SAMPLE PREPARATION EQUIPMENT

Pharmaceutical Formulations as 7mm and 13mm KBr Pellet Preparations for comparison

Introduction

IR spectroscopy is a useful tool for group chemical species identification of a wide variety of sample materials, particularly for the classification of "organic" chemical materials based upon carbon atoms being present in the molecular structure.

A sample can exist in solid, liquid and gaseous states and be analysed by IR spectroscopy from the radiant light interaction with the sample as a transmission technique. (Light passes through the bulk of the sample for a certain pathlength). Reflectance IR spectroscopy techniques can be used for specific sample types, but only in their solid or liquid states at particular pressure and temperature conditions.

Application

A classic sampling technique for the study of solid sample types by IR transmission spectroscopy is to prepare the solid sample as a potassium bromide (KBr) disc or pellet to mount appropriately and correctly within the sampling area of an infrared spectrometer. This application shows how a few examples of pharmaceutical formulations as tablets/pills and capsules can be prepared to form both 7mm and 13mm diameter pellets from a "common" concentration of pre-ground mixture of sample with KBr. Subsequent IR transmission spectra were taken of both the 7mm and 13mm KBr sample pellets produced for comparison.

Equipment and Method

For this study the Specac Basic Solid Pack (p/n GS01150) for the 7mm diameter size pellets and the 13mm Evacuable Pellet Die (p/n GS03000) with a 15 Ton Manual Hydraulic Press (p/n GS15011) for the 13mm diameter size pellets were used. Instructions for identification and use of this equipment were followed as provided with the equipment itself.

Specac's Mini-Pellet Press (p/n GS03940)

The samples prepared as both 7mm and 13mm KBr pellets were subsequently analysed to produce an IR transmission spectrum for each sample using a Nicolet iS5 IR spectrometer. All spectra were collected over the spectral range between 4000cm^{-1} to 400cm^{-1} using the standard room temperature detector system set at a resolution of 4cm^{-1} for 32 scans.

The samples chosen for this study were eight different types of pharmaceutical formulations, presented in tablet/pill or capsule form for oral introduction into humans. The samples are tabulated in table 1.

Table 1

Sample	Name	Sample Form/Description	Chemical Formula (*)
1	Topiramate	Orange coloured tablet	$C_{12}H_{21}NO_8S$
2	Gabapentin	White coloured capsule	$C_9H_{17}NO_2$
3	Buscopan	White coloured pill (glossy coating)	$C_{21}H_{30}NO_4(+)$
4	Cyclizine	White coloured pill	$C_{18}H_{22}N_2$
5	Omeprazole	Yellow coloured capsule	$C_{17}H_{19}N_3O_3S$
6	Amlodipine	White coloured tablet	$C_{20}H_{25}ClN_2O_5$
7	Imipramine	Red coloured pill	$C_{19}H_{24}N_2$
8	Levothyroxine	White coloured pill (small)	$C_{15}H_{11}I_4NO_4$

(*) Note: Chemical formula for the active pharmaceutical (substance) in formulation

The pharmaceutical formulation samples as selected for study are mixtures of a variety of different chemicals. The typical make up of a tablet or pill for oral introduction as a compressed form of the formulation consists of:-

5 to 10% of the active sample.
80% of fillers, disintegrants, lubricants, gliders and binders.
10% of compound types that help to ensure easy disintegration, disaggregation and dissolution of the tablet/pill in the stomach or intestine.

Special coatings can make the tablet or pill more resistant to stomach acids, to localise for a specific efficacy and certain coatings of sugars, varnishes or waxes can help to disguise the taste. A capsule is a gelatinous outer envelope that encloses the active substance and formulation mixture.

The method of sample preparation involves a small proportion of the solid sample to be ground and uniformly dispersed in a potassium bromide (KBr) support matrix prior to formation of the 7mm and 13mm diameter discs or pellets. For all of the sample types, a pre-crushing of the tablet, pill or capsule was carried out to grind the sample mixture to a fine powder itself using an agate pestle and mortar, (p/n GS03600).

The samples varied in ease of preparation to form a powder. The results for their preparation are shown in table 2.

After pre-grinding of the sample, a small proportion of the powdered sample was added to an excess of KBr powder and this mix was further ground together using the pestle and mortar. Up to 2% weight of the sample to 98% weight of KBr as a mix is perfectly adequate to produce an acceptable concentration strength IR spectrum for specific signal intensity and resolution of spectral bands from the sample. The overall quality of a pellet is largely dependant upon the quality of the KBr powder used, which should always be of a spectroscopic grade purity.

When the sample and KBr had been ground to a uniform consistency, an appropriate amount of the same powder mix was transferred to fill the 7mm pellet die assembly (p/n GS03950), as used with the 2T Mini-Pellet Press (p/n GS03940), and the 13mm evacuable pellet die (p/n GS03000), used with the 15T Manual Hydraulic Press (p/n GS15011).

7mm Diameter KBr Disc/Pellet Preparation Procedure

A load of 1.9 tons for each sample was applied to the powder mix in the 7mm die assembly for compression from the 2T Mini-Pellet Press and held for 10 seconds. Release of the load and dis-assembly of the die parts resulted in a 7mm diameter KBr pellet being formed and held within the circular die frames. The circular die frame with pellet was placed in its dedicated 3” x 2” slide mount holder located within the spectrometer sample

Table 2

Sample	Method of Powdering Preparation	Resultant Powder Appearance
1	Tablet held with forceps and cut with a blade. Orange colour outer coating. White powder within. Entire tablet fragments ground in agate mortar.	White powder with fine orange flecks
2	White capsule casing cut. White powder within emptied into agate mortar and ground.	Fine white powder
3	Pill held with forceps and cut with blade. Entire pill fragments ground in agate mortar.	Fine white powder
4	Pill held with forceps and cut with blade. Entire pill fragments ground in agate mortar.	Fine white powder
5	Yellow capsule casing cut. Hard white spheres within emptied into agate mortar and ground.	Fine white powder
6	Tablet held with forceps and cut with a blade. Entire tablet fragments ground in agate mortar.	Fine white powder
7	Pill held with forceps and cut with blade. Red colour outer coating. White powder within. Entire tablet fragments ground in agate mortar.	Fine pink powder
8	Pill held with forceps and cut with blade. Entire pill fragments ground in agate mortar.	Fine white powder

compartment for correct positioning and an IR spectrum was collected for the 7mm diameter pellet sample.

13mm Diameter KBr Disc/Pellet Preparation Procedure

A load of 7.0 tons for each sample was applied to the powder mix in the 13mm die assembly for compression from the 15T Manual Hydraulic Press and held for 10 seconds. Release of the load and dis-assembly of the die parts resulted in a 13mm diameter KBr pellet being formed. The pellet was carefully transferred to be held in a 3" x 2" slide mounted pellet holder (p/n GS03410) for correct positioning and location within the spectrometer sample compartment and an IR spectrum was collected for the 13mm diameter pellet sample.

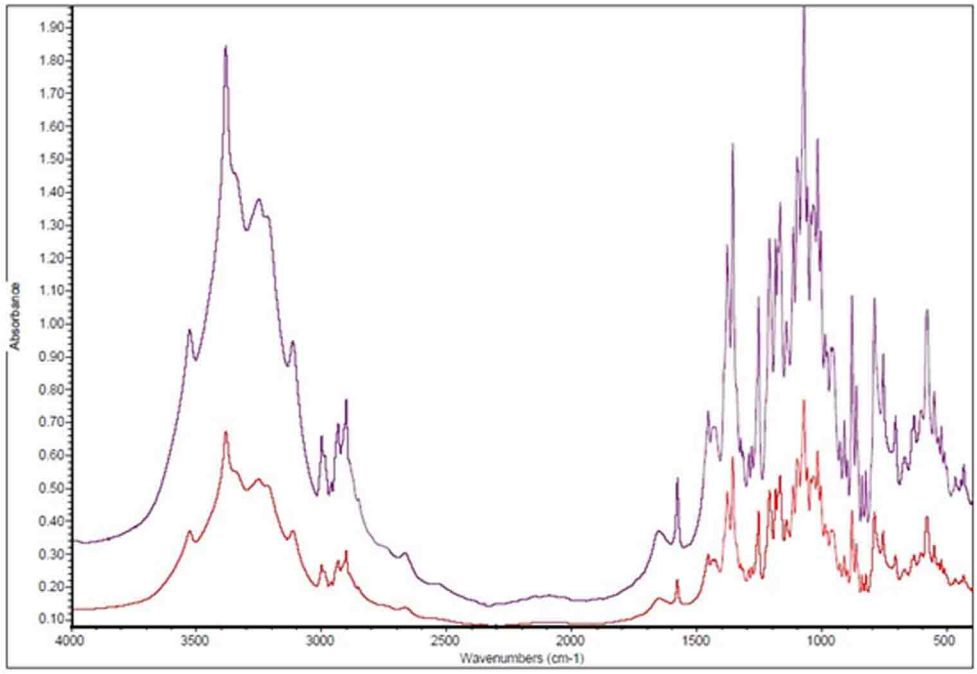
In the case of the 7mm diameter pellets, the KBr pellet was held within its own circular die frame of the 7mm die assembly of parts to present an aperture of 7mm diameter for the IR sample beam. For the 13mm diameter pellets, these were supported in a 3" x 2" slide mounted 13mm pellet holder (p/n GS03410), which provides an overall aperture of 11mm diameter for the sample beam.

Note:

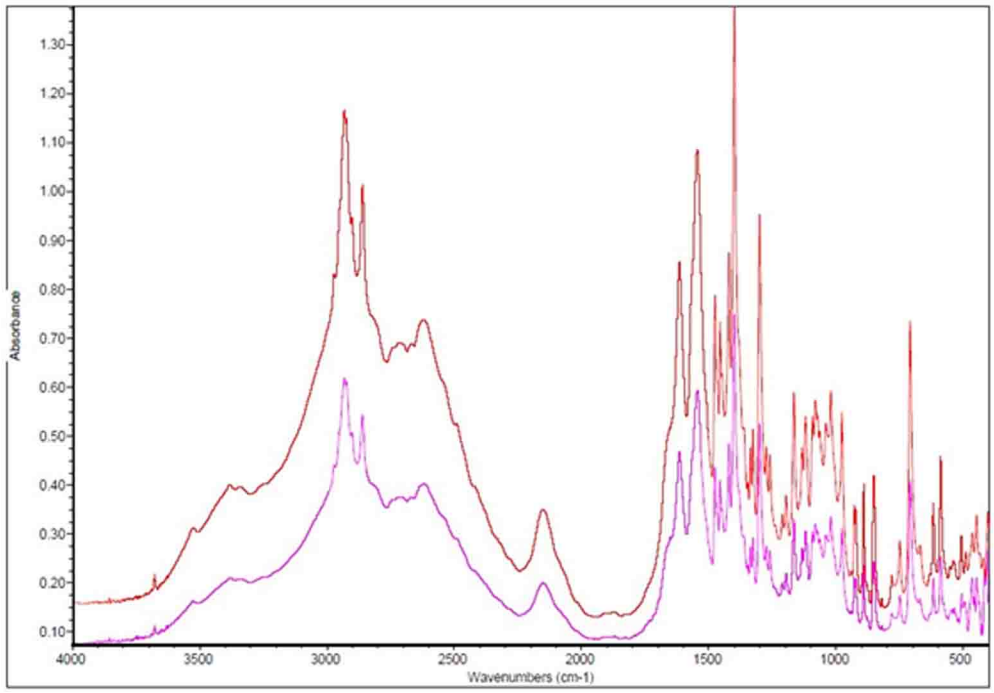
As a suitable reference background for spectral collection of the 7mm diameter pellet sample, an empty circular die frame (no sample), was placed into position in the spectrometer sample compartment to present a similar aperture size for better spectral sample and reference subtraction matched conditions. Reference background spectra collected for the 11mm diameter aperture for the 13mm diameter pellet samples were taken without the holder (p/n GS03410) in position in the sample compartment.

Spectral Data

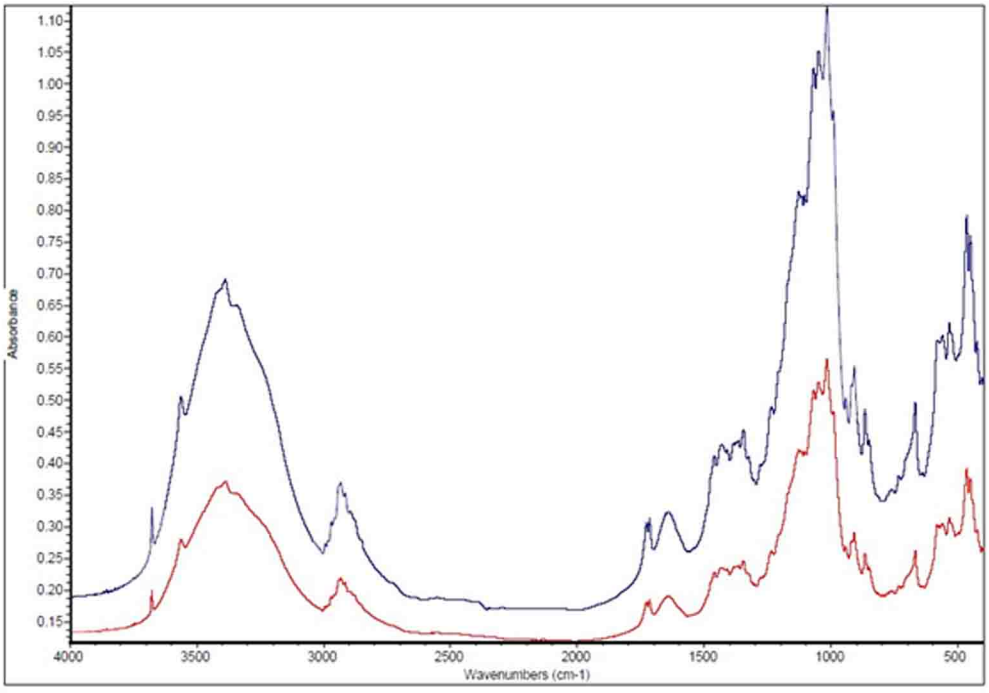
The transmission spectra collected for the eight pharmaceutical formulation samples prepared as 7mm and 13mm diameter KBr pellets are presented as follows. Sixteen pairs of spectra are compared. Each sample (1 to 8) has been shown for their individual 7mm pellet and 13mm pellet IR spectrum over the range of 4000cm^{-1} to 400cm^{-1} overlaid against a common absorbance value scale for the first eight sets of spectral comparisons. The same spectrum pairs are then shown again, but with each spectrum overlaid against a full absorbance value scale representation of the data for the second eight sets of spectral comparisons.



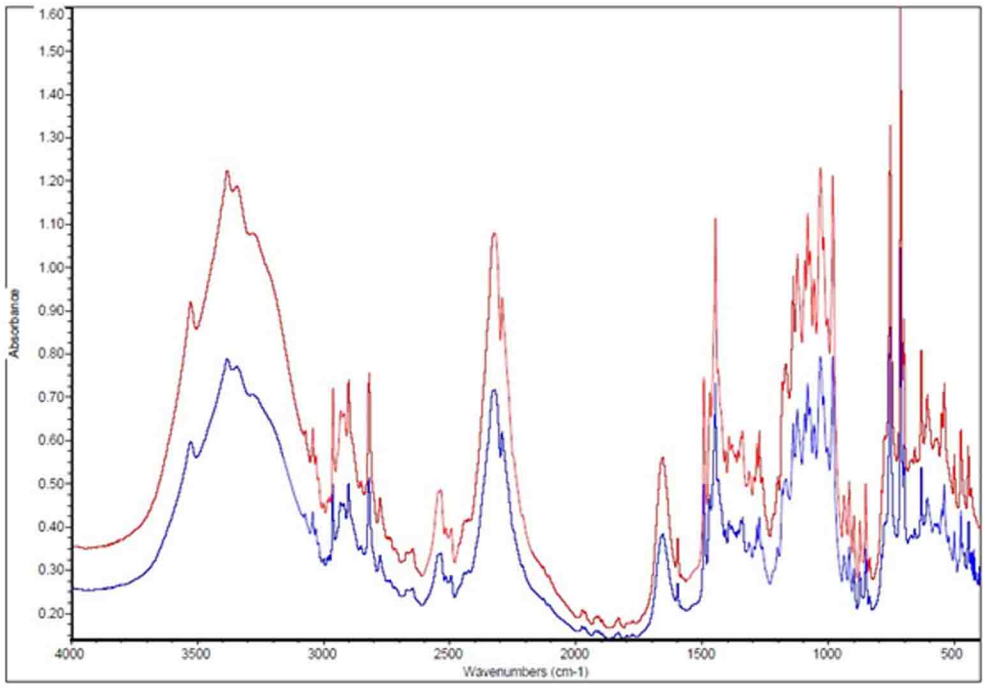
Sample 1 - Topiramate Tablet - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for Comparison on Common Absorbance Scale (7mm pellet - red trace)



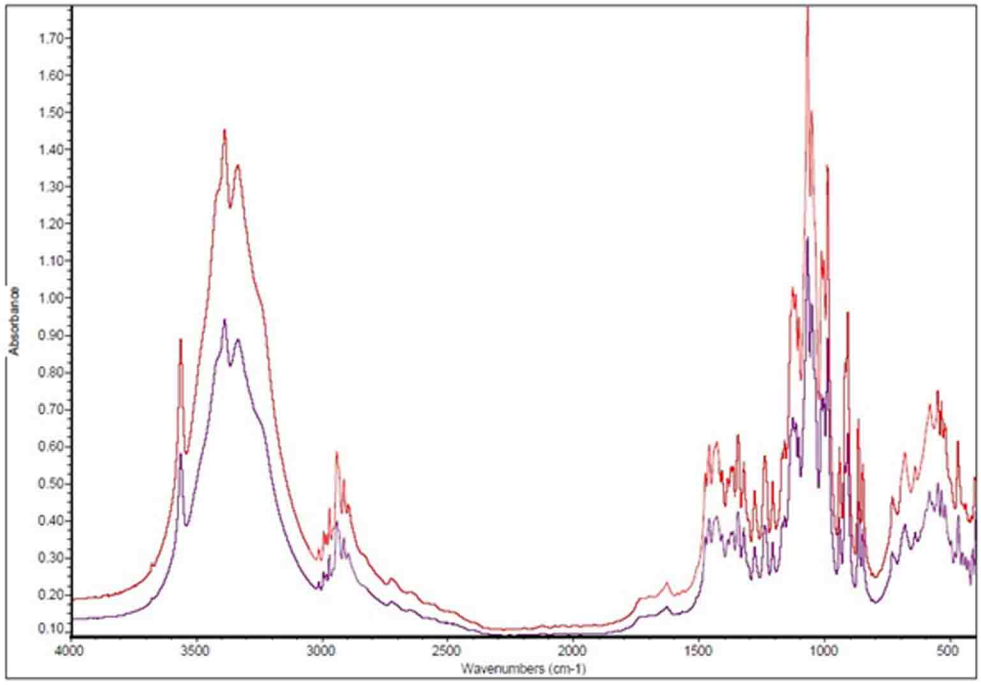
Sample 2 - Gabapentin Capsule - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Common Absorbance scale (7mm pellet - pink trace)



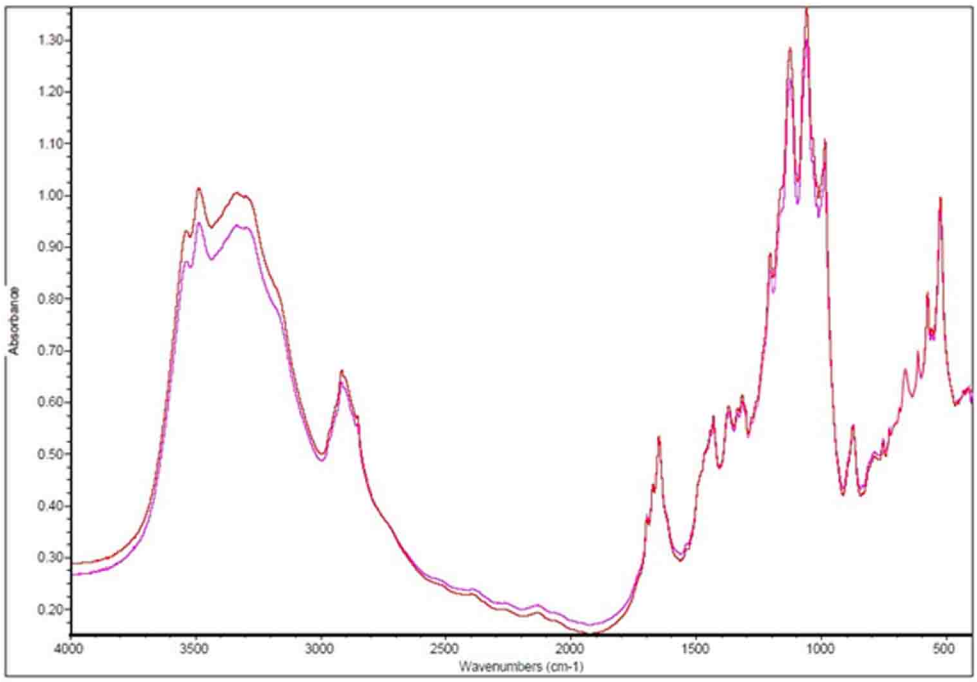
Sample 3 - Buscopan Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Common Absorbance scale (7mm pellet - red trace)



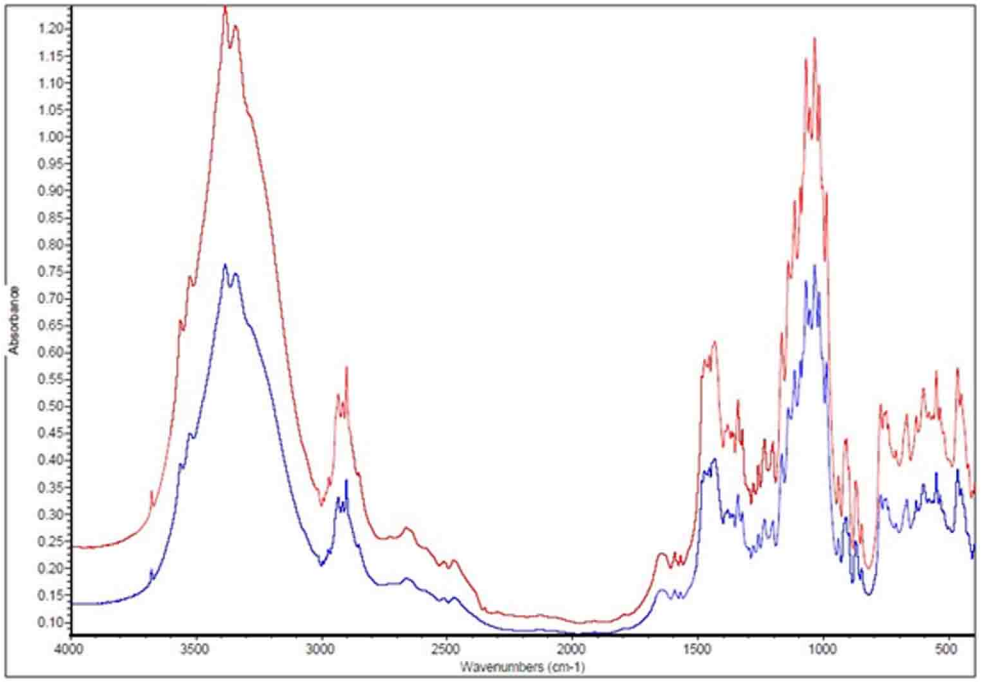
Sample 4 - Cyclozine Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Common Absorbance scale (7mm pellet - blue trace)



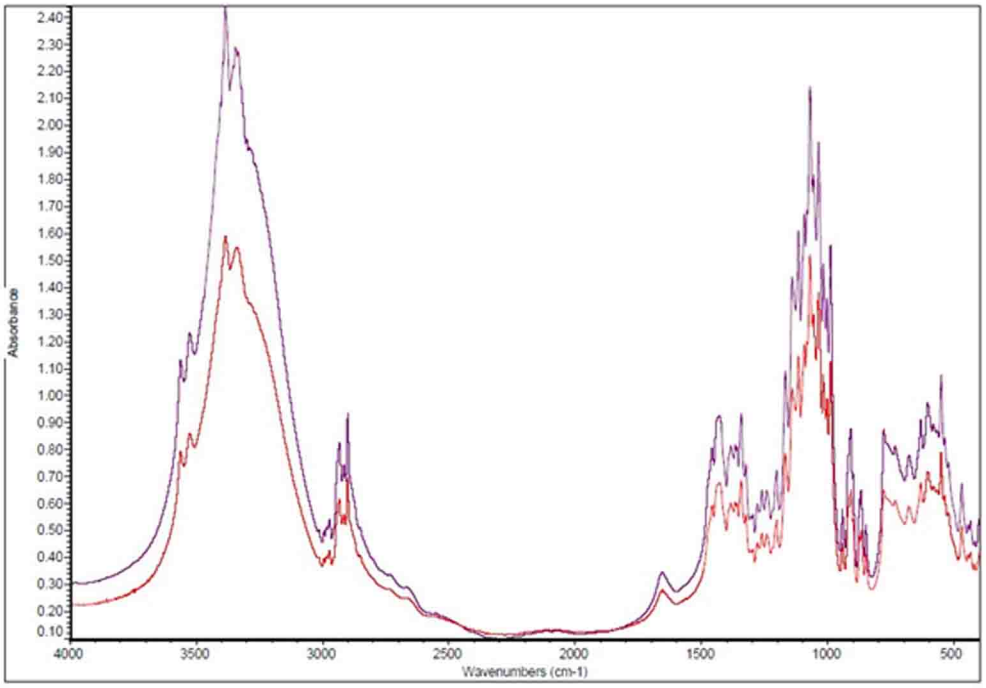
Sample 5 - Omeprazole Capsule - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Common Absorbance scale (7mm pellet - blue trace)



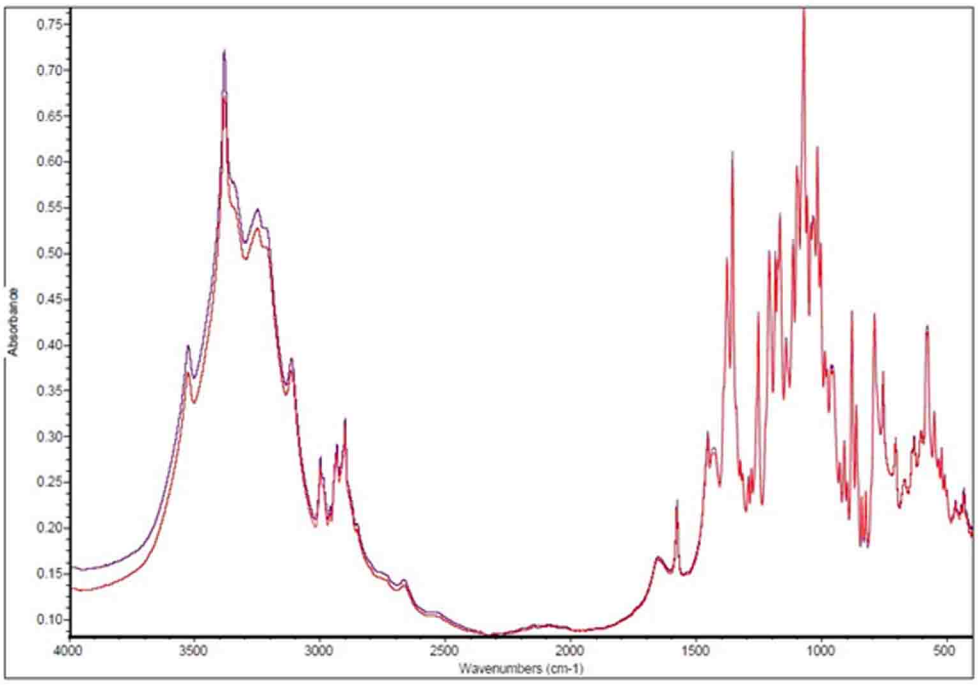
Sample 6 - Amlodipine Tablet - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Common Absorbance scale (7mm pellet - pink trace)



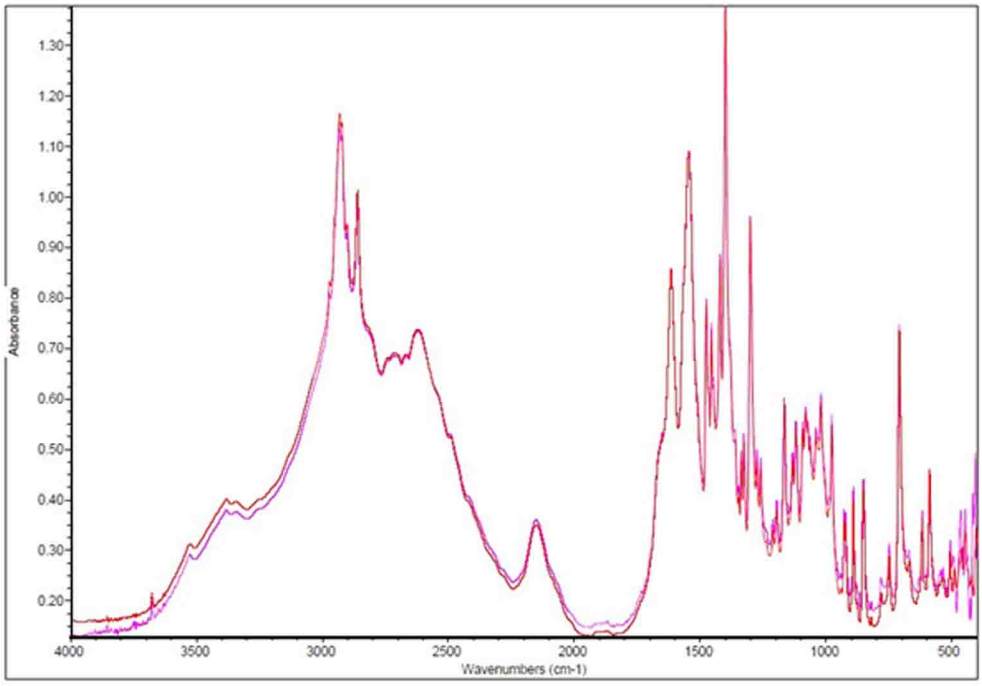
Sample 7 - Imipramine Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Common Absorbance scale (7mm pellet - blue trace)



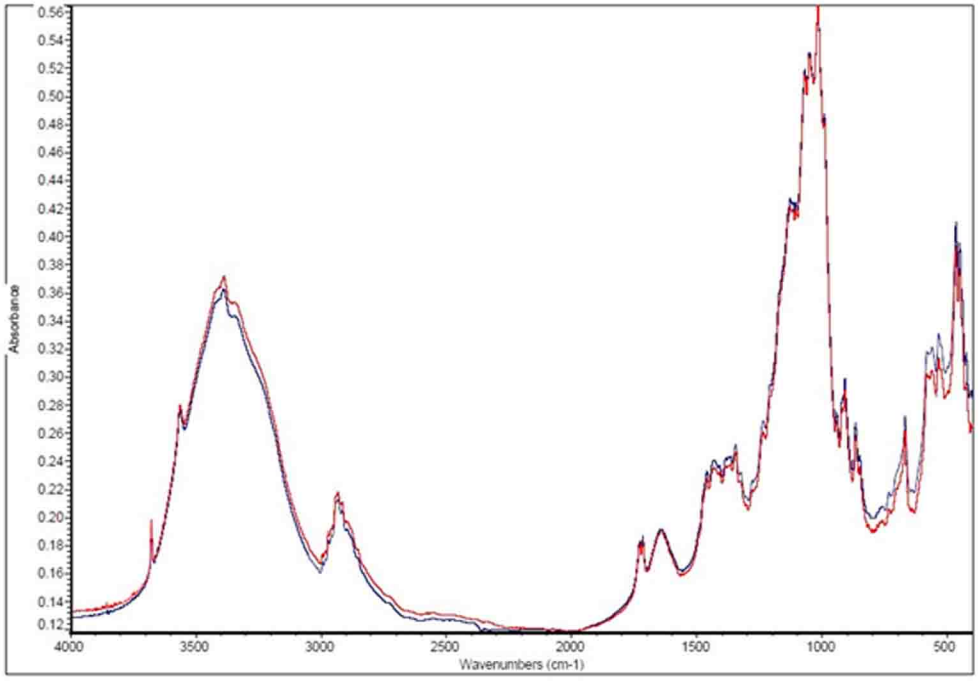
Sample 8 - Levothyroxine Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Common Absorbance scale (7mm pellet - pink trace)



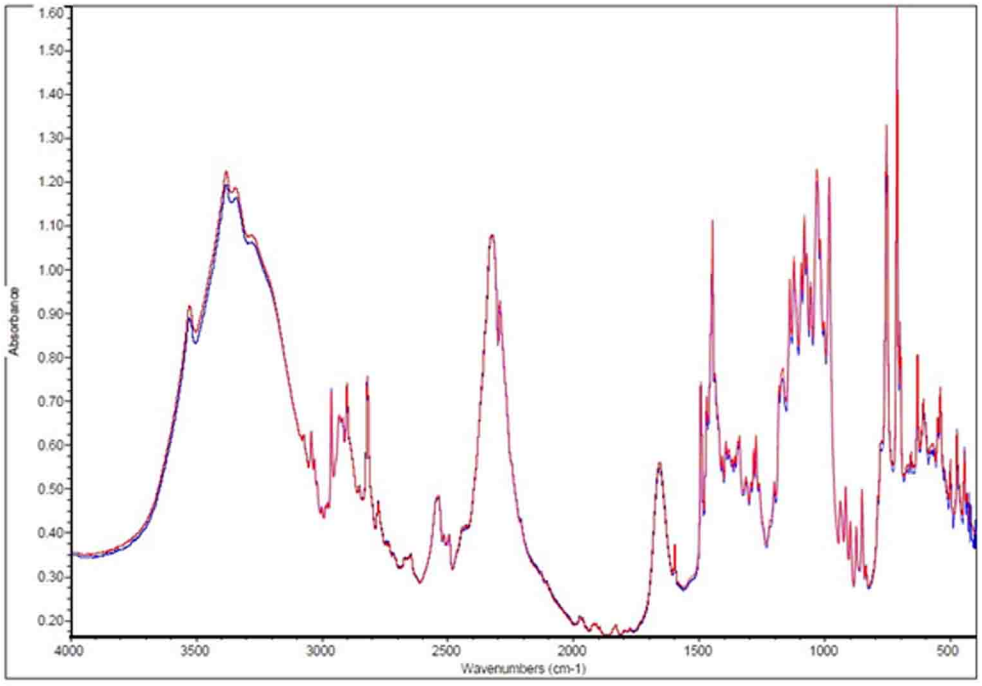
Sample 1 - Topiramate Tablet - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Full Absorbance scale (7mm pellet - red trace)



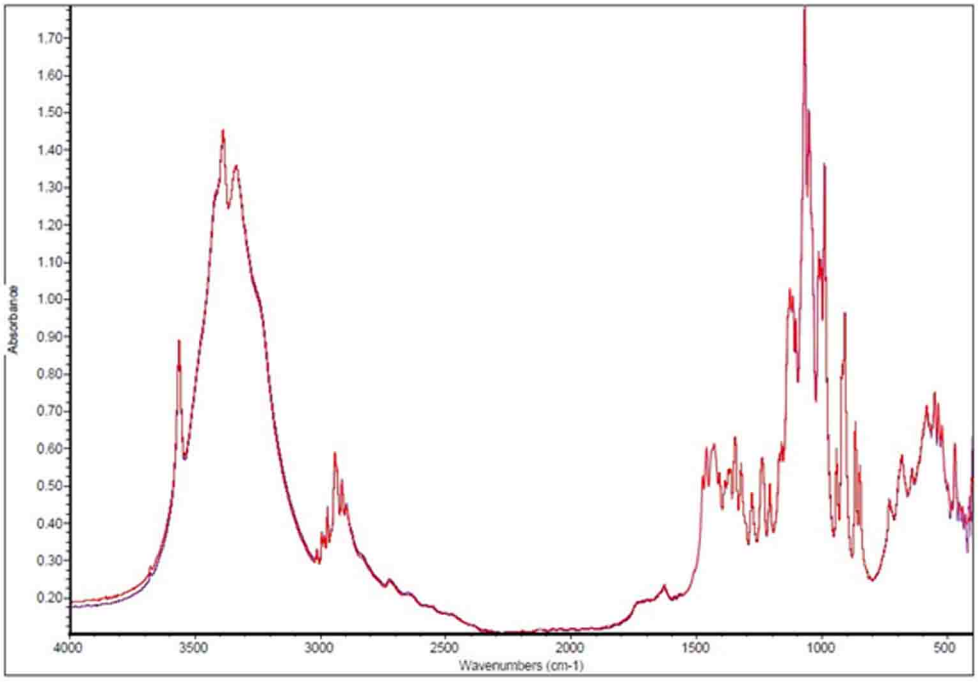
Sample 2 - Gabapentin Capsule - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Full Absorbance scale (7mm pellet - pink trace)



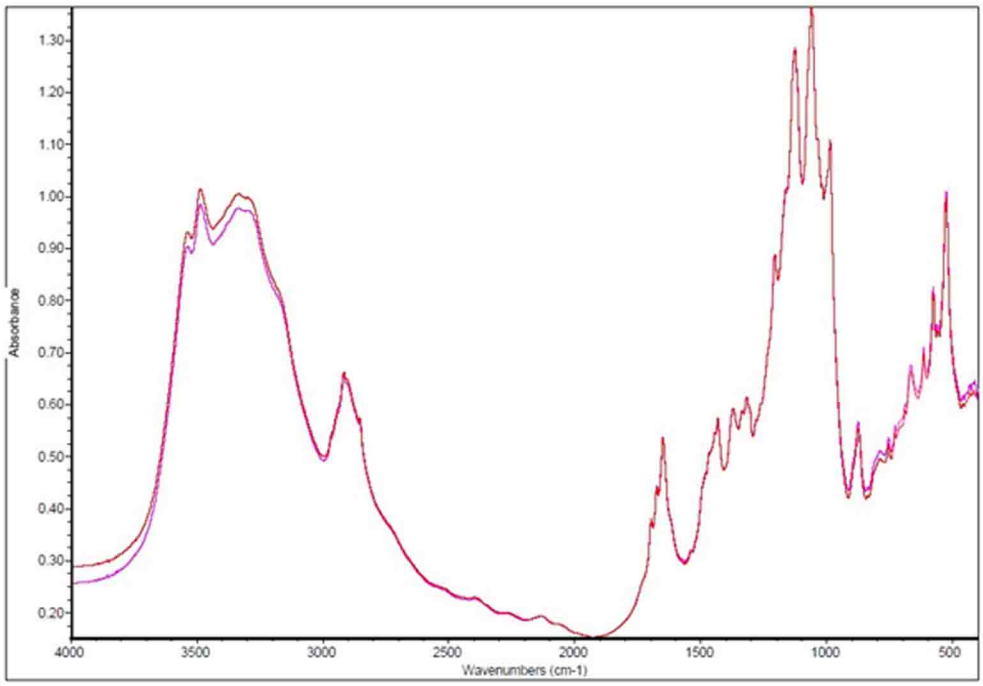
Sample 3 - Buscopan Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Full Absorbance scale (7mm pellet - red trace)



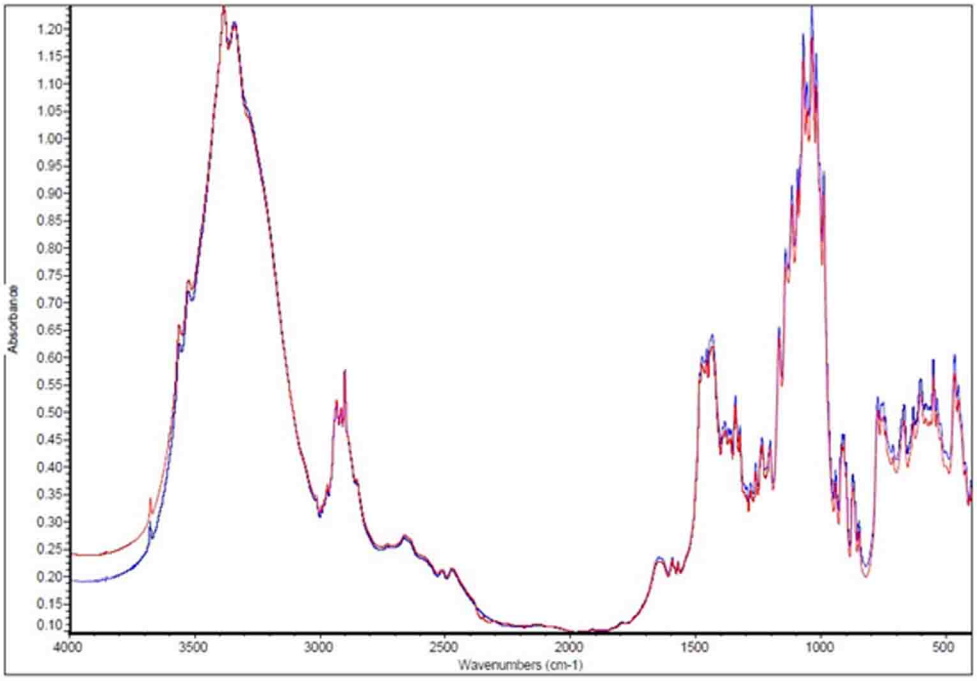
Sample 4 - Cyclozine Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Full Absorbance scale (7mm pellet - blue trace)



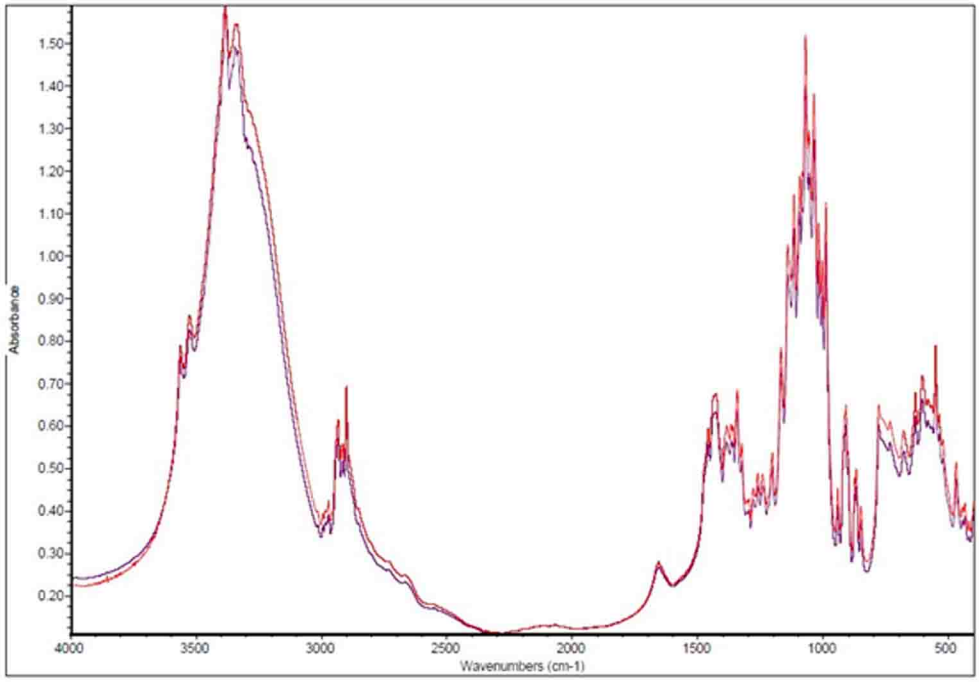
Sample 5 - Omeprazole Capsule - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Full Absorbance scale (7mm pellet - blue trace)



Sample 6 - Amlodipine Tablet - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Full Absorbance scale (7mm pellet - pink trace)



Sample 7 - Imipramine Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for comparison on Full Absorbance scale (7mm pellet - blue trace)



Sample 8 - Levothyroxine Pill - Powdered Formulation Prepared as 7mm and 13mm KBr Pellets and Overlaid for Comparison on Full Absorbance Scale (7mm pellet - red trace)

Discussion

Very good quality IR transmission spectra for sufficient signal intensities and overall concentration strength to resolve for the strongest spectral bands have been produced for each sample formulation. Each sample has been well ground and distributed throughout the KBr support matrix from the grinding stage in sample preparation to allow for the overall quality of spectrum to be produced from a subsequent formulation into both 7mm and 13mm diameter solid KBr pellets.

The resultant spectra are all unique for the specific sample from this method of preparation, but there are certain overall similarities, consistent with common binders and filler materials being present as an excess in the overall mixture concentration and dominating over the potential relative signal intensities that may be seen in contribution from the active substance alone. Comparison of the spectra for Samples 3 (Buscopan), 5 (Omeprazole), 7 (Imipramine) and 8 (Levothyroxine), show particularly close agreement for their overall spectral profiles, but there are fine discriminations potentially attributable to the presence of the differing active substance and specific components in the particular formulation mixture.

Comparison of the spectra for the same sample type and KBr mixture on a common absorbance scale show the intensities of the absorption bands to be generally weaker for a 7mm diameter pellet than for a 13mm diameter pellet. This must be expected because of the different relative concentration of the sample present interacting with the IR light beam over a given surface area and different pathlength for the pellet size, albeit the pellets were formed from a consistent method of sample preparation steps for relative tonnage loads to apply a constant force per unit area and for load hold duration.

Like for like, the relative sample pellet surface areas being presented to the light beam are $(7mm)^2$ to $(11mm)^2$ i.e. a factor of circa 0.4 in relative energy

intensity throughput for a 7mm diameter pellet (aperture) compared to a 11mm diameter pellet (aperture). In actuality the focussed light beam diameter for the instrument used has been calculated to be circa 8mm diameter, so the beam is "overfilling" the 7mm diameter aperture for a sample, but not covering completely the 11mm diameter aperture for the sample surface. Therefore any discrepancies in absorbance intensity reduction for the spectral peaks of a 7mm aperture pellet for a relative energy intensity throughput were more likely to be on a factor basis of $(7mm)^2$ to $(8mm)^2$ - circa 0.76. However, comparison overlay of the spectra taken for Sample 6 (Amlodipine) as presented on the common absorbance scale, show the spectra intensities for the peaks to be very similar for both the 7mm and 13mm pellets. Given the overall relative energy throughput reduction for a 7mm pellet and because the same sample mixture was used to form both the 7mm and 13mm sizes of pellets, closeness in intensity of the peaks can be attributed to the 7mm pellet having a slightly longer pathlength than the 13mm pellet in terms of relative concentration of the sample mix being presented to the sampling IR beam.

When the spectra are represented as comparative overlays on a full absorbance scale, they can be shown to be an almost identical match, which would also be expected considering that aliquots of the same overall concentration of a bulk mixture have been used to form both the 7mm and 13mm diameter pellets. Any slight discrepancies seen in the general spectral profile and background position etc. from a full absorbance scale comparison can be attributed to a potential change in the sample mixture itself from the time taken in the sample preparation stage from formation of one pellet at 7mm diameter to the other at 13mm diameter and prior to their subsequent analysis using the common equipment and methodology procedure for spectral data collection.

Conclusion

To study solid samples by IR spectroscopy using the transmission technique, the sample can be made to form a KBr pellet, provided the sample itself is prepared specifically, usually from grinding and mixing into a fine powder with KBr, and lends itself to such a method of preparation. Solid samples that pick up water from the atmosphere etc., are not suitable candidates to form into KBr pellets for such IR study using a transmission technique. A reflectance technique may be required instead for specific sample handling.

From establishment of a suitable sample grinding preparation stage for a solid sample. 7mm diameter KBr pellets can be made using the 7mm die assembly with its dedicated Specac 2T Mini-Pellet Press (p/n GS03940) - the whole equipment needed being supplied as the Basic Solid Pack (p/n GS01150).

Following the same sample preparation steps and an established methodology, similarly 13mm diameter KBr pellets can be made using a 13mm Evacuable Pellet Die (p/n GS03000) within a 15T Manual Hydraulic Press (p/n GS15011). The subsequent IR spectra produced for both pellet sizes of 7mm and 13mm diameter of the same sample mixture show no differences in their qualitative and quantitative interpretations.

The equipment need for making a suitable sized KBr pellet at 7mm or 13mm diameter for IR transmission study have their particular merits and potential advantages. For making a typical 13mm diameter size KBr pellet using the 13mm Evacuable Pellet Die, a typical tonnage load of circa 7 tons is required to be applied to produce a good quality pellet, hence the need for a suitable size Press such as the 15T Manual Hydraulic Press. Access for use of such a large size of Press allows for flexibility in the preparation of a wide range of sample types. Different size Evacuable Pellet Dies, Heated Platens (p/n GS15515) and High Temperature/Constant Thickness Film Makers (p/n GS15800/GS15640), can be used in this Press. In many senses, there is a requirement to site such a Press and related equipment in a permanent location for use because of the physical size and weight considerations.

To make a 7mm diameter KBr pellet typically requires a tonnage load of circa 2 tons to compare directly for a force per unit area as applied from a 7 tons load for a 13mm diameter KBr pellet. The equipment offered as the Basic Solid Pack (p/n GS01150) for specific 7mm KBr pellet production, in

comparison to a 15T Manual Hydraulic Press and 13mm Evacuable Pellet Die for KBr pellet making is more compact and also potentially easier to use. The 2T Mini-Pellet Press itself may be considered small and light enough in weight to be portable and not site specific. It offers the capability of being able to take this method of KBr pellet production pressing system locally for any sample preparation if it is problematic to bring the sample itself to the pressing system. An example can include preparation of samples in a controlled environment (inert atmosphere) and require manipulation using a glove-box.

The choice of which particular KBr pellet preparation system needs to be employed may depend on other factors such as cost, but any IR transmission spectral data collected from a consistent method of sample preparation will not differ if either a 7mm or 13mm diameter pellet has been produced.

Acknowledgement

The study was carried out by: C. Moss, Specac Limited, Orpington, Kent, UK.

Specac's Manual Hydraulic Press (p/n GS15011)

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