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The microbial resistance of polymer dispersions and the efficacy of polymer dispersion biocides – A statistically validated method

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ABSTRACT

The biodeterioration of water-based manufactured formulations, such as polymer dispersions, is a major problem for the producing companies and the users of such products. Industrial preservatives, also known as biocides, are therefore used to protect these and similar products from the effects of micro-organisms, predominantly bacteria and yeasts. In the absence of internationally recognised standard methods for determining the resistance of polymer dispersions to microbial growth and the efficacy of biocides used in them, protocols for testing other products, e.g., paints, have been adapted, and other methods produced by manufacturing companies, test laboratories, and academic institutions have been used. Often these do not take into account the specific nature of the materials being tested, the types of organism commonly causing contamination, and the conditions that the polymer dispersions will be exposed to during manufacture, storage, and use. By conducting a series of round-robin, collaborative tests, the member organisations of the International Biodeterioration Research Group Polymer Dispersion Working Group have identified the bacteria that commonly infect polymer dispersions, defined the main parameters necessary for a standard method of test, and developed a protocol that is robust, repeatable, and reproducible. The recommended test involves three repeat inoculations of the material with a previously determined mix of seven Gram-negative bacteria and evaluation of living cells by a simple plating technique. The work reported here, carried out by nine participating laboratories, is a final statistical validation and suggests that the method is eminently suitable as a standard test method.

1. Introduction

Polymer dispersions, also referred to as polymer emulsions, latices, latex emulsions, latex dispersions, or binders, are used in a wide variety of applications including production of emulsion paints, adhesives, paper and textile coatings, non-woven fabrics, and carpet-making compounds (Gillatt, 1990). In composition they are fine dispersions or suspensions of synthetic polymer particles (0.1–6 μm) in aqueous media and their pH varies greatly, from acidic in the case of some ethylene vinyl acetate (EVA) and polyvinyl acetate (PVA) types to relatively alkaline in the case of some acrylic, styrene acrylic, and styrene butadiene products.

A common feature of most types of polymer dispersion is that they are susceptible to spoilage by microorganisms (Gillatt, 2005), and an earlier study by the IBRG Polymer Dispersion Group (Gillatt, 1995) identified a large number of bacteria, moulds, and yeasts

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causing such contamination (Table 1). Doménech-Carbó et al. (2009) found that biodeterioration of polymer dispersions, especially those based on polyvinyl acetate (PVA), could be brought about by microbial attack on the plasticisers, often based on phthalates, present in such products, and that such degradation was more likely to be brought about by fungi than by bacteria. However, they were investigating cast films of polymer dispersions rather than liquid products and it is in the latter that the effects of viscosity changes, production of gases and odours, colour changes, and enzyme production are noted, with concomitant effects on manufactured end products such as water-based paints and adhesives (Cheroni et al., 2012; Ravikumar et al., 2012).

The prevention of microbiological contamination of manufactured products requires an integrated approach involving plant hygiene and monitoring, coupled with the use of effective, broad-spectrum antimicrobial agents (biocides). Such products and the active substances they contain are regulated by many national and international bodies and, in particular, will require registration under Product Type 6 (in-can preservatives) of the European Union Biocidal Products Regulation (BPR - European Parliament, 2012).

The need for standardised methodology was recognised by Cresswell and Holland (1995) and the role of the International Biodeterioration Research Group (IBRG, Hueck van der Plas, 1962) in developing such a protocol was important in that it enabled the production of efficacy data for biocidal active agents being registered under the then proposed Biocidal Products Directive (BPD), enacted in 1998 (European Parliament, 1998).

Gillatt (1991) pointed out that few nationally or internationally recognised methods exist for the evaluation of industrial biocides or for testing the microbiological resistance of susceptible liquid products. Although there are a small number of standard test methods for products containing polymer dispersions, such as ASTM D2574–06 (ASTM, 2012) and ABNT NBR 15821 (ABNT, 2010) for paints and ASTM D 4783–01 (ASTM, 2008) for adhesives, no specific nationally or internationally recognised test method exists for determining the efficacy of biocides used in polymer dispersions.

Members of the Polymer Dispersion Working Group (PDG) have carried out eight multi-laboratory tests, several with a number of phases, to determine the most important parameters in a standard test. The most recent, Collaborative Experiment Eight, involving nine laboratories, was a statistical validation of the method that had evolved as a result of the previous seven, and is reported here.

Table 1
Microorganisms isolated from polymer dispersions.

Organism	Number of species	Of which:
Bacteria		
Pseudomonas	30	12 were *Pseudomonas aeruginosa* 6 were *Pseudomonas putida* 5 were *Pseudomonas fluorescens* 5 were *Pseudomonas stutzeri*
Escherichia	11	all were *Escherichia coli*
Alcaligenes	11	6 were *Alcaligenes faecalis*
Proteus	9	6 were *Proteus vulgaris* 2 were *Proteus morganii*
Flavobacterium	6	various species
Klebsiella	5	3 were *Klebsiella pneumoniae*
Micrococcus	5	4 were *Micrococcus luteus*
Mycelial fungi		
Aspergillus	10	5 were *Aspergillus niger* 2 were *Aspergillus oryzae*
Geotrichum	7	5 were *Geotrichum candidum*
Penicillium	7	2 were *Penicillium ochrochloron*
Yeast-like fungi		
Candida	7	3 were *Candida albicans* 2 were *Candida valida*
Rhodotorula	4	2 were *Rhodotorula glutinis* 2 were *Rhodotorula rubra*
Saccharomyces	2	both were *Saccharomyces cerevisiae*

2. Materials and methods

2.1. Polymer dispersion

A fifty-five percent dispersion of Vinnapas® RE 5010N (Wacker Chemie AG), a water-dispersible copolymer powder of vinyl acetate and ethylene, was prepared in sterile distilled water by slowly adding the powder with efficient mixing to produce a model polymer dispersion. This had been shown in previous IBRG PDG collaborative experiments to be readily susceptible to growth of bacteria isolated from contaminated polymer dispersions.

2.2. Biocide

A methyl-isothiazolinone (MIT – CAS 2682-20-4)/benz-isothiazolinone (BIT – CAS 2634-33-5) biocide (Acticide® MBS – Thor Specialities [UK] Ltd.) was added to duplicate 100-g aliquots of the dispersed powder polymer to give: (a) 12.5 ppm MIT, 12.5 ppm BIT; (b) 18.75 ppm MIT, 18.75 ppm BIT; and (c) 25 ppm MIT, 25 ppm BIT – referred to below as "low," "medium," and "high" biocide additions, respectively. These biocide/dispersed polymer samples plus duplicate biocide-free blanks were equilibrated at 30 °C for 24 h.

2.3. Test method

The samples thus prepared were evaluated against bacteria only since these are the main causative organisms of polymer dispersion biodeterioration (Gillatt, 1995). Evaluation was done following A Method for the Evaluation of Biocidal Compounds in Aqueous-Based Polymer Dispersions; Version 5.6-2011/04, IBRG document IBRG/PD11/005 (IBRG, 2011).

Test organisms for this study were among those isolated from contaminated polymer dispersions and their components (Gillatt, 1995). They were identified by the culture collection of Bundesanstalt für Materialforschung und – prüfung, Berlin (BAM) and were shown in previous IBRG collaborative tests to grow readily in the model polymer dispersion used in this experiment.

Aeromonas hydrophila (sorbia)	BAM 485
Alcaligenes faecalis	BAM 486
Alcaligenes faecalis	BAM 487
Providencia rettgeri	BAM 488
Pseudomonas aeruginosa	BAM 489
Pseudomonas sp.	BAM 490
Serratia marcescens	BAM 491

Use of 16SrRNA gene sequencing and BIOLOG phenotyping showed that isolates BAM 486 and BAM 487 were both *Alcaligenes faecalis*. Further genotypic characterisation by BOX PCR fingerprinting showed them to be two different strains of the same species (Koeuth et al., 1995; Rademaker et al., 2000; Tacao et al., 2005).

The organisms were lodged with the culture collection of Bundesanstalt für Materialforschung und – prüfung, Berlin (BAM), from which they were obtained for this study.

Individual species were grown on tryptone soya agar slants (Oxoid Ltd, UK) at 30 °C for 24 h and washed off into 10 ml of quarter-strength Ringer's solution. Each suspension was enumerated using a counting chamber and diluted with quarter-strength Ringer's solution to 0.50–5.0 × 10^6 ml^{-1}. Equal volumes of the individual suspensions were pooled and a total viable count (TVC) of the inoculum thus prepared was performed on tryptone soya agar (Table 2).