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POLYMORPHISM AND CRYSTAL GROWTH OF PHENYLBUTAZONE IN SEMISOLID PREPARATIONS. PART ONE: CHARACTERISATION OF ISOLATED CRYSTALS FROM COMMERCIAL CREAMS OF PHENYL-BUTAZONE.

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ABSTRACT

It has been observed that a commercial product of phenylbutazone cream (o/w type) shows gritty appearance. Large Crystals up to 1000 μm in diameter have been isolated from unexpired batches. New batches from the same product show crystal size up to 150 μm in diameter. Investigation of the physical characters of the isolated

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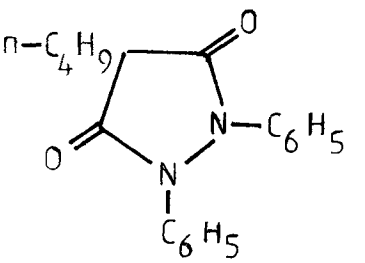
crystals have been done in comparison with a pure phenylbutazone sample (99.5%). UV-Spectrophotometric analysis of the isolated crystals indicates phenylbutazone content of 90%. However, TLC examination and UV, IR and NMR Spectra examinations indicate the absence of decomposition products. Oily material has been separated from the isolated crystals by phosphate buffer solution (pH. 6.90).

DSC examinations of the isolated crystals show two endothermic peaks at 76°C and 100.2°C and one exothermic peak at 81°C. The endothermic peak at 76°C is interpreted as a result of the interaction between phenylbutazone and the oily material.

The examination of the isolated crystals before and after isothermal transformation and during melting by both hot stage microscope, ESM, (IR) and DSC indicates that the presence of phenylbutazone in the crystals is not a mechanical entrapment. It suggests that phenylbutazone interacts with the fatty base on the molecular level and both crystallise out from the cream.

INTRODUCTION



$C_{19}H_{20}N_2O_2$. Mol. Wt. 308.4

Phenylbutazone is 4-butyl-1,2-diphenylpyrazolidine-3,5-dione. The drug has analgesic and anti-inflammatory effect (1). It is formulated as tablets, injections, suppositories and creams.

Phenylbutazone has a well-known polymorphic behaviour as reported by many studies (2-10). These studies have dealt with the mechanism and kinetics of transformation, dissolution characters and compression behaviours of phenylbutazone polymorphs. Recent studies carried on phenylbutazone suppositories (11 and 12) have reported crystal growth of drug particles on storage which is dependent on the temperature of the production.

The polymorphic form of the drug is one of the major parameter which is considered during the formulation of pharmaceutical dosage forms, because it has a significant effect on the physicochemical characters and bioavailability of the dosage form (13 and 14). A recent study shows that the absorption of phenylbutazone in dogs is dependent on the polymorphic form of the drug (15).

In semisolid preparations, the use of improper polymorphic form and improper preparation technique may lead to polymorphic changes and crystal growth of drug particles. Consequently, both the physical appearance and bioavailability of the preparation will be substantially affected (13 and 16).

It has been observed that a commercial product of phenylbutazone cream (o/w type) currently marketed in Jordan shows gritty appearance. Large crystals up to 1000 μm in size have been isolated from unexpired batches. The new batches of the same product show crystal size up to 150 μm in diameter. Thus, the first part of this study investigates the physicochemical characters of these isolated crystals.

EXPERIMENTAL

Materials

Twenty four tubes of phenylbutazone containing (5% w/w) cream from the same manufacturer (Imported Product) have been purchased locally. Pure phenylbutazone powder is obtained from A.P.M. (Jordan). Solvents are spectroscopic (Merck, W. Germany) and analytical grade (May and Baker, UK) reagents.

Methods

1- Isolation of phenylbutazone crystals from creams:

Wet sieving technique has been used in order to separate phenylbutazone crystals from creams after dilution with distilled water. Vacuum has been applied to speed up the filtration process. Washing with large amount of distilled water is done to remove other ingredients of cream. Cleanliness of the isolated crystals

is checked by microscopical examination. The isolated crystals have been dried by keeping in vacuum oven at 900 m bar at room temperature for 2 days. Drying at 60°C induces some degree of transformation.

2- UV-Spectrophotometric Examinations:

UV spectra of solutions of isolated crystals in 0.1N sodium hydroxide and ethanol have been obtained by using Uvikon 810 spectrophotometer (Kontron, Switzerland). Spectra of phenylbutazone standard solutions are obtained at the same time. Calibration curve is constructed and the content of phenylbutazone in isolated crystals is determined.

3- TLC Examinations:

Two systems have been empolyed to check the presence of decomposition products in the isolated crystals.

First System (17): TLC Plate 20x20 cm with silica gel G (0.25 mm thickness). Sample solution: 1% in 2N acetic acid. Solvent: Strong ammonia solution: methanol(1.5:100). Time of run, 30 minutes. Spraying reagent: Potassium permanganate solution.

Second System (18): TLC Plate 20x20 cm with silica gel GF (Merck), 0.25 mm thickness. Sample solution:Chloroform. Solvent: Cyclohexane-chloroform-methanol and glacial acetic acid (60:30:5:5). Detection: Short-wavelength UV light.

4- Nuclear Magnetic Resonance Examination:

The NMR spectra of isolated phenylbutazone crystals and pure phenylbutazone powder have been recorded using Varian T60 NMR Spectrometer (Varian Analytical Instrument Division, USA). The setting conditions are: Spectrum amplitude 1.6x10, Sweep time 250 sec., Sweep width 500 Hz, Filter 1, Solvent $CDCl_3$ containing 1% tetramethylsilane as internal standard.

5- Elemental Analysis:

Elemental analysis (C, H, N and O) of isolated phenylbutazone crystals and pure phenylbutazone powder has been carried out by Mikroanalytisches Labor Pascher (Buschstr. 54, D-5300 Bonn 1, W. Germany).

6- C-13 NMR Examination:

The spectra of C-13 NMR of isolated phenylbutazone crystals and pure phenylbutazone powder have been done at Eidgenossische Technische Hochschule, Pharmazeutisches Institut, Zentrum, CH-8092 Zurich using 75 MH13C-NMR, Bruker Instrument.

7- Thermal Analysis:

Differential scanning calorimetry (DSC) examinations have been performed using Mettler TA3000, DSC 20 unit (Mettler, Switzerland) calibrated with pure indium (m.p 155^{o}C). The samples are heated in covered pans.

Isothermal treatment has been done by heating the isolated phenylbutazone crystals in DSC cell at 81°C for 10 minutes.

Thermogravimetric analysis of the isolated phenylbutazone crystals is also performed using Mettler TA3000, TG50 unit (Mettler, Switzerland) by heating at a rate of 10°k/min. in the range of 35°-120°C.

Purity of phenylbutazone powder (APM) has been determined by DSC method at heating rate of 5°K/min. Effect of compression on the isolated phenylbutazone crystals has been studied by compressing crystals using IR KBr disc preparation Kit and hydraulic press at compression force of 10 tons for 15 minutes. The compressed crystals are then examined by DSC.

8- Infrared Spectrophotometric Examination:

Nujol mulls of the powdered isolated crystals and pure phenylbutazone have been examined and the spectra determined using a double beam infrared spectrophotometer (Jasco IR810-Japan Spectroscopic Co.Ltd),with % T mode, scan speed 2 and slit M. Isothermally treated sample (See TA Examination) and compressed isolated crystals (10 tons for 15 minutes by IR KBr disc hydraulic press) have been examined similarly.

9- X-ray Powder Diffraction Examination:

The powder diffraction patter of isolated phenylbutazone crystals and pure phenylbutazone powder

is recorded by Philips PM 9920/05 X-ray diffractometer (Philips, The Netherlands). Experimental conditions are: 25 mA, 30KV, 1° Slit width, Ka Cu radiation with Nickel filter and scan speed 2 theta per minute.

10- Electron Scanning Microscopy Examination:

ESM Photomicrographs have been made using Lietz 1000A, AMR electron scanning microscope (Lietz,W.Germany). The samples have been coated with gold using a direct sputter coating technique.

11- Hot-Stage Microscopy Examination:

A stereomicroscope (Olympus, Japan) equipped with a hot-stage has been employed. Appearance changes during heating up the isolated phenylbutazone crystals have been examined. Isothermal treatment at approximately 81°C has been applied to examine any transformation stages.

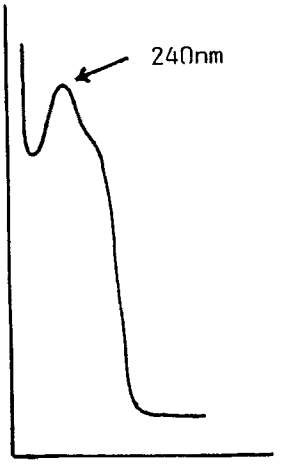
RESULTS AND DISCUSSION

1- Purity of the standard phenylbutazone powder obtained from APM (Jordan) has been determined by DSC and found to be 99.54% Mol. for 9 replicates with standard deviation of ± 0.303. Thus, the drug powder is considered pure enough to carry out determinations required for the current study.

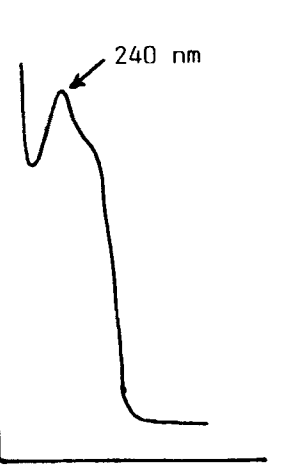
2- UV spectra of the isolated phenylbutazone crystals and pure drug powder in 0.1N NaOH and ethanol are shown in Fig.1. The spectra are identical with respect to the absorbing moieties rather than to the concentrations. The maximum wavelengths in 0.1N NaOH and ethanol solutions are 264 nm and 240 nm which are identical to the reported data (5). The quantitative determination of phenylbutazone in isolated crystals is found to be 89-93.8% w/w. The spectra in 0.1N NaOH and ethanol indicate absence of foreign absorbing moieties and decomposition products.

3- The TLC result of the first system shows one main spot at the same retention time for both isolated crystals and pure phenylbutazone powder. However, the second system which is reported to be characteristic for separation of decomposition products (18) shows two spots for each sample solution whether contains isolated crystals or pure drug powder. A major spot at Rf value 0.80 indicates phenylbutazone and a very faint small spot at Rf value 0.59 indicates possible decomposition product have been observed for both isolated crystals and pure drug powder.

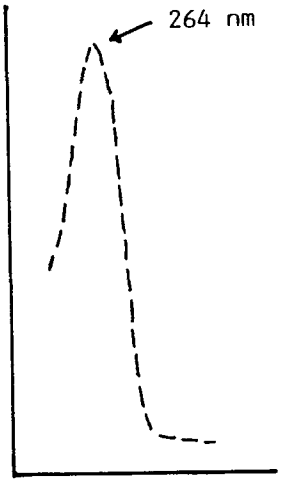
4- N.M.R. spectra are shown in Fig.2. The doublet and multiplet peaks at 0.95-2 ppm in the spectrum of pure drug are indicative to the protons of n-butyl rest of



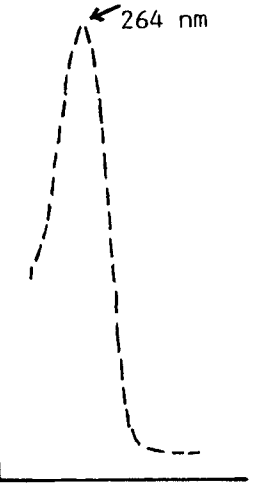
Isolated crystals in ethanol



Pure phenyl butuzone in ethanol



Isolated crystals in 0.1 N NaOH



Pure phenyl butazone in 0.1N NaOH

Fig, 1 . UV Spectra of the isolated crystals and pure phenylbutazone .

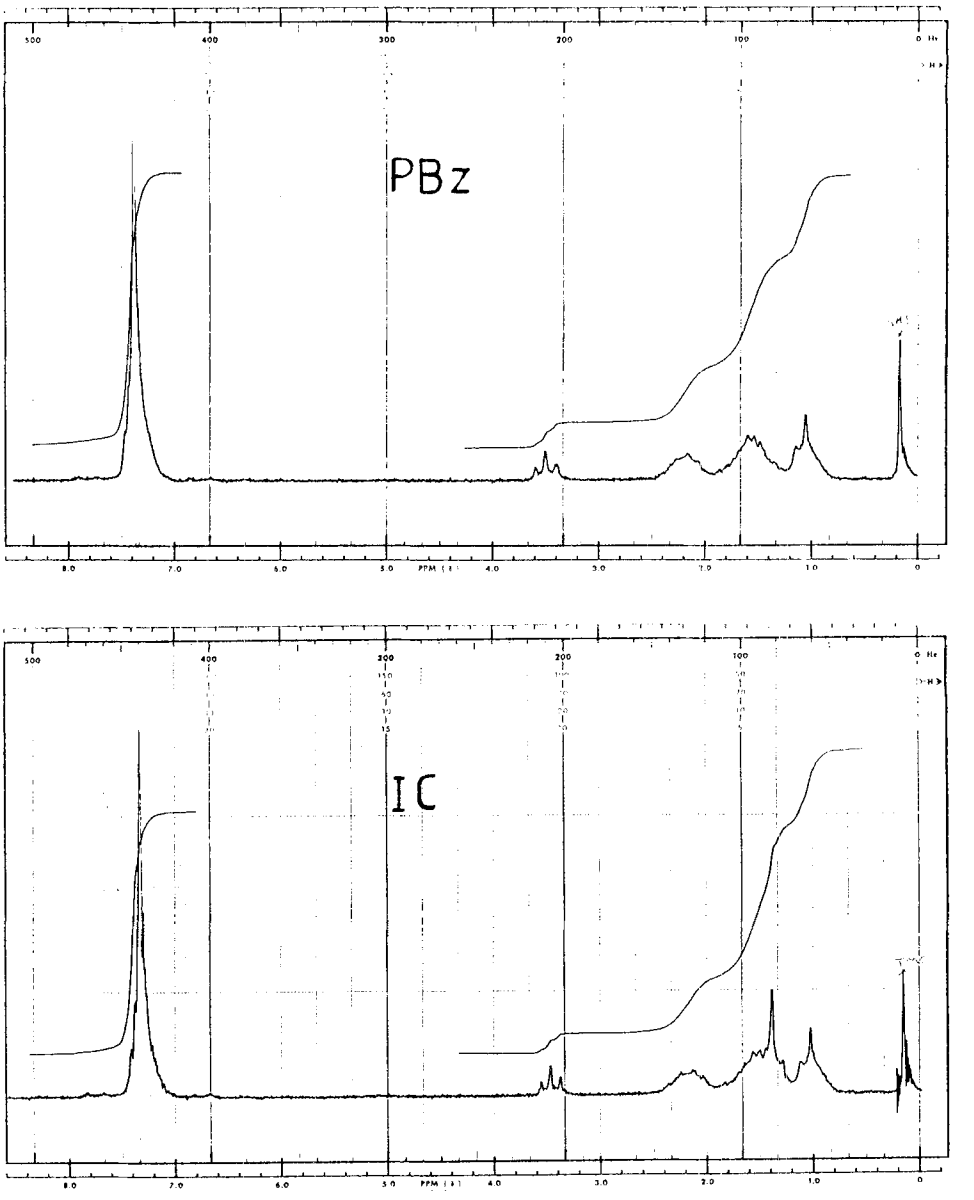


Fig.2. NMR Spectra of the isolated crystals(IC) and pure phenylbutazone powder (PBz).

phenylbutazone molecule. Isolated crystals spectrum shows similar peaks. However, the peak at 1.33 ppm indicates presnce of higher proton number. This higher proton number may be interpreted as an indication of the presence of extra CH_2 groups. The triplet peaks at 3.35 ppm in both spectra are due to proton at C_4 position in phenylbutazone molecule. The multiplet peaks at 7.30 ppm are assigned for aromatic phenyl protons in phenylbutazone molecule as shown in both spectra. The spectrum of the pure drug is in a good agreement with the reported data in literatures (5).

5- 13C-NMR spectra are shown in Fig.3 and 4. The assignments are given in Tables 1 and 2.

The results obtained from UV-spectra, TLC, NMR spectra and 13C-NMR spectra all indicate that the isolated phenylbutazone crystals donot contain any decomposition products. Moreover, the phenylbutazone molecules donot have any sort of chemical combinations with the unknown material present in the isolated crystals.

6- Elemental analysis results for C,H,N and O of the isolated crystals and pure phenylbutazone are given in Table 3. If the decomposition products are present, the percentage of N is expected to be approximately

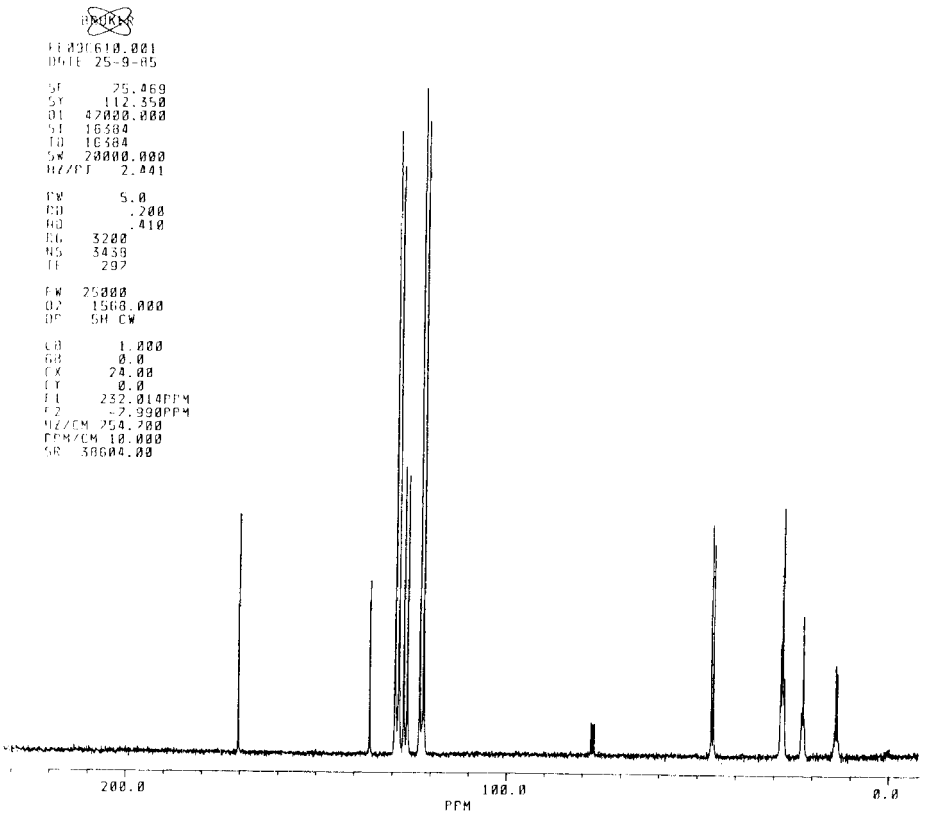


Fig.3. 13C NMR Spectrum of pure phenylbutazone powder.

the same. The percentage of N is less by 1.1% in the isolated phenylbutazone crystals. Consequently, the amount of approximately 10% w/w in the isolated crystals is due to molecules which are not containing N atoms. These molecules are composed mainly of C,H and O.

7- ESM photomicrographs of the isolated phenylbutazone crystals (Fig.5) show that the crystals are not aggregates of small crystals. The crushed crystals of fres-

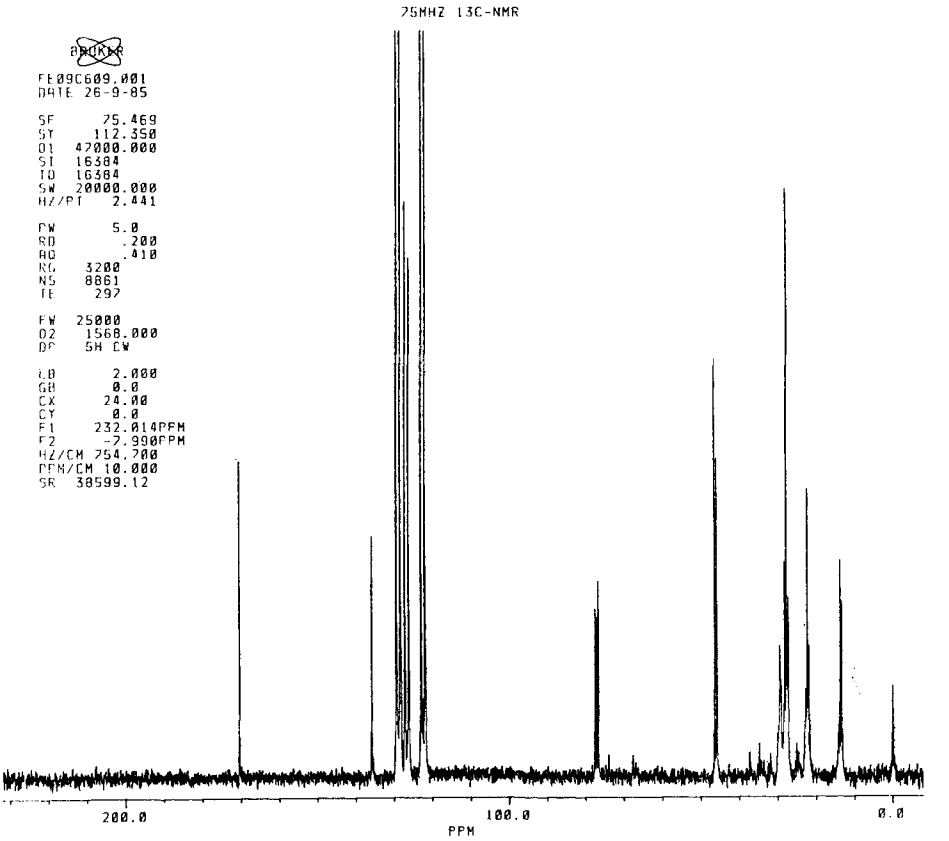


Fig.4. 13C NMR Spectrum of the isolated phenylbutazone crystals.

hly isolated and vacuum dried sample indicate that the body of the crystals is a homogeneous mass. The crystal size isolated from old unexpired batch is large up to 1 mm in length as shown in Fig.5 and 6. While the crystal size isolated from recently manufactured batches is smaller approximately 150-200 μm in length (Fig.7). However, it is much larger than the size of the pure phenylbutazone crystals (Fig.6).

Table 1. Assignments of 13C-NMR spectrum of pure phenylbutazone.

Chemical Shift ppm.	Assignment
170.29	Carbonyl group.
135.88	Quaternary carbon of aromatic ring.
128.79-122.09	Ortho, meta and para carbons of phenyl group.
46.04	Carbon at position 4.
27.85-5.27	n-butyl rest.

Table 2. Assignments of 13C-NMR spectrum of the isolated phenylbutazone crystals.

Chemical Shift. ppm.	Assignment.
170.32	Carbonyl group.
135.80	Quaternary carbon of aromatic ring.
128.88-122.44	Ortho, meta and para carbons of phenyl group.
67.22	Carbon attached to electron withdrawing group
46.16	Carbon at position 4
27.86, 25.02, 22.42 and 13.62	Carbons of n-butyl rest.

Table 3. C,H,N and O Elemental Analysis of the isolated crystals and pure phenylbutazone.

Sample	%			
	C	H	N	O
Pure drug.	74.34	6.61	9.21	10.3
Isolated Crystals(1)	74.29	7.09	8.11	11.0
Isolated Crystals(2)	73.94	7.07	8.11	10.9

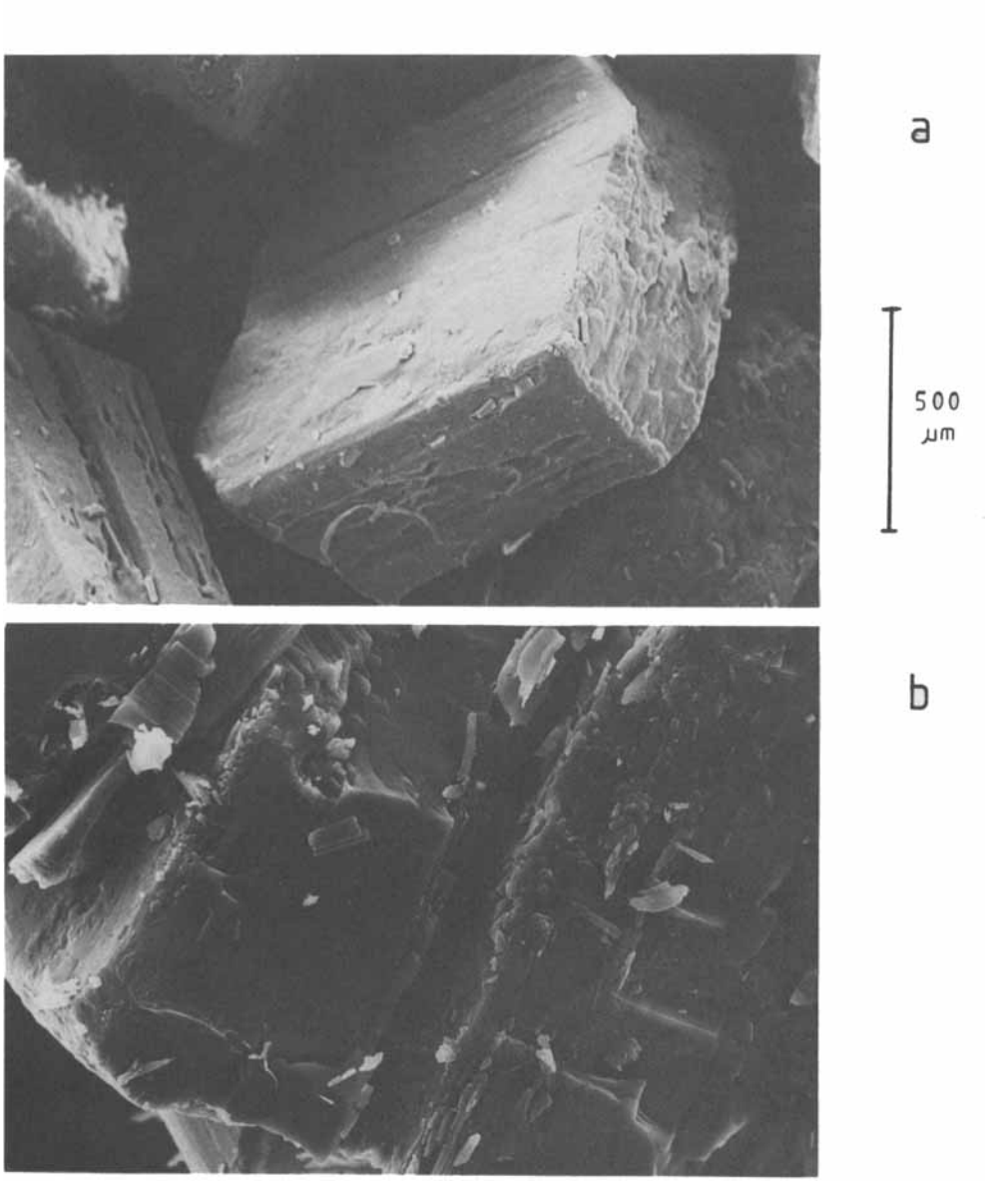


Fig.5. ESM Photomicrographs of the isolated phenylbutazone crystals before (a) and after crushing (b).

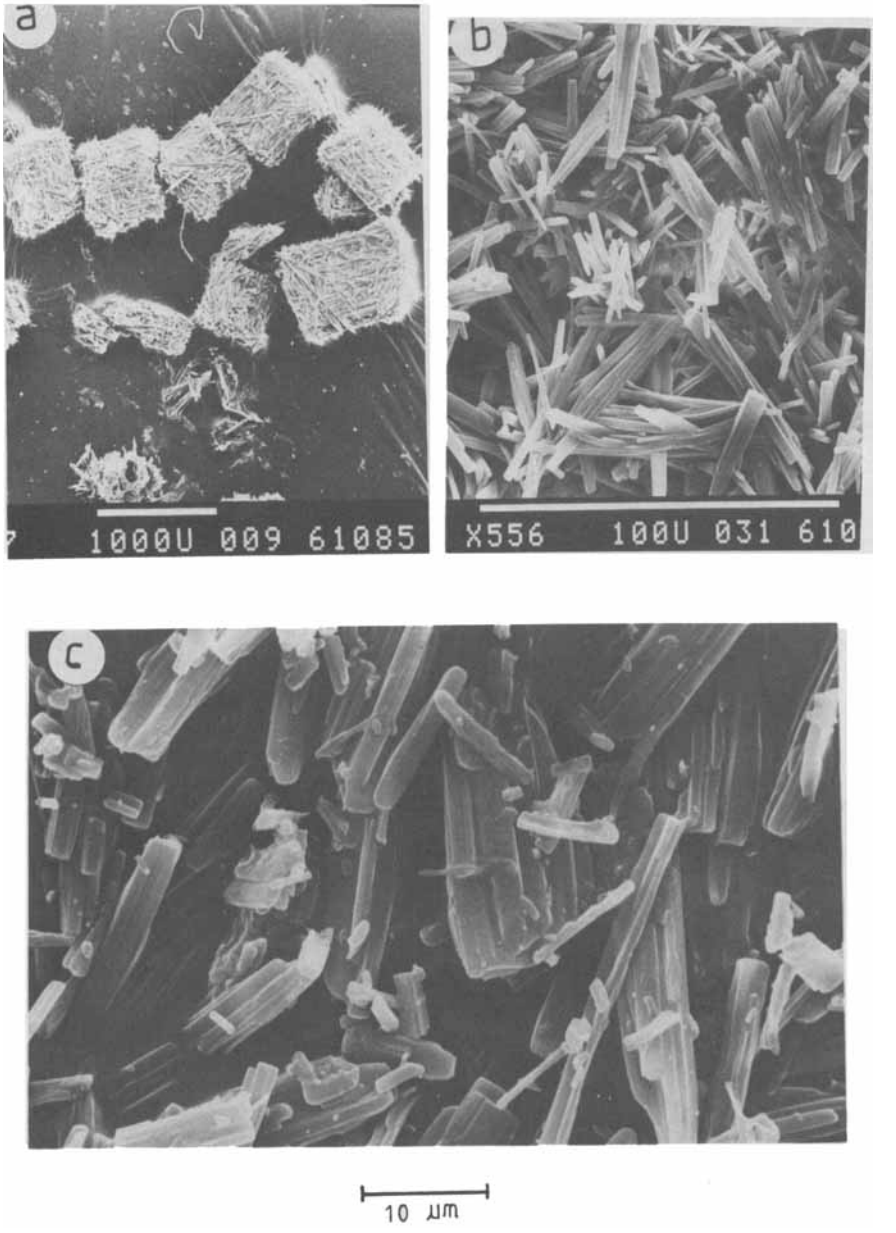


Fig.6. ESM Photomicrographs of the isolated crystals (a and b) after isothermal treatment at 81°C for 10 min. and pure phenylbutazone powder (c).

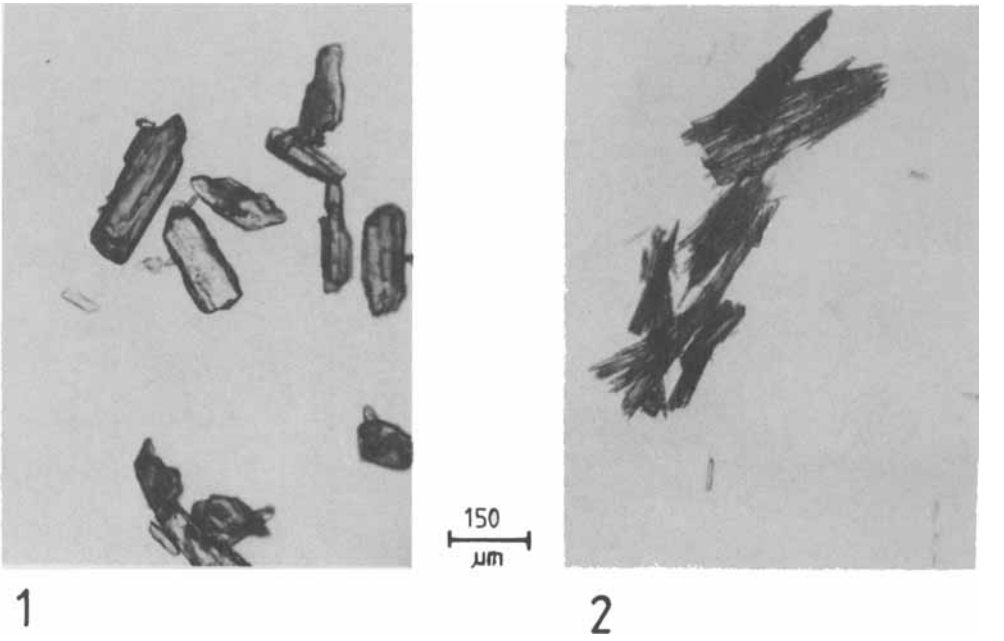


Fig.7. a. Photomicrographs of the isolated crystals(new batch) before(1) and after(2) isothermal treatment at 81°C for 10 minutes.

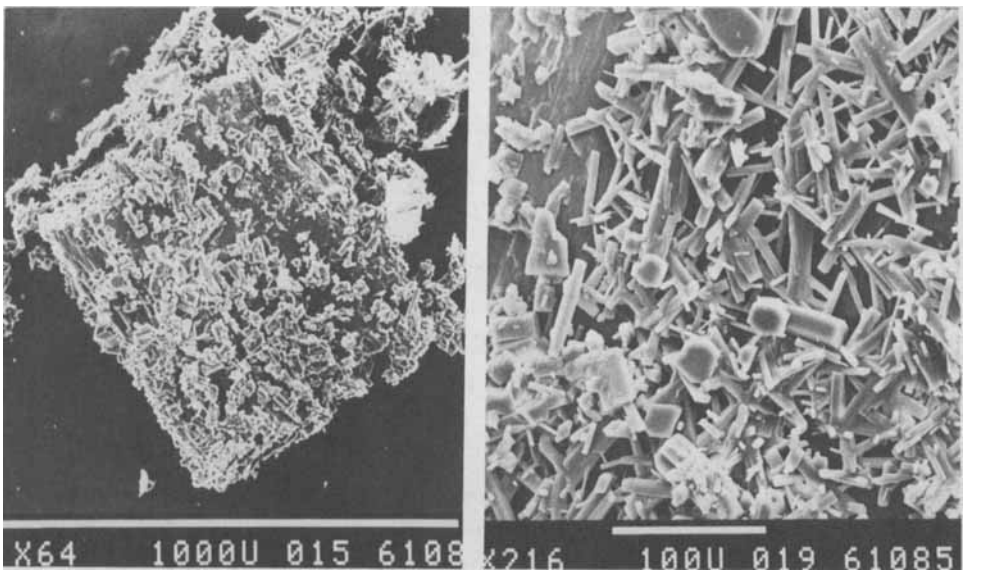


Fig.7. b. ESM Photomicrographs of the isolated crystals dried at 60°C overnight.

The presence of approximately 10% w/w of non phenylbutazone content in the isolated crystals changes significantly the size and the shape of these crystals (Fig.5,6 and 7). Consequently, it is expected that the behaviour of the crystals under thermal treatment will be different.

8- Thermogravimetric analysis indicates the absence of solvates. The weight of isolated phenylbutazone crystals has not been changed significantly at a heating rate of $10K^{\circ}$/min. in the range of 35°-120°C.

9- X-ray powder diffraction patterns of the isolated crystals and pure phenylbutazone powder which represents the most stable polymorph are shown in Fig.8. The diffraction patterns are different which suggests that the two samples have different crystalline structures.

10- DSC examination of the isolated phenylbutazone crystals shows two endothermic peaks even at a high heating rate of $30^{\circ}K$/min. When the sample is heated at $5^{\circ}K$/min. two endothermic peaks appear at 76°C and 100.2°C and one exothermic peak at 81°C as shown in Fig.9. The pure phenylbutazone which represents the most stable polymorph exhibits one endothermic peak at 106.5°C when heated at $5K^{\circ}$/min. as shown in Fig. 9.

The examination under microscope equipped with hot stage, on heating it reveals that the crystals start to

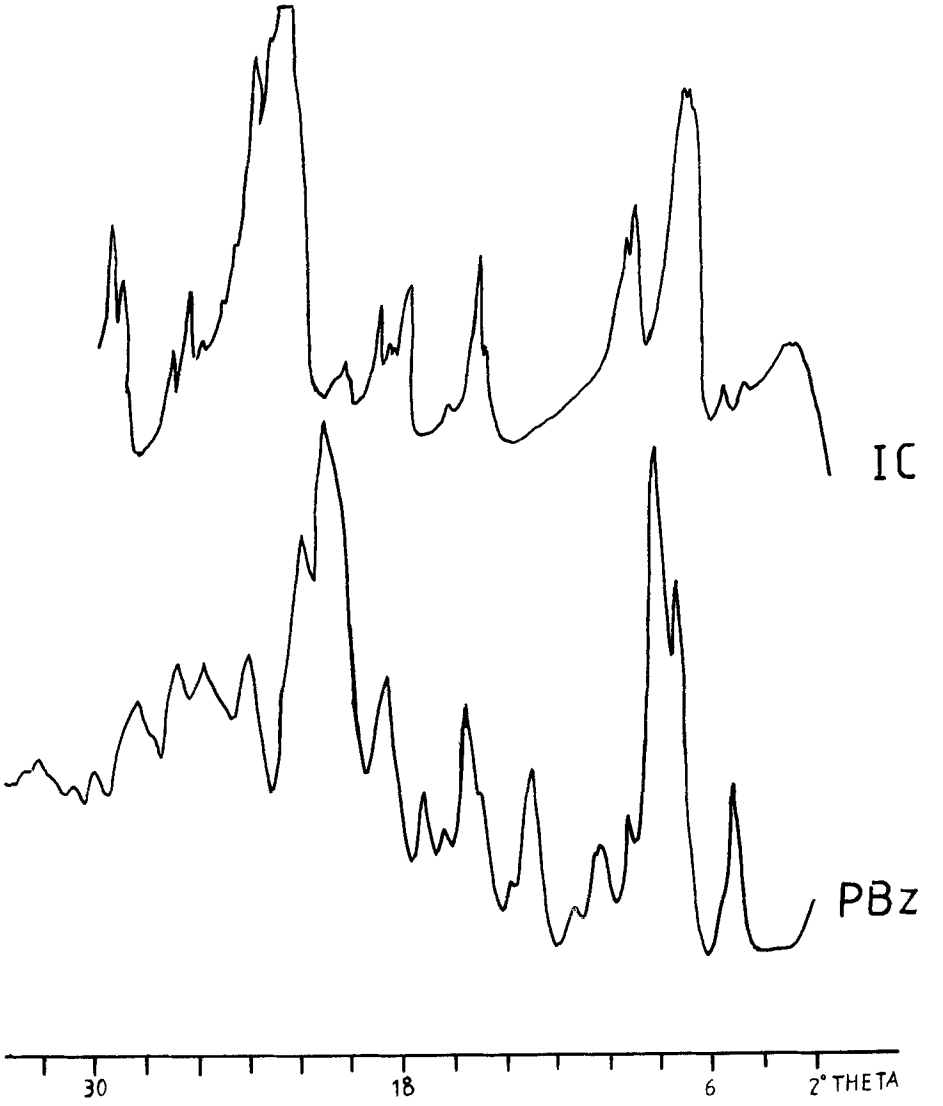


Fig.8. Powder X-ray diffraction patterns of the isolated crystals (IC) and pure phenylbutazone powder (PBz).

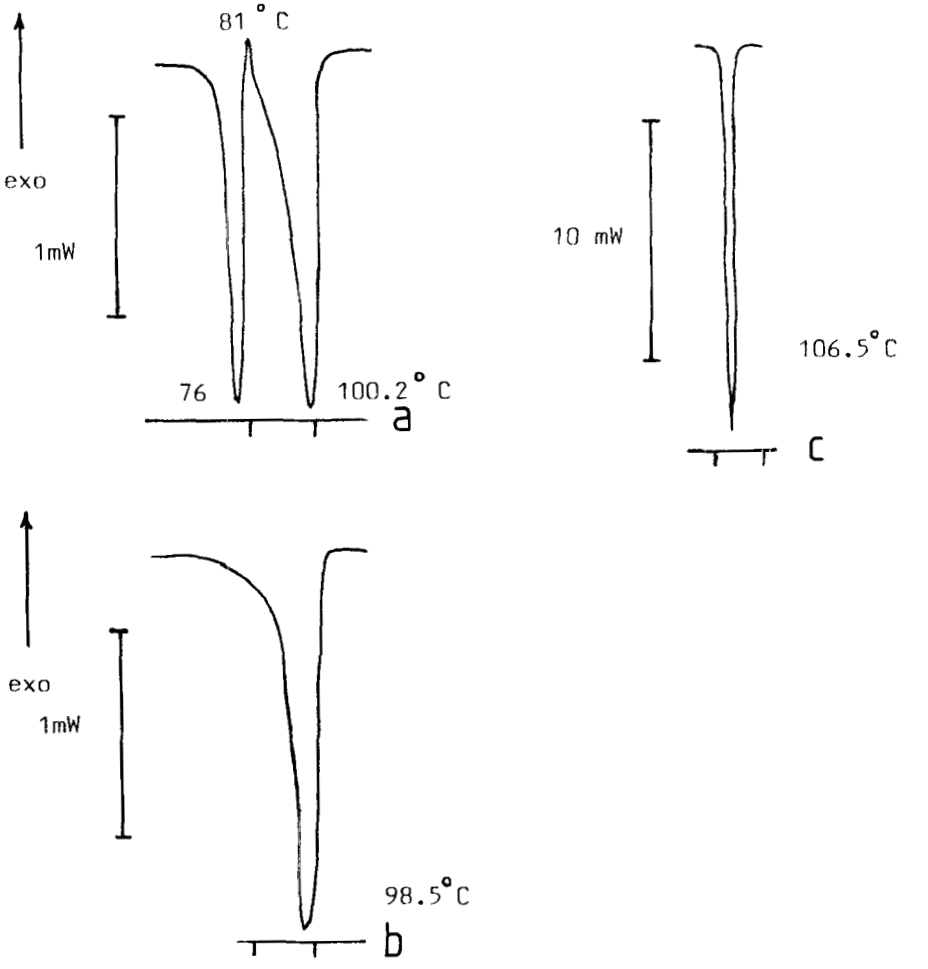


Fig.9. DSC Thermograms. Heating rate 5K°/min. a-Isolated crystals. b- Isolated crystals after isothermal treatment at 81°C for 10 min. c- Pure phenylbutazone powder.

soften without melting at 75°C and transform to needle shape crystals before melting at approximately 100°C. When another sample of crystals is heated at a constant temperature 80°-81°C which corresponds to the exothermic peak temperature of DSC thermogram, the crystals

are transformed to aggregate of needle shape crystals as shown in Fig.6. and Fig.7. This behaviour indicates that the isolated crystals are sensitive to heat. Samples which are dried at 60°C overnight show some degree of transformation particularly on the surface of crystals (Fig.7).

When the isolated crystals are treated in DSC cell under isothermal condition at 81°C for 10 minutes the crystals as pointed out before, are transformed to aggregates of needle shape crystals (Fig.6 and 7). Subsequent heating of these crystals at 5°K/min (35°-120°C) the DSC thermogram exhibits one endothermic peak at 98.5°C (100.4°C) as shown in Fig.9. This peak corresponds to the second endothermic peak in the thermogram of untreated sample. The crystals are transformed, most probably to the most stable polymorph. The depression in the melting point is due to the presence of approximately 10% w/w component other than phenylbutazone.

11- The examination of IR spectra of pure drug powder, isolated phenylbutazone crystals and isothermally treated crystals results in interpretation of the thermal behaviour of isolated crystals. The IR spectra are shown in Fig.10 and 11. The characteristic peaks of phenylbutazone are present in IR spectra of pure drug and isolated crystals as follows:

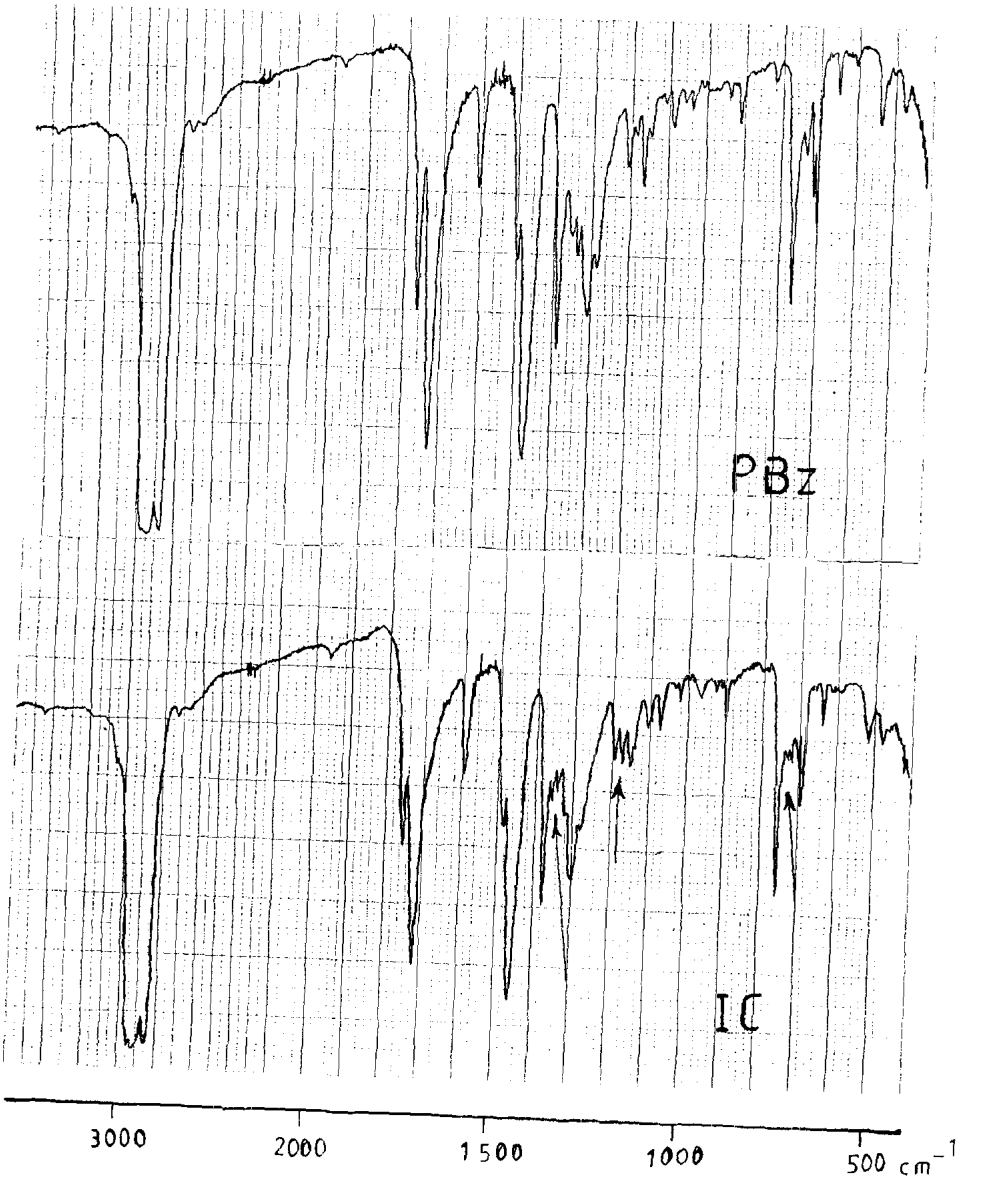


Fig.10. IR Spectra of the isolated crystals (IC) and pure phenylbutazone powder (PBz).

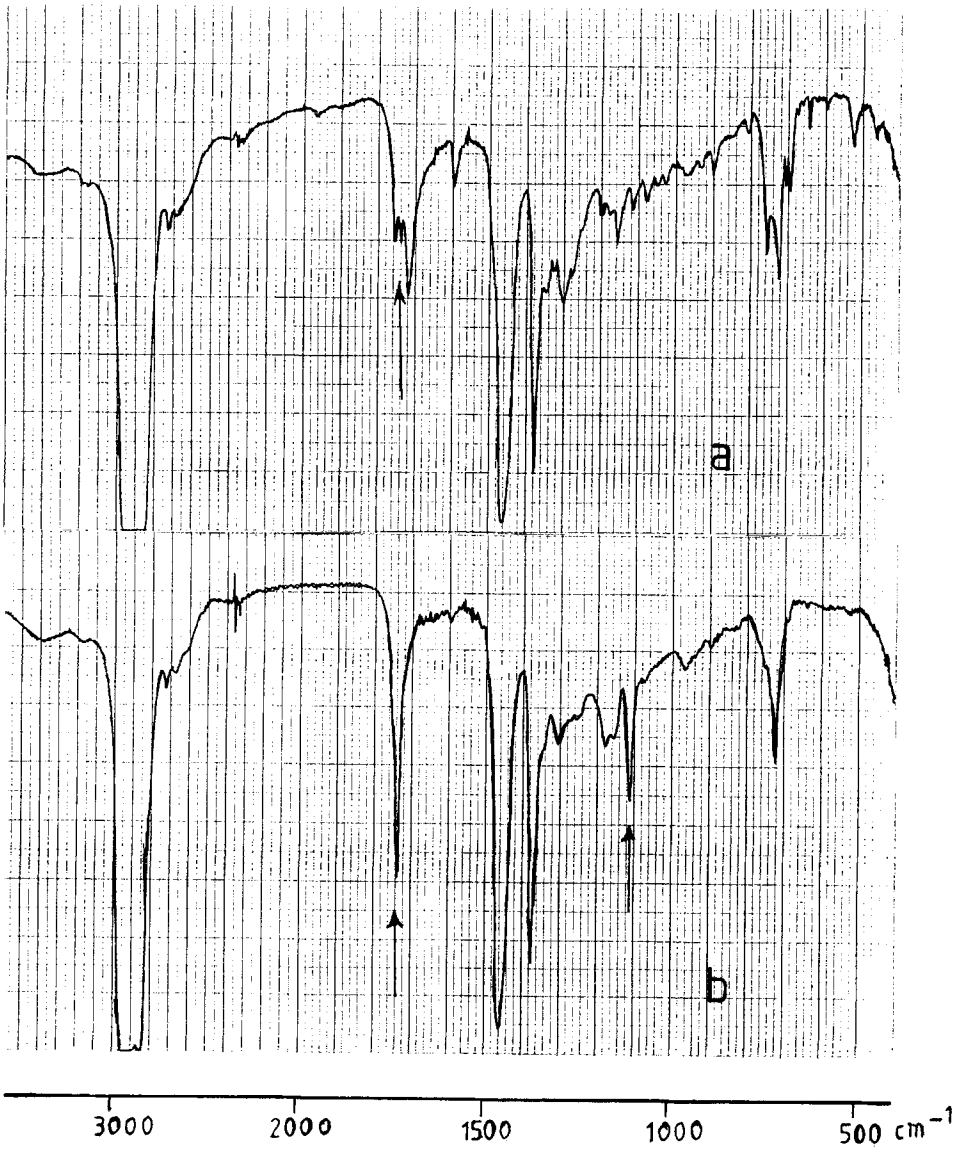


Fig.11. IR Spectra of the isolated crystals after isothermal treatment at 81°C for 10 min. (a) and the isolated oily material (b).

1- Characteristic stretching vibrations of carbonyl groups at frequencies 1720 and 1755 cm^{-1}.

2- Characteristic band of dioxopyrazolidine compounds at frequency 1300 cm^{-1}.

3- Bands of monosubstituted phenyl at frequencies 760-695 cm^{-1}.

However, there are differences in IR spectrum of the isolated crystals as indicated by arrows in Fig.10. which may be due to the difference in crystal structure.

After isothermal treatment of the isolated crystals the IR spectrum (Fig.11) exhibits extra peak which appears at a frequency 1740 cm^{-1}. This peak is characteristic for ester carbonyl group. The IR spectra of isolated crystals before and after compression at 10 tons for 15 min. donot exhibit any difference which indicates that the isolated crystals are only sensitive to the effect of heat.

Extraction of phenylbutazone from isolated crystals by shaking gently, for 24 hours with phosphate buffer (pH 6.90) at 37°C leaves oily droplets. The IR spectrum as shown in Fig.11 exhibits the following frequencies:

1- A one single peak appears at frequency 1740 cm^{-1} which is characteristic for ester carbonyl functional group.

2- A sharp strong bands at frequencies 1110 and 1160 cm^{-1} are characteristic for C-O bending frequencies.

Thus, the peak at frequency 1740 cm^{-1} which appears after isothermal treatment corresponds to the liberation of the oily material. The oily material is liquid at 37°C.

The isolated crystals are containing oily material in association with phenylbutazone rather than as a mechanical entrapment. This association is indicated by the first endothermic peak at 76°C. However, this sort of association is broken down by heat and the oily material is liberated as pointed out before. Further investigations are undertaken to study the chemical nature of the oily material and to study the type of interaction or association which exists between the oily material and phenylbutazone.

CONCLUSION

The isolated crystals from commercially available phenylbutazone creams are composed of phenylbutazone as a major component and fatty material as a minor component. The presence of both components in the crystals is not a mechanical entrapment. The fatty component interacts with phenylbutazone and both together crystallise out from creams.

ACKNOWLEDGEMENT

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