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## RESEARCH PAPER

### A COMPARATIVE STUDY ON THE RATE OF BONE DECALCIFICATION AT VARYING TEMPERATURES, FLUID TYPE AND FLUID CONCENTRATIONS

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## ABSTRACT

This study evaluates the rate of bone decalcification at varying temperatures, decalcification fluid and fluid concentrations. A decalcification methodology was adopted using modern household microwave oven to accelerate the decalcification rate of rabbit compact bone sample. Bone biopsy was obtained from a rabbit limb and fixed immediately for 24hrs in 10% formalin. Traditional decalcification was carried out at room temperature (RT) with 5% Jenkins fluid (JK) and 5% Gooding and Stewart (GS) fluid as controls. Microwave oven decalcification with 5% JK and 7% JK, as well as 5% GS and 10% GS served as the tests at 30<sup>o</sup>C, 40<sup>o</sup>C and 50<sup>o</sup>C in microwave oven. Results showed that at 30<sup>o</sup>C, 5%-GS and 10%-GS as well as 5%-JK and 7%-JK presented a decalcifying time of 11hrs:30mins and 10hrs:30mins as well as 15hrs and 13hrs respectively. At 40<sup>o</sup>C, a decalcifying time of 8hrs, 6hrs:30mins, 10hrs:30mins and 12hrs were recorded for the respective concentrations of decalcifying fluid. Also, at 50<sup>o</sup>C, the decalcifying times were 6hrs, 5hrs, and 9hrs:30mins and 8hrs respectively. The observed differences between RT and microwave oven decalcification was significant while the rate between the two decalcifying fluid were not. Our findings further encourage the use of microwave oven for bone decalcification.

**Key Words: Bone, Decalcification rate, fluid concentration, Temperature**

## INTRODUCTION

Histologically, bone and bone-containing specimens are inherently difficult tissues to work with (Janneke *et al.*, 1999). Specifically, routine bone decalcification requires long period of time and has been a major deterrent to many types of morphological studies (Keithley *et al.*, 2000). In fact, literature has it that prolonged periods of exposure to decalcifying fluids cause tissue swelling and hydrolysis to the bone matrix (Pitol *et al.*, 2007).

Similarly, problems arise in cutting sections of bone marrow for biopsy specimens, bone tumour sample or biopsies of metastases to the bone because of the intimate mixture of hard tissues (bone) and soft tissue (marrow, fat, or neoplastic tissue) which has resulted in long time traditional demineralization process to soften bone before sectioning (Janneke *et al.*, 1999). Consequently, several attempts have been made to accelerate the decalcification process and this gave rise to the histological use of laboratory microwaves (Mayers, 1970).

Since then, microwaves have become widely used in histology laboratories for fixation, decalcification and processing (Marr and Wong, 2009). This study therefore, intends to use household microwave oven to determine the rates of bone decalcifications at varying temperature and concentration of decalcifying fluid as it is speculated, that household microwave oven can be used to speed-up the rate of bone decalcification, since it operates like a the laboratory microwave, but has no thermo-regulator.

## MATERIALS AND METHODS

**Study Area:** The study was conducted at the histopathology laboratory, department of Medical Laboratory Science, Ambrose Alli University and University of Benin Teaching Hospital (UBTH), Edo State, Nigeria.

**Sample Size:** The total sample of 14 pieces of Rabbit bones was used, 2 pieces for room temperature decalcification and 12 Pieces for the main project work microwave decalcification.

**Study Design:** This is a study with pieces of bone samples from the shaft of the limb of a Rabbit and fixed immediately for 24hrs in 10% formalin. The bone samples were cut into smaller sizes with a saw after 24hours fixation and the pieces of compact bone were fixed after cutting. It was rinsed in distilled water and transferred to 70% alcohol before decalcification. It was properly labeled. Decalcification was performed using different concentrations of Jenkins (JK) fluid and Gooding and Stewart's (GS) fluid at different range of temperatures using a modern house hold microwave oven. Prior to the commencement of the test decalcification procedures, a control decalcification procedure was conducted at room temperature with 5% Jenkins fluid and 5% Gooding and Stewarts.

**Study Materials and Equipment:** The study materials and equipments used for this study includes a house hold microwave oven, dissecting set, thermometer, small plastic container, universal bottle, test tubes, saw and red litmus paper.

**Room Temperature Decalcification Procedure:** The pieces of tissue were suspended in a universal container containing 5% JK and 5% GS. The decalcifying fluids were changed once daily until decalcification was completed. The bone tissue were rinsed in distilled water and transferred to 70% alcohol before suspending in fresh decalcifying fluid. The chemical method of testing for end point of decalcification was carried out daily to evaluate the progress of decalcification until decalcification is completed. After decalcification was completed, the bone tissues were rinsed in distilled water and transferred to 70% alcohol.

**Microwave Decalcification Procedure:** The microwave oven is operated at different power and time. The power ranges from p10-p100 and thermometer was used to determine the temperature at each power (p). At power p10, p20, p30, the temperatures were determined to be 30<sup>0</sup>C, 40<sup>0</sup>C and 50<sup>0</sup>C respectively.

The decalcification were carried out as follows; Pieces of the bone tissues were suspended in 5% and 10% JK and 5% and 10% GS respectively. It was microwave at 30<sup>0</sup>C for 1hour intervals until decalcification is completed. The chemical method of checking end point of decalcification was carried out at 1hour intervals to evaluate the progress of decalcification. The bone tissues were rinsed in distilled water and transferred to 70% alcohol after each 1hour interval of decalcification. The washed bone tissues were suspended in fresh decalcifying fluid at each 1hour intervals. The same processes were carried out with 5% and 10% JK and GS fluids at 40<sup>0</sup>C and 50<sup>0</sup>C respectively. After the decalcification has been completed, the tissues were rinsed in water and transferred to 70% alcohol.

**Data analysis:** The time of bone decalcifications were recorded for the different temperature, fluid and concentrations employed. The data were then subjected to statistical analysis (SPSS) and presented with simple descriptive statistic and the student t test was performed ( $p < 0.05$ ).

## RESULTS

**Decalcification Rate:** Table 1 shows the comparison between bone decalcification rate at different concentration of Jenkins fluid and Gooding and Stewart fluid and at different temperatures. It was observed that at room temperature (control, 25<sup>0</sup>C), 5% GS fluid presented a decalcifying time of 120hrs (5days) while 5% JK presented a decalcifying time of 168hrs(7days).

At 30<sup>0</sup>C, 5% GS, 10% GS, 5% JK and 7% JK presents a decalcifying time of 11hrs:30mins, 10hrs:30mins, 15hrs and 13hrs while at 40<sup>0</sup>C, were 8hrs, 6hrs:30mins, 10hrs:30mins and 12hrs respectively. Also, at 50<sup>0</sup>C, the decalcifying times were 6hrs, 5hrs, 9hrs: 30mins and 8hrs respectively.

The observed changes in bone decalcifying time between the two fluid was faster in G&S fluid. Similarly, increased temperature were observed also to increase rate of decalcification. In other words, as temperature increases,

decalcification time become faster. This was observed in both fluids. However, an increase in concentration of decalcifying fluid brought about a decrease in decalcifying time on each fluid.

Comparatively 5% GS was observed to be faster than 5% JK at both temperatures. The differences were between, 48hrs in normal room temperature, 4hrs at 30°C, 4hrs at 40°C and 3hrs at 50°C and these difference in time were not statistically significant. Similarly, 10% GS concentration was observed to be faster in decalcification time than 7% JK and these differences were between 48hrs in normal room temperature, 3hrs at 30°C, 4hrs at 40°C and 3hrs at 50°C which is not statistically significant.

Comparatively 5% GS and 5% JK in microwave decalcification were observed to be faster than RT decalcification. The differences were 108:30mins, 112hrs and 114hrs for 5% GS at 30°C, 40°C and 50°C respectively while 153hrs, 156hrs and 159hrs:30mins for 5% JK at 30°C, 40°C and 50°C respectively which is statistically significant.

**Table 1: The comparison between bone decalcification rate at different concentration of Jenkins fluid and Gooding and Stewart fluid and at different temperatures.**

Temperature	Concentration/ Time (hours/mins)		Concentration/ Time (hours/mins)	
	5% G&S	10% G&S	5% JK	7% JK
S				
RT	120hrs(5days)	-	168hrs(7days)	-
30°C MW	11hrs:30mins	10hrs:30min	15hrs	13hrs:30min
40°C MW	8hrs	6hrs:30mins	12hrs	10hrs:30min
50°C MW	6hrs	5hrs	9hrs:30mins	8hrs

MW = microwave, RT= Room temperature, GS= Gooding and Stewart fluid, JK= Jenkins fluid, Hrs= Hours and - = Not conducted at room temperature.

## DISCUSSION

A comparative analysis shows that microwave decalcification was faster and in line with Pitol *et al.*, (2007); Ekuni *et al.*, (2006); Lillie, (1948) and Russell, (1963) who reported that bone decalcification was accelerated by microwave treatment and that the periodic manual or even mechanical agitation, materially speeds the decalcification process while greatly increasing the rotation of tissue blocks in the decalcifying fluid at 1-rev/min also increases the rate of decalcifications. Similarly, Richman *et al.*, (1947) and Willis, (2002) supported these assertions based on the fact that increase in temperature, non-ionizing radiation agitation and the exposure of calcified tissue specimens to microwave energy affects the entire specimen instantaneously and simultaneously, facilitating the exchange of solutions and accelerating the reaction rate due to internal heat.

Our result also suggests that type of decalcifying fluid affects the rate of decalcification as JK and GS fluid at room temperature gave different decalcification rates. This is in line with the study of Ochi and Kpchatkar (2008), who reported a decalcification rate 2 – 7 days for GS, and that of Jenkins and Keren (2011) who reported a decalcification rate of 4 – 10 days for JK. Similar differences were observed for the decalcification rate in microwave oven as cited by Guntz and English, (2011), who stated that microwave decreases the overall processing time for bone decalcification. However, GS fluid in this study presented a faster decalcification rate than the microwave induced decalcification rate reported by Guntz and English; probably due to differences in the source of bone samples.

Comparatively, the decalcification rate became faster as temperature increased and this is in line with Carleton, (1979) and Tinling *et al.*, (1992), who reported that increased temperature accelerates many chemical reactions, including decalcification and that the microwave enhances the rate of demineralization process using decalcifying fluid at a setting temperature.

Meanwhile, the comparative differences in the decalcification rates following variations in fluid concentrations indicated that decalcification is faster as concentrations increases and this is in line with reports by Brain *et al.*, (1966) who says that the higher the concentration of acid used, the faster the rate of decalcification of tissue.

Our finding therefore, encourages the use of microwave oven for moderated temperature variations alongside moderated increases in fluid concentration in order to hasten the decalcification rate of bone samples. This will also reduce the prolonged exposure of such tissues to the damaging effects of decalcifying fluids as well as preventing the attendant architectural tissue distortions.

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**Author(s) contribution**

Bankole J.K., supervised this study with assistance from Okoro C.J., Anyanwa R.A. and Osiagwu D. Eloka C.C. observed the decalcification process with assistance from Uwigbe M. All authors were involved in the preparation of this article.