NOTE ON A LIGHT MICROSCOPE TECHNIQUE TO PREVIEW SPECIMEN MOUNTINGS FOR SCANNING ELECTRON MICROSCOPY

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The scanning electron microscope (SEM) is fast becoming an indispensable tool for examining particulate structures of interest in biological and food systems. Although many laboratories do not have sufficient needs to justify having their own SEM facilities, consulting laboratories are available to provide this service. Our experience with this type of service indicated a distinct advantage was derived from mounting the specimens in our laboratory before sending them to the SEM service laboratory for examination. However, it soon became apparent that a method was needed to monitor gross sample properties and specimen distribution on the mounting. The present light microscopic technique was devised for previewing specimen mountings to select those which were satisfactory for SEM examination. The technique of dual examination of lymphoid cells by light microscopy (LM) and SEM was previously used by Wetzel *et al.* (1), but for a different purpose.

MATERIALS AND METHODS

Sample Materials

Two types of samples were examined: 1) commercial spray-dried soy protein isolate (Edi-Pro N, Ralston Purina Co., St. Louis, Mo.) and 2) finely ground, defatted soy flakes. No special fixation or drying treatments were used for these materials.

Specimen Mounting

Samples were suspended in hexane with a Vortex mixer, and 1 drop of the suspension was transferred to a 5 × 5-mm section of a LM slide. After evaporation of the hexane, the particles adhered sufficiently to the glass section to permit careful handling, LM examination, coating, and SEM examination. After LM examination, the glass sections were attached to aluminum SEM stubs with double-stick tape, then coated and examined by SEM.

Hexane-dispersed samples were also mounted directly onto aluminum SEM stubs, then coated and examined by conventional procedures.

Light Microscopy

The glass-mounted specimens were placed on a LM slide and examined with a Zeiss bright field binocular microscope equipped with a Polaroid Land Camera. Regions of special interest, identified at various magnifications, were photographed and circumscribed with a needle to provide demarcation lines for easy recognition under SEM.

Scanning Electron Microscopy

Specimens were coated with gold using an ISI Model PE-5000 sputter-coating

apparatus (International Scientific Instruments, Inc., Palo Alto, Calif.). SEM was done with a Model MSM-5 Mini-SEM (International Scientific Instruments, Inc., Palo Alto, Calif.) operated at 15 kV. Specially outlined regions on the specimen mountings were located in the SEM at 50× magnification. They were subsequently examined at up to 5000× magnification and at a 35° to 40° tilt angle.

RESULTS AND DISCUSSION

The present preview technique was found suitable for routine examination of

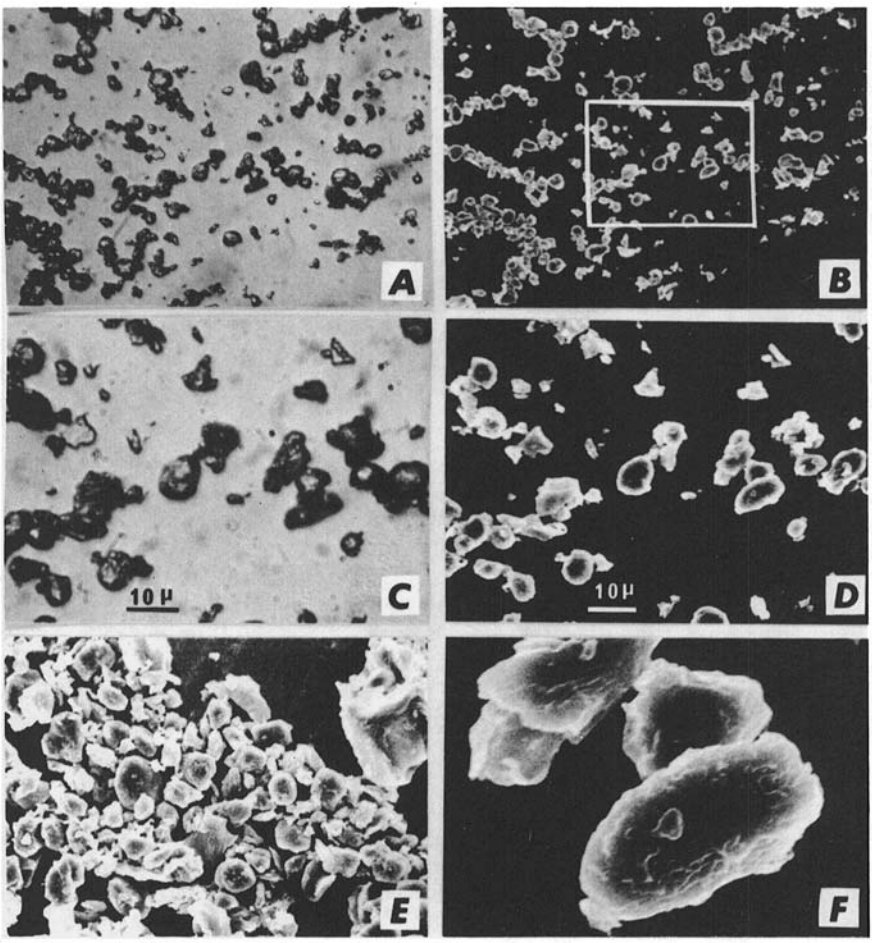

Fig. 1. LM and SEM micrographs of ground, defatted soy flakes. A and B, LM and SEM micrographs (400×) of the same specimen region within demarcation lines; C and D, LM and SEM micrographs (1000×) of outlined region of B; E, SEM micrograph of direct stub-mounted specimen (1000×); and F, SEM micrograph (5000×) of particles located in the center right area of D. All except E were mounted on glass sections.

particles by the LM and SEM procedures. This technique enables us to examine the specimen mounting rapidly and locate special regions which possess interesting properties for further SEM examination. Two examples are shown in Fig. 1 and 2 to stepwise demonstrate the results obtained from this preview technique.

The regions within the demarcation lines located by LM at 400× and 1000× magnifications are presented in micrographs A and C of both figures. The same regions were easily identified and examined by SEM, using the demarcation lines plus these LM micrographs as reference (micrographs B and D of both figures). Selected particles of interest within these regions were then further examined at

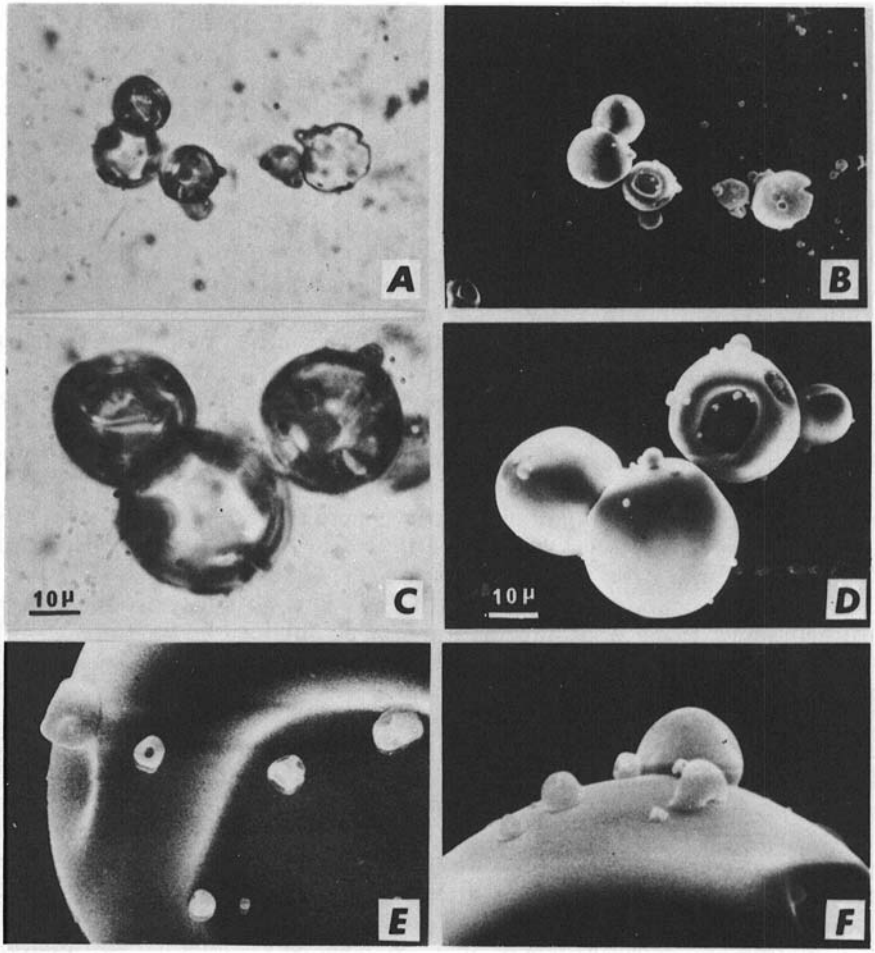

Fig. 2. LM and SEM micrographs of spray-dried soy protein isolate. A and B, LM and SEM micrographs (400×) of the same specimen region within the demarcation lines; C and D, LM and SEM micrographs (1000×) of the particles in the center of A and B; E and F, SEM micrographs (5000×) of the particles in the upper right corner of D.

higher magnification by SEM (micrographs F of Fig. 1; E and F of Fig. 2). An additional benefit from using glass slides for specimen mounting is that they provide a smoother background than metal SEM stubs (micrographs D and E of Fig. 1).

The particles in finely ground, defatted soy flakes (Fig. 1) are similar to those previously reported for defatted soy flour (2). They are mostly less than 10 μ in size and are smaller and less regularly shaped than the spray-dried protein isolate particles (Fig. 2). The latter particles appear as hollow spheres with indentations and attached smaller particles of similar shape (2).

Comparison of the LM and SEM data for both samples reveals that these two techniques provide totally different information regarding the surface characteristics of the particles. SEM obviously provides much more meaningful information than LM in this regard.

As indicated in the procedure, the dry particle specimens were first suspended in hexane and then applied to the glass section. This method assures a more uniform specimen distribution than that achieved by spreading the dry particles directly onto the specimen support. Other volatile solvents besides hexane, which retain structural integrity of the specimens, may also be used to disperse the specimens. Normally, fine particulate specimens adhere well enough on the glass surface to allow reasonable handling and SEM examination. Otherwise, they may be transferred from the glass surface to a transparent double-stick tape by gently pressing the tape over them. The tape is then secured to a new glass section and examined by LM and SEM. Also, the specimens may be gold-coated immediately after LM previewing to provide complete stability during handling for subsequent SEM examination. Modifications along these lines are always possible.

The present LM preview technique has the following advantages over conventional SEM specimen handling techniques:

1. The researcher can mount his own specimens and examine them by LM to assure proper sample selection and particle distribution. He can also select regions of mounting for SEM examination.
2. The SEM operator can preview the LM micrograph to study its gross properties and decide what information may be obtained by SEM.
3. Considerable time and money can be saved by selecting only those specimen mountings that have been satisfactorily prepared.
4. In process and product development where the SEM is needed to evaluate proper processing conditions, this LM preview technique provides immediate screening and selection of samples that demonstrate significant change.
5. Differences between LM and SEM micrographs can be reliably compared for the same particles on the specimen mounting.

In conclusion, results demonstrate that the present LM preview technique allows better specimen selection prior to SEM examination and provides additional information on particulate materials, such as those in plant and protein structures, which cannot be obtained by the SEM alone. The information gained is useful to the SEM operator for obtaining the best possible data in the shortest time and at a reduced cost.

Literature Cited

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