(MM-302; Table 14.2) is being developed clinically by Merrimack Pharmaceuticals. This is a doxorubicin liposome conjugated to an anti-HER2 scFv antibody targeting cancer cells that express HER2, and is in Phase II clinical trials in patients with locally advanced/metastatic breast cancer. Another immunoliposomal delivery system in clinical development is SGT-53 (Table 14.2), which uses an anti-transferrin receptor single-chain antibody fragment to target liposomes encapsulating a p53 DNA plasmid to cancer cells *via* the transferrin glycoprotein receptor (Senzer et al., 2013).

Another targeting approach involves coupling a molecule such as folate or transferrin to the liposome surface; folate and transferrin receptors are expressed to a higher extent in cancer cells, and thus such receptor-targeted liposomes would be expected to have increased uptake in cancer cells (Sapra and Allen, 2003; Felnerova et al., 2004). MBP-426 (Table 14.2) is a pegylated liposome encapsulating oxaliplatin targeted with transferrin to cancer cells expressing transferrin receptors; it is currently in Phase II trials (Suzuki et al., 2008).

Another method to enhance the release of liposomal drugs at specific locations is by use of pH- and thermosensitive liposomes. These are destabilized by an acidic environment or by an elevated temperature, respectively, and release their contents at specific tissues meeting the criteria (Simoes et al., 2004; Andresen et al., 2005). Thermodox (Table 14.2) is a thermally responsive liposomal doxorubicin that releases drug at induced temperatures above 37°C in Phase III trials for liver cancer (Miller, 2013).

### Other Structures

Although not strictly within the scope of this chapter, a few other delivery systems are worth mentioning by virtue of their similarity to liposomes. Bilayer vesicle structures are not limited to phospholipids. For example, cholesterol hemisuccinate vesicles have been proposed as a delivery system for poorly soluble substances (Janoff et al., 1988). Similarly, an Amphotericin B/cholesterol sulfate (1:1) complex, Amphotericin B Colloidal Dispersion, was originally developed by Sequus Pharmaceuticals and now marketed by Three Rivers Pharmaceuticals as Amphotec® (Hiemenz and Walsh, 1996; Noskin et al., 1998; AHFS Drug Information, 2006) (Table 14.1). Niosomes are lamellar structures composed of certain nonionic surfactants, and also have the potential for formulation of water-insoluble drugs.

A related consideration is that incorporation of a hydrophobic drug into a phospholipid bilayer may lead to formation of nonbilayer structures, if high drug/lipid ratios destabilize the lipid bilayer. In general, in situations in which bilayers are destabilized by drug, there will be a threshold lipid/drug ratio below which alternate structures (e.g., micelles, mixed micelles), will be formed. Size exclusion chromatography is probably the best method to measure the relative amounts of liposomes and alternate structures, and to determine the optimum lipid/drug ratio for liposome formation. For example, the metalloporphyrins heme and tin mesoporphyrin form micellar structures at lipid/porphyrin ratios of less than 5 (Cannon et al., 1984; Cannon et al., 1993). Formation of such nonliposomal structures does not preclude their clinical use and commercial development, provided that the structures are well characterized. For example, Amphotericin B destabilizes the liposome bilayer structure when present at a lipid/drug ratio of less than 5:1, giving rise to unusual ribbonlike lipid complexes. A DMPC/DPMP/Amphotericin B (7:3:1) formulation, called Amphotericin B Lipid Complex (ABLC®), is currently marketed as Abelcet® (Table 14.1) (Hiemenz and Walsh, 1996; Physicians Desk Reference (PDR), 2006).

## PREPARATION OF LIPOSOMES

The driving force behind the formation of liposomes is the interaction between water and phospholipids, which have an amphiphilic nature. On one hand, the hydrophilic headgroup prefers the water phase through the hydrophilic interaction. For example, the zwitterionic headgroup of phosphatidylcholine has approximately 15 water molecules weakly bound to it. On the other hand, the