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Thermoanalytical methods applied to medicine*

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Abstract: Thermoanalytical methods have found increasing application in medicine due to the improved sensitivity and usability of the available instrumentation. Studies have identified important findings applied to medicine, including information on the thermal properties of the skin and the effect of insertion into body cavities of implants and prosthetics. These studies have explored the thermal stability of various materials to provide insight into drug penetration in order to design drug delivery systems, which are not only safe but capable of delivering improved and predictable therapeutic outcomes for patients. Differential scanning calorimetry (DSC) has also been applied to the study of disease states such as diabetes, where changes in the collagen structure of the skin, which may lead to long-term complications in these patients, can be detected. Although these results may at this stage not have significant clinical implications, they do provide medical researchers with a starting point for future investigations. The application of these techniques has been further extended to examinations of body systems and other disease states. The key for the future will be the ability of these techniques not only to provide information on alterations in these biological systems, but also to determine whether these alterations are clinically relevant.

Keywords: disease states; implants; medicine; prosthetics; skin; thermoanalytical methods.

INTRODUCTION

Thermoanalytical methods find applications in medicine because of the ability of these techniques to examine changes in biological systems, ranging from the application of a cream or ointment for the transdermal delivery of a drug substance for systemic absorption, to the monitoring of implants or prosthetics inserted into the body. These techniques thus provide information on thermal events in biological systems that allow not only medical research to be advanced, but also any therapeutic benefits for patients to be predicted.

Thermoanalytical methods have, for example, been extensively applied to the investigation of drug penetration of the skin and have provided some understanding of the skin as a site for drug delivery. These methods have also found application in the study of implants used to deliver drugs to targeted sites in the body. As medical science has advanced, there has been an increased tendency to insert "foreign" objects into the body, whether they be metal or biodegradable polymeric stents, orthopedic or breast implants. It is important to understand not only the effects of these "foreign" objects on the body, but also the effect of the biological system on such factors as their mechanical strength. Thermoanalytical methods are also being used to detect physiological changes in various body

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systems such as cartilage and also to monitor the progression of disease states, such as diabetes and cataract formation.

This paper reviews the role of thermoanalytical methods in these many and varied medical applications, primarily in terms of their ability to detect change. However, there are limitations, because much of this research is in its infancy and it is often not clear as to whether these changes are clinically relevant or statistically valid in terms of sample size.

THERMOANALYTICAL METHODS: TRANSDERMAL DRUG DELIVERY

In recent years, the skin has been increasingly used to provide an entry point for transdermal delivery and subsequent systemic absorption of drugs, as opposed to the traditional topical application of creams and ointments to treat various skin diseases [1].

Transdermal drug delivery: The skin

Differential scanning calorimetry (DSC) has been used to investigate thermal transitions in the skin [2]. The heat capacity of the skin is very sensitive to the temperature, and transitions are observed between 30–120 °C, due to lipids and proteins in the human stratum corneum (SC), which forms the outer horny layer of the skin. Such studies can provide understanding of the changes caused by heat on the structure of the skin and also insight into its functioning in terms of drug delivery.

Guia et al. [2] attributed the three major transitions observed in DSC experiments on all samples at 65, 80, to lipids, and 95 °C to keratin, while a small peak (not observed in all samples) seen at 35 °C was attributed to loosely bound lipids. Since the hydration of the SC facilitates percutaneous absorption, it is an important factor to consider. The effect of hydration on the thermal profiles may be investigated by examining changes in the thermal transition midpoint (T_m) and its thermal sharpness. Results showed that T_m decreases with increased hydration for those transitions near 65 and 80 °C, with corresponding decreases in sharpness of the DSC peaks. The role of water in decreasing T_m is due to its insertion amongst the polar heads and loosening the packing and lowering the attraction of the hydrocarbon chains for each other [2].

Transdermal delivery: Penetration enhancement

The ability of a drug to penetrate the skin and exert a therapeutic effect depends initially on its ability to diffuse from the formulation to the skin surface, followed by penetration of the skin [1]. Skin transitions at temperature ranges above 0 °C have been extensively investigated. Some penetration enhancers, such as propylene glycol (PG), have low melting points (PG at –100 ± 1 °C), and therefore exhibit sub-zero transitions requiring thermal analyses at lower temperatures [3]. Endothermic transitions between –20 and 0 °C for the SC are related to bound water. An additional peak was observed at –9 °C, which has been assigned to lipids and is referred to as the sub-zero lipid peak [3]. Pretreatment of the SC with penetration enhancers, such as oleic acid and PG, resulted in the appearance of two new transitions at –5 and –12 °C, with no evidence of the peak at –9 °C. The peak at –12 °C, was attributed to the formation of an eutectic mixture between the oleic acid and the SC, and the peak at –5 °C was due to excess oleic acid. Penetration enhancers may exert their action by either chemical or physical means, so thermal analysis may be able to reveal whether the mode of action of penetration enhancers in increasing drug solubility occurs by altering the partitioning of the drug between the SC and the formulation, or by enhancing the fluidity of the lipid bilayers [4].

Substances that have proved to be effective penetration enhancers include: acetone, azone, dimethyl acetamide, dimethyl formamide, dimethyl sulfoxide, ethanol, oleic acid, polyethylene glycol, and sodium lauryl sulfate [5,6]. Choice of a penetration enhancer involves more than just consideration of penetration, because absorption into the skin and any resulting toxicity and skin irritation must also

be considered [7]. Aioi et al. [7] have reported on the use of vitamin E and squalene to alleviate the skin irritation caused by these enhancers. Both vitamin E (30 %) and squalene (10 %) improved penetration by factors of 1.5 and 2.5, respectively [8].

Differential thermal analysis (DTA) was used in a study of the effects of fatty acids as penetration enhancers on permeation of piroxicam through rat skins. Fatty acids have been proven to disorder the lipid bilayers, thus explaining their ability to act as penetration enhancers [9]. Endotherms occurred at 65, 75, and 105 °C due to skin transitions, and an additional broad endotherm was observed at 57.5 °C that was not observed in skin that had not been treated with the enhancer. Linoleic acid showed the greatest enhancing effect in this study with an enhancement factor (EF) of 1.76 at 57.5 °C. A broad endotherm at the lower temperature of 44.6 °C occurred in the skin treated with linoleic acid.

Laurocapram (or azone) is reported to be an effective penetration enhancer for both hydrophilic and lipophilic drugs because it solubilizes the intercellular lipids and/or disrupts the lipid lamellae [10]. While there has been some controversy about the use of chemical penetration enhancers, this information as to their mechanism of action is positive due to the fact that only part of the SC lipids is disturbed and the corneocytes are unaffected. This allows the skin to continue to fulfil its very important barrier function. Tenjarla et al. [11] used DSC to investigate a series of *N*-acetylproline esters as penetration enhancers, with hydrocortisone as a model drug applied to the skin in PG, using azone as a control. The transdermal penetration of ibuprofen has also been investigated. This route has the obvious advantage for this drug of avoiding the gastrointestinal (GI) side effects due to the primary insult of the acidic functionalities on the molecule. Ibuprofen disrupts the SC and, through its ability to act as an anionic surfactant, gives rise to self-permeation enhancement [12]. Eutectic mixtures of ibuprofen and terpene penetration enhancers have resulted in superior penetration compared to that of an aqueous solution when the skin was pretreated with terpenes. In addition to these results with ibuprofen [13], screening by thermal analysis has shown a number of other drug classes, which, because of the formation of eutectic mixtures or solid solutions and resulting melting point depression, may be suitable for enhanced drug delivery. These drugs include angiotensin-converting enzyme (ACE) inhibitors, β-blockers, and other anti-inflammatory drugs [14,15]. Further work has been undertaken to investigate the effect of terpenes as penetration enhancers, and DSC studies have provided information on their thermotropic behavior [16]. Iontophoresis, a technique which has obvious application in the increased migration of ionic drugs across the skin, is becoming increasingly accepted by the pharmaceutical industry.

DSC studies have also been undertaken to investigate the effect of iontophoretic transport of the β-blocker, propranolol hydrochloride, on the lipid bilayers of the SC, pretreated by two penetration enhancers, sodium lauryl sulfate (NaLS), and hexadecyl trimethylammonium bromide (CTAB). DSC results indicated that NaLS caused the temperatures of the two higher transitions in the SC to be lowered, an indication that the bilayer has increased in fluidity. Thus, pretreatment of the SC with NaLS proved to be successful in enhancing the flux of propranolol hydrochloride across the SC [17]. DSC studies, undertaken to investigate the effect of PG, glycerol, and isopropyl myristate (IPM), and combinations thereof on the SC, showed a decrease in transition temperatures of the lipid fraction [18].

Nicardipine, used in the treatment of hypertension, has proved to be an excellent candidate for transdermal delivery because its bioavailability is only 20–33 % due to the "first pass" effect in the liver, and its short half-life requires frequent dosing, resulting in poor patient concordance. The effect of menthol on the transdermal delivery of nicardipine hydrochloride from a hydroxypropyl cellulose gel formulation was investigated. DSC results showed increased penetration of nicardipine hydrochloride due to the ability of menthol to partially extract the lipids from the SC [19]. Broadening of the peaks is observed on treatment with menthol, with further broadening associated with increasing concentrations of menthol. This suggests that there is a pronounced effect on extraction of the lipids in the SC, and there is potential for improved drug penetration. The use of menthol is supported by an excellent safety profile.

THERMOANALYTICAL METHODS: EFFECTS OF "FOREIGN" OBJECTS ON THE BODY

Thermoanalytical methods have been used to monitor the thermal and mechanical properties of "foreign" objects, which may be implants or prosthetics, prior to and after insertion into body cavities, and for determining the effects of these devices on biological systems.

Implants

Thermal analyses have been used for over 20 years to characterize implants [20] and to evaluate the in vivo absorption of lipids and related compounds from the body that results in changes in their hardness and other mechanical properties, which may, in turn, compromise their functioning in the body. Implants have been used in the treatment of conditions of the esophagus, the heart, and in orthopedics. Benko et al. [21] investigated two different metal stents, 1 and 2, implanted into the esophagus. DSC curves for stent 1 showed higher melting points, compared to the control and stent 2. This confirmed its improved thermal stability in the esophagus and assisted in its regeneration after surgery. Bioresorbable stents have the advantage of only being present in the body during the healing process and, therefore, do not require a second operation for removal. Poly(L-lactic acid) (PLLA) is commonly used when high mechanical strength and toughness are required. Stents may have a coating to incorporate drugs, which then undergo controlled release. Thermal methods were used [22] to determine the melting temperature (T_m), the heat of fusion (ΔH_m), and the degree of crystallinity (%C), which affect the mechanical properties of these stents, which need to be shown to be capable of supporting blood vessels for at least 20 weeks.

DSC has also been used to examine the thermal and thermo-oxidative properties of high-molecular-mass polyethylene [23], used in components of an orthopedic implant. These implants not only undergo changes during processing, but also during clinical use post-implantation. Conditions after implantation are generally different from those experienced under storage conditions, with higher temperatures and oxidizing compounds present as a result of the inflammation in the patient. These changes are reflected in the thermal properties, melting, crystallinity, and oxidation. Implanted cups, retrieved later from patients, were compared with the properties of controls and showed changes due to the shear and compression forces in vivo, experienced by the person in conducting their normal life of walking, etc. Changes in the implants were shown to continue with the length of time of the implant.

Although coronary stents have been a breakthrough in the treatment of obstructive coronary disease, there have been adverse effects in patients [24]. The design of these stent coatings presents a challenge to the pharmaceutical scientist because the structural integrity must be preserved, while they must provide the correct kinetics of dosing. Ranade et al. [24] describe the paclitaxel-eluting coronary stent, including the methodology to characterize drug delivery from this stent. DSC has been used in the characterization of the polymer, providing information on thermal properties, such as its glass transition, crystallization, and melting range. An important development of orthopedic implants has been the development of artificial composites, which when combined with biomolecules will induce osteogenesis. The properties of hydroxyapatite materials have been studied over the years with a view to increasing elasticity [25], and it has been suggested to be used in a composite in combination with a polymer of hydroxybutyric acid. The physicochemical properties and biocompatibility of this biodegradable composite have been reported [25]. DSC results indicated that the crystallinity of the composite was higher, at 81–89 %, than that of polymer of hydroxybutyric acid at 67–73 %. DTA results indicated that the hydroxyapatite fraction did not influence the melting temperature of the polymer of hydroxybutyric acid at 166 °C, irrespective of the hydroxyapatite content, but did affect the temperature for the onset of decomposition. Composites with higher percentages of hydroxyapatite thus had lower thermal stabilities.

Biodegradable or absorbable implants have value over metal implants because no surgical removal is required. Poly(lactide) thus has application for bone plates and screws for bone fixation.

Because it degrades in the body to lactic acid, a natural intermediate in carbohydrate metabolism, it is suitable for implants and blood vessels which are replaced eventually by the body tissue [26].

Because of their biological tolerance, silicone breast-implants, consisting of an elastic silicone envelope filled with a silicone gel and saline solution, have been used. The earliest manufactured implants tended to undergo gel bleeds, which is the removal of low-molecular-mass silicones by diffusion. Other processes taking place over time are the penetration of body compounds into these envelopes, causing changes in the polymer networks and resulting in decreases of the tensile strength. Thus, patients with implants should be monitored carefully because they have a high risk of exposure to silicone. DSC has provided useful insight into the aging processes of implants through determinations of glass transitions, crystallization points, and melting points, with shifts in these parameters indicating increased mobility of the gels compared to the shells. The time since implantation and the generation of the manufacture affect the DSC parameters [27].

Prosthetics

The biodegradability and antigenicity of the bioprostheses used in heart valves have been most successfully decreased by glutaraldehyde (GA) fixation. Because tissue length may be shortened as much as 80 % within a fairly narrow temperature range, it is important to determine the shrinkage temperature of these tanned biological tissues. DSC is the obvious choice because shrinkage is accompanied by absorption of heat and hence gives rise to a measurable endotherm over the temperature range. The stability of the tissue is high if either the shrinkage temperature is relatively high, or the enthalpy of shrinkage is relatively large. Loke et al. [28] used DSC to determine the shrinkage temperature of the animal tissues for heart-valve applications and presented detailed information on the methodology, including instrumental and sample considerations. Instrument and method validation have played an important role in the reproducibility of these results.

The ideal aortic valve bioprosthesis should consist of a collagen-elastin matrix. Lack of success in achieving such a system has been attributed to factors such as calcification and degradation. Calcification may be due to the cell membrane and/or the GA treatment. It is important to be able to remove cellular remnants and maintain the structural integrity of this collagen-elastin matrix. To mimic the properties of the aortic valve, an extraction process was developed [29]. The authors [29] investigated the effect of sodium dodecyl sulfate and Triton-X 100 on the structural parameters of this extracellular matrix.

Thermogravimetry (TG) and DSC were used to characterize the biopolymers obtained after extraction from porcine aortic valves. TG and DSC indicated the release of bound water from the biopolymer, while the DSC scans of the aortic wall proved to be complex. DSC revealed the effects of sodium dodecyl sulfate on the complex resulting in a shift to lower temperature values for all transitions. However, the denaturing action of sodium dodecyl sulfate on collagen was related to changes in structural parameters, while for Triton-X 100, destabilization was not observed and the structural integrity of the collagen-elastin networks in the aortic wall was maintained. The clinical use of bioprosthetic heart valves has been limited because of calcification. Although ethanol has been suggested as a suitable anti-calcification agent, both clinically and nonclinically, there is still need for further investigation into the anti-calcification activity of bioprosthetic heart valves in the aortic valve. Chelating agents, such as ethylenediaminetetracetic acid (EDTA), in addition to ethanol, may decrease calcification in the aortic wall. DSC was used to determine the denaturation temperatures of the collagen-elastin matrix and to elucidate the calcium-binding properties of the collagen-elastin matrix. The thermal denaturation temperatures for GA-treated collagen-elastin matrix, pretreated with EDTA and ethanol, have been reported to be all in the narrow range of 95–105 °C. This suggests that these pre-treatments did not affect the stability of the collagen-elastin matrix. However, even within this range, there was a difference between the untreated collagen-elastin matrix, that pretreated with ethanol only, and that pretreated with ethanol and EDTA. The study found that sequential treatment with ethanol and EDTA decreased the cal-

cification rate of collagen-elastin matrix, and this has value in the future therapeutic application strategy of bioprosthetic heart valves.

Thermoanalytical methods: Physiological aspects of disease states

DSC studies have been used to study physiological aspects, such as the effects of drugs on membranes, and the progression of disease states, such as intervertebral disc degeneration, arthritis, diabetes, cataract formation, and even to monitor the effects of shoulder electrothermal arthroscopic capsulorrhaphy (ETC).

Physiological aspects

Because of the ability of local anesthetics, such as tetracaine, to affect both lipids and proteins of erythrocytes, their mechanism of interaction has been investigated using thermoanalytical methods. DSC results have confirmed that tetracaine affects not only lipids and proteins (transition at 55–56 °C) but also hemoglobin (68–70 °C). Although the transitions indicate an involvement of the tetracaine with the proteins in the membrane, results confirm that the packing of the lipids is affected to a much greater extent [30]. Degeneration of intervetrabral discs is attributed to mainly environmental effects, including mechanical injuries as a result of the normal aging process. The use of DSC represents a new approach in this field and is suggested as a means of characterizing the different stages of intervertebral disc degeneration (IVDD) [31]. DSC results show a gradual increase in the main transition temperature from 60.5 to 62.7 °C from stages I to V attributed to mechanical overload, resulting in the structure being more tightly packed and extra energy required for disintegration, resulting in the transition at a higher temperature. This study, however, confirmed that IVDD is a continuous process, rather than a disease with distinct stages.

Disease states

Thermoanalytical methods can be used [32] to monitor the destruction of the cartilage in patients with bacterial arthritis over a period of time. DSC results for control samples indicated melting temperatures of 49.7 and 55 °C, which shifted to 57 and 63.15 °C on day 1 of the infection. This was accompanied by a decrease in the total enthalpy from 0.55 J g^{-1} to 0.375 J g^{-1} on the first day. These findings highlight the usefulness of DSC to detect infection of these joints in the early stages [32].

Collagen denaturation in patients with diabetes mellitus (DM) was investigated by DSC, with results showing an elevation of the heat flow per unit mass in diabetic patients with a result of 65.7 °C as opposed to 64.2 °C. DSC parameters increased in samples from diabetic patients compared to those of control patients [33]. The alterations in these thermal properties can be attributed to improved collagen stability due to glucose-mediated cross-linking. Clinical applications of these findings may, however, be limited because they do not provide a quantitative measure of the severity of the disease state, and because of the small sample size.

DSC has also been used to monitor the thermal effects of ETC. This was a feasibility study undertaken to determine whether there were any structural changes in the cartilaginous tissue in the shoulder treated by ETC [34]. DSC results showed a significant difference in the melting transitions, which are reported as 1.5 °C for the untreated tissue compared to 8 °C for the treated tissue, indicating much less cooperativity and lower thermal stability. DSC can thus be used to investigate the effects of ETC.

Formation of cataracts is a significant problem worldwide due to resulting blindness placing a monetary burden on most societies [35]. DSC was used to investigate these lenses to compare the melting temperatures of the lens proteins of diabetic and nondiabetic patients with cataracts. Findings indicate that the sex of the patient and the stage of the cataract have an effect on the melting of the lens pro-

tein. This is explained by the fact that hyperglycemia results in oxidative stress and that in nondiabetic patients the formation of cataracts related to age is related to an abundance of free radicals.

CONCLUSIONS

Thermoanalytical methods have been used widely to provide information on the effects of changes in biological systems and as such have a wide application to medicine [36], from predicting drug penetration of the skin to providing an explanation for the formation of cataracts in patients, in addition to monitoring their progress. DSC studies of the thermal transitions in the human SC can provide some understanding of the role of penetration enhancers used to facilitate the transdermal delivery of drug substances for systemic absorption. The effects of insertion of "foreign" objects, such as implants and prosthetics, on the body can be monitored, and information on the effect that biological systems have, for example, on their mechanical strength, can be obtained. Thermoanalytical methods, such as DSC, have also proved useful for monitoring physiological events and following the progression of disease states in the body. Despite the limitations of the clinical relevance and problems of statistical validity, the use of thermoanalytical techniques and their application to medicine will continue to increase, because they are able to provide important information on the effects of changes in biological systems, which ultimately allow the prediction of better therapeutic and medical outcomes in patients.

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