Hence, a method to associate the protein to the preformed nanoparticle surface by adsorption has been investigated. This technique can be performed in an aqueous solution and at a low temperature, thus improving the prospects for preserving the activity of sensitive protein and peptide molecules [261].

Polymeric materials used for the formulation of nanoparticles include synthetic polymers such as PLA, PLGA [148,192], poly(ε-caprolactone) (PCL), poly(methyl methacrylates), and poly(alkyl cyanoacrylates) [262,188], or natural polymers as albumin, gelatin, alginate, collagen, or chitosan. Research has also been extended to PEGylation of nanoparticles to achieve prolonged circulation [263,264]. PEG-coated nanoparticles have been found to be of great potential in therapeutic application for controlled release of drugs and site-specific drug delivery, as investigated with the TNF-α delivery using gold nanoparticles [265,266]. These nanoparticles exhibit stearic repulsion, which results from a loss of configurational entropy of the bound PEG chains [267]. In addition, the hydrophilic PEG can form a hydrated outer shell, which protects the nanoparticles from being quickly uptaken by the RES [268], extends the half-life of drugs, and alters their tissue distribution. These nanoparticles have been highly investigated for protein and peptide encapsulation for therapeutic delivery [265,266].

Poly(vinyl alcohol) (PVA) hydrogel nanoparticles have been prepared by using a water-in-oil emulsion technology plus cyclic freezing–thawing process. The PVA hydrogel nanoparticles prepared by this method are suitable for P/P drug delivery because formation of the hydrogel does not require crosslinking agents or other adjuvants and does not involve any residual monomer. Particularly, there is no emulsifier involved in this new method [269]. Nanoparticles for hormone delivery can be prepared from smart polymers which shows pH, or temperature-responsive drug release profile, exemplified by poly(acrylic acid), PMAA, a pH-responsive polymer due to its pH-dependent ionization and thereby hydration degree. The maximum volume change in PMAA occurs at a pH around its p*K*a [270–274]. In vaccine development, protein-coated, wax-based nanoparticles have also been shown to increase immune responses significantly [275,276], thus demonstrating high protein delivery. Table 11.7 summarizes examples of proteins and peptides delivered in various polymeric nanoparticles discussed in this section.

In general, one of the drawbacks associated with the formulation of nanoparticles is the initial burst and the incomplete release of the encapsulated protein, which may influence its potential as a drug delivery system [279]. Although to some extent the drawback can be controlled by optimizing particle size and the manufacturing techniques applied [279], it is still a challenge to formulate nanoparticulate drug delivery systems for parenteral protein administration.

11.5.5 Solid Lipid Nanoparticles

SLNs (Fig. 11.9) are colloidal particles composed of a biocompatible/biodegradable lipid matrix that is solid at body temperature and exhibits size range between 100 and 400 nm. SLNs combine the advantages of colloidal drug carrier systems like liposomes, polymeric nanoparticles, and emulsions, but avoid or minimize the drawbacks associated

Table 11.7 List of P/P Encapsulated Polymeric Nanoparticles

Protein/Peptide Encapsulated	Characteristics of the System	Reference
Urokinase	Targeted drug delivery. Alternative copolymers of maleic anhydride and *n*-butylvinylether hemiesterified with 2-methoxyethanol and grafted with PEG segments and MAb-single chain fragment specific for fibrin clot. Increased bioavailability observed at the site of action	[277]
VEGF	PLGA nanoparticles promote vascular growth	[278]
Leuprolide, insulin, lysozyme	poly(*N*-isopropylacrylamide-*co*-methacrylic acid) of various NIPAAm:MAA ratios dispersed in a matrix of a hydrophobic polymer. Crosslinked PNIPAAm shrinks or swells when temperature is raised above or reduced below its phase transition temperature, with a sharp volume change	[271]
BSA	PEG-PLGA nanoparticles prepared by double emulsion method. Extends BSA half-life and distribution	[197]

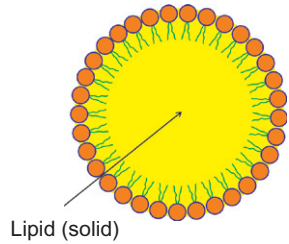

Figure 11.9 Schematic representation of SLNs.

with those systems [280,281]. The various advantages such as the particulate nature of SLNs, opportunity to encapsulate hydrophilic and hydrophobic drugs; ability to sustain the release of the incorporated drug; ability to prevent chemical, photochemical, or oxidative degradation of drug; ability to immobilize the drug in the solid matrix; ease of scale-up and manufacturing; and low cost of solid lipids as compared to phospholipids and biodegradable polymers give SLN an edge over other colloidal carriers [281]. Also, P/P drug delivery from SLN shows improved protein stability, avoids proteolytic degradation, and provides sustained release of the incorporated molecules, improved bioavailability and targeting ability, enhanced cytotoxicity against multidrug-resistant cancer cells, enhanced AUC (area under the plasma concentration curve) and MRT (mean residence time), enhanced anticancer efficacy, and enhanced brain targeting [281].

Common lipids and excipients used in preparation of SLN and other lipid carriers are poloxamers, PEGs, stearic acid-PEG 2000, F68 and Brij78 (stealth agents), cetyl alcohol/polysorbate, trymyristin, egg phosphotidylcholine, pegylated phospholipids (stabilizers), tripalmitin, acetyl palmitate (lipid matrix), *N*-[1-(2, 3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (cationic detergent), cetyltrimethylammonium

bromide (CTAB, stabilizer), DOTAP, and DDAB (cationic surfactants), monostearin (SLN with or without PEG 2000), sterylamine, emulsifying wax, and thiamine. These agents are well studied in research and show little or no systemic toxicity. They are widely used for P/P drug delivery.

The second generation of lipid nanoparticles has been introduced with the term nanostructured lipid conjugates (NLC). These particles are prepared from a blend of a solid lipid with a liquid lipid (oils) in such a proportion that the mixture has to be solid at a minimum temperature of 40°C [282,283]. Another modification of SLN has been introduced as lipid drug conjugates (LDC), which were developed especially for the hydrophilic drug molecules [281,284]. These advanced lipid carriers have shown advantages over the conventional SLNs in being more flexibile for modulation of drug release, increasing the protein and peptide loading, and preventing its leakage and degradation during release.

The main factors influencing P/P release from solid lipid particles are the physicochemical characteristics of the P/P drug itself: particle size, lipid matrix composition, surfactants used, drug distribution throughout the matrix, method of preparation, and production parameters [285–287].

Further improvement of tissue selectivity can be achieved by engineering the surface of lipid carriers with hydrophilic polymers or coupling targeting ligands; for example, by modifying surface characteristics. Lipid nanoparticles coated with PEG or chitosan have been developed and reported [288]. Some of the P/P associated with SLN, their production procedure, and the association efficiency are tabulated in Table 11.8.

Although future application of SLN in P/P drug delivery is inevitable, researchers should also check the product for cytotoxicity of lipid carrier in *in vivo* evaluation. However, many research articles report that cytotoxicity of the SLN can be mainly attributed to components of the aqueous phase, especially to nonionic emulsifiers and preservatives rather than lipid. Lipid carriers prepared with several different lipids and emulsifying agents did not exhibit any cytotoxic effects *in vitro* up to concentrations of 2.5% lipid [299]. In fact, it has been shown that even concentrations higher than 10% of lipid phase led to a viability of 80% with cultured human granulocytes in culture [299].

More formulation aspects must be considered while formulating lipid carriers, such as the presence of alternative colloidal structures (micelles, liposomes, mixed micelles, drug nanocrystals) in the aqueous dispersion; the complexity of the physical state of the lipid (transformation between different modifications, possibility of supercooled melts), which causes stability problems during storage or administration (e.g., gelation, particle size increase, drug expulsion); and sample dilution or water removal, which might significantly change the equilibrium between the different colloidal species and the physical state of the lipid [300].

11.5.6 Hydrogels

Hydrogels (Fig. 11.10), as the name suggests, are gelled networks of hydrophilic polymers. They may exist as a covalently crosslinked chemical gel network or as physical gel without covalent crosslinking. Physical gels form by linking polymer chains

Table 11.8 Reported P/P Encapsulated in SLNs as Therapeutic Agent or Targeting Moiety Along with their Techniques of Manufacturing

S. No.	Peptide/Protein Encapsulated	Characteristics of the System	Reference
1.	D-Trp6, LHRH, thymopentin	Water–oil–water microemulsion-based technique, low encapsulation efficiency of 2–5%	[281,289]
2.	Insulin, thymocartin, somatostatin	SLN prepared by solvent emulsification-evaporation method, high encapsulation efficiency	[290,291]
3.	BSA	BSA crystals were coated using supercritical fluid technology either with tripalmitin or Gelucire®, and the latter resulted in prolonged release lipid microcapsules (80% of intact BSA in 24 h)	[292]
4.	BSA	Hot high-pressure homogenization and microemulsion technique. Association efficiency ~100	[293]
5.	Calcitonin	Water–oil–water double emulsion. Association efficiency 75–90%	[287]
6.	Cyclosporin A	Microemulsion technique. Hot high-pressure homogenization technique. Association efficiency 6–13%, prolonged drug release	[294]
7.	Gonadorelin	Solvent diffusion. Association efficiency 50–69%	[295]
8.	Human recombinant epidermal growth factor	Microemulsion technique; effective *in vivo* targeting	[296]
9.	Insulin	Supercritical fluid technology—association efficiency 75%, water–oil–water double emulsion—association efficiency 35–45%	[292,290]
10.	Streptavidin	Microemulsion technique—association efficiency ~100	[296]
11.	Lysozyme	Cold high-pressure homogenization technique—association efficiency ~100	[297]
12.	TAT peptide	Hot high-pressure homogenization technique. Effective intracellular targeting	[298]
13.	Leuprolide	By combining hydrophobic ion pairing and Oil–Oil emulsion evaporation. Increased entrapment efficiency 74%	[286]

through entanglements, ionic forces, or hydrophobic interactions. Biodegradable hydrogels may be prepared using synthetic or natural polymers. Hydrogels may biodegrade due to the presence of degradable polymer backbones, degradable crosslinks, or pendant chains that can be cleaved from the polymer backbone. Water-soluble