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Evaluation of a prototype micro-electronic autoclave cycle integrator

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Instability and non-uniformity of spore preparations and the non-conformity of chemical indicators to the temperature coefficients of spore inactivation are problems associated with current methods of autoclave cycle monitoring. A prototype micro-electronic instrument, which largely overcomes these problems, is described. It monitors autoclave cycles in terms of the integral F_0 and Nabla functions. Its thermometric and integrating accuracy is demonstrated. A discussion of the problems associated with the use of sensitive electronic instruments in the autoclave room environment reveals the need for independent monitoring areas when such devices are used. Inactivation constants were determined for spores of the organism *Bacillus stearothermophilus*, NCTC 10 003 and were described using D_{115} (12.0 min) and Z (9.0 °C) values and the first order reaction Arrhenius constants A ($10^{41.2}$ min^{-1}) and E_a (74.4 kcal mol^{-1}, 311 kJ mol^{-1}). These have been compared with recently published values. The standardization of F_0 and Nabla is discussed with reference to the setting of minimum values related to product bioburden and maximum values related to an acceptable degree of product degradation.

The official methods of assessing the efficiency of steam sterilization cycles rely on the measurement of temperature and time (P.C. 1979, B.P. 1973), the criteria for successful sterilization being a minimum time at a specified temperature. In practice a Master Temperature Recording (MTR) (Hospital Technical Memorandum 1979) is produced for each product and loading pattern at commissioning which is then used as a standard against which routine cycles can be evaluated.

Additional monitoring methods include the use of Biological Indicators (Bühlmann et al 1973) in the form of heat resistant spores, and Chemical Indicators in the form of chemicals which change colour when subjected to certain patterns of heat, time and moisture, with post process sterility tests as a final 'safeguard'. The reliability of these methods is debatable. The variability of commercial biological indicators in terms of the number of organisms per device and their resistance to heat is widely documented (Mayernik 1972; Bühlman et al 1973) and studies relating to the retention of viability and resistance under differing storage conditions have demonstrated the instability of such devices (Cook & Brown 1965; Smith et al 1976; Reich et al 1979).

A study of the kinetics of the colour changes occurring in commercially available chemical indicators (Lee et al 1979) has shown that many exhibit temperature time profiles which bear no relation to the kinetics of death of spore-bearing microorganisms subjected to steam sterilization cycles.

Sterility testing is retrospective and its value in terms of the statistical significance of the results is questionable particularly where the incidence of contaminated units in a batch is low (Brown & Gilbert 1977).

With any of the above methods, the results in terms of the lethal effectiveness of the process are not available until completion of the cycle, and with biological indicators and sterility tests may not become available until several days after completion of the autoclaving process. Furthermore, unless the monitoring device evaluates and indicates the overall lethal effectiveness of the autoclave cycle in terms of the inactivation of any potential contaminants, no assurance is given as to the probability of post process survival of these organisms. Under the present system of cycle validation no account of the lethal effects of temperatures above and below that of nominal, is taken. Using the F_0 (Akers et al 1978; Stumbo 1965) or Nabla (∇) (Deindoerfer 1957;

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Deindoerfer & Humphrey 1959) systems this becomes possible, however, the manual integration of temperature/time recordings is difficult and time consuming, particularly when high resolution is required. This paper describes a micro-processor based instrument capable of integrating temperature/time directly from a thermocouple probe placed within the autoclave chamber, in terms of the F_0 and Nabla parameters and discusses the problems associated with the calibration of this instrument.

MATERIALS AND METHODS

Temperature-time integrator

The integrator (Hoskins 1979a,b; M. C. Fitzgerald personal communication) is based on a Motorola 6802 microprocessor which is controlled by software contained in 2Kbyte of EPROM (Erasable programmable read only memories). During normal operation five functions may be indicated on the digital displays: 1) the accumulating Nabla (∇) value; 2) the accumulating F_0 value; 3) the probe temperature with a resolution of 0·1 or 0·5 °C; 4) the time at which the probe has been above 80 °C; 5) the time at which the probe has been above 121 °C.

The e.m.f. produced by a thermocouple probe in the autoclave chamber is converted into digital format (temperature). Compensation for changes in ambient temperature is achieved by a separate cold junction compensation unit. The data are then used to address a Table stored in the memory containing F_0 and Nabla values equivalent to temperatures from 80 to 130 °C in 0·5 °C increments. This value is summed into a memory location which is then used to update the displays. This cycle is performed every second so giving a continuous evaluation of the progress of the autoclave cycle.

THERMOCOUPLE PROBE

The instrument's thermocouple was carefully prepared so as to be sealed against the entry of steam. Single stranded copper-constantan (type T) wire conforming to B.S. 1828 (Fothergill and Harvey, Tygadure Div., Littlebord. Lancs) was used. The insulation was stripped and each wire cleaned with abrasive paper, then degreased with carbon tetrachloride. The wires were twisted together, tin soldered (resin flux), re-washed with carbon tetrachloride and then cut to a length of 0·6 cm. The junction was then coated with a thin layer of Araldite epoxy resin, which was allowed to harden at ambient temperature overnight. The junction was then encapsulated in a length of thin walled brass tubing (2 cm long. 0·3 cm external diameter, 0·05 cm wall thickness) sealed at one end with tin solder. The thermocouple tip was sealed into position with epoxy resin.

The thermal lag of this thermocouple was estimated by heating from ambient to 100 °C and was found to be about 10 to 15 s.

Integrator calibration

Calibration experiments were carried out using an electrically heated oil bath. Temperature was regulated by an electronic control unit (Radio Spares Ltd) which powered a heating element, this was found to give variations in oil temperature of no more than ±0·1 °C of nominal. Temperatures were measured using a mercury in glass thermometer (BS 593/54) with a range of 100–130 °C and graduated in 0·1 °C increments, this was used as a standard to which the integrator was calibrated. In a later experiment the accuracy of temperature measurements was checked using a precision thermometer bridge with platinum resistance probe (Leeds and Northrup, Type 8078, Warfdale Rd, Birmingham) which had been calibrated at the National Physics Laboratory.

The method of calibration was that described by the NPL (National Physics Laboratory 1967) for mercury in glass thermometers. Briefly, the integrator was switched to the 0·1 °C resolution mode and allowed to equilibrate over 1 h at room temperature (ca 20 °C). A thermocouple probe was secured to the mercury bulb of the thermometer which was then placed in the oil bath to the indicated immersion mark. The oil bath was heated to several static temperatures, at each, several readings were taken simultaneously from the mercury/glass thermometer and the integrator display and the means determined. The oil bath heater was then switched off and the measurements repeated as the oil cooled to ambient temperature.

Integrator validation

(i) *Thermometric accuracy.* The thermometric accuracy of the integrator was, essentially determined during calibration. The integrator was adjusted so as to be in exact agreement with the standard thermometer. The calibration was then carried out at various temperatures and differences in indicated temperature between the integrator and the standard thermometer noted.

(ii) *Integrating accuracy.* The thermocouple probe was detached from the integrator and a series of fixed voltages applied to the input from a precision millivolt source (Time Electronics Ltd Model 404 0·05%

Grade), so as to produce the required temperatures on the digital displays. The temperatures for steam sterilization of the PC (1979) were used as models. The accumulating integrals were noted at specific times and compared with calculated ∇ and F_0 values for similar temperature-time profiles.

The stability under working conditions

Three studies were carried out in order to assess the effects of routine use, of changes in ambient temperature and of continuous 'power up', on the integrator's calibration.

The integrator was used to monitor the routine sterilization cycles of a Gëtinge autoclave (Model: SAR 450, Gëtinge AB, Halmstad, Sweden). The calibration was periodically checked against the standard thermometer.

The effects of gross changes in ambient temperature were determined by maintaining the integrator at a series of elevated ambient temperatures whilst calibration studies were carried out. The integrator plus cold junction compensation were placed in a hot air oven, the power and thermocouple cables being introduced through the ventilation port. The oven was heated to the required temperature then the integrator switched on and allowed to equilibrate over 1 h. The calibration was then checked against the standard thermometer.

In a second series of experiments the cold compensation junction remained at room temperature whilst the integrator was heated.

The effect of continuous power up was carried out over 288 h the calibration being periodically checked against the standard thermometer.

Inactivation kinetics of Bacillus stearothermophilus *spores*

A suspension of *B. stearothermophilus* spores NCTC 10003 (carbon and magnesium depleted—Brown MRW, Microbiology Research Lab, Pharmacy Dept, Aston University, Costa Green, Birmingham) in ¼ strength phosphate buffer was used. Before use the suspension was diluted with sterile glass distilled water to ca $8{\cdot}5 \times 10^5$ viable spores ml^{-1}.

Thermal inactivation of B. stearothermophilus *spores*

The method of Hambleton & Allwood (1976) was used to determine thermal inactivation curves using an enumerating medium composed of glucose 0·5% w/v (Fisons Ltd,) Tryptone 0·7% w/v (Oxoid L42), agar 1·5% w/v (Oxoid, L13) (sterilized at 121 °C for 20 min) and an incubation time of 48 h at 57 ± 1 °C.

Five replicate plates were prepared at each dilution level and the inactivation experiments were carried out in triplicate.

RESULTS

Integrator performance

The integrator's calibration data are presented as plots of the difference between the integrator's indicated temperature and that shown by the standard thermometer *vs* the standard thermometer's reading (Benedict 1969). Thus an integrator reading which is greater than that of the standard thermometer is shown as a positive difference.

The initial calibration is shown along with the calibration data determined after the integrator had been used to monitor autoclave cycles (Fig. 1). No

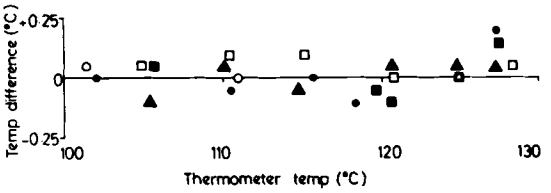

FIG. 1. The accuracy of the integrators calibration originally (●) and after being used to monitor autoclave cycles.

marked changes in calibration occurred after routine use, the maximum deviations observed being no more than + 0·15 to −0·1 °C.

A similar result was obtained when the integrator was continually used over 288 h. A maximum deviation of ± 0·2 °C was observed throughout this period, no obvious trends in calibration drift occurred.

The effects of ambient temperature changes on the integrator's calibration were pronounced (Fig. 2). When the cold compensation junction was held at

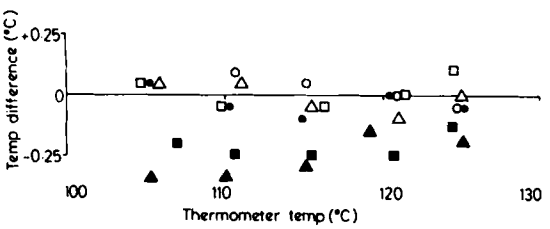

FIG. 2. The effect of ambient temperature on the integrators thermometric accuracy, when the cold compensation junction remained at room temperature, ca 18 to 20 °C (open symbols), and when the cold compensation junction was heated at the same temperature as the integrator (closed symbols). Ambient temperatures at which calibration was checked was 25 (○ ●), 30 (△ ▲) and 36 °C (□ ■).

room temperature while the integrator was heated (open symbols) no significant calibration drift was noticed. However, when the cold compensation junction was heated at the same temperature as the integrator a marked, negative calibration drift resulted which reached as much as −0·35 °C at 30 °C. No obvious correlation between ambient temperature and thermometric accuracy was observed.

The accuracy of the integrated function are shown in Table 1. F_0 and Nabla values were calculated from equations 1 and 2.

$$F_0 = 10^{T-121/10}.dt \quad (1)$$

$$NABLA = A\, e^{-E/RT}.dt \quad (2)$$

where T = temperature under consideration at time dt. A = Arrhenius constant (Table 3). E = Energy of activation (Table 3). R = Universal gas constant 1·9858 cal (8·3144J) $mol^{-1}\ K^{-1}$ for the temperatures shown. Two experiments were carried out at each temperature.

Table 1. The accuracy of the integrated functions, F_0 and Nabla, at the PC sterilization temperatures. Voltages required to produce the indicated temperatures were 4·318 mV (115 °C), 4·615 mV (121 °C) and 4·869 mV (126 °C).

Indicated temp. °C	Time Min	F_0 Calc.	F_0 Indicated		Nabla Calc.	Nabla Indicated	
115	5	1·25	1·26	1·26	3·42	3·37	3·39
	10	2·5	2·51	2·51	6·85	6·78	6·78
	15	3·75	3·77	3·77	10·2	10·1	10·1
	20	5·00	5·03	5·04	13·7	13·5	13·5
	26	6·50	6·53	6·54	17·8	17·5	17·5
	30	7·50	7·54	7·55	20·5	20·3	20·2
121	3	3·00	3·00	3·00	7·83	7·73	7·73
	6	6·00	6·00	6·00	15·6	15·5	15·5
	9	9·00	9·00	9·02	23·5	23·2	23·2
	12	12·0	12·0	12·0	31·3	31·0	31·0
	15	15·0	15·0	15·0	39·1	38·8	38·8
126	2	6·32	6·37	6·32	15·4	15·3	15·2
	4	12·6	12·6	12·6	30·8	30·5	30·5
	6	19·0	18·9	19·0	46·2	45·7	45·6
	8	25·3	25·3	25·4	61·6	60·9	61·0
	10	31·6	31·6	31·6	77·1	76·2	76·1

Thermal death curves

The results from the three replicate thermal inactivation experiments were combined and analysed using a two variable linear regression model (Model 10 'STAT PAC' III—1) programmed into a bench calculator (Hewlett Packard 9810A). The means of the combined counts at each sample time were plotted as log surviving fraction *vs* time (Fig. 3) and the results from the statistical analysis used to calculate the inactivation constants for the test organism (Table 2).

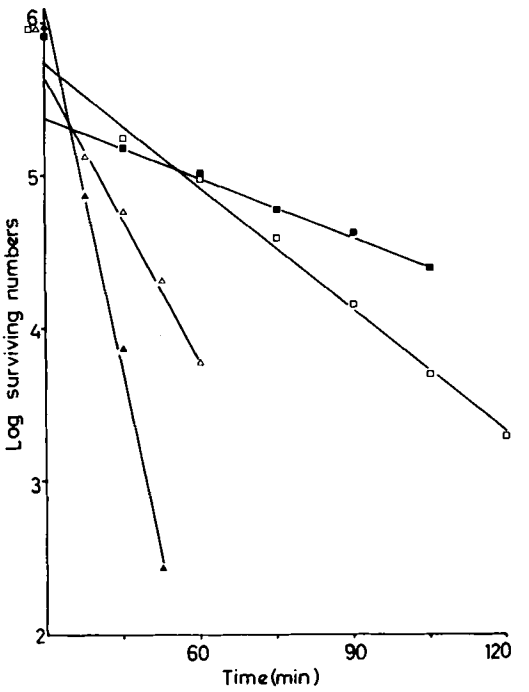

FIG. 3. Thermal inactivation curves for spores of *Bacillus stearothermophilus* NCTC 10 003 at 105 (■), 109 (□), 112 (△) and 115 °C (▲).

Table 2. The inactivation constants for *Bacillus stearothermophilus* NCTC 10003 spores.

Temp. °C	D value min	Z °C	$K \times 10^{-4}$ s^{-1}	A	E k cal mol^{-1}
105	155	9·0	2·476	$10^{39·38}$	74·44
109	75		5·118		
112	33		11·630		
115	12		31·986		

DISCUSSION

The experimental work was carried out in two phases. The first involved an appraisal of the integrator's performance under bench and working conditions. The second involved a study of the inactivation kinetics of a 'typical' biological indicator used to assess the efficiency of steam sterilization processes, with the purpose of comparing the inactivation rate constants, derived from these, with those used to program the integrator.

The integrator's calibration was found to deviate by no more than ca ± 0·1 °C from the standard

Table 3. Published inactivation constants for strains of *Bacillus stearothermophilus* spores in various heating menstrua. * Calculated values from authors' original data.

Strain	Heating medium	A	E_a k cal mol^{-1}	E_a kJ mol^{-1}	Source
		$10^{38\cdot0}$	67·7	285	Deindoerfer 1957
FS 7954	Phosphate buffer, pH 7	$10^{47\cdot2}$	83·6	350	Wang 1964
NCIB 8919	Water	$10^{55\cdot9}$	100·9	428	Briggs 1966*
ATCC 7953	Phosphate buffer, pH 7	—	82·1	345	Jonsson 1977
FS 7954	Phosphate buffer, pH 6·9	—	91·2	382	Jonsson 1977
NCTC 1518	Phosphate buffer, pH 7	$10^{37\cdot8}$	68·7	288	Wallace 1978*
NCTC 10003	Water	$10^{41\cdot2}$	74·4	311	Present study

thermometer. During routine monitoring of autoclave cycles this deviation increased slightly (ca ± 0·2 °C) but no marked calibration drift with time was observed (Fig. 1). When used over an extended period of time the maximum deviation from the standard was slightly greater at ± 0·25 °C, again no marked drift with time occurred. The integrator is designed to have a working tolerance of ± 0·5 °C and this is easily met under controlled environmental conditions.

The integrated functions (F_0 and Nabla) were studied at the initial calibration stage and these showed excellent agreement with theoretical values calculated for similar temperature time intervals (Table 1), and it was assumed that the integrated functions would remain as accurate as the thermometric accuracy.

The effects of changes in ambient temperature on thermometric accuracy and hence integrating accuracy were pronounced. Any deviation in ambient temperature produced a calibration drift, this became unacceptable when the temperature deviated by more than ± 5 °C of that at calibration. When the cold compensation junction was maintained at room temperature while the integrator was heated, only a slight, acceptable discrepancy in calibration resulted. However, when the cold compensation junction was heated under the same conditions as the integrator, a marked negative deviation in calibration resulted (Fig. 2). This shows that the actual digital circuitry of the instrument is only marginally affected by the increasing ambient temperature (providing it is within the circuitry's working tolerance), whereas the cold compensation junction over compensates for high ambient temperatures therefore producing a low indicated temperature. This effect is of considerable importance if one considers the 'seasonal' variations encountered in autoclave plant rooms where ambient temperatures can range from ca 10° to 60 °C. In practice to overcome such problems it would be necessary to provide a monitoring instrument area with suitable environment conditioning. This is borne out by the fact that temperatures greater than 45 to 50 °C caused a total shut down of the circuits.

Several unquantifiable spurious effects occurred when the integrator was used to monitor autoclave cycles, these presented themselves as fault state indications, the most common was that resulting from activation of a high voltage switching relay, this was thought to be due to electrical noise and was largely overcome by the inclusion of a voltage surge suppressor in the mains supply (Radiospares Ltd.) Steam entering the thermocouple sheath produced a similar effect (Benedict 1969) and several thermocouple designs were tried before this problem was eliminated.

The bench tests successfully demonstrated the integrator's thermometric and integrating accuracy and stability. When used to monitor autoclaves cycles, the integrator proved reliable once an adequately stable thermal environment was provided.

The semi-log thermal death rate plots (Fig. 3) show a characteristic biphasic response which is typical of a spore suspension of mixed heat resistance (Stumbo 1965). In this case the heat resistances of the two populations differed markedly as is demonstrated by the initial steep gradient, followed, after the inflexion by the more gradual gradient. With this in mind, the initial results were excluded from the linear regression and so, derived constants may be considered representative of the population of greater resistance.

The inactivation constant for various strains of *B. stearothermophilus* derived by the cited authors (Table 3) show a good correlation with those calculated

here. Deindoerfer's (1957) results, which were used as a basis for the integrator's program, differ considerably from more recently published work and are representative of a spore population of much lower heat resistance and it can be argued that these constants are a poor model for the inactivation constants of the more common strains of *B. stearothermophilus* used as biological indicators.

Kelsey (1958) introduced the concept of the ideal chemical indicator as one which '. . . should follow a temperature/time curve which runs parallel to that of pathogenic sporebearing organisms but offset by a distance which allows a reasonable margin of safety . . .' Only recently has a device meeting these criteria been introduced (Thermalog S, Biomedical Sciences Inc. Fairfield N.J. 07006, U.S.A.). These have been described by Beck (1976) and Witonsky (1977). The integrator would appear to provide an alternative means of meeting these criteria, in that the 'temperature/time' kinetics of the ideal indicator could easily be incorporated into the instruments program.

The controlling authorities in England accept the measurement of temperature and time as an adequate assessment of the efficiency of autoclave sterilization cycles, this takes the form of the Master Temperature Recording (MTR). Marked variations from MTR will lead to failed batches even though an adequate 'safe' cycle may have been achieved. By integrating temperature and time in terms of one fundamental variable (F_0 or Nabla) the assessment of autoclave cycles becomes much easier and more discerning.

The lethal effectiveness of the heating and cooling portions of the cycle are accounted for and variations in temperature or time are accounted for in terms of the nominal holding temperature.

Thus upper and lower acceptable limits can be set, the lower being representative of an acceptable reduction in viability of the bioburden, the upper being an acceptable degree of degradation occurring in the product.

The integrator would provide a versatile means of assessing sterilization cycles in terms of the fundamental parameters, F_0 and Nabla. Because of the nature of the programme format it is possible to reprogram the device with constants representative of any degradation pattern, e.g. the constants representing the Kelsey ideal chemical indicator or the first order inactivation constants of a typical bioburden or biological indicator organism.

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REFERENCES

Akers, M. J., Attia, I. A., Avis, K. E. (1978) Pharmaceutical Technology International, October 1978, 45–48

Beck, W. C. (1976) Med. Instrum. 10(6): 293–296

Benedict, R. P. (1969) Fundamentals of Temperature, Pressure and Flow Measurements. J. Wiley and Son, London

Briggs, A. (1966) J. Appl. Bacteriol. 29(3): 490–504

British Pharmacopoeia (1973) HMSO, London

Brown, M. R. W., Gilbert, P. (1977) J. Pharm. Pharmacol. 29: 517–523

Bühlmann, X., Gay, M., Schiller, I. (1973) Pharm. Acta Helv. 48: 223–244

Cook, A. M., Brown, M. R. W. (1965) J. Pharm. Pharmacol. Suppl. 17: 1S–11S

Deindoerfer, F. H. (1957) Appl. Microbiol. 5: 221–228

Deindoerfer, F. H., Humphrey, A. E. (1959) Appl. Microbiol. 7: 256–264

Hambleton, R., Allwood, M. C. (1976) J. Appl. Bacteriol. 41: 109–118

Hospital Technical Memorandum 10 (1979) HMSO London

Hoskins, H. T. (1979a) J. Clin. Pharm. 4: 9–18

Hoskins, H. T. (1979b) J. Pharm. Pharmacol. 30 Suppl. 101P

Kelsey, J. C. (1958) Lancet I (7015) 306–309 (8 Feb. 1958)

Jonsson, V., Snygg, B. G., Harnulv, B. G., Zachrisson, T. (1977) J. Food Sci. 42(5): 1251–1252, 1263

Lee, Cherl-Ho, Montville, T. J., Sinskey, A. J. (1979) Appl. Environ. Microbiol. 37(6): 113–117

Mayernik, J. J. (1972) Bull. Parent. Drug Assoc. 26: 205–211

Pharmaceutical Codex (1979) Pharmaceutical Press, 11th Edition, London

Reich, R. R., Whitbourne, J. E., McDaniel, A. W. (1979) J. Parent. Drug. Assoc. 33(4): 228–234

Smith, G. M., Pflug, I. J., Chapman, P. A. (1976) Appl. Environ. Microbiol. 32(2): 257–263

Stumbo, C. R. (1965) Thermobacteriology in Food Processing, Academic Press, London

Wallace, M. J., Nordsiden, K. L., Wolf, I. D. (1978) J. Food Sci. 43: 1738–1740

Wang, D. C., Scharer, J., Humphrey, A. E. (1964) Appl. Microbiol. 12(5): 451–454

Witonsky, R. J. (1977) Bull. Parent. Drug Assoc. 31(6): 274–281