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Black ceramic spheres as marker grains for microfossil analyses, with improved chemical, physical, and optical properties

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ABSTRACT

Marker grain method is commonly used by palynologists to quantify absolute pollen concentration in sediments. DuPont NEM plastic microbeads and *Lycopodium* spore tablets are two of the most commonly used marker grains. The DuPont NEM series has an obvious advantage over *Lycopodium* tablets because *Lycopodium* is not guaranteed 'exotic' in many ecosystems (especially in the past). Moreover, NEM also has high visibility, easily identifiable spherical shape, and availability in different size ranges. However, the production of NEM has been discontinuous since more than a decade, and palynologists of the world are using the precious remainders of their own stock in very small (=suboptimal) quantities. Here we propose a solution. Microspheres of black ceramic can be used as marker grains for a wide range of microfossil analyses (in this paper, however, we mainly discuss the use of the material for pollen analysis because of the expertise of the authors). The ceramic spheres are tolerant to all chemicals commonly used during pollen extraction processes and is extremely resistant to physical stresses. The particles are matt black, spherical, available in a wide range of size clusters, have a density very close to that of fossil pollen grains (1.424 g/cm^3 vs. 1.494 g/cm^3 in average), and don't change color and density over a long storage in acidic liquids. The behavior of ceramic spheres was closer to the pollen grains than the *Lycopodium* spores regardless of the pollen concentration and composition in sediments. In combination, these properties make the black ceramic spheres an even better solution for palynologists than DuPont NEM microbeads or *Lycopodium* spore tablets. The absolute pollen concentrations estimated from ceramic spheres and those estimated from volume method were indistinguishable within errors. On the other hand, when the samples were relatively poor in fossil pollen grains, the concentrations calculated by *Lycopodium* method tended to be significantly overestimated. The ceramic microbeads are available either as a dry powder in different size ranges, or as a mixture of two size ranges blended in a defined ratio and dispersed in a buoyancy-neutral liquid, ready to be added to sediment samples. Mixing two different size ranges in a known ratio serves to detect any laboratory failures that differentially favor recovery of larger or smaller pollen types.

1. Introduction

The absolute quantity of microfossils is a very useful parameter (Bonny, 1972 and references therein). For instance, with quantitative data we can discuss pollen productivity, presence or absence of sorting or ultraviolet decomposition of organic matter and biomass in sparse vegetation (e.g. boreal forest or arid zone). Because the absolute pollen concentration is independent from frequencies of pollen grains of other taxa, it often reflects environmental changes more directly than percentage values. Adding known-amount of marker grains, such as *Lycopodium* spores and DuPont NEM plastic microbeads series was making those scientific approaches possible (Stockmarr, 1971; Ogden, 1986).

The DuPont NEM microbeads series has been, however, sorely missed since its production was discontinued. The microbeads method was first proposed in 1986 (Ogden, 1986) and, since that time, the DuPont product has attracted a number of enthusiastic users (e.g. Wang and Geurts, 1993; Turetsky et al., 2004; Fedotov

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et al., 2012; Sugaya et al., 2016). Unfortunately, since the DuPont NEM series was discontinued, no alternative marker grain product of comparable quality has been introduced. The DuPont NEM microbeads were supplied in various size ranges and had high-visibility under the microscope due to their uniform spherical shape and matt black color. With an average density of 1.369 g/cm^3, they were also close to that of fossil pollen grains (1.494 g/cm^3 in average; Regnéll and Everitt, 1996). The microbeads were also inert and tolerant of the chemical treatments used in conventional palynological procedures (HCl, KOH, $ZnCl_2$, acetolysis, and even conc. HF). The synthetic microbeads from DuPont NEM therefore offered what seemed to be an ideal marker grain for palynological research and so the discontinuation of the product line sent shock waves throughout the palynology community.

Here we report a high quality alternative to the DuPont NEM microbeads. As well as being tolerant of all palynological processing chemicals, including HF, this novel marker grains does not decolorize or swell after lengthy storage in liquid, which was something of an issue with the DuPont NEM materials. Its specific gravity is also closer to that of fossil pollen grains than the DuPont microbeads, which ensures more coherent movement of pollen and marker grains during pollen extraction. This paper aims to assess the performance of this new product in comparison with the conventional *Lycopodium* spore tablets.

2. Materials and methods

We tested the black ceramic spheres (PALYNOSPHERES; Fig. 1a–b) obtained from a UK-based company called Palynotech (http://www.palynotech.com/). The density of the microbeads is 1.424 ± 0.008 g/cm^3 (2σ) (Table 1), which, according to our measurements by Archimedes method, does not depend on their size clusters. The ceramic spheres are available in five size ranges centered at ca. 23 μm (coded “Orange”), 27 μm (“Blue”), 34 μm (“Green”), 38 μm (“Yellow”) and 46 μm (“Red”) (Table 2). (N.B. The color codings are simply for convenience; the actual color of the powder is matt black in all size ranges). Two marker grains, coded “Red” and “Orange”, are easily distinguishable by sizes under the microscope (Fig. 1a, b, d). Using the mixture of small and large marker grains enables to check the size dependency of the microfossil recovery rate after the treatment of sediments.

In this research, PALYNOSPHERES SG06 Special Blend (Batch no. SG0605; Fig. 1a and b) was used for the experiment. The SG06 Special Blend is a mixture of two easily distinguishable size clusters of ceramic microbeads (coded “Orange” and “Red” – see above) suspended in a buoyancy-neutral carrying media. The Orange/Red mixing ratio is ca. 2.9 (exact ratio is variable across batches; the product label provides more precise and lot specific value), which counterbalances the higher visibility of larger grains. The carrier media (sodium bromide; NaBr) has specific gravity of 1.40 ± 0.05 (measured at 20 °C; temperature dependence unknown). This media density has been chosen to be quasi-buoyancy neutral with the marker grains so that re-dispersal is easily achieved. The media contains non-ionic detergent, Tween 20. The marker grain concentrations differ to a small degree from lot to lot, but are generally in the region of $1.8 \pm 0.1 \times 10^4$ grains/ml and $6.2 \pm 0.4 \times 10^3$grains/ml for the small and large grains, respectively. Exact concentrations to 3 decimal places are provided by the manufacturer for each production lot and are detailed on the product label. The lot No. SG0605, the one that we used in this experiment, contains $6.17 \pm 0.42 \times 10^3$ large (‘Red’) grains and $1.78 \pm 0.13 \times 10^4$ small (‘Orange’) grains per ml, according to the lot-specific product label. The number of grains in 1 ml of the formulated material is equivalent to approximately two *Lycopodium* tablets manufactured by Lund University (Stockmarr, 1971), which makes it easy to convert between *Lycopodium* tablets and PALYNOSPHERES in the laboratory procedures.

The pollen analysis was carried out to compare the behavior of the black ceramic spheres and *Lycopodium* spores. The DuPont NEM

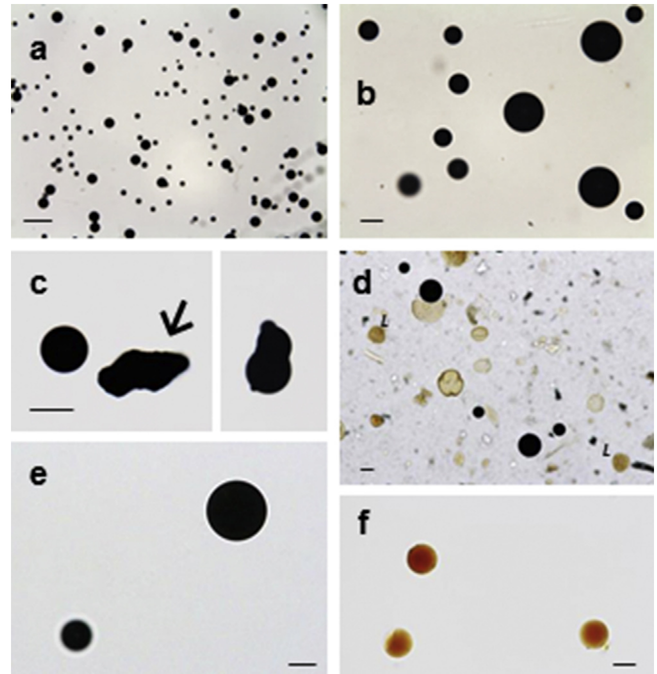

Fig. 1. Photos of (a, b) the black ceramic spheres. (c) Amorphous (i.e. non-spherical) grains. (d) The marker grains and *Lycopodium* spores added to the sediment, treated and extracted by a normal preparation protocol (Nakagawa et al., 1998) along with fossil pollen grains. *L* denotes the *Lycopodium* spore. (e–f) New and conventional microbeads after storage in suspension liquid for long-term. DuPont NEM microbeads (f) are loosing its color after storage in suspending solution for 13 years. The marker grains proposed in this paper (e) were developed very recently and hence such lengthy stability check has not been carried out. However, the oldest available lot, which has been stored in the buoyancy neutral medium described in this paper for almost 5 years, is not showing any recognizable changes in optical properties. Scale bars represent 100 μm and 20 μm in (a) and (b–e), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of density.

	Density (g/cm^3)
Fossil pollen grains[a]	1.494 ± 0.028
Black spherical ceramic powder[b]	1.424 ± 0.008
DuPont NEM series	1.369 ± 0.013
Lycopodium spore[c]	1.377 ± 0.016

[a] After Regnéll and Everitt (1996).
[b] The product (PALYNOSPHERES) discussed in this paper.
[c] *Lycopodium* tablet (Batch no. 3862) made by Lund University.

Table 2
Available size clusters of black ceramic spheres.

Color code	Particle size (μm)
Orange	22.6 ± 2.0
Blue	27.2 ± 3.6
Green	33.7 ± 3.7
Yellow	38.0 ± 3.2
Red	45.5 ± 3.86

The color codes are simply for convenience. The color of microspheres is matt black in all size clusters.