Vol. 15 No. 2 DEC 2023
Journal of The Indonesian Society of Integrated Chemistry
P-ISSN 2085-1715 & E-ISSN 2621-5543
Published by Jambi University, Chemistry Education Study Program
JISIC

Verification Method for Determination of Melting Temperature and Enthalpy Changes (ΔH) Using Differential Scanning Calorimeter

Warmiati[1], Wijayanti1*[1]

[1] Department of Rubber and Plastic Processing Technology, Polytechnic of ATK Yogyakarta, 55188, Indonesia.

ABSTRACT

Verification of measurement method for determination of melting temperature and enthalpy changes using Differential Scanning Calorimeter has been carried out. This study was done to ensure that the method used can be applied in the laboratory with valid results and uncertainty. The verification method in this research consists of accuracy and precision. Accuracy was done by comparing measurement results with reference values using the t-student test. Precision can be seen in its repeatability and reproducibility. In this study, method verification of melting temperature measurement was carried out by thermal analysis using Differential Scanning Calorimeter (DSC) based on ISO 11357-3: 2018 method concerning determining temperature and enthalpy changes in melting and crystallization. The results showed that the method used to determine melting temperature and enthalpy changes (ΔH) had met the repeatability and reproducibility requirements. The RSD (Relative Standard Deviation) value was less than 2%, and the accuracy met the acceptance requirements with a t-count smaller than the t-table in a 95% confidence level. It means that the results are not significantly different from reference values, so the method can be used in the laboratory.

Keyword: *verification method, melting temperature, differential scanning calorimetry.*

INFO ARTIKEL

Received: 13 Jul 2023; *Revised: 10 Dec 2023;* *Accepted: 20 Dec 2023*	* coresponding author: wijayanti@atk.ac.id DOI: https://doi.org/10.22437/jisic.v15i2.27198

INTRODUCTION

Differential Scanning Calorimetry (DSC) is a measurement technique of the difference between the heat flow rate to the sample and the reference vessel. It is derived as a function of temperature and time while the specimen and reference are subjected to the same controlled temperature program at a given atmosphere using a symmetric measurement system (Menczel et al., 2023). Metals, alloys, and ceramics can be analyzed for heat capacity and enthalpy using DSC (Szécsényi & Menczel, 2023). A known mass sample is heated or cooled. The changes in heat capacity are shown as changes in heat flow. This method implies some parameters, such as melting point, glass transitions, and curing. In material processing, an enthalpy value can be used to estimate the process efficiency (Sedov et al., 2016; Szécsényi & Holló, 2023). It is the reason why the DSC become the most common thermal analysis in laboratories.

Method verification must be carried out to confirm that the method used in the laboratory has valid results (Duong & Wang, 2007). Method verification is one of the requirements for implementing the ISO 17025: 2005 quality assurance system in testing laboratories. It shows that the laboratory has standards competency to testing a sample. Verification of the right method will produce the right analysis result, thereby increasing consumer confidence in laboratory performance (Riyanto, 2019). In laboratories that regularly employ a standard procedure to track the levels of an analytical substance in a specific product, the method's precision, accuracy, and uncertainty are predominantly assessed at critical concentrations. This is because the decision-making process can present certain difficulties, leading to a focus on crucial concentration ranges for evaluation (Aliakbarzadeh et al., 2023)

Verification of a test method can be seen from the accuracy results and its precision (repeatability and reproducibility), based on standard method of ISO 17025: 2005. Accuracy itself is an agreement between the test result and the acceptable reference value. The reference value comes from Certificate Reference Material (CRM) or Standard Reference Material (SRM). Some ways can be used to determine the accuracy of an analytical method, namely,

1. Comparing the analysis results with the CRM or SRM
2. Recoverability test by adding the analyte into the blank matrix (spiked placebo)
3. Standard addition to the sample matrix containing analytes (standard addition method)

Accuracy test can be done by at least 10 repetitions of reading of the standard material. Repeatability is the accuracy of the method if it is carried out repeatedly by the same analyst under the same conditions and in a short time interval. Reproducibility is the exactness of the method if carried out under different conditions. These differences include equipment, solvents, analyzers, and time intervals (Riyanto, 2019).

The aim of this research is to ensure that the method used in laboratory can be applied with valid results, based on its accuracy and reproducibility.

METHOD

Materials

This study uses equipment such as Differential Scanning Calorimeter (Perkin Elmer DSC 4000), analytical balance, chiller, crimper, scissors, and tweezers. The materials used are standard alum pans and covers, SRM Indium standard with lot number BD147, and nitrogen gas (UHP).

Procedures

Instrument Conditioning

Nitrogen flowed at a 19.8 ml/min flow rate, then the instrument and the computer were turned on. After 30 minutes, the chiller was turned on and set at ± 20 °C.

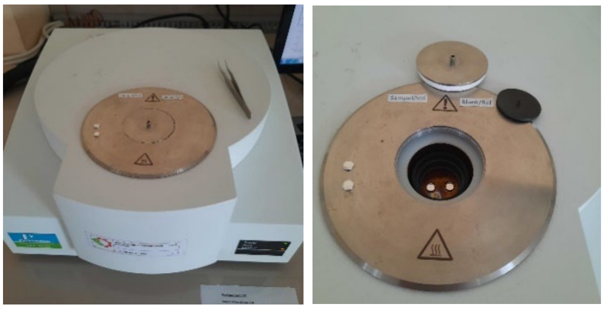

Figure 1. Differential Scanning Calorimeter (DSC 4000)

Instrument Operating

The indium standard was weighed in the range of 5 – 10 mg. Then, put the indium and the pressed empty pan (as a reference) into the DSC for analysis. SRM indium was analyzed at a temperature rise rate of 10 °C/min from 100 °C to 180 °C. This research method refers to ISO 11357 – 3: 2018 concerning determining temperature and enthalpy changes of melting and crystallization.

Accuracy test

The accuracy test was performed by 10 repetitions of reading of the indium standard. After that, the accuracy value was determined by comparing the reference value with the result analysis using the t-test. It is accepted if t count ≤ t-test.

Precision test

Repeatability

The repeatability test was determined by repeating the test 10 times at the same interval of time, by the same analyst, and in the same laboratory. The Relative Standard Deviation (RSD) percent value expresses the repeatability value. If the RSD value of the test is less than or equal to 2%, this repeatability test is accepted.

Reproducibility

The reproducibility test measured the melting and enthalpy values 10 times at random intervals over a long period. The Relative Standard Deviation (RSD) percent value expresses the reproducibility value. If the RSD value of the test is less than or equal to 2%, this reproducibility test is accepted.
The RSD value in repeatability and reproducibility can be calculated by:

$$\%\,RSD = \frac{Standard\ deviation\ of\ average\ value}{average\ value}\ x\ 100\%$$

RESULT AND DISCUSSION

This verification method was standard based on ISO 17025: 2005. Differential Scanning Calorimeter is used to determine melting point, transition glass, enthalphy changes, and curing. This instrument was the most common thermal analysis because it could be provided specific results. Some of the sample requirements for analysis using DSC were as follows: the sample should be dry and free from water, with an amount of 5 – 10 miligrams, and should not react with the alumunium pan. The indium standard was used in this study as a standard material. The indium standard thermogram provided melting point and enthalpy changes (ΔH), as shown below in Figure 2 below. Figure 2 showed that the melting point of indium was 156,54 °C meanwhile, the reference value of indium was 156,6 °C. It means that the test value is similar to the reference value. Based on standard indium thermogram measurement results, an endothermic reaction occurs in the standard indium material during the heating process. An endothermic reaction is indicated by a positive value of enthalpy changes (ΔH). The system's temperature (sample material) generally decreases in an endothermic reaction. This decrease in temperature causes the system to absorb heat from the surroundings. The reaction indicates the melting process of the sample material (Hidayanti et al., 2016).

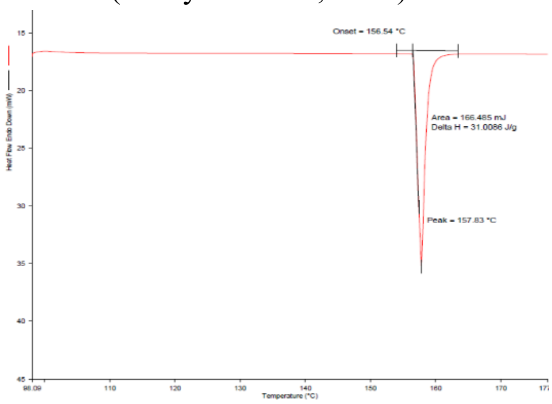

Figure 2. The thermogram of indium standard

Accuracy test

Accuracy refers to the degree of agreement between the test results produced by a particular method and the actual value. There are various means to determine the true value for assessing accuracy. A possible option involves comparing the outcomes of the method with those obtained from a well-established reference method. This method assumes that we know the uncertainty associated with the reference method(Mark & Workman, 2007). The accuracy test and repeatability are done by reading 10 times of indium standard. The results are shown in Figure 3 below.

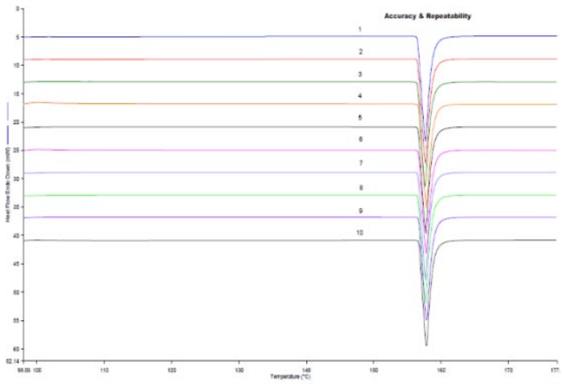

Figure 3. Thermogram of indium standard for accuracy and repeatability test

Figure 3 and Table 1 below show no significant differences in each measurement, or it gives a similar value of melting temperature.

Table 1. Accuracy test

No	Standard	Melting (°C)	ΔH
1	indium 1	156.46	28.47
2	indium 2	156.46	28.46
3	indium 3	156.43	28.20
4	indium 4	156.67	28.07
5	indium 5	156.54	28.43
6	indium 6	156.62	28.46
7	indium 7	156.61	28.56

No	**Standard**	**Melting (°C)**	**ΔH**
8	indium 8	156.63	28.49
9	indium 9	156.66	28.52
10	indium 10	156.68	28.33
Total		1565.76	283.97
Average		156.58	28.40
Reference Value		156.60	28.45
SD		0.10	0.15
t count		0.6643	0.9024
t table n-1 (u95%)		2.2620	2.2620

The accuracy test shows the similarity between the test value and reference value. Based on Table 1, the t-count value in melting point is 0.6643, and the t-count in enthalpy change is 0.9024. The t table (n-1) in confidence level 95% is 2.2620, so both the t count in melting and enthalpy change are accepted because the t count ≤ t table. It means the test value is not significantly different from the reference value.

Precision test

Repeatability

Repeatability, also known as intra-assay precision, refers to the level of precision achieved when performing the same procedure under consistent operating conditions within a brief time interval (Mark & Workman, 2007). The thermogram of the repeatability test is shown in Figure 3 above. It shows that there are no significant differences for each measurement.

The repeatability test in this study was done by repeated measurement of the indium standard in a short period. Based on Table 2, the %RSD value is 0.06 for melting and 0.54 for enthalpy change. The results indicate % that the RSD test is ≤ 2%, so both of the test values are acceptable in the repeatability test.

Table 2. Repeatability test

No	**Standard**	**Melting (°C)**	**ΔH**
1	Ind. 1	156.46	28.47
2	Ind. 2	156.46	28.46
3	Ind. 3	156.43	28.20
4	Ind. 4	156.67	28.07
5	Ind. 5	156.54	28.43
6	Ind. 6	156.62	28.46
7	Ind. 7	156.61	28.56
8	Ind. 8	156.63	28.49
9	Ind. 9	156.66	28.52
10	Ind. 10	156.68	28.33
Total		1565.76	283.97
Average		156.58	28.40
Reference Value		156.60	28.45
SD		0.10	0.15
%RSD		0.06	0.54

Reproducibility

The reproducibility test measured the melting and enthalpy values 10 times at random intervals over a long period. The result is shown in Figure 4 and Table 3. The reproducibility test in this study was done by repeated measurement of the indium standard over a long period. Based on Table 3, the %RSD value is 0.04 for melting and 0.63 for enthalpy change. The results indicate % the RSD test is ≤ 2%, so both test values are acceptable in the reproducibility test.

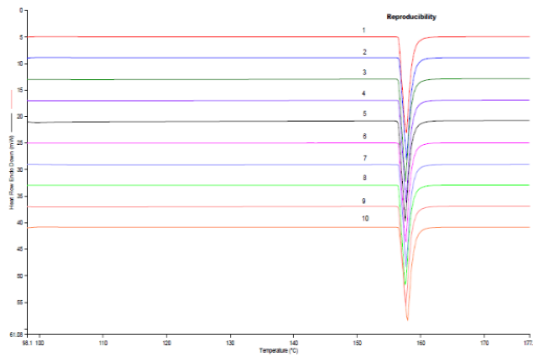

Figure 4. Thermogram of indium standard for reproducibility test

Table 3. Reproducibility test

No	Sample	Melting (°C)	ΔH
1	Rep indium 1	156,64	28,70
2	Rep indium 2	156,51	28,13
3	Rep indium 3	156,55	28,50
4	Rep indium 4	156,45	28,27
5	Rep indium 5	156,54	28,30
6	Rep indium 6	156,60	28,26
7	Rep indium 7	156,50	28,43
8	Rep indium 8	156,58	28,38
9	Rep indium 9	156,63	28,60
10	Rep indium 10	156,53	28,57
Reference values		156,60	28,45
Average value		156,55	28,42
SD		0,06	0,18
%RSD		0,04	0,63

These genes, namely iron superoxide dismutase (FeSOD) and cytosolic copper/zinc superoxide dismutase (SaCSD1), have an essential role in preventing plant tissue from free radical exposure and extreme high saline environmental conditions. FeSOD and SaCD1 genes showed enzyme activity with stable acidic and neutral pH that could be beneficial for industrial product use (Wang et al., 2013; Yang et al., 2015).

High antioxidant activity of *mangrove* plant *S. alba* to inhibit free radical has been reported in several studies. Considering high antioxidant potency and biochemical constituents, *S. alba* leaf could be useful in food and beverage industries. Functional tea could be a useful industrial product, for which *S. alba* leaf is processed with several methods to produce tea products (Mandang et al., 2021).

Another potential use of *S. alba* is in active fish gelatin packaging food and nutrition aspects, due to high antioxidant and antibacterial activity (Nurdiani et al., 2022).

CONCLUSION

The verification for melting and enthalpy change measurement method using Differential Scanning Calorimeter has met the requirements of verification parameters, namely accuracy, repeatability, and reproducibility. The accuracy test results imply that t count ≤ t table in the confidence level of 95%, so it has met the acceptance requirement. The repeatability and reproducibility results show that %RSD is less than 2%. It means that the results are not significantly different from reference values, so the method can be used in the laboratory.

ACKNOWLEDGE

We would like to express our sincere gratitude to Poliytechnic of ATK Yogyakarta which has provided its valuable support, facilities, resources, and guidance.

REFERENCES

Ansari, Z., Mohandass, C., Sahoo, G., & Mulla, N. S. S. (2012). Antibacterial activity of mangrove leaf extracts against human pathogens. Indian Journal of Pharmaceutical Sciences, 74(4), 348. https://doi.org/10.4103/0250-474X.107068

Aseer Manilal, Behailu Merdekios, Tigist Gezmu, & Akbar Idhayadulla. (2015). An in vitro Antibacterial and Anticandidal Activity of Sonneratia alba (J. Smith). An International Journal of Marine Sciences, 31(2), 67–73.

Assaw, S., Mohd Amir, M. I. H., Khaw, T. T., Bakar, K., Mohd Radzi, S. A., & Mazlan, N. W. (2020). Antibacterial and antioxidant activity of naphthofuranquinones from the twigs of tropical mangrove Avicennia officinalis. Natural Product Research, 34(16), 2403–2406. https://doi.org/10.1080/14786419.2018.1538220

Friess, D. A. (2016). Mangrove forests. Current Biology, 26(16), R746–R748. https://doi.org/10.1016/j.cub.2016.04.004

Gawali, P & Jadhav, BL. (2011). Antioxidant Activity and Antioxidant Phytochemical Analysis of Mangrove Species Sonneratia Alba. Asian Jr. of Microbiol. Biotech. Env. Sc, 13(2), 257–261.

Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2010). Identification and Concentration of Some Flavonoid Components in Malaysian Young Ginger (Zingiber officinale Roscoe) Varieties by a High Performance Liquid Chromatography Method. Molecules, 15(9), 6231–6243. https://doi.org/10.3390/molecules15096231

Grattan, S. R., & Grieve, C. M. (1998). Salinity–mineral nutrient relations in horticultural crops. Scientia Horticulturae, 78(1–4), 127–157. https://doi.org/10.1016/S0304-4238(98)00192-7

Hanani, Z. A. N., Yee, F. C., & Nor-Khaizura, M. A. R. (2019). Effect of pomegranate (Punica granatum L.) peel powder on the antioxidant and antimicrobial properties of fish gelatin films as active packaging. Food Hydrocolloids, 89, 253–259. https://doi.org/10.1016/j.foodhyd.2018.10.007

Harizon, Pujiastuti, B., Kurnia, D., Sumiarsa, D., Shiono, Y., & Supratman, U. (2015). Antibacterial Triterpenoids from the Bark of Sonneratia alba (Lythraceae). Natural Product Communications, 10(2), 1934578X1501000. https://doi.org/10.1177/1934578X1501000215

Hassan, H. M. (1989). Microbial Superoxide Dismutases. https://doi.org/10.1016/S0065-2660(08)60223-0

Kaewpiboon, C., Lirdprapamongkol, K., Srisomsap, C., Winayanuwattikun, P., Yongvanich, T., Puwaprisirisan, P., … Assavalapsakul, W. (2012). Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants. BMC Complementary and Alternative Medicine, 12(1), 217. https://doi.org/10.1186/1472-6882-12-217

Kaleh, A. M., Singh, P., Mazumdar, P., Chua, K. O., & Harikrishna, J. A. (2022). Halotolerant rhizobacteria isolated from a mangrove forest alleviate saline stress in Musa acuminata cv. Berangan. Microbiological Research, 265, 127176. https://doi.org/10.1016/j.micres.2022.127176

Kimura, W., Muralidhar, S., Canseco, D. C., Puente, B., Zhang, C. C., Xiao, F., … Sadek, H. A. (2014). Redox Signaling in Cardiac Renewal. Antioxidants & Redox Signaling, 21(11), 1660–1673. https://doi.org/10.1089/ars.2014.6029

Kjer, J., Wray, V., Edrada-Ebel, R., Ebel, R., Pretsch, A., Lin, W., & Proksch, P. (2009). Xanalteric Acids I and II and Related Phenolic Compounds from an Endophytic Alternaria sp. Isolated from the Mangrove Plant Sonneratia alba. Journal of Natural Products, 72(11), 2053–2057. https://doi.org/10.1021/np900417g

Latief, M, Utami, A., Amanda, H., Muhaimin, & Afifah, Z. (2019). Antioxidant activity of isolated compound from perepat roots (Sonneratia alba). Journal of Physics: Conference Series, 1282(1), 012088. https://doi.org/10.1088/1742-6596/1282/1/012088

Latief, Madyawati, Muhaimin, M., Amanda, H., Tarigan, I. L., & Aisyah, S. (2021). Isolation of alkaloids compound of ethanol extract of mangrove perepat (S. alba) root and its antibacterial activity. Jurnal Ilmiah Farmasi, 17(1), 9–18. https://doi.org/10.20885/jif.vol17.iss1.art2

Latief, Madyawati, Nelson, N., Amanda, H., Tarigan, I. L., & Aisyah, S. (2020). POTENTIAL TRACKING OF CYTOTOXIC ACTIVITIES OF MANGROVE PEREPATE (Sonneratia alba) ROOT EXTRACT AS AN ANTICANCER CANDIDATE. Pharmacology and Clinical Pharmacy Research, 5(2). https://doi.org/10.15416/pcpr.v5i2.26790

Muhaimin, M., Latief, M., Dwimalida Putri, R., Chaerunisaa, A. Y., Aditama, A. Y., Pravitasari, N. E., & Siregar, J. E. (2019). Antiplasmodial Activity of Methanolic Leaf Extract of Mangrove Plants against Plasmodium berghei. Pharmacognosy Journal, 11(5), 929–935. https://doi.org/10.5530/pj.2019.11.148

Newbold, C. J., El Hassan, S. M., Wang*, J., Ortega, M. E., & Wallace, R. J. (1997). Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. British Journal of Nutrition, 78(2), 237–249. https://doi.org/10.1079/BJN19970143

Nurdiani, R., Ma'rifah, R. D. A., Busyro, I. K., Jaziri, A. A., Prihanto, A. A., Firdaus, M., Huda, N. (2022). Physical and functional properties of fish gelatin-based film incorporated with mangrove extracts. PeerJ, 10, e13062. https://doi.org/10.7717/peerj.13062

Rahayu, SM., & Sunarto. (2020). Mangrove Plants Use As Medicine In Gedangan Village, Purwodadi District, Purworejo Regency, Central Java Province. Jurnal Jamu Indonesia, 5(2), 76–84. https://doi.org/10.29244/jji.v5i2.116

Reef, R., Feller, I. C., & Lovelock, C. E. (2010). Nutrition of mangroves. Tree Physiology, 30(9), 1148–1160. https://doi.org/10.1093/treephys/tpq048

Saad, S., Taher, M., Susanti, D., Qaralleh, H., & Awang, A. F. I. B. (2012). In vitro antimicrobial activity of mangrove plant Sonneratia alba. Asian Pacific Journal of Tropical Biomedicine, 2(6), 427–429. https://doi.org/10.1016/S2221-1691(12)60069-0

Suh, S.-S., Hwang, J., Park, M., Park, H.-S., & Lee, T.-K. (2014). Phenol content, antioxidant and tyrosinase inhibitory activity of mangrove plants in Micronesia. Asian Pacific Journal of Tropical Medicine, 7(7), 531–535. https://doi.org/10.1016/S1995-7645(14)60089-4

Suryaningrum, F. D. (2021). The Effect of Mangrove Leaf Extract Dosage Sonneratia Alba On Hela Cell Viability. Journal of Stem Cell Research and Tissue Engineering, 5(1), 30. https://doi.org/10.20473/jscrte.v5i1.29383

Wang, F., Wu, Q., Zhang, Z., Chen, S., & Zhou, R. (2013). Cloning, Expression, and Characterization of Iron Superoxide Dismutase in Sonneratia alba, a Highly Salt Tolerant Mangrove Tree. The Protein Journal, 32(4), 259–265. https://doi.org/10.1007/s10930-013-9482-5

Wetlands International Indonesia Programme. (2008). Sonneratia alba. https://www.wetlands.or.id/mangrove/mangrove_species.php?id=41 Accessed: 12 November 2023

Yang, E., Yi, S., Bai, F., Niu, D., Zhong, J., Wu, Q., … Wang, F. (2015). Cloning, Characterization and Expression Pattern Analysis of a Cytosolic Copper/Zinc Superoxide Dismutase (SaCSD1) in a Highly Salt Tolerant Mangrove (Sonneratia alba). International Journal of Molecular Sciences, 17(1), 4. https://doi.org/10.3390/ijms17010004

Zhu, J.-K. (2003). Regulation of ion homeostasis under salt stress. Current Opinion in Plant Biology, 6(5), 441–445. https://doi.org/10.1016/S1369-5266(03)00085-2