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CONFERENCE CONTRIBUTION

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The production of powdered candidate biological and environmental reference materials in the laboratories of the Joint Research Centre

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Abstract The production of candidate biological and environmental reference materials in the laboratories of the Joint Research Centre, mainly on behalf of BCR, started in 1972 in Ispra and became concentrated in 1984 in Geel. Today, dedicated facilities for the transformation of biological and solid or liquid materials into dry powder samples are in use. The major guidelines followed in the conception of these facilities are described. Some typical examples of facilities developed or adapted to fulfil the specific requirements of professional CRM preparation are discussed, e.g. the whole-Teflon cryo-grinding equipment, the in-line controlled freeze-drier, the special facility for clean and dry handling of powders, the milling with classification equipment and the automation of bottling and labelling operations.

1 Introduction

As H. Muntau described in his presentation on 25 years of environmental reference material production, the Joint Research Centre [1] (JRC) in Ispra was already active in this field since 1972. Meanwhile, the Central Bureau for Nuclear Measurements in Geel (now IRMM) was active in the non-ferrous metal field. In 1984 it was decided to concentrate the JRC CRM activities in Geel. The success of this operation was largely due to the good cooperation between the two laboratories, so that the experience and know-how accumulated in Ispra could be transferred in an optimal way. These activities are developed in close cooperation with the research programmes of the European Commission.

Today, facilities for the preparation of biological and solid or liquid environmental materials into dry powder samples exist at IRMM.

The major guidelines followed in the conception of these facilities are [2]:

- *to be able to handle large quantities of base material in a short time*, as this is an important prerequisite to be able to produce CRMs at a unit price which is economically acceptable; IRMM facilities are conceived to handle typically 200 to 500 kg fresh material in order to produce up to 2000 samples of 20 to 50 g dry powder;
- *to prepare samples of the correct sample size*, which often means larger series of smaller samples (to avoid spoilage, contamination or conservation problems in the end user's laboratory); this requires a fair amount of automation to avoid lengthy and costly bottling and labelling operations;
- *to prepare samples under safe conditions*, which means that preparations must take place under a clean atmosphere, cold temperature, that dry products are prepared and packed under inert gas. Care must be taken that particle size distributions are adequate. Such products offer better guarantees of stability. This may have a beneficial influence on the cost of both storage and distribution.

Some typical examples of facilities developed or adapted to fulfil these specific requirements for CRM preparation are discussed below.

2 Whole-Teflon cryo-grinding equipment

The Teflon cryo-grinding equipment for the preparation of quantities up to 200 kg of biological materials was built from existing commercial equipment. It consists of a *jaw-crusher* and *two upgraded ball mills*.

The jaw-crusher is used for continuous pre-crushing the material down to a particle size of 1 to 10 mm [3]. The width of the gap between the two jaws and the angle of in-

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Table 1 Summary of recent production of BCR reference materials using cryo-grinding and jet milling techniques

BCR reference material	Description	Applied preparation technique
CRM 422	trace elements in cod muscle	cryo grinding
CRM 487	vitamins in pig liver	
CRM 185R	trace elements in bovine liver	
CRM 474 & 475	bovine liver for trenbolone analysis	
CRM 648 & 649	bovine liver for β-agonist analysis	
CRM 462	TBT and DBT in coastal sediment [9]	jet milling
CRM 481	PCBs in industrial soil [10]	
CRM 427 & 428	various organic constituents in soil	
CRM 529 & 530	PCDD/Fs in soil	
CRM 535 & 536	PAHs and PCBs in harbour sediment	

clination of the moving jaw are important parameters for the efficient use of the jaw-crusher. Under optimal conditions, a material of a grain size smaller than 4 mm can be produced at a rate of 14 kg/h. Two ball mills equipped with Teflon bowls of 1.25 L, filled with Teflon balls of 20 and 30 mm, are used in parallel for the batchwise fine milling. The system allows to produce per hour 3 kg of powder of less than 125 μm.

However, it was recently observed, that if a biological material is freeze-dried before ball milling, the production rate can be increased by a factor of 4, as liquid nitrogen cooling can be omitted [4].

The BCR references materials prepared according to these techniques are presented in Table 1.

3 In-line controlled freeze-drying

The freeze-drier used at IRMM is a Martin Christ type Epsilon apparatus [4]. It has been adapted to present a shelf surface area of 5.4 m^2, a product shelf temperature adjustable from –60 to +60° C, and an ice condenser temperature of –85° C. A vacuum pump system with a flow rate of 68 m^3/h provides a final vacuum of 0.4 Pa.

An automatic load measuring appartus was developed at IRMM to be particularly used for monitoring the weight loss of a sample during freeze-drying at temperatures of –30 to 40° C and vacuum up to 0.4 Pa. This important measurement provides information on the stage of the sample during the freeze-drying process. It allows the calculation of the moisture content without stopping this process. For moisture-sensitive materials, e.g. orange juice powder (CRM 478), to be certified for sugars and amino acids, an interruption of the freeze-drying process to determine the residual water content would cause the material to loose its solid structure (collapse) by taking up moisture from the air.

The automatic load cell measuring system is shown in Fig. 1 and consists of [5] a load cell model 4021 (Sensy USA), two microswitches mounted on two shelves, an electro-hydraulically movable device inside the chamber, an industrial electronic indicator Model T-10 (Ohaus) with RS 232 receptacle and a personal computer with printer and two timers outside.

The tested load cell was screwed on a home-constructed stable stainless steel holder and mounted on the shelf above the electro-hydraulically movable device in the freeze-drying chamber of the Epsilon freeze drier (Fig. 1).

The equipment is also used for re-drying materials sampled in penicillin vials. It can also be applied to batchwise closing of large numbers of vials after filling them with pure argon. The latter procedure is of the utmost importance, especially for biological matrices. It allows the preparation of powdered CRMs with moisture contents between 0.3 and 3%. It was demonstrated on numerous occasions that, if handled as above, sensitive matrices like fruit juices, milk-, curd-, or cod muscle powders show no alteration even after several years of conservation at room temperature [6, 7].

4 Facility for clean and dry handling of powders

Clean handling of materials to be processed into reference materials not only avoids contamination of materials by atmospheric or equipment pollution (e.g. by heavy metals), but also prevents microbiological and chemical activity in the samples. This is “always” achieved by working under clean conditions. Also important for long-term stability is that the produced material remains dry.

Therefore, handling and bottling must be done under perfectly controlled and protective atmospheric conditions. For that purpose IRMM has set up a versatile set of interconnected glove boxes linked to a gas purification system [4].

In general the system is operated under dry air conditions (< 77 mg/m^3 H_2O), but depending on the product protection required it may also be used, under dry N_2 or Ar atmosphere. In extreme cases a liquid nitrogen supply may be arranged to carry out cryo-grinding.

5 Milling with ultrafine particle size segregation

Reference materials with selected particle size distributions allow to achieve homogeneity even when very low sample intakes are used for measurements (some μg).

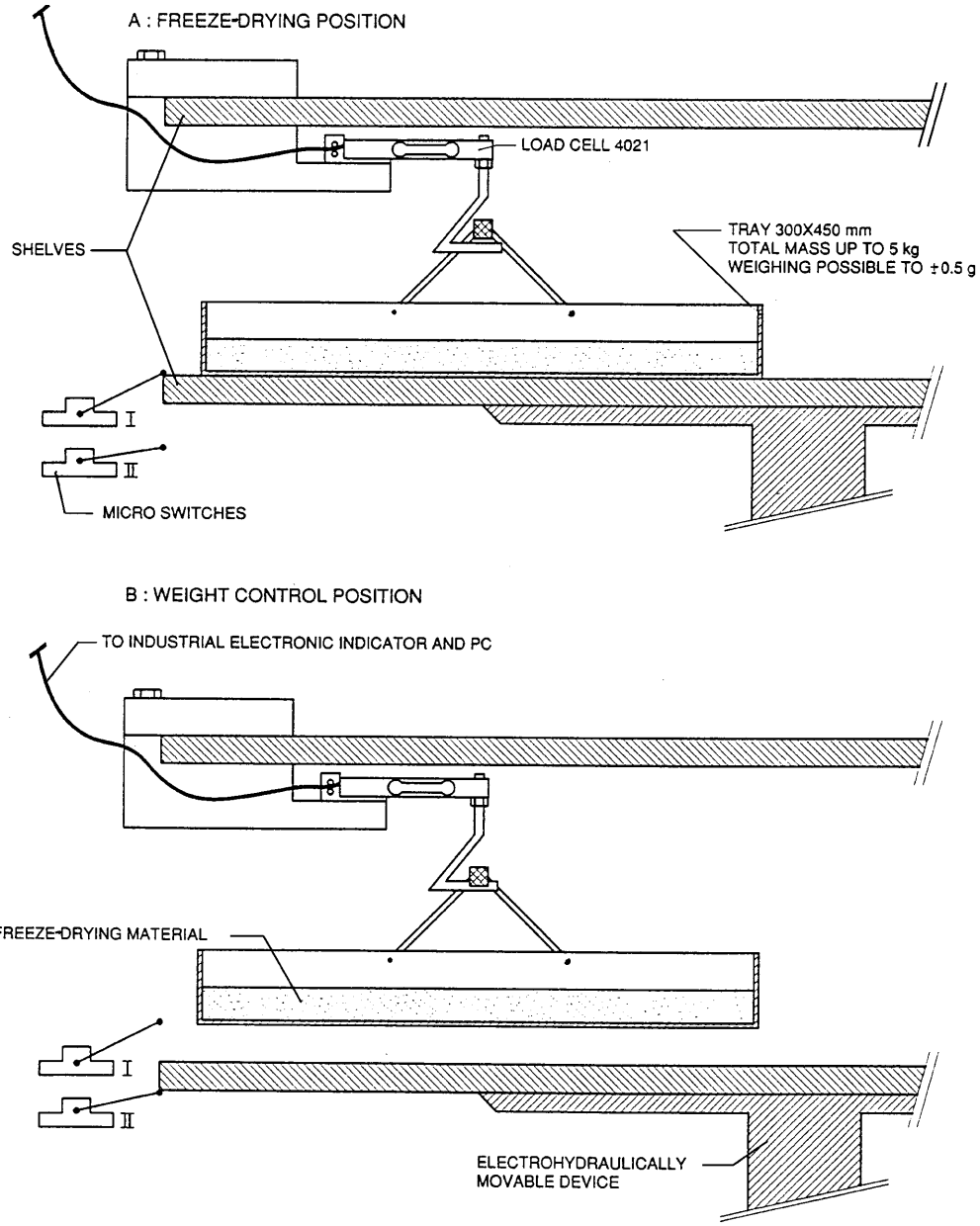


Fig. 1 A, B Automatic weight control system during freeze-drying at shelf temperatures of –30° C to 40° C and vacuum up to 2 Pa. The design shows under **A**: "freeze-drying position", a tray filled with drying material and a mass up to 5 kg placed on the movable device. Weighing is possible to ± 0.5 g. The automatic mass control system is fitted with two timers. A typical weight control cycle is: weight control position 10 s, freeze-dry position 1 h. The movable tray moves to the "weight control position" at microswitch II (see under **B**). The mass is measured and after 10 s the tray moves back to microswitch I "freeze-drying position". After a given time (e.g. 1 h) the cycle restarts. The load cell is connected by a vacuum tight connection to the electronic indicator outside the freeze-drying chamber. The RS 232 receptacle of the indicator is connected to a personal computer

Milling with ultra-fine segregation of particles can be achieved using the Multi-Processing System from Alpine, Augsburg (D) [8]. It is a non-contaminating, fast and well-controlled jet or impact mill. In jet milling the material is ground by impact of particles on each other accelerated from three high-pressure air streams. It generates a size reduction of material without significant heat generation. Fine powders can be produced with closely defined particle size distributions with throughputs of 5 to 25 kg/h. Several recent BCR reference materials (Table 1) or candidate reference materials have been prepared using the jet mill procedure [9, 10].

In the impact mill, a better disc equipped with two beaters rotating at 12 000 to 20 000 rpm is mounted in the bottom section of the grinding chamber. Tests on hay powder and rye grass revealed that well selected particle size distributions and, consequently, homogeneities even for very small test sample intakes could be obtained [11].

6 Automated mixing and dispensing

In general, certified reference materials are expensive. Therefore, analysts prefer CRM units which allow multiple determinations and can be used over a long period of time. However, under such circumstances the risk that samples suffer instability or contamination is not negligible. Therefore, it is preferable to prepare samples in single use or at least limited portions (few repetitions). This increases production cost, which might become unacceptable. However, applying automated preparation procedures limits preparation costs.

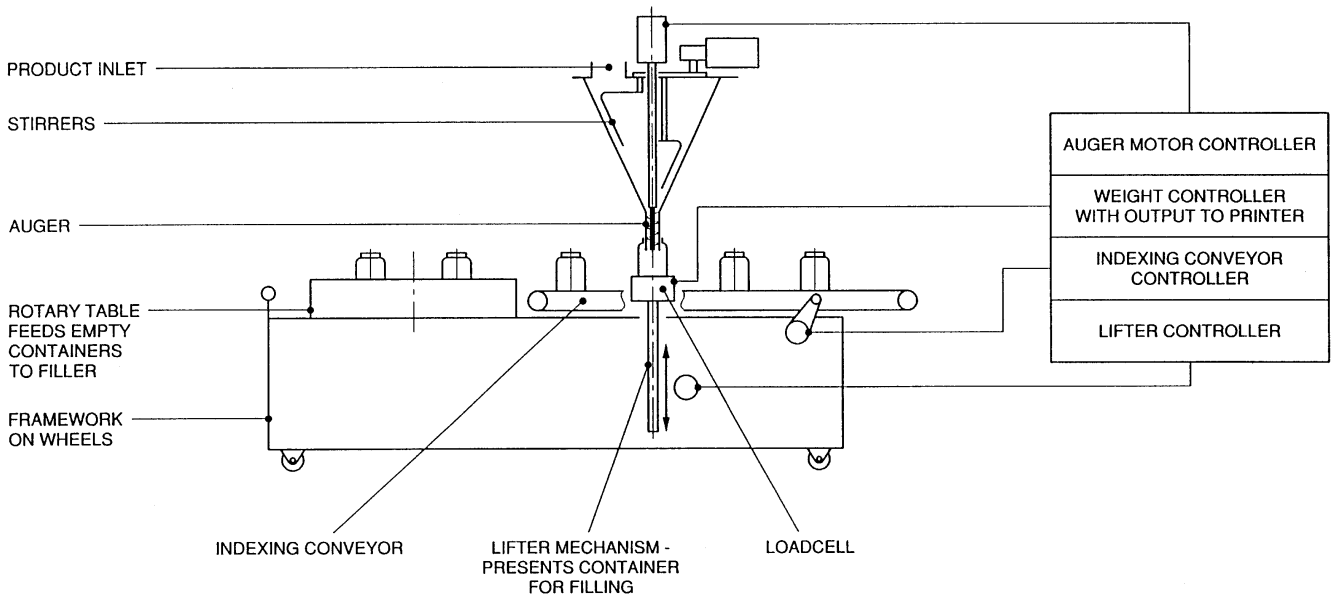
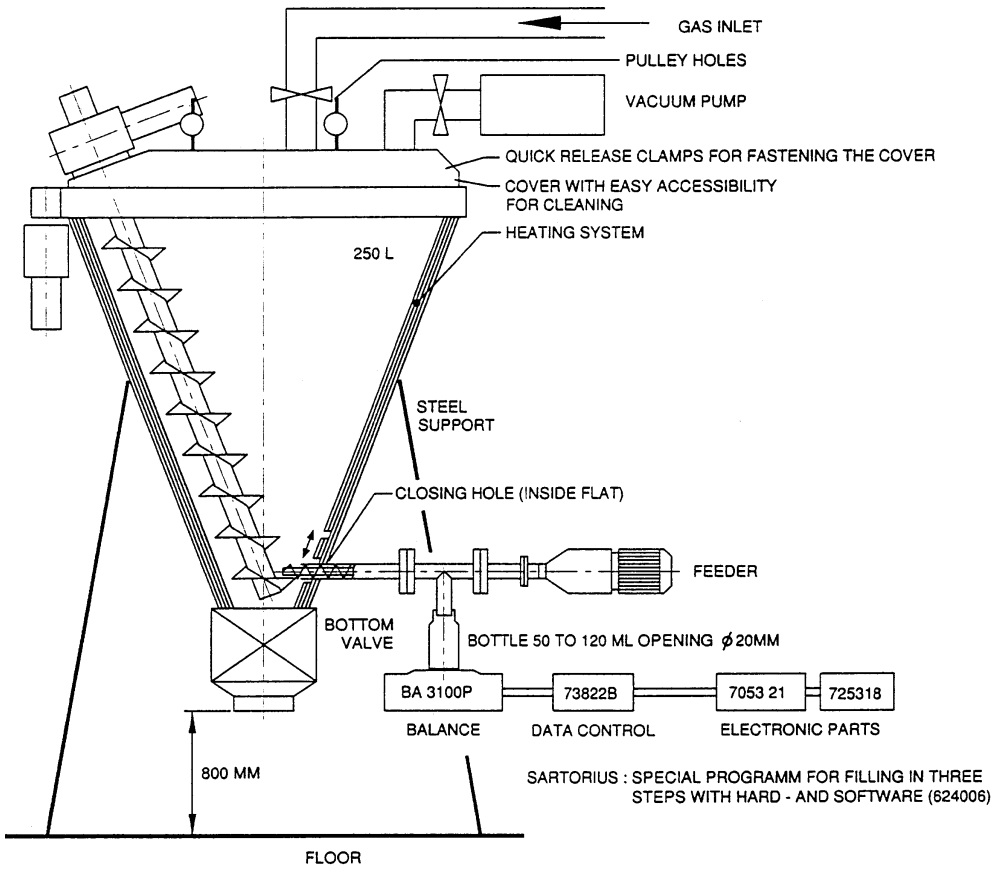


Fig. 2 Fully automatic filling machine for powders type DM-1 of the company Transmatic Fyllan

Fig. 3 Multipurpose cone mixer with filling equipment



For example, the time consuming filling procedures for powders can be drastically reduced with automatic filling devices.

IRMM has ordered a tailor-made fully automatic filling machine for powders from Transmatic Fyllan Limited, Bedford, England (type DM-1). It can be installed in a glove box and can operate under dry air or inert gas conditions (< 77 mg/m^3 moisture). The machine (Fig. 2) is composed of a turntable connected to an indexing belt to the bottom lift and load cell. Above the load cell a well closed 28 L hopper is mounted. The powder is dispensed using a vertical screw driven by a stepper motor into the

vial or bottle. It can be adapted to various types of vials (5 to 100 mL) or bottles (30 to 120 mL). The powder is continuously mixed during filling procedure. The production rate for filling 2.8 g powder/vial of BCR CRM 606 (pesticides in freeze dried water residue) was about 200 vials/h. The mass of powder for each vial is monitored and printed. All parts in contact with the powder are made of stainless steel.

A second filling device in which all the spare parts in contact with the material are made of or protected with Teflon is under construction. This will guarantee automatic bottling of CRMs for trace metals without risk of contamination.

Homogenization and sampling of "dirty" materials such as soils, sediments, fly ashes or sludges are carried out in a multipurpose cone mixer of 250 L with a semi-automatic filling device (see Fig. 3).

After jet milling and sterilization the material is directly given into the cone mixer by a central filling nozzle on top of the mixer. All drives and gearing are placed outside the mixing chamber so that contamination by oil is excluded. All parts of the mixer in contact with the product are made of polished stainless steel. Homogenization and heating under inert gas is possible. A feedscrew at 100 mm of the bottom of the mixer allows filling of vials without stopping mixing with given masses of the material.

Directly after filling of a bottle, the feeder turns into the opposite direction to push the powder back into the cone mixer. The software (Sartorius) allows production rates of about 100 bottles/h. BCR CRMs 530, 535 and 536 have been successfully bottled with this equipment.

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