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Invited review

Electron microscopy of pharmaceutical systems

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ABSTRACT

During the last decades, the focus of research in pharmaceutical technology has steadily shifted towards the development and optimisation of nano-scale drug delivery systems. As a result, electron microscopic methods are increasingly employed for the characterisation of pharmaceutical systems such as nanoparticles and microparticles, nanoemulsions, microemulsions, solid lipid nanoparticles, different types of vesicles, nanofibres and many more. Knowledge of the basic properties of these systems is essential for an adequate microscopic analysis. Classical transmission and scanning electron microscopic techniques frequently have to be adapted for an accurate analysis of formulation morphology, especially in case of hydrated colloidal systems. Specific techniques such as environmental scanning microscopy or cryo preparation are required for their investigation. Analytical electron microscopic techniques such as electron energy-loss spectroscopy or energy-dispersive X-ray spectroscopy are additional assets to determine the elemental composition of the systems, but are not yet standard tools in pharmaceutical research. This review provides an overview of pharmaceutical systems of interest in current research and strategies for their successful electron microscopic analysis. Advantages and limitations of the different methodological approaches are discussed and recent findings of interest are presented.

Contents

Abbreviations: EM, electron microscopy; TEM, transmission electron microscopy; SEM, scanning electron microscopy; CCD, charge-coupled device; SE, secondary electron; BSE, backscattered electrons; TSEM, transmission scanning electron microscopy; STEM, scanning transmission electron microscopy; FIB, focus ion beam; ESEM, environmental scanning microscopy; FF-EM, freeze-fracture EM; AEM, analytical electron microscopy; EELS, electron energy-loss spectroscopy; EDS, energy dispersive X-ray spectroscopy; EFTEM, energy-filtered TEM; AES, Auger electron spectroscopy; DLS, dynamic light scattering; FE-SEM, field-emission SEM; HEED, high energy electron diffraction; HAADF, high angle annular dark field; SLN, solid lipid nanoparticles; NLC, nanostructured lipid carriers; FFDI, freeze-fracture direct imaging; PLGA, poly lactic-co-glycolic acid.

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1. Introduction

Electron microscopy (EM) is an important asset to modern pharmaceutical technology. Complex drug delivery systems, but also individual compounds such as adjuvants, drugs and possible impurities, can be directly characterised with different EM techniques, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), cryo EM, analytical EM techniques or combinations thereof. Although optical light microscopy may provide a rapid overview especially in case of conventional pharmaceutical systems with morphologies in the micrometre range, a detailed characterisation of most modern systems is not possible in this way. During the last decades, pharmaceutical research has increasingly focused on nano-scale drug delivery systems such as nanoparticles or lipid nanocarriers. This trend necessitates the concomitant development of suitable techniques to obtain reliable information about the morphology of the systems with near atomic resolution. Moreover, the elemental composition of the observed systems is required to prevent erroneous interpretations or to confirm the successful design of multicomponent systems.

EM offers numerous possibilities for both visualisation and elemental analysis of pharmaceutical systems with dimensions in the nano-scale range. Both solid and hydrated samples can be characterised after appropriate preparation. Classical methods of TEM and SEM require less effort in this respect while cryo preparation methods are more time-consuming and costly. Analytical EM methods allow not only for the characterisation of the structural appearance of different samples, but also their chemical composition. These methods are likely to be of great importance in future research of nanomaterials in pharmaceutics.

The different EM techniques employed for analysis of pharmaceutical systems today will be briefly presented in the following chapters. Since the specific terminology in the field of pharmaceutical technology may lead to misunderstandings regarding the required methods of sample preparation for EM, we provide a short but helpful overview of important pharmaceutical systems and possibilities for their characterisation by EM. In this context, focus is laid on findings published within the last years, since many technological developments of interest are comparatively new. We hope that this article will instigate future research by presenting successful examples of EM analysis adapted for pharmaceutical preparations.

2. Electron microscopy of pharmaceutical systems

An electron microscope uses electrons instead of light for the imaging of objects. The resolving power of a microscope is a linear function of the wavelength. Thus, the use of electrons, which have wavelengths about 100,000 times shorter than the photons of visible light, allows for a resolution better than 50 pm (Erni et al., 2009) While light microscopes are constrained to a resolution of around 200 nm, the spatial resolution achieved with multi-purpose TEMs today is generally around 0.2 nm (Kane and Sternheim, 1978; Perez-Arantegui and Mulvey, 2005); thus, much smaller structural details can be visualised.

The development of the electron microscope was based on theoretical work done by Louis de Broglie, who found that wavelength is inversely proportional to momentum. In 1926, Hans Busch discovered that magnetic fields could act as lenses by causing electron beams to converge to a focus. A few years later, Max Knoll and Ernst Ruska made the first modern prototype of an electron microscope. When electrons enter a material, they interact with the constituent atoms via electrostatic, i.e. Coulomb forces. As a result of these forces, some electrons are scattered; the direction of their momentum is changed and in many cases they transfer an appreciable amount of energy to the specimen (Williams and Carter, 2009). After the interaction of electron beam with the specimen, the electrons are focused, collected, processed and a two dimensional projected image of the three dimensional sample structure is obtained.

There are two major types of electron microscopes: transmission electron microscopes and scanning electron microscopes. The different working principles are explained briefly in the following sections. In all conventional electron microscopes, the specimens are viewed at room temperature under high-vacuum since gas molecules may scatter the electrons and bias the analysis. Under these conditions, only solid pharmaceutical systems such as powders or nanoparticles can be viewed with satisfying accuracy. Hydrated pharmaceutical systems, such as emulsions,