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Note

Use of back-scattered electron imaging as a tool for examining matrix structure of calcium pectinate

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Abstract

The internal structure of pharmaceutical solid dosage forms is commonly revealed by secondary electron imaging using standard scanning electron microscopy (SEM) technique. In this work we propose a back-scattered electron imaging (BEI) as a new tool for examining the matrix structure of calcium pectinate beads. Imaging samples with back-scattered electrons in the SEM is based on material or atomic number contrast. High atomic number elements, such as calcium, reflect more electrons and appear bright on electron micrographs. The BEI–SEM images of calcium pectinate matrix beads clearly showed net-like structure of calcium pectinate and uniform distribution of drug particles. The matrix compositions were confirmed by energy dispersive analyzer. The result demonstrates the advantageous of BEI for examining the matrix structure of calcium pectinate.

Keywords: Scanning electron microscopy (SEM); Back-scattered electron imaging (BEI); Matrix; Calcium pectinate; Pectin

The polysaccharide pectin is an inexpensive, non-toxic product extracted from citrus peels or apple pomaces. Pectin consists mainly of linearly connected α-(1 → 4)-D-galacturonic acid residues which have carboxyl groups. The degree of esterification (DE), which is expressed as a percentage of carboxyl groups (esterified), is important mean to classify pectin. Low methoxy pectin (with DE < 50%) form rigid gels by the action of calcium, which cross-links the galacturonic acid chains (Rolin, 1993). Since pectin can react with calcium ions, it is being investigated as a carrier material for different controlled release systems. Recently, matrix beads of calcium pectinate have been investigated as sustained release drug delivery system (Sriamornsak and Nunthanid, 1998, 1999).

Imaging an internal structure of matrix dosage forms is important to understanding how solid drug particles disperse in the matrix structure, which influences the mechanism of drug release. High resolution images of the morphology or topography of a specimen can be achieved by scanning an electron beam across a specimen. The incident electrons cause low energy secondary electrons to be generated, and some escape from the surface. The emitted secondary electrons are detected and provide information on the topographical nature of the specimen. Excellent depth of field at high or low magnifications can be achieved. Some of the incident electrons may strike an atomic nucleus and bounce back into the vacuum. These electrons are known as primary back-scattered electrons and provide compositional information. By far,

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the most common, however, is image formation by means of the low-energy secondary electrons, known as secondary electron imaging (SEI) or standard SEM imaging.

Back-scattered electron imaging (BEI), unlike SEI, uses high-energy elastic electrons to detect differences in atomic number. Higher atomic number elements reflect or highly deflect more electrons along the primary electron axis, where the detector is located. Lower atomic number elements absorb more electrons and appear dark on electron micrographs. Images are the result of atomic number contrast. On flat specimens, this technique may be quite informative, but BEI is sensitive to topography, so this method is suited to specific types of specimens.

In this study, we prepared calcium pectinate matrix beads by ionotropic gelation method as described in previous reports (Sriamornsak and Nunthanid, 1998, 1999). The aqueous solution (5%, w/w) of pectin (GENUpectin type LM-104 AS-FS, Copenhagen Pectin, Denmark) containing metronidazole was extruded into 0.3 M calcium chloride and allowed to stand for 20 min, then washed and dried at 37 °C for 12 h. The dried samples were either uncoated or coated with gold to a thickness of 30 nm. The scanning electron microscope (Model Maxim, CamScan, UK) equipped with secondary electron detector and back-scattered electron detector was used to examine the surface and cross-section of matrix beads. The energy dispersive X-ray spectroscopy (Model Econ-4, EDAX, USA) connected to the SEM was used to analyze metal elements in the structure.

Fig. 1 shows images of the surface and cross-section of gold-coated sample, formed using SEI. These images yielded some information on morphology (i.e. size and shape) and topography of matrices. However, they did not demonstrate a detailed structure of the chemically formed matrix of drug-loaded calcium pectinate. Moreover, the images, which have been metalized with gold in order to avoid charge effects, did not show differences in color. This is probably due to the equivalent emission of secondary electrons (from gold atoms) when an electron beam hit a gold-coated sample.

Fig. 2 shows back-scattered electron images of drug-loaded calcium pectinate matrix beads. When an electron beam hits a sample, back-scattered electrons, among other signals, are emitted. The quantity of back-scattered electrons is highly dependent on the atomic number of the sample. Material with a larger atomic number will give off more back-scattered electrons. Calcium has a much higher atomic number than carbon atoms of drug molecule and therefore gave off many more electrons. The calcium signal was much more intense, and in this figure the calcium pectinate structure appeared brighter than drug particles.

The application of BEI to biological samples has been mainly conducted at relatively high primary beam accelerating voltages (25 kV in this study). This procedure can cause considerable radiation damage to sample surfaces (Pawley and Erlandsen, 1988). This must be considered if applying BEI technique to similar preparations which their melting point is fairly low. When the accelerating voltage applied to the primary beam is lowered, however, the primary electron range within the sample is decreased, thereby reducing the interaction volume. The net charge deposition is also reduced. This may actually increase radiation damage due to the same number of electrons being present in a smaller volume.

Fig. 3 demonstrates a SEM view of calcium pectinate structure and the energy spectrum of the characteristic X-rays emitted from the calcium. A close look in Fig. 3a indicated that no crystal of calcium was found in the structure. This is probably due to the calcium was cross-linked with pectin or entrapped in calcium pectinate structure. Inter-molecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules called an ‘egg-box’ conformation with interstices in which the calcium ions may pack and be coordinated (Grant et al., 1973).

A modern trend in electron microscopy is to fit X-ray analysis equipment as a bolt-on accessory. Bombarding a specimen with electrons causes X-rays of characteristic wavelengths and energies to be emitted from the spot where the beam strikes the specimen. Computer analysis of the wavelength or energy spectra makes it possible to measure accurately the nature and quantity of different elements in the material. The technique is of great value in elucidating of metal-contained matrix structure because the metals, such as calcium, produce an X-ray signal whereas light elements (e.g. carbon) produce a weak X-ray signal. In the energy spectrum (Fig. 3b) the X-ray peaks from calcium (Ca) and chloride (Cl) are iden-

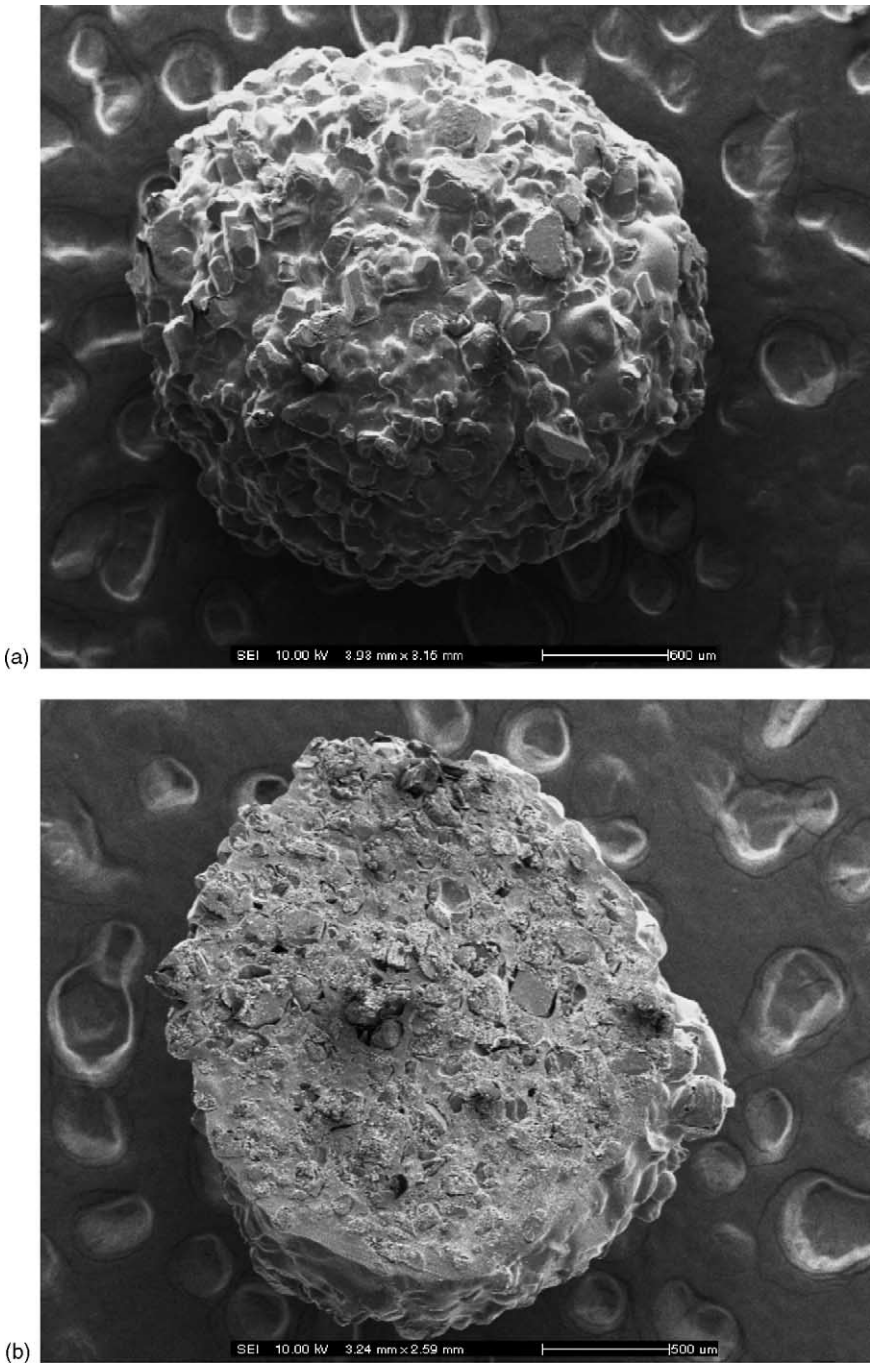

Fig. 1. Images of (a) external and (b) internal structure of drug-loaded calcium pectinate matrix bead formed using secondary electron imaging. The samples were metallized with gold using a cathodic pulverizer.

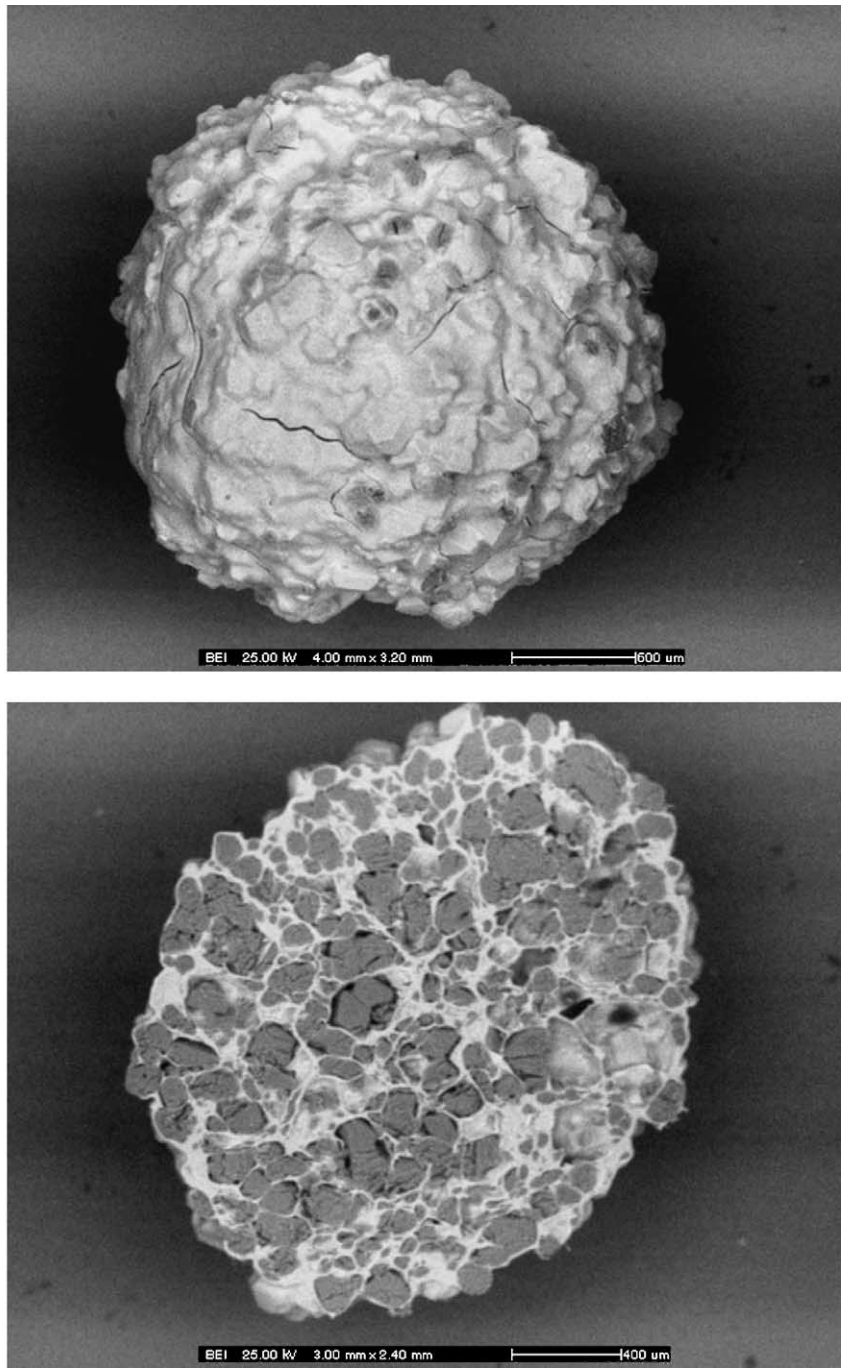

Fig. 2. Images of (a) external and (b) internal structure of drug-loaded calcium pectinate matrix bead formed using back-scattered electron imaging.

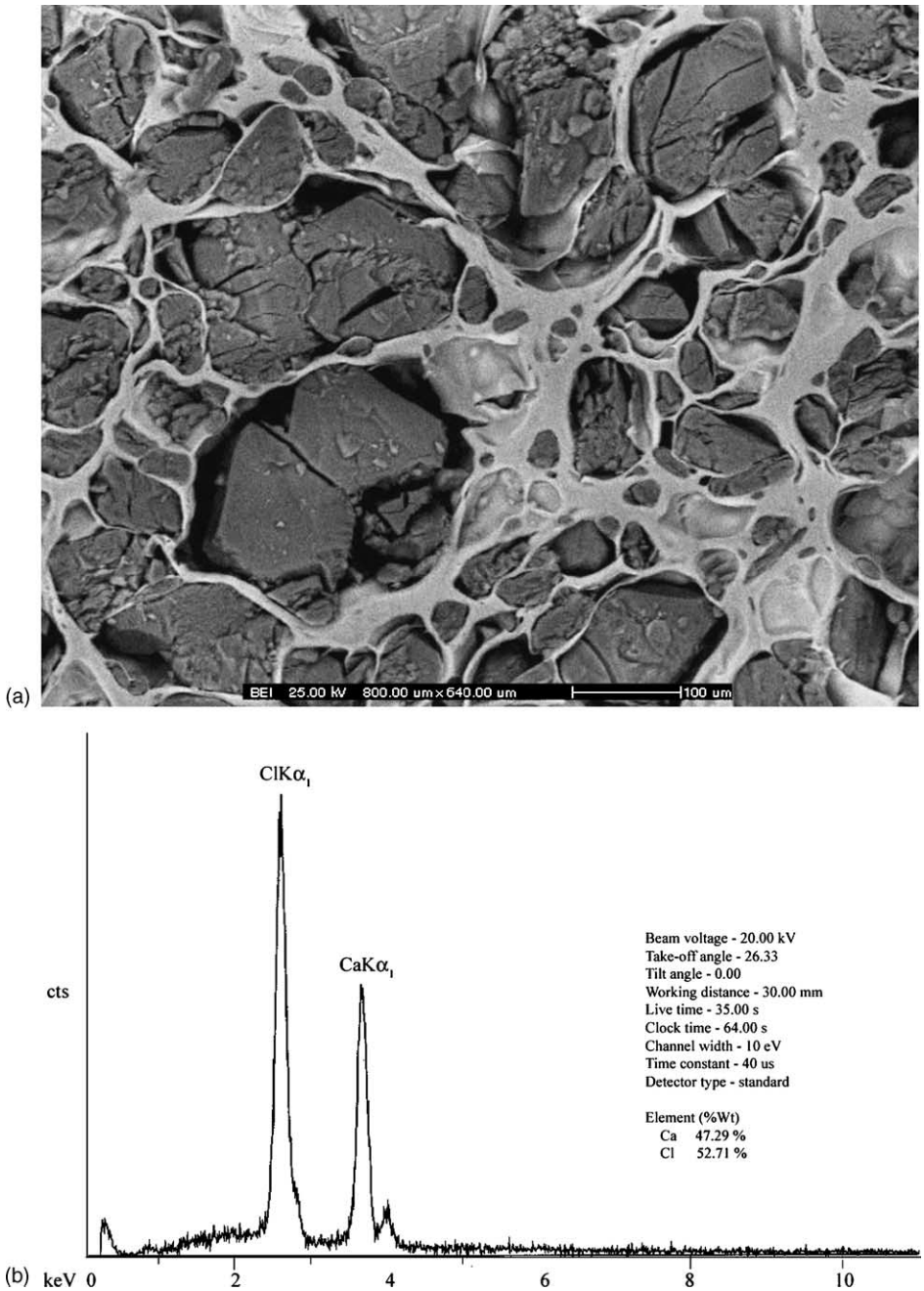

Fig. 3. (a) Internal structure of drug-loaded calcium pectinate matrix showing net-like structure of calcium pectinate and unifomly distributed drug particles and (b) the energy spectrum of the characteristic X-rays emitted from the metals of calcium and chloride. In the energy spectrum the X-ray peaks from calcium (Ca) and chloride (Cl) are identified.

tified when an electron beam hit a bright (white) area of Fig. 3a. The calcium salts presented in the matrix beads are likely to be the composition of the bead skeleton, i.e. calcium pectinate. The chloride residues found in the spectrum may come from calcium chloride that used in the gelation process.

In conclusion, both methods (i.e. SEI and BEI) of high-resolution microscopy are complementary and lead to access different information. This is due, in particular, to the different sample preparation methods used for these techniques. The advantage of BEI is the simple sample preparation as no gold metallization is needed before operation. In addition, the BEI allows observation of the structure of metal-contained sample. In the present study, the BEI images of calcium pectinate matrix beads clearly showed net-like structure of calcium pectinate and uniform distribution of drug particles. The matrix compositions were confirmed by energy dispersive X-ray spectroscopy.

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