

The Use of Negative Stain Electron Microscopy for the Examination of Pharmaceutical Preparations

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Negative stain electron microscopy is a quick and simple technique suitable for examining pharmaceutical preparations such as vaccines. It is especially appropriate when those preparations contain, viruses, subviral particles, virus-like particles, and particles of a size similar to viruses. A variety of chemicals can be used as negative stains. Routinely we use sodium phosphotungstate as a negative stain to examine preparations of influenza virions and sub-viral particles, and ammonium molybdate for preparations containing other virions.

ISCOMATRIX™ adjuvant consists of saponin, cholesterol and phospholipid, which under defined conditions form cage-like structures typically 40nm in diameter. We use sodium phosphotungstate at pH 7.0 to stain samples of ISCOMATRIX™ adjuvant (Fig. 1). A variety of antigens can be formulated with ISCOMATRIX™ adjuvant to produce ISCOMATRIX™ vaccines[1]. Various ISCOMATRIX™ vaccines have been tested in animal models and in human clinical trials. Some antigens are stable at acid pH while other antigens are only stable at alkaline pH. Cage-like structures can be observed in these vaccines using sodium phosphotungstate at pH 7.0. We investigated whether the cage-like structures existed in vaccines at the pH at which the vaccines were formulated. ISCOMATRIX™ vaccines in acid pH were stained with uranyl acetate and uranyl formate, and ISCOMATRIX™ vaccines in alkaline pH were stained with sodium tetraborate. Cage-like structures were observed in the ISCOMATRIX™ vaccine formulated at acid pH and stained with uranyl acetate (Fig. 2) and also in an ISCOMATRIX™ vaccine formulated at alkaline pH and stained with sodium tetraborate (Fig. 3).

Apo A-1 is the major protein constituent of the antiatherogenic HDL and is a primary candidate for the development of pharmaceuticals for the treatment of cardiovascular diseases [2]. Plasma HDL are spherical particles with a diameter of 7-13 nm. These spherical particles are derived from discoidal “nascent” HDL. To visualise discoidal complexes in rHDL sodium phosphotungstate is used as a negative stain [3] (Fig. 4). Harris [4] recommends a combination of cobalt nitrate and ammonium molybdate as an interesting negative stain for the study of liposomal suspensions. We evaluated this stain with ISCOMATRIX™ adjuvant and rHDL. Using this stain the discoidal complexes of rHDL were not observed but more complex structures were apparent (Fig. 5). Typical cage-like structures were observed with ISCOMATRIX™ adjuvant (Fig. 6).

Negative stain electron microscopy is a quick and simple technique for examining pharmaceutical preparations. Some samples show a consistent morphology no matter what stain is used. In other samples the morphology observed depends on the negative stain that is used.

References

- [1] M.J. Pearse and D. Drane, *Advanced Drug Delivery Reviews* 57 (2005) 465
- [2] C.R. Sirtori et al. , *Atherosclerosis* 142 (1999) 29
- [3] A.V. Nichols et al. , *Biochim. Biophys Acta* 750 (1983) 353
- [4] J.R. Harris *Negative Staining and Cryoelectron Microscopy*, Bios Scientific Publishers Limited, Oxford, 1997

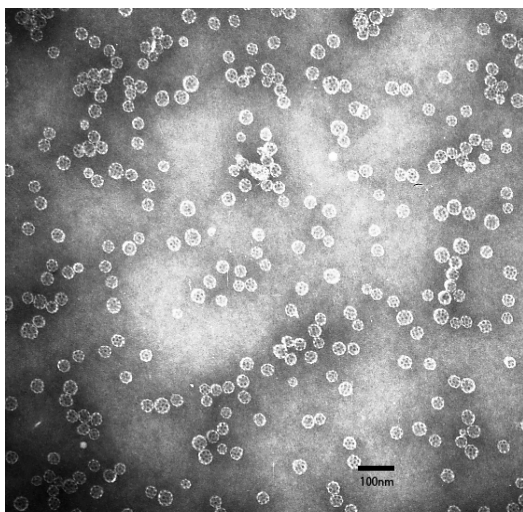


FIG 1: ISCOMATRIX™ adjuvant stained with sodium phosphotungstate at pH 7.0

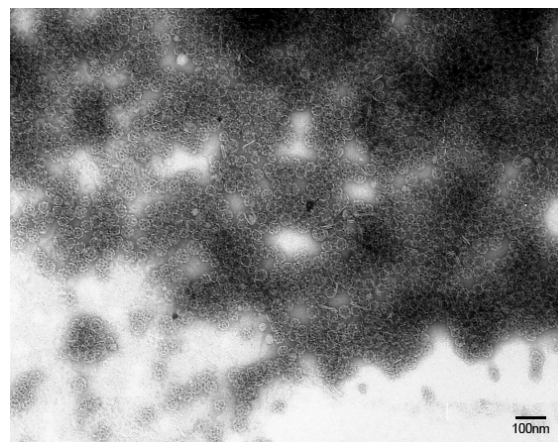


FIG 2: ISCOMATRIX™ vaccine formulated at acid pH and stained with uranyl acetate

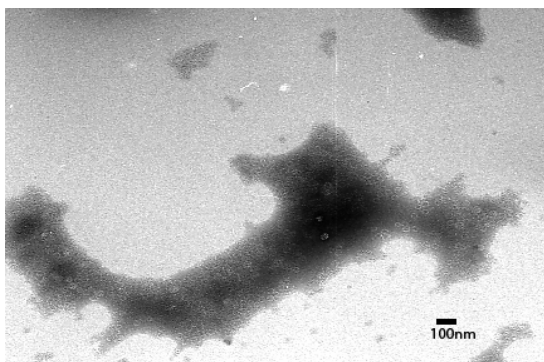


FIG 3: ISCOMATRIX™ vaccine formulated at alkaline pH and stained with sodium tetraborate

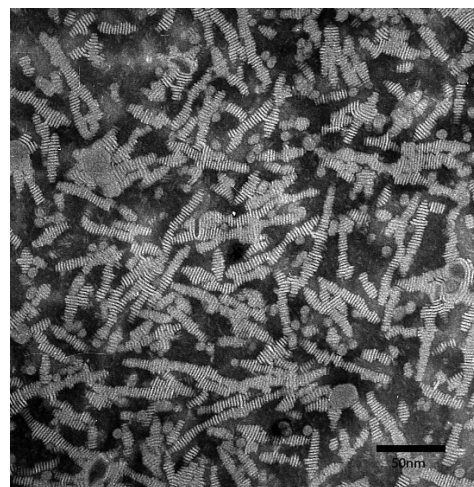


FIG 4: rHDL stained with sodium phosphotungstate, pH 7.0

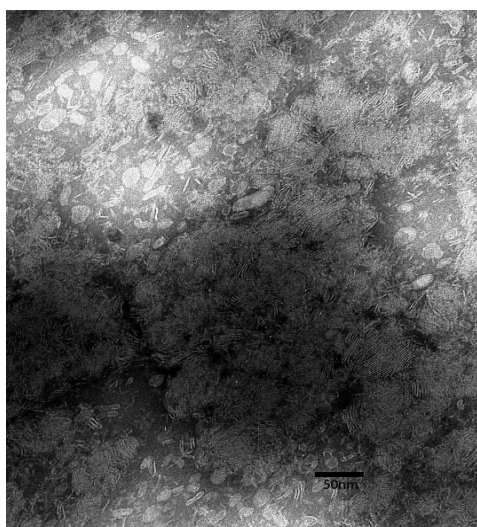


FIG 5: rHDL stained with a combination of cobalt nitrate & ammonium molybdate

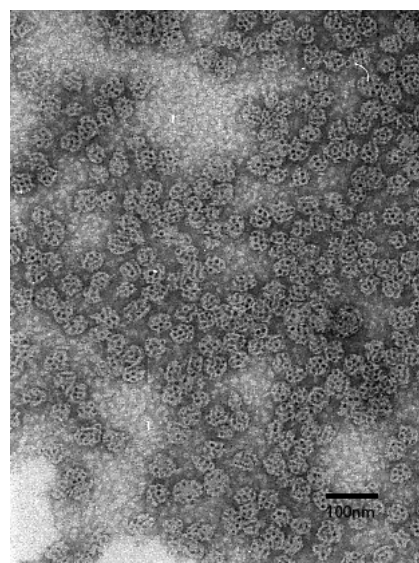


FIG 6: ISCOMATRIX™ adjuvant stained with a combination of cobalt nitrate and ammonium molybdate.