, Engelhardt B et al. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs

to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharm Res 2003; 20: 409-16.

31. Gelperina S, Maksimenko O, Khalansky A, Vanchugova L, Shipulo E, Abbasova K et al. Drug delivery to the brain using surfactant-coated poly(lactide-co-glycolide) nanoparticles: influence of the formulation parameters. Eur J Pharm Biopharm 2010; 74: 157-63.
32. He M, Zhao Z, Yin L, Tang C, Yin C. Hyaluronic acid coated poly(butyl cyanoacrylate) nanoparticles as anticancer drug carriers. Int J Pharm 2009; 373: 165-73.
33. Win KY, Feng SS. Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. Biomaterials 2005; 26: 2713-22.
34. Jain A, Jain SK, Ganesh N, Barve J, Beg AM. Design and development of ligand-appended polysaccharidic nanoparticles for the delivery of oxaliplatin in colorectal cancer. Nanomedicine 2010; 6: 179-90.
35. Chalasani KB, Russell-Jones GJ, Yandrapu SK, Diwan PV, Jain SK. A novel vitamin B12-nanosphere conjugate carrier system for peroral delivery of insulin. J Control Release 2007; 117: 421-9.
36. Cui F, Qian F, Yin C. Preparation and characterization of mucoadhesive polymer-coated nanoparticles. Int J Pharm 2006; 316: 154-61.
37. Kaur A, Jain S, Tiwary AK. Mannan-coated gelatin nanoparticles for sustained and targeted delivery of didanosine: in vitro and in vivo evaluation. Acta Pharm 2008; 58: 61-74.
38. Barichello JM, Morishita M, Takayama K, Nagai T. Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method. Drug Dev Ind Pharm 1999; 25: 471-6.
39. Gupta VK, Karar PK, Ramesh S, Misra SP, Gupta A. Nanoparticle Formulation for Hydrophilic & Hydrophobic Drugs. Int J Res Pharm Sci 2010; 1: 163-9.
40. Borges O, Cordeiro-da-Silva A, Tavares J, Santarem N, de Sousa A, Borchard G et al. Immune response by nasal delivery of hepatitis B surface antigen and codelivery of a CpG ODN in alginate coated chitosan nanoparticles. Eur J Pharm Biopharm 2008; 69: 405-16.

41. Pan X, Guan J, Yoo JW, Epstein AJ, Lee LJ, Lee RJ. Cationic lipid-coated magnetic nanoparticles associated with transferrin for gene delivery. Int J Pharm 2008; 358: 263-70.
42. Dung DTK, Hai TH, Phuc LH, Long BD, Vinh LK, Truc PN. Preparation and characterization of magnetic nanoparticles with chitosan coating. Journal of Physics: Conference Series 2009; 187: 012036.
43. Hoa LTM, Dung TT, Danh TM, Duc NH, Chien DM. Preparation and characterization of magnetic nanoparticles coated with polyethylene glycol. Journal of Physics: Conference Series 2009; 187: 012048.
44. Ahmad Z, Pandey R, Sharma S, Khuller GK. Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential. Indian J Chest Dis Allied Sci 2006; 48: 171-6.
45. Jin X, Xu Y, Shen J, Ping Q, Su Z, Yo W. Chitosan–glutathione conjugate-coated poly(butyl cyanoacrylate) nanoparticles: Promising carriers for oral thymopentin delivery. Carbohydr Polym 2011; 86.
46. Thote AJ, Gupta RB. Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release. Nanomedicine 2005; 1: 85-90.
47. Vines T, A. FT. Assessing the safety and cost-effectiveness of early nano drugs. J Law Med 2007; 16: 822-45.
48. Seaton A, Tran L, Aitken R, Donaldson K. Nanoparticles, human health hazard and regulation. J R Soc Interface 2010; 7 Suppl 1: S119-29.
49. Bawa R. Nanoparticle-based Therapeutics in Humans: A Survey. Nanotechnology Law & Business 2008; 5: 133-55.