Use of Isothermal Heat-Conduction Microcalorimetry (IHCMC) for the Evaluation of Synthetic Biomaterials

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Abstract: Isothermal heat-conduction microcalorimetry (IHCMC) allows measurement of extremely small rates of heat flow—on the order of 0.1 microwatt. This provides, for example, the ability to directly observe—and quantitate in a few days—rates of degradation as low as 1% per year at body temperature, in solid material samples of a few grams. Also, one method of IHCMC data analysis allows direct determination of the reaction-rate constant at the temperature of interest, thereby avoiding possible errors due to rate mechanism changes with temperature, an issue that needs to be considered when the Arrhenius method is used. IHCMC can also be used to measure transient phenomena, such as heat of adsorption, and initial metabolic responses of cellular entities to biomaterials. The purposes of this review article are to (a) explain the basic principles, attractive features, limitations, and methods of IHCMC; (b) describe biomaterials applications to date—including studies of the stability of ultra-high-molecular-weight polyethylene and implant-grade calcium sulfate, setting reactions of dental adhesives, and macrophage response to biomaterial particles; (c) provide a discussion of issues and concerns that should be addressed in order to maximize the utility of IHCMC in biomaterials studies; and (d) suggest a number of possible future biomaterials applications for this technique. © 2003 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 66B: 487–501, 2003

Keywords: isothermal heat-conduction microcalorimetry; degradation rate, ultra-high-molecular-weight polyethylene; calcium sulfate; macrophage response

INTRODUCTION

Calorimetry is a well-known and widely used method for measuring the exothermic or endothermic heat resulting from reaction(s) occurring in a given system. Although there are many types of calorimetry, the heat-conduction type is well recognized as being the best for studying systems in which the heat flow rate is very small,[1] as is expected to occur, for example, in oxidative degradation or hydrolysis of many synthetic biomaterial components during shelf storage or *in vivo*. Isothermal heat-conduction microcalorimetry (IHCMC) is a type of heat-conduction calorimetry in which the detection sensitivity is very high (of the order of ± 0.1 μW) and the test sample has a small mass (typically, 1–3 g) or a small volume (typically, 20–30 ml). (Note that, following the proposal by Hansen,[2] the nomenclature used in this article is IHCMC, rather than the more popular one; namely, IMC-isothermal microcalorimetry.) Until recently, the majority of studies utilizing IHCMC have been in the field of solid-state pharmaceutics; specifically, for the determination of the stability, compatibility, amorphicity, and other changes in physical form of pharmaceutical products or formulations.[3] Although the recognition of the usefulness of this technique for studying other materials is rising steadily, IHCMC is not as widely known to biomedical materials scientists as it could be. It thus seems appropriate and useful, at this point in the growth of the range of applications of IHCMC, to review critically its uses, to date, for studying synthetic biomaterials, highlighting its attractive features and versatility but also indicating issues and concerns that should be addressed in order to improve the quality of data collected in future studies.

This article is organized into four main sections. In the first, a background to IHCMC is provided in the form of a detailed primer, covering basic principles, attractive features, limitation, calorimeter designs and choices, experimental procedures, and, finally, principal uses and treatment of data. The second section provides summaries of relevant literature reports. In the third, a number of issues or concerns related to the use of IHCMC are discussed, these being divided into

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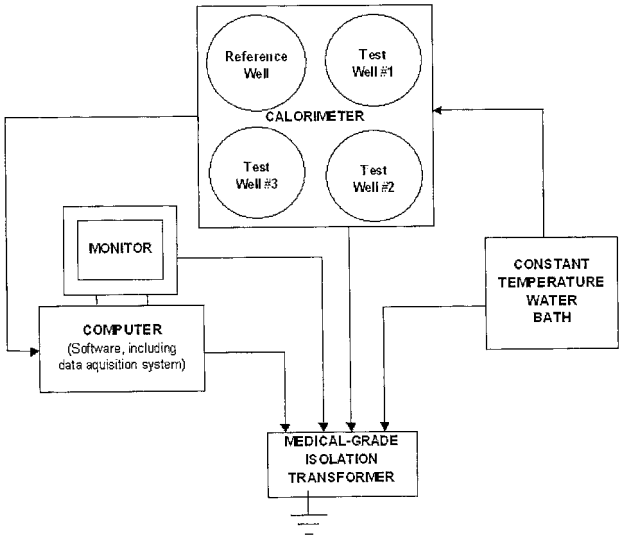

Figure 1. Block diagram of experimental setup of the connection of an isothermal heat-conduction microcalorimeter with associated equipment.

four categories dealing with experimental design, calorimeter performance, standard protocol, and test information reporting. The fourth section presents a number of ideas for future research. A summary of the most salient points of the review follows these sections.

AN IHCMC PRIMER

Basic Principles

IHCMC involves differential measurement of the temporal changes in the enthalpy (in J) between a test material–test medium system on the one hand and a reference material–reference medium system on the other. The time integral of this record, called the heat flow [Q (in W) or q (in W per test sample mass)] is directly proportional to the exothermic or endothermic heat resulting from process(es) occurring in the test material–test medium system.[1] In turn, the rate of heat flow with time is directly proportional to the rate of the process(es) occurring. Because changes in enthalpy accompany all chemical and physical reactions, in principle, then, the progress of all processes can be monitored with the use of IHCMC.

In a typical experiment, the test sample and a suitable reference material contained in two separate, identical ampoules are kept at constant temperature in separate, identically constructed wells of the calorimeter (Figure 1). Ideally, the reference material is identical or very similar to the test sample in mass, heat capacity, and thermal conductivity, but, unlike, the test sample, is thermally inert. That is, the reference material will not undergo changes that result in heat production or absorption under the conditions of the experiment. One example is a small quantity of ordinary glass beads in air at room temperature used as reference for the same amount of a hydrated ceramic material, expected to lose water under the same conditions.

A feedback temperature control system between the wells (a) serves to ensure that the temperature difference between the wells is zero, and (b) provides an output that measures any difference in electric power requirement of one well relative to the other, needed to keep the temperature of both wells the same. This power difference, as a function of time, is the output from the calorimeter, which is recorded continuously or intermittently over the duration of the test.

Attractive Features

IHCMC has six key attractive features for determining process kinetics and energetics.

Sensitivity. The sensitivity of IHCMC is extremely high. Therefore, measurements can be carried out at ambient temperatures if desired. This is a feature that is particularly useful in stability (shelf storage) studies. The high sensitivity also means that, in most cases, a few grams of sample are sufficient even for measuring very slow processes, for example, room-temperature oxidation of relatively stable polymers.

Rapidity. Because of the high sensitivity, it is often possible to capture the rate constant for extremely slow processes in only a few days ($\leq$ 200 h) from a few grams of sample. In contrast, at such low reaction rates, measurement methods relying on the accumulation and quantitation of degradation products frequently require months before sufficient product is available for analysis from a specimen of this size.

Experimental Simplicity. The combination of sensitivity and rapidity also provides another advantage. Experiments frequently can be carried out in simple sealed ampoules, with no need to monitor or replenish the experimental environment. This is because with slow processes, small samples, and short experimental times, there is often no significant consumption of the environment in the ampoule (e.g., oxygen in air) or accumulation of degradation products. There is thus little effect on the rate process being studied.

Universal Detection. Most rate techniques record a change in only one property of a process, as the process proceeds (e.g., in potentiometry, only a change in pH is recorded and in spectrophotometry, only a chromophoric change is recorded). In contrast, IHCMC is receptive to a universal property (namely, the heat produced or consumed in all reactions). Thus, IHCMC may be used to study a process in which there may be either as-yet-uncharacterized reactions or multiple interactions too complex to be captured with the use of a technique that is receptive to only one specific characteristic of the process. Consequently, IHCMC is especially useful as a screening tool for such things as (a) assessment of the effect of fabrication process changes on the stability of a given material, and (b) batch-to-batch quality control of material stability.

Direct Determination of Kinetics and Energetics. *Process kinetics* can be determined directly from IHCMC data because the time rate of heat flow is directly proportional to the rate of a process. As in conventional kinetics studies, if the process fits the Arrhenius model, rate data can be taken at several temperatures and used to compute an activation energy and a rate constant. Of course, if formal values for a specific process (e.g., a particular chemical reaction) are needed, then a model must be proposed, and validated with data from an analytical technique that identifies reaction products (e.g., high-pressure liquid chromatography combined with mass spectrometry). A typical example of this approach is the work of Koenigbauer et al.,[4] described later. In addition, an effective rate constant can also be determined directly from the IHCMC data obtained at a single temperature, using a relatively new method of data analysis described by Willson et al.[5] and also discussed later. The Willson method is especially useful for reactions that are not zero order, and also when multiple temperatures are not appropriate to the subject of study (e.g., a process at body temperature) or may lead to complications, due to changes in reaction mechanisms with temperature. *Process energetics* can also be determined directly, because IHCMC data are a record of enthalpy changes with time. For example, in studies of adsorption of molecules (or biologic entities, such as platelets or cells) on surfaces, the entire process may be complete in minutes or hours. In this case, the complete heat energy evolved or absorbed in the process can be obtained from the integral of the heat flow data.

Availability of Specimens for Other Studies. To perform an IHCMC study, it is not necessary to sample the material before, during, or after a test. However, prior to IHCMC, a portion of a specimen may be removed for study by other methods. Afterward, the entire specimen is available for other studies. Of course, some or all of it may have been converted to another chemical or physical form by the processes that took place during the exposure in the test ampoule.

Main Limitation

The main limitation to the use of IHCMC is a consequence of the fourth attractive feature described above; namely, universal detection. This limitation is that the information obtained from the Q (or q) -t record provides an aggregate or nondiscriminating record of all the reactions taking place in the test material. Thus, IHCMC results cannot be used independently to identify specific phenomena or provide unambiguous interpretation of the molecular details of a reaction. Also, it is possible that a system being investigated may simultaneously exhibit both exothermic and endothermic processes. In that case, the IHCMC signal will be the net heat flow rate and not a true representation of either process. Careful consideration of the specifics of the specimen–environment–temperature system being studied is necessary to avoid such problems.

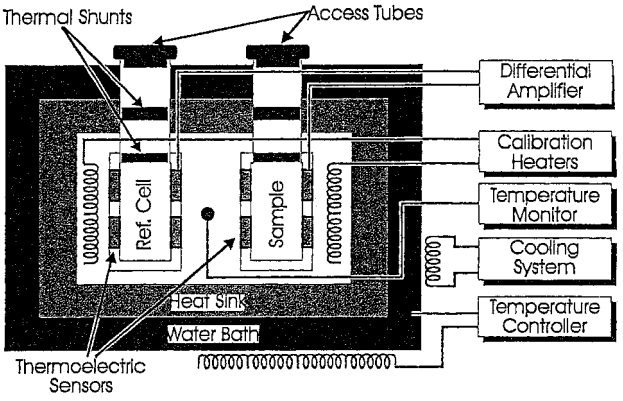

Figure 2. A schematic diagram of the CSC 4400 isothermal heat-conduction microcalorimeter and ancillary systems. (Adapted from Calorimetry Sciences Corporation.[10])

Calorimeter Designs and Choices

Three designs of commercially available microcalorimeters have been mentioned in the recent literature as being used in studies on specimens of synthetic biomaterials: the TA 2277 (Thermoelectric AB, Stockholm, Sweden), the CSC 4400 (Calorimetry Sciences Corporation, Spanish Fork, UT), and the Tian-Calvet (Setaram, Lyons, France). [As an example, the various parts of the CSC 4400 are detailed schematically in Figure 2, from which it is seen that it (a) is, essentially, a large aluminum heat sink that incorporates one reference well and three test sample wells; and (b) has built-in, integral, internal electrical calibration heaters in close proximity to the wells.]

One general aspect of calorimeter design is the incorporation of a separate fluid bath or reservoir to aid in maintaining steady set temperatures in the block containing the test wells, with a minimum cycling of the control system. This is particularly important if the test-well temperature is to be set at or near the temperature of the laboratory surroundings. The bath is kept at a temperature a few degrees below the selected experimental set temperature, and the fluid from the bath circulates around the block containing the test wells. The purpose of the differential temperature of the fluid is to give the test block temperature control system a load against which to work. Without the differential temperature, if, for example, the temperature of the laboratory is 24 °C and the test temperature is 25 °C, the control system will rapidly cycle on and off, as often as every minute. The result is an almost unavoidable increase in the background noise of the differential current required to keep the test well at the same temperature as the reference well. In practice, one important consequence of this situation is a decrease in the calorimeter's detection limit.

It is worth pointing out that the differences in the various features of calorimeter designs, while interesting, are not as important as the differences in many key characteristics between them (for example, see Table I), which, it is expected, will result in differences in performance between them, for the study of a given test material–test medium system. Thus,

TABLE I. Some Performance Characteristics of Three Commercially Available Models of Isothermal Microcalorimeter

Characteristic	Model		
	Thermal Activity Monitor 2277[a]	Tian-Calvet MS80[b]	CSC 4400[c]
Range of operational temperature (°C)	5–90[d]	22–200	−40–200
Accuracy of temperature reading (°C)	$\pm 2 \times 10^{-2}$	$\pm 1 \times 10^{-3}$	$\pm 5 \times 10^{-1}$
Stability of temperature (°C)	$\pm 2 \times 10^{-4}$[e]	$\pm 1 \times 10^{-3}$	$\pm 5 \times 10^{-4}$
Stability of baseline heat flow measurement (μW)	$\pm$ 0.2		$\pm$ 0.1
Short-term baseline heat flow noise (μW)	$\pm$ 0.05[f]		
Range of heat flow measurement (μW)	3–3000		
Minimum detectable heat flow, Q_m (μW)	0.15	0.40	0.20
Volume, V (cm^3)	5[g]	100	100
Practical heat-flow detection limit[h] (mW m^{-3})	30	4	2

[a] Source of data: References 6–8.
[b] Source of data: References 7 and 9.
[c] Source of data: References 7 and 10.
[d] In some high-temperature versions of the calorimeter, maximum operational temperature is 150 °C.
[e] Over an 8h- period, if the room temperature is stabilized to within 1 °C.
[f] Over a 1- h period, when calorimeter temperature is 85 °C.
[g] For each of the four units (some versions of the calorimeter have as many as four units).
[h] Defined as Q_m/V.

attention should be paid to the various aspects of a test system when selecting a calorimeter to use. For example, if the system is to be studied over a wide temperature range, the CSC 4400 would be the best choice. The Tian-Calvet MS80 or the CSC 4400 would be more suitable if the test sample size is very large. If the heat flow (reaction) rate in the test system is expected to be very low, the CSC 4400 would be ideal. (This design has a potential practical heat flow detection limit that is 2- and 15-fold better than that of the Tian-Calvet and Thermal Activity Monitor 2277 designs, respectively.)

Experimental Procedures

Typically, after selecting a type of specimen for study (e.g., an implantable polymer), five steps are involved in performing an experiment: (a) selection of experimental conditions, that is, test sample size, reference material, test ampoule and test medium; (b) preparation and handling of calorimeter wells, specimens, and test ampoules; (c) calibration of the calorimeter; (d) adjustment of the calorimeter's baseline; and (e) initiation of the experimental study.

Selection of Experimental Conditions. Specimen size (i.e., weight and volume) is a first consideration. Larger specimens result in a larger heat signal and, therefore, effectively improve the detection limit and signal-to-noise ratio. However, a large specimen size may require an unacceptably long time to achieve initial temperature equilibrium. Selection of a reference material was discussed at the beginning of this primer, under "Basic Principles."

A typical glass ampoule with its metallic and elastomeric seal components is shown in Figure 3. The selection of both the type of test sample ampoule and the environment to which the sample will be exposed in the ampoule should be guided by the conditions to be simulated. Thus, for example, it might be intended to simulate either shelf storage or body-implantation conditions for ultra-high-molecular-weight polyethylene (UHMWPE) used for joint replacement components. In the first case, the test ampoule could be filled with ambient air. In the second case, a solution (e.g., phosphate-buffered saline, PBS) simulating synovial fluid could be used. However, any tendency for the environment in the ampoule (gas or liquid) to undergo exothermic or endothermic reactions, must, of course, be taken into account.

Preparation and Handling of Calorimeter Wells, Specimens and Ampoules. Extreme care is required in (a) calorimeter well, specimen, and ampoule preparation and handling, (b) introducing specimens into ampoules, (c) sealing ampoules, and (d) introducing the loaded ampoules into the calorimeter wells. This is because of the ability of an IHCMC instrument to sense extremely small amounts of heat flow from any source introduced into the calorimeter well. The required care consists of doing everything necessary to eliminate or minimize any phenomena that may produce extraneous heat flow. Four sets of these requirements are now discussed in more detail.

1. First, calorimeter wells, specimens, and ampoules must be free of water, oil, or other deposits that might be transferred to their surfaces from the experimenter's hands or other sources. Such deposits may evaporate or oxidize during an experiment and produce detectable heat flows. Also, methods of specimen preparation that produce residual stresses in solid material specimens should be avoided (or the stresses removed before IHCMC), because stress relaxation during an experiment can produce detectable exothermic heat. Depending on the sensitivity of detection needed, it also may be advisable to flush the wells with a slow constant flow of dry, relatively inert gas

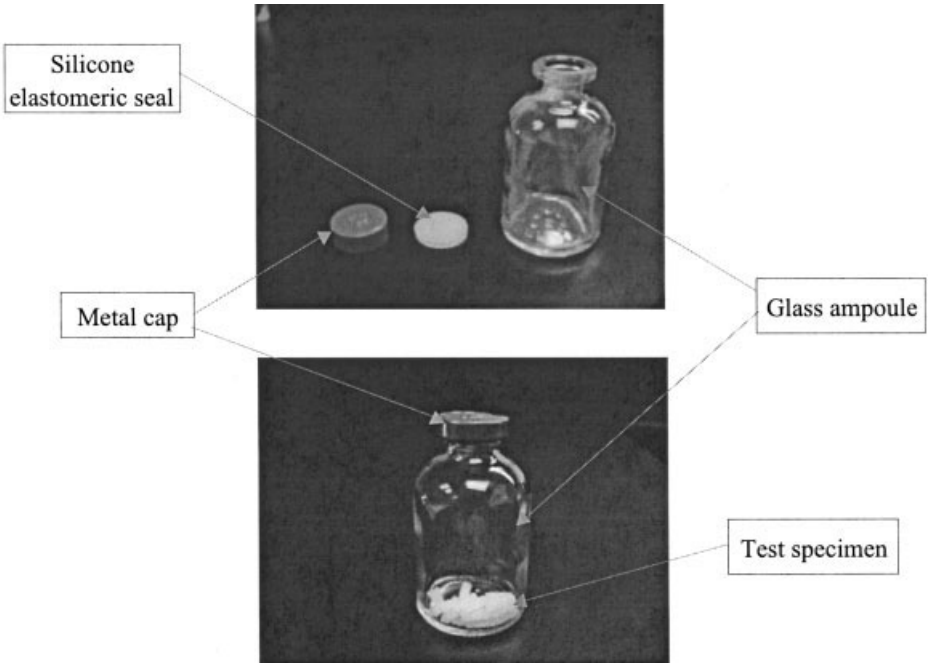

Figure 3. Photograph of pellets of ultra-high-molecular-weight polyethylene sealed in an IHCMC test ampoule.

(e.g., N_2) during calibration, baseline determination, and operation.

2. Similarly, mechanically stressing a specimen or disturbing its surface while introducing it into an ampoule (e.g., by forcibly packing it into the ampoule) can also create subsequent extraneous sources of heat flow.
3. Sealing an ampoule can pose problems. As with specimens, residual stresses can be created in either the metallic or elastomeric portion of a seal during the sealing process. In addition, if a seal is faulty and experimental temperature is much different than the temperature at which sealing is accomplished, very slow leakage of ambient gas (air) into or out of the ampoule will occur, and this will have enormous effects on the observed heat flow. Furthermore, if an ampoule containing air or another gas is sealed at one temperature (e.g., ambient), and the test is performed at a much different temperature, the pressure of the gas in the ampoule will be different than before sealing, and this may also affect reaction (and heat flow) rates.
4. Introducing the loaded specimen ampoules into the calorimeter wells can produce transient heat effects. These stem from air currents created by opening the well and lowering the specimen, and also from mechanical contact of the ampoules with the well interior. It is best to wait for these transients to dissipate before beginning to record heat flows. Usually, the waiting time required is determined while adjusting the calorimeter's baseline.

Calibration. The calorimeter must be calibrated at the temperature(s) of interest. As mentioned in the previous section on calorimeter design, the experimenter must also select (in addition to the experimental temperature) a differential temperature for the calorimeter bath, which must remain the same for baseline adjustments and the test series at the temperature of interest. Calibration can be accomplished using electrical, chemical, or radioactive methods. The electrical method (using internal or external electrical resistors) is, currently, the most popular approach. With this method, the electric resistor in the calorimeter well acts as an exothermic specimen, producing a known steady-state flow of heat.

Baseline Determination. Baseline determination (or adjustment to zero) is accomplished by placing ampoules containing the previously described inert reference material in both the reference well and the test well(s). The procedure is initiated by lowering an ampoule slowly and gently into a well (preferably, using metal harnesses attached to the top of the ampoule). The ampoule is then placed first in a temperature equilibration position for a few minutes, slightly above the base of the well (with thermal shunts and the cover of the well in place) and then lowered to the measurement position. In some cases, an hour or longer in the measurement position may be required to dissipate transient heat effects, some of which have been mentioned earlier. When the observed heat flow rate no longer varies significantly with time, this level is taken as zero (relative to the reference well) for the subsequent experiments.

Test Procedures. The test sample is carefully placed in the test sample ampoule. Next, the reference material, having the same mass as the test sample, is carefully introduced into the reference material ampoule, and the ampoules are sealed.

Depending on the test temperature, it may be advisable to bring the loaded ampoules to the approximate test temperature in an external oven, prior to introducing them into the calorimeter. This can markedly decrease the time required to achieve specimen/test well temperature equilibrium. Then, the test sample and reference material ampoules are lowered gently into the respective wells. After thermal equilibrium is achieved (depending on the thermal condition of the test sample, this could take any time from a few minutes to several hours), the measurement of the heat flow, Q (in W) over the test time t commences. The results may be normalized with respect to the test sample mass M. Thus, the final result format may be Q versus t or q ($= Q/M$) versus t. Typically, IHCMC test runs are conducted for times ranging from about 5 to 200 h, depending on the rate of the process under study.

Uses and Treatment of Data

To date, the principal uses of Q-t or q-t data generated in IHCMC studies of synthetic biomaterials specimens have been to estimate (a) the degradation rate constant (k), at a specified temperature, of the process(es); and (b) the activation energy, E_a.

Two methods are available for the estimation of k, and have been presented respectively by Koenigbauer et al.[4] (the Koenigbauer method) and Willson et al.[5] (the Willson method). With the Koenigbauer method,

$$k_1(\text{in } \%\ y^{-1}) = k_2/[\exp\{E_a(T_2 - T_1)/(T_1T_2R\}],$$

where k_1 is the rate constant at the temperature of interest, T_1 (in K); k_2 is the rate constant for the process (in $\%\ y^{-1}$) at another temperature, T_2 (in K); E_a is the process activation energy (in J mole^{-1}); and R is the molar gas constant ($=$ 8.314 J mole^{-1} K^{-1}). The drawback of this method is that the values of k_2, E_a, and T_2 have to be determined (with the use of either IHCMC or, as is usually the case, some other technique) before k_1 can be computed.

In contrast, Willson et al.[5] have shown, in theory and practice, that for solid specimens of degradable materials, IHCMC data can be used to predict directly the percent degradation over time at the temperature of interest, without any knowledge of either the order of reaction or of the rate constants. Briefly, an equation that fits relatively short-term IHCMC heat flow rate data ($\sim$200 h) with a high correlation coefficient is found empirically, with the use of computer software. The equation is then integrated from time, $t = 0$ to $t = \infty$ to give the entire or total predicted heat output (i.e., the available heat) of the specimen under the experimental conditions used (Q_T or q_T). The equation is then reintegrated from $t = 0$ to the time of interest—for example, 1 year, to give Q_t or q_t. The percentage degradation that occurs over the period of interest is then given by $100(Q_t/Q_T)$ or $100(q_t/q_T)$.

In the estimation of an activation energy, E_a, a set of Q (or q)-t data should be obtained at a series of (at least three) calorimeter test sample well temperatures, T. At each value of T, the "initial" heat flow (Q_{in} or q_{in}) [defined as the value of Q or q at a time at which the test sample and the calorimeter test well are at the same temperature] could be read off either directly from the Q (or q)-t record or extrapolated from that record (if the record is available only for periods of time greater than 1 h). Alternatively, the heat flow averaged over a specified test period (Q_m or q_m) or that achieved at steady state (Q_{ss} or q_{ss}) may be used instead of Q_{in} or q_{in}. Assuming that (a) the process(es) being studied are Arrhenius in nature [which, in effect, means the all reaction(s) are of zero order]; and (b) the rate-determining step is invariant over the range of test well temperatures used in the study, E_a may then be calculated from the slope (m) of either the ln [Q_m (or q_m)]-versus $1/T$ plot or the ln[Q_{ss} (or q_{ss})]-versus $1/T$ plot; that is, $E_a = -mR$.

LITERATURE STUDIES

To date, literature IHCMC studies involving synthetic biomaterials may be grouped into three categories: solid-state stability or degradation, monitoring of *in situ* setting reactions, and responses to cellular species.

Dynamic Physico-Chemical Stability. Stability studies have been conducted on: calcium sulfate (without and with an antibiotic, tobramycin sulfate),[11–13] UHMWPE (unsterilized and sterilized with different methods),[12–19] various commercial formulations of acrylic bone cement,[20] and urethane dimethyl-based composites.[9]

Daniels and co-workers[11–13] used IHCMC to investigate the storage stability of dry tobramycin sulfate (TS) powder, pellets of medical-grade, fully hydrated calcium sulfate ($CaSO_4$), and $CaSO_4$ + TS pellets, in air at 27% relative humidity. With the use of the IHCMC measurements and the Willson method,[5] Daniels et al.[11] determined the degradation processes for these materials to be as follows. First, for $CaSO_4$ alone, an endothermic process was detected, which increased with temperature and was attributable to loss of physical or hydrated water from the fully hydrated $CaSO_4$. Estimated degradation per year was 1% for TS alone, in air at 30 °C and $<$ 10% relative humidity; and less in the $CaSO_4$ carrier: 0.12% and 0.25% for $CaSO_4$ + TS, in air at 40 and 50 °C, respectively, and relative humidity of 27%. Charlebois[13] also obtained an estimate of the activation energy of $CaSO_4$ + TS degradation, in the test temperature range of 30–50 °C, to be 81.34 kJ mole^{-1} (Figure 4). From these results, Daniels et al.[11] and Charlebois[13] concluded that $CaSO_4$ appears to be a carrier for tobramycin with a stable shelf life.

Daniels, Charlebois and co-workers[12–16] used IHCMC to measure the exothermic heat flow from two control and four sterilized states of Hospital for Special Surgery (HSS) Reference Grade UHMWPE (GUR 1150) under simulated shelf storage conditions (in air, at 25 °C and 30% relative humidity) and simulated body implantation conditions (in PBS, at 37 °C). The controls were unsterilized and stored until testing

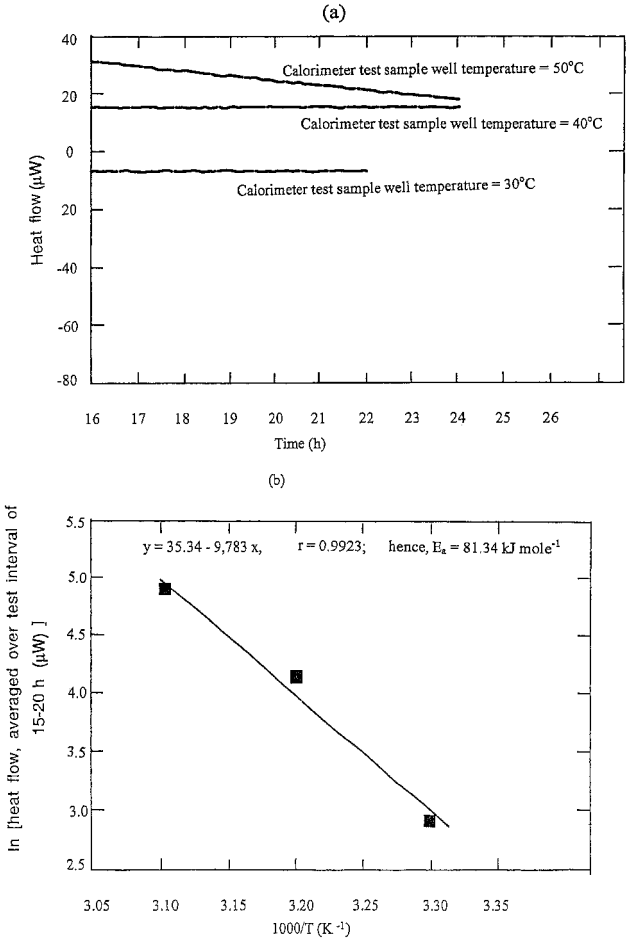

Figure 4. The variation of heat flow with time, for $CaSO_4$-tobramycin sulfate composite (2.6 mg of pure tobramycin per 100 mg of the composite), at test sample well temperatures of 30, 40, and 50 °C. (b) Results in (a) presented on an Arrhenius plot. (Data taken from Charlebois and co-workers.[11,13])

in a double-oxygen-barrier pouch [Control 1], and unsterilized and stored until testing in an air-permeable package [Control 2]. Experimental states were unsterilized [UNS]; γ-irradiated, in air, at 2.5–3.2 Mrad [γ-air]; γ-irradiated, in nitrogen gas, at 2.5–3.2 Mrad [γ-N2]; sterilized using ethylene oxide, EtO [ETO]; and sterilized using gas plasma [GP].

There were three main findings of these studies. First, for both simulation conditions, the exothermic heat flow data for the Control 1, Control 2, ETO, and GP specimens were low (1–5 μW), practically the same, and essentially constant over the duration of the tests (up to 200 h). Second, the initial exothermic heat flow from γ-air or γ-N2 specimens were about 7–10 times higher under simulated shelf storage and 2–3 times higher under the simulated body implantation conditions (even after 1 month) compared to the ETO or GP specimens. Daniels, Charlebois and co-workers[12,13,16] suggested that these results imply that γ irradiation of UHMWPE produces many more unstable (hence, oxidizable) bonds than sterilization using EtO or gas plasma. Third, the initial reactivity of γ-N2 was higher than that for γ-air. However, the heat flow results for these two groups were coincident when the results were shifted to account for the time over which initial exposure to oxygen gas occurred. Based on these three main findings, Charlebois and co-workers [12,13,16] came to two conclusions. First, that their results confirmed the reason for the widely reported higher oxidation rate of UHMWPE specimens sterilized using γ radiation in air compared to ETO- or GP-sterilized ones.[21] Second, that γ sterilization and storage in nitrogen gas does not, per se, prevent the creation of oxidizable bonds but, rather, delays the oxidation of these bonds until the UHMWPE is exposed to oxygen gas.

In another study, Daniels, Charlebois and co-workers[13,17] measured the exothermic heat produced by specimens from the following sources: Unsterilized HSS reference grade UHMWPE [URM]; HSS reference grade UHMWPE, γ sterilized, and shelf-aged for 3 months [GRM]; virgin UHMWPE tibial inserts, γ-irradiated, in air, and shelf-stored for times varying from 90–120 months [GSS]; virgin UHMWPE tibial inserts, sterilized in EtO and shelf-stored for times varying from 110–116 months [ESS]; and retrieved UHMWPE tibial inserts that had been γ sterilized and shelf-stored, for times of between 30 months and 86 months, prior to implantation, with time *in vivo* varying from 7 to 60 months [GCR]. The IHCMC tests on these specimens were run under simulated shelf storage and body implantation conditions. As in the previous work,[12,16] it was found that, irrespective of the simulation conditions, the heat flow from URM and ESS specimens was low (< 3 μW), and remained essentially constant with time. In contrast, the GRM, GSS, and GCR specimens were about an order of magnitude more reactive. These workers[13,17] suggested that these results showed that, several years after shelf storage alone or shelf storage followed by implantation, the γ-sterilized material would still be undergoing physico-chemical change(s).

Hardison and co-workers[18,19] measured the exothermic heat flow from the following three groups of specimens fabricated from HSS reference grade UHMWPE, at calorimeter test well temperatures (T) of 20, 25, 35, and 45 °C: unsterilized [UNSTERA], γ-irradiated in air, at 2.5–4 Mrad [γ-AIRA]; and sterilized using EtO [ETO]. For each specimen, the heat flow (q, in μW g^{-1}) was measured over a test time of about 200 h; whence, the value of q averaged over the test time interval of 15–20 h (q_m) was computed. As an example, q_m results and the Arrhenius plot of these results for UNSTERA are presented in see Figures 5(a) and 5(b), respectively. The Arrhenius plots of the q_m versus $1/T$ results reported by Hardison and co-workers[18,19] yielded activation energies of 47.3, 11.8, and 41.0 kJ $mole^{-1}$ for the UNSTERA, γ-AIRA, and ETO materials, respectively. Based on the postulate of Michaels and Bixler,[22] the value of E_a estimated for UNSTERA suggests that oxygen transport into this material is the rate-determining step. Hardison[19] suggested that the low value of E_a for γ-AIRA is further indication of its high susceptibility to oxidation and, hence, likely rapid degradation *in vivo*.

In another study, Hardison[19] measured the exothermic heat flow from the following groups of specimens fabricated from GUR 1150 UHMWPE: unsterilized [UNSTERB]; and

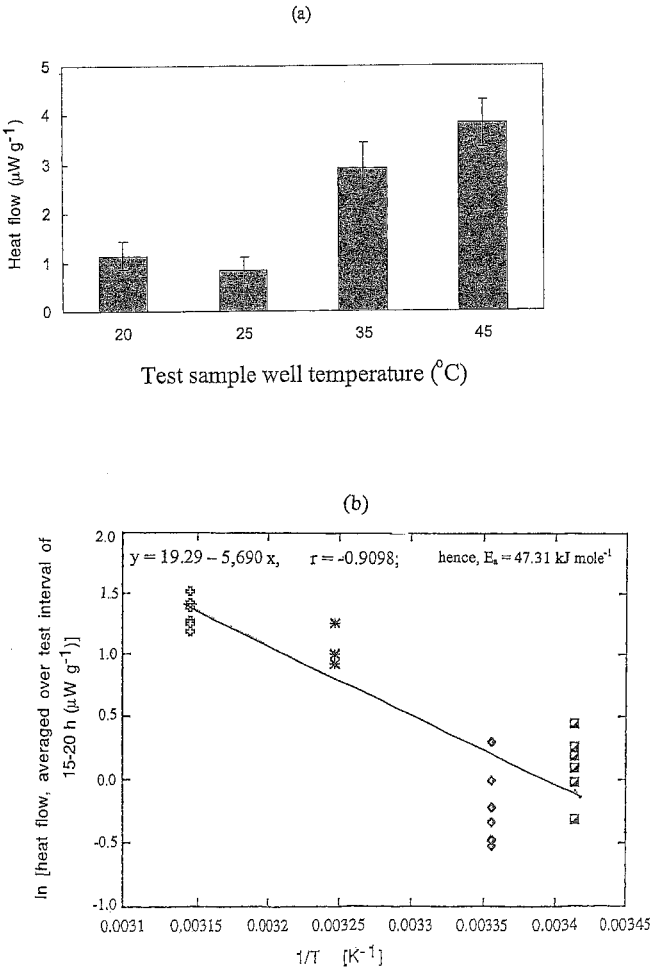

Figure 5. (a) The variation of heat flow, for unsterilized GUR 1150 UHMWPE, with test sample well temperature; (b) results in (a) presented on an Arrhenius plot. (Data taken from Hardison.[19])

γ-irradiated, in air (at 2.5–5 Mrad), and then shelf-aged for a period (τ) ranging from 1 to 8 months before the IHCMC tests were run [γ-AIRB1 to γ-AIRB8]. For the UNSTERB specimens, the heat flow averaged over the test time interval of 15–20 h (q'_m) was very low (mean = 0.65 μW g^{-1}) and increased very marginally with increase in τ. In contrast, the q'_m for the γ-AIRB specimens was about 3–9 times higher than that for UNSTERB, and dropped markedly with increase in τ (Figure 6). Curve fits to these results (Figure 6) showed that q'_m for γ-AIRB equals that of UNSTERB at τ = 28.5 months.

Thomas and Smith[20] reported on an IHCMC study of the effect of the method of mixing the powder and monomer liquid constituents of an acrylic bone cement material (at ambient temperature of 21 ± 1 °C) on the heat flow characteristics of the fully cured cement, under simulated body implantation conditions (in PBS, at 37 ±1°C). Two mixing methods (uncontrolled hand and vacuum) and three commercially available formulations (Palacos® R, Osteopal®, and CMW™3) were used in the study. These workers found that, for a given formulation, the mixing method had no significant effect on the heat flow, suggesting that the mixing methods produced equally stable materials.

Mohsen, Craig, and Filisko[9] measured the isothermal enthalpy changes with time for urethane dimethacrylate (UDMA) -based composites in order to determine the influence of (a) filler type [zirconia-silica (ZS) powder and 75 wt.% 3-methacryloxypropyltrimethoxysilane, MAPM-silanated ZS], (b) ZS filler concentrations (between 0 and 75 wt%), and (c) curing times (ranging from 13 to 300 s) on the chemical aging of these composites. There were three main findings of this study. First, for any combination of test time and temperature, the heat flow of these composites increased with increase in curing time, suggesting that the cross-link density of these materials when cured for a short time does not increase compared to when a long cure time is used. Second, for the ZS-filled UDMA composites, heat flow increased with increase in filler concentration up to 25%, after which it dropped. Third, for the 75 wt.% composites, the heat flow for specimens containing silanated filler (MAPM-silanated ZS) was lower than for those in which the filler was unsilanated (ZS).

Monitoring Setting Reactions. Zimehl, Drews, and Fischer-Brandies[23] used IHCMC to monitor the setting reaction of various dental adhesives; namely, two conventional glass ionomer cements (GICs), two resin-modified glass ionomer cements (RMGICs), and one conventional composite material (CCM). They found that, over both the short term ($0 < t < 20$ h) and long term ($t > 90$ h), the ranking of the magnitude of the measured heat flows from specimens fabricated from these materials was as follows: GIC > RMGIC > CCM. These results, taken in conjunction with others from water uptake measurements, led Zimehl et al.[23] to conclude that, of the three types of materials studied, the RMGIC is the most suitable for the anchoring of orthodontic brackets to teeth.

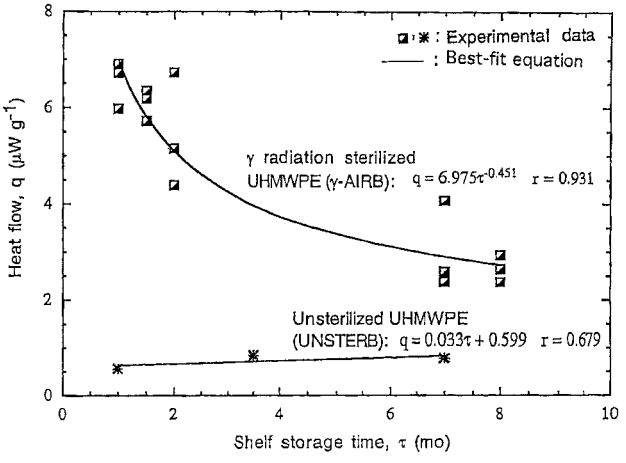

Figure 6. The variation of the heat flow (averaged over a test time interval of 15–20 h), obtained with the two sets of test specimens (made of GUR 1150 UHMWPE; for one set, specimens are unsterilized, and for the other, they are γ–radiation sterilized, in air), with shelf storage time. (Experimental data taken from Hardison.[19])

Metabolic Responses of Cells. In the field of the interaction between cellular species and synthetic biomaterials, studies have been reported on the *in vitro* response of (a) human granulocytes to different dialysis membrane materials,[24] (b) human platelets and granulocytes to a vascular graft,[25] and (c) mouse peritoneal macrophages to various polymeric and metallic biomaterials.[26]

Ikomi-Kumm, Ljunggren, and Lund[24] measured the metabolic response of human granulocytes *in vitro* to four different materials used to fabricate membranes for dialysis machines: fluorinated ethylene-propylene (FEP), polyacrylonitrile (PAN), polyether-polycarbonate (PEPC), and regenerated cellulose (RC). The results were given as metabolic heat production for 2 h after introduction of zymosan particles to the test sample ampoule containing the cells and the test material (Q_g). It was found that the ratio of Q_g to the baseline level (which was the heat flow without any zymosan particles) was 1.47, 3.15, 5.48, and 8.81 % for FEP, PAN, PEPC, and RC, respectively. This indicated that the granulocyte responded the strongest to RC, making it the least suitable material for a dialysis membrane. The order of intensity of granulocyte response to the membranes correlated perfectly with clinical measures of adverse effects of the membranes on granulocytes.

Parsson et al.[25] incubated platelets and granulocytes with plain and collagen-coated Dacron® vascular grafts *in vitro*, and then used IHCMC to measure the metabolic heat response. They reported that (a) for each cell type, there was a rapid increase in heat flow initially, followed by a gradual decrease; and (b) the heat flow for the collagen-coated material was significantly higher than that for the plain material.

Charlebois, Daniels, and Smith[26] used IHCMC to gauge the *in vitro* metabolic heat response of a transformed peritoneal macrophage cell line (*MØ*) to particulates from two biomaterials, meant to simulate wear debris from articulating joint replacement prostheses. The *MØ*s were also exposed to bacterial debris—lipopolysaccharide (LPS) endotoxin, which is readily adsorbed on many surfaces and may be a source of *MØ* response to particles.[27] The groups of specimens studied were: *MØ* (negative control); *MØ*+LPS (positive control); *MØ* + clean particles of high-density polyethylene, HDPE; *MØ* + LPS-bound particles of HDPE; *MØ* + clean particles of Co-Cr alloy; and *MØ* + LPS-bound particles of Co-Cr alloy. It was found that (a) the heat flow from the negative control was lower than from cases where *MØ* was exposed to LPS or the particles of HDPE or Co-Cr alloy; (b) there was no statistically significant difference in the heat-flow response observed for MØs cultured with HDPE particles versus those cultured with Co-Cr alloy particles; and (c) in cases involving Co-Cr alloy particles, the presence of LPS led to a higher, though not significant, increase in heat flow.

ISSUES AND CONCERNS

As shown in the preceding section, IHCMC has been used successfully in a number of studies involving synthetic biomaterials. There are, however, a number of issues and concerns regarding the use of IHCMC for this type of work, which, once addressed, should enhance the technique's appeal and contribute to an improvement of the quality of the results obtained. Ten of these issues and concerns are discussed here, these being grouped into four categories: experimental design issues, calorimeter performance issues, standard protocol issues, and test data reporting issues.

Experimental Design

In this category, there are three issues: the effect of test sample preparation methods on the test results, the optimum test sample mass, and the medium to which the test sample should be exposed.

Sample Preparation. There is a dearth of discussion in the literature on the effects of test sample preparation methods on the heat-flow results. For example, in the case of polymer samples, tests have been carried out on specimens that were molded from the stock,[8] cut, machined, and punched from the stock,[13,16,18,19] or simply cut from the stock.[20] For UHMWPE, it may be feasible to obtain test samples through microtoming the stock. (This would allow investigations as a function of depth below the specimen surface, or would provide, by increasing the surface area per unit mass of sample, an increase in the heat signal.) Each of these preparation methods may well produce samples with different thermal characteristics.

Sample Mass. There is little discussion in the literature on how test sample mass M is selected or how to optimize it. A plethora of values of M have been used (Table II). In fact, even for the same test synthetic biomaterial, no consensus exists as to what M should be. For example, for studies on UHMWPE, M of 0.5 g,[17] 1 g,[18,19] and 5 g[16] have been used. To be fair, when heat-production rates are low (e.g., with relatively stable materials) larger masses are sometimes necessary in order to obtain sufficient heat signal. For interstudy comparison, heat-flow results can be normalized to a unit mass basis. Nonetheless, M is important from the perspective that heat flow is a mass-dependent phenomenon.

Test Medium. There is also a paucity of discussion in the literature on the effect of the medium to which the test sample is exposed during IHCMC measurements. *In vivo*, all synthetic biomaterials are exposed to various body fluids, depending on where the material is placed. Thus, the expectation is that IHCMC tests on these materials should use an appropriate test medium. Simple simulations of body fluids include PBS or 90 vol.% Ringer's solution + 10 vol.% calf serum. However, care must taken with fluids containing biologic materials, in that such fluids may degrade or otherwise change and can be a source of heat in addition to the specimen.

The significance of this issue may be illustrated with the use of heat-flow results for unsterilized UHMWPE. Over the test time span of 15–20 h after test sample ampoule insertion

TABLE II. Summary of Various Experimental Details and Format of Results in Some Literature IHCMC Reports

Ampoule Material	Test Material Amount	Reference Material	Time to Achieve Thermal Equilibrium (h)	Time between Achievement of Thermal Equilibrium and Commencement of Test (h)	Results Format[a]	Was Information Given On: Instrument Calibration Procedure?	Was Information Given On: Baseline Correction Method?	Reference
Steel	1 ml	NS[b]	1-0-2.0	NS	q; q'	No	No	Nassberger et al.[28]
Glass	2.4 mg	0.1 M HCl	0.5	NS	Q-t	Yes	Y/N[c]	Angberg et al.[8]
S/S[d]	1.0 g	Glycine or water	0.5-1.0	5–10	q''-t	Yes	No	Pikal et al.[29]
Glass	1-2 ml	Glycine or water	0.5-1.0	5–10	q''-t	Yes	No	Pikal et al.[29]
S/S	2 cm^3	PBS	0.8	NS	q'	No	No	Kemp[30]
NS	5 ml	Water	NS	NS	Q-t	No	No	Thoren et al.[31]
NS	1.0-3.0 g	NS	NS	NS	Q	No	No	Hansen et al.[32]
S/S or Glass	0.5-1.0 g	Nothing (reference ampoule left empty)	0.5	2	Q	No	Y/N[c]	Koeningbauer et al.[4]
S/S or Glass	0.002-0.50 g	Nothing (reference ampoule left empty)	NS	NS	q''-t	No	No	Tan et al.[33]
NS	2.7 ml	NS	NS	NS	Q-t	No	Y/N[c]	Backman et al.[34]
S/S	1.5–2.5 g	Zirconia-silica	0.5; 2.5; 3.3	Test started immediately	q''-t	Yes	Y/N[c]	Moshen et al.[9]
NS	0.2–0.4 g	NS	2	NS	q''	Yes	Y/N[c]	Forstrom et al.[6]
Glass	0.5–12.5 g	Glass	2-4	3–7	Q-t	No	Y/N[c]	Charlebois[13]
Glass	0.5 or 5.0 g	Glass	NS	NS	Q-t; Q	No	No	Daniels et al.[16, 17]
Glass	1.0 g	NS	NS	NS	q''	No	Y/N[c]	Hardison et al.[18, 19]

[a] q: heat flow (in μW ml^{-1}); q: heat flow (in pW $cell^{-1}$); Q: heat flow (in μW); q'': heat flow (in μW g^{-1}).
[b] Information was not provided in the report.
[c] Baseline was stated as having been determined but details were not provided.
[d] Stainless steel.

in the calorimeter's test sample well, the heat flow was about 1.8 ± 0.9 μW when the test sample (that had been stored in room-temperature air, τ, for 4 yr) was in 10 ml PBS (pH, 7.4; temperature, 37 °C) to simulate implantation.[17] (When normalized based on test sample mass of 0.5 g, the mean heat flow was, thus, about 3.6 μW g^{-1}.) In contrast, when the test sample was in air (temperature, 25 °C; relative humidity, 47%) the heat-flow value was essentially invariant with τ, and was about 1.14 ± 0.28 μW g^{-1}.[19]

Calorimeter Precision and Accuracy

In this category, two issues are discussed, these dealing with the inherent precision and accuracy of the test results.

Precision. The extent to which, in a calorimeter with more than one test sample well, the precision of heat-flow results is affected by combining results from the test sample wells needs to be established. In other words, the question to answer is: Does it matter which test sample well is used? An answer to this question is important because it will elucidate the issue of inherent variability in the heat-flow results recorded by the calorimeter. The result is likely to be different for different designs of calorimeters and, to a lesser extent, different from one calorimeter of the same brand and model to the next.

A study of this aspect has been conducted by Hardison[19] with the use of one specific example of the CSC 4400 calorimeter and 1 g of UHMWPE, with the q-t results being collected over a time period t, of 200 h in ambient conditions (mean temperature and relative humidity of 25 °C and 47%, respectively). The heat-flow results, averaged over the test time interval of 15–20 h (q_a) with the test sample ampoule being located in each of the three test sample wells of the calorimeter sequentially, were as follows: 1.17 ± 0.27, 1.14 ± 0.33, and 0.89 ± 0.29 μW g^{-1}, for test sample wells 1, 2, and 3, respectively ($n = 3$). A statistical analysis found that q_a was not affected significantly by the test sample well selected for placing the test sample ampoule (Student's t-test; $p < 0.05$). It is not clear, though, the extent to which this result is affected by other variables, such as test material and test sample well temperature.

Information on another aspect of the precision of the heat-flow measurements for a given calorimeter could be

obtained by measuring the heat flow from a given test sample medium system on different days, utilizing identical protocols. (Preferably, these protocols should be standard ones, as are discussed in the section that follows.)

Accuracy. The issue of accuracy is very rarely addressed in the literature. The question to address is: to what extent is a reported heat-flow rate absolutely correct, that is, gives the actual quantity of heat or rate of heat flow emanating from the specimen? Accuracy is not of much importance when IHCMC is used for such things as comparisons of heat flows for the degradation of differently prepared specimens of a given material. In those cases, the differential heat is of primary interest, and the only concern is precision. However, accuracy is important if, for example, the intention is to make a fundamental measurement of the metabolic heat produced by a given type of cultured cell. Determination of accuracy requires comparison with both theory and with data obtained by other types of measurements. Accuracy is also obviously tied to the issue of calorimeter calibration discussed in the next section.

Standard Protocols

At the moment, there are no standard protocols for (a) calibration procedures; (b) baseline determination; and (c) other procedures, notably preparing and sealing the test sample ampoule.

Calibration Procedures. There are a multitude of methods available for calibrating an isothermal microcalorimeter, these being classified into three broad groups: electrical, chemical, and radioactive. As expected, each of these approaches has its share of attractions and drawbacks. As for the electrical method, there are two types, which may be termed internal and external. Both involve producing a known rate of heat flow by passing a constant electrical current through a resistance coil or calibration heater (located very close to or at one of the test sample wells of the calorimeter). In the internal method, this heater is built into the calorimeter, whereas in the external method, separate calibration heaters are provided. Electrical calibration methods are convenient to use and the electrical power used can be measured with high accuracy. However, the heat-flow patterns obtained can be very different from that in the process to be investigated.

Chemical methods of calibration involve the determination of the heat generated by the reaction between carefully quantified amounts of chemicals that are placed in the test sample ampoule. Among reactions that have been utilized are the hydrolysis of a triacetin-imidazole-acetic acid-water system, the aqueous dissolution/dilution of propan-1-ol in water, and the dissolution of octan-1-ol.[35] The main attraction of chemical calibration methods is that the amount and pattern of heat flow produced can easily be made to match that of the process to be investigated quite well. Their main drawback is that the calibration depends on the degree to which the values of the relevant thermodynamic properties, such as enthalpy and specific heats, of the actual chemicals used are accurately known.

In one radioactive method of calibration, americium radioactive probes inserted into stainless-steel test sample ampoules have been used.[35] Concerns about safety and the very tight controls on supply of suitable probe materials are the main drawbacks of this approach.

An example of the steps to be followed in calibrating a calorimeter has been provided by Mohsen et al.[9] for the Tian-Calvet calorimeter, in which the external electrical calibration method is used. Calibration is accomplished by (a) inserting the calibration cells (which are, in effect, electrical heating elements of known resistance, R) into the test sample and the reference material wells of the calorimeter; (b) applying a known level of electrical power P_a (this simply means passing a known current I_a, through the elements; $P_a = I_a^2 R$), to the heating element in the well; (c) allowing thermal equilibrium to be achieved; and (d) recording the calorimeter output (Y). This procedure is repeated for a series of values of I_a, thereby allowing a graph of P_a versus Y to be plotted. The expectation is that this calibration graph will be linear, with the slope (C) being the calorimeter's calibration constant. The unit of C depends on the unit of P_a and Y. For example, if P_a and Y are in nW and nV, respectively, then the unit of C is nW nV^{-1}.

No matter which calibration method is used, the issue of identifying the sources of systematic errors in the heat-flow data collected from the calorimeter, even after calibration, and how to deal with them, is important. (For example, in electrical calibration methods, one major source is the change in the heater's resistance over time.) This matter seems to be intractable, leading some calorimeter manufacturers to suggest applying an approximation factor (usually, of the order of 4%) to the heat flow results.[36] Recently, however, an absolute calibration method has been presented.[36] The method uses a well-characterized physico-thermal property of a stable, pure material (such as alumina) of known mass (m_{ac}) placed in the test wells of a calorimeter that has previously been calibrated by, for example, one of the methods mentioned above. This absolute method has three steps. First, the heat gain or loss (Q_{ac}) from a sample of the calibration material following a small, but rapid, change in the operating temperature of the calorimeter (ΔT), is recorded. Second, the apparent specific heat of the calibration material, $C_{p(app)}$, is calculated as the ratio of Q_{ac} to the product of m_{ac} and ΔT. Third, the correction factor to be applied to the heat-flow data is then calculated as the ratio of $C_{p(true)}$ (which is the correct specific heat of the calibration material at the test temperature, a value that could be obtained from the literature) to $C_{p(app)}$.

In spite of the significance of calorimeter calibration, the details are provided in only a few literature reports (Table II), and even in those cases, there are gaps, with no information being provided on a number of parameters; for example, the number of I_a values and the range of I_a values, when the internal electrical method is used.[16,17]

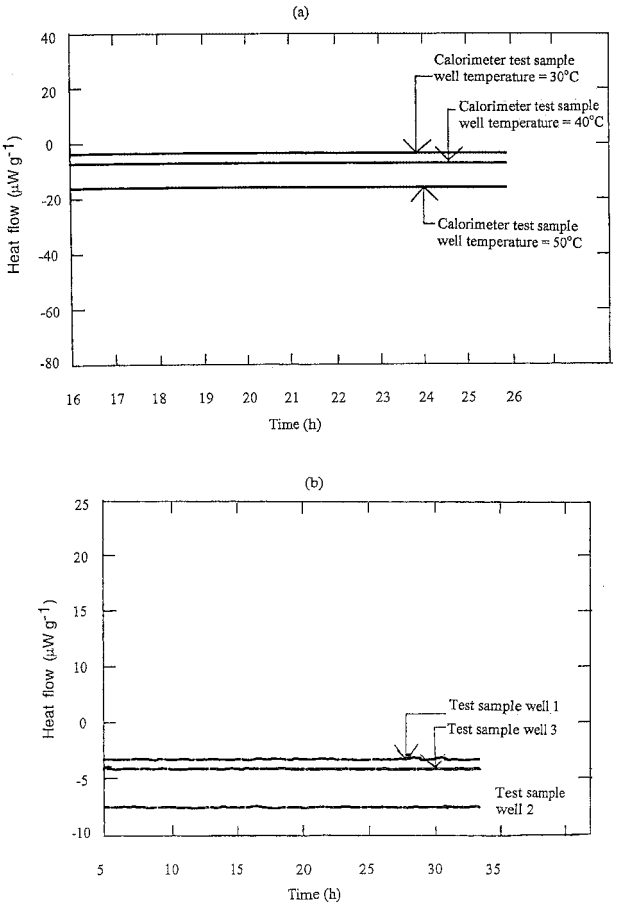

Figure 7. The effect of (a) test sample well temperature and (b) test specimen well location on baseline heat flow results obtained with the use of the CSC 4400 calorimeter. Note that 12.5 g and 5.0 g of glass beads were used in parts (a) and (b), respectively. (Data taken from Charlebois.[13])

The discussion in this section underscores the need for the development of a standard protocol for calibration that will include all the germane details.

Baseline Determination. Determination of the calorimeter's baseline is important because in the final format of the heat-flow results, all Q (or q) values must be baseline corrected. Details of the method used for baseline determination have been given in only a few reports (Table II). In none of these reports has any of the following relevant issues been addressed in an explicit manner: (a) the temporal stability of the baseline (for a given test sample well temperature T_s, and thermally equivalent material), (b) the influence of the thermally equivalent material used on baseline stability (for a given T_s), (c) the influence of T_s on baseline stability (for a given thermally equivalent material), and (d) the influence of the test sample well selected (in the case of calorimeters with more than one test sample well) on the baseline results. Baseline results presented by Charlebois[13] for 12.5 g of glass beads, sealed in air, at various test sample well temperatures [Figure 7(a)] and for 5 g of glass beads, sealed in air, at test sample well temperature of 25 °C [Figure 7(b)] provide some insight into the latter two of these matters. Thus, at the moment, there is no information on the factors that exert the greatest influence on baseline stability. Such information would be useful in the development of a standard protocol for baseline determination.

Test Ampoule Concerns. Even for relatively simple studies of material degradation, using sealed ampoules, ampoule design and materials of construction can affect the measured heat-flow results and, hence, impact the precision and accuracy of the test results. For example, investigators have found that leakage of miniscule amounts of air or water vapor from the test sample ampoule affects the heat flow value measured and is dependent on how well the ampoule is sealed. There are, in fact, a number of related issues. In current literature reports, there is little or no discussion of the impact on the Q (or q) -t results of the (a) heat capacity and thermal conductivity of the specimen ampoule material (glass versus metal, for example); (b) extraneous reaction(s) between the ampoule material and the fluid or gas in the ampoule; (c) type of test sample ampoule sealing (for example, in the case of glass ampoules, elastomeric seal plus metallic crimp cap) (elastomeric seals can cause spurious heat effects through the loss or absorption of volatile species and/or mechanical relaxation); and (d) the level and dispersion of residual mechanical stress from the sealing process. Clearly, a sealing device or method should at least produce reproducible amounts of such stress.

Concluding Remarks. The significance of completely described protocols is illustrated with two sets of heat flow measurements obtained, using the same calorimeter (CSC 4400) and baseline determination method, from specimens of GUR 1150 UHMWPE γ sterilized (2.5–4.0 Mrad), in air, and tested in air (temperature, 25 °C; relative humidity, 30–47%). Over an IHCMC test duration of 15–20 h, the mean q value (q_m) was 15.12 μW g^{-1} (for specimens shelf-aged for 90–120 months before the IHCMC test was performed),[16] whereas q_m was 2.65 μW g^{-1} (for specimens shelf-aged for 8 months before the IHCMC tests were performed).[19]

Reporting of Test Information

A study of the current IHCMC literature shows that there is lack of consistency in (a) reporting details of the test procedures and (b) the format used for presenting the results (Table II). Thus, in many of these reports, many key experimental details are not given, and in most of them, there are no details on (a) whether and how the test sample ampoule is sealed; (b) the protocols used for (i) determining the inherent precision and accuracy of the heat flow measurement; (ii) preparing and cleaning the test sample, (iii) cleaning the test sample and reference ampoules, and (iv) handling of the test sample and reference ampoules prior to their placement in the wells of the calorimeters; and (c) steps taken to create a laboratory environment in which the inherent heat-flow variability of the calorimeter is minimized. For example, one calorimeter man-

ufacturer recommends housing the calorimeter in a room in which the temperature and humidity are kept as constant as possible, and the electric power supply to the calorimeter is provided by a separate low-noise line.[37] In many institutions, laboratory environmental control typically takes the form of ensuring that the room heating/cooling/humidification system settings are kept the same night and day, and that doors are routinely kept closed.

FUTURE BIOMATERIALS APPLICATIONS OF IHCMC

Although IHCMC can be used for diverse types of biomaterials studies, including metabolic response of cells,[26,27] it is contended that its greatest potential is for stability studies of synthetic biomaterials in simulated-use environments. This is for two reasons. First, the sensitivity of IHCMC makes rapid, quantitative studies of stability possible, even for materials as stable as UHMWPE. Second, such studies (especially comparisons) are relatively simple to perform, although, as described previously, the sensitivity of IHCMC makes careful experimentation of great importance.

Discussions of three types of IHCMC stability or degradation studies are presented: comparisons of stable materials (e.g., UHMWPE), comparisons of less stable materials (e.g., bioabsorbable polymers), and general use of IHCMC for formal determination of degradation rate and/or laws.

Stable Biomaterials. In recent years, much research attention has been paid to studying cross-linking of UHMWPE. Although there is abundant evidence that this process improves the polymer's *in vitro* wear performance markedly, its impact on many germane material properties—notably fatigue life, fatigue crack propagation, and physico-chemical stability—has not been fully characterized.[38] IHCMC may be used to correct this deficiency as far as stability is concerned. Thus, future IHCMC work may focus on the influence of a number of variables of cross-linked UHMWPE on the stability under both simulated shelf storage and body implantation conditions. Examples of such variables are type of cross-linking method (γ radiation versus electron-beam radiation versus chemical-induced) and process parameters (for example, packaging medium, radiation dosage, and post-cross-linking stabilization method, in the case of radiation-induced cross-linking).

Long clinical experience has shown that acrylic bone cements are extremely stable biomaterials.[39] However, differences in formulations and polymerization routes may well result in substantial stability differences. IHCMC has seen only initial use for bone-cement studies and seems well suited to this purpose.[20] For example, the relationship between physico-chemical stability of these cements, as measured by heat flow, and their fatigue performance could be investigated. Similarly, IHCMC could be used to study *in situ* polymerized materials and composites used in dentistry.

Degradable Biomaterials. Many implantable biomaterials are intended to degrade and be absorbed by the body and are currently of special interest because of their use as, for example, scaffolds in tissue engineering applications. These materials are deliberately designed to be orders of magnitude less stable than UHMWPE or acrylic bone cements. As a consequence, the degradation-related heat flow from such materials must also be much greater, and this should make their stability exceptionally easy to study by IHCMC.

Such studies could be of great importance, since the rate of degradation is often one of the most critical factors determining the *in vivo* performance of such materials. The only reported IHCMC study of stability of a degradable biomaterial found by the authors in preparing this review was one of implantable calcium sulfate (with and without added tobramycin).[11,13] Also, this was only a study of stability under storage conditions.[11–13] IHCMC should be useful for stability studies not only of synthetic bioabsorbable polymers and ceramics, but also of natural biomaterials, such as bone allograft. For degradable biomaterials, IHCMC could also prove useful for, among other things, performing comparisons of the effects of changes in materials processing variables and as a quality control tool.

Rate/Order Laws. Determination of the rate order/law for the reaction(s) occurring in a given test biomaterial–medium system would involve utilizing the initial portion of the experimental Q (or q) -t data for the system to compute the values of x and y in the general expression for the reaction rate, which was given by Ng et al.[40] to be

$$d\alpha/dt = k'\alpha^{1-x}(1 - \alpha)^{1-y},$$

where α is the fraction of the reaction which has occurred up to time t ($\alpha = 0$ at $t = 0$, and $\alpha = 1$ at $t = \infty$); k' is the rate constant; and x and y are constants characteristic of the reaction rate law. (If $x = y = 1$, the reaction is zeroth order; if $x = 1$ and $y = 0$, the reaction is first order; and if x and y are fractions, the reaction is autocatalytic.) Knowing the order of a reaction makes it possible to propose specific molecular and environmental mechanisms for degradation, and to verify them by changing key variables in subsequent experiments. The resultant more complete understanding of stability makes it possible to propose ways in which stability can be increased or decreased, as desired, by changes in, for example, material composition, molecular weight, and porosity.

SUMMARY

1. Isothermal heat-conduction microcalorimetry, IHCMC, is an attractive method for measuring, in simulated implantation or storage environments, either (a) the rate of physico-chemical change or degradation of biomaterials or (b) the magnitude of the response of living cells to contact with biomaterials. The attractive features of

IHCMC include (a) the ability to rapidly quantify slowly occurring or low-energy phenomena, (b) direct determination of kinetics at the temperature of interest, and (c) for degradation studies, simplicity of experimental setup and rate measurement. Also, given the fact that IHCMC detects all exotherms, it is (d) an excellent screening tool compared to techniques in which, for example, only one degradation product can be detected and measured.

2. The attractions of IHCMC stem principally from the fact that the technique can measure extremely small heat flow rates—on the order of 0.1 μW. However, this extreme sensitivity raises a number of experimental issues and concerns. In general, these take the form of a requirement for scrupulous experimental techniques, which are necessary in order to avoid spurious sources of exothermic or endothermic heat and to ensure reproducibility of results.
3. In the biomaterials field, IHCMC has been used to date mainly for rapidly determining the stability (i.e., degradation rate) of highly stable biomaterials (e.g., UHMWPE) and for determining the metabolic response of cells (e.g., granulocytes and macrophages) to biomaterials contact. For the future, IHCMC holds particular promise for rapidly and accurately determining the degradation rates of bioabsorbable polymers and ceramics (e.g., scaffold materials for tissue engineering), and also biologically obtained materials, such as bone allograft.

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