DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 4(6), 537-539 (1978)

A FLUORESCENT TECHNIQUE FOR THE OBSERVATION OF POLYVINYLPYRROLIDONE BINDER DISTRIBUTION IN GRANULES

M.E. Aulton, M. Banks and I. ab I. Davies
School of Pharmacy,
Leicester Polytechnic,
Leicester, U.K.

A technique is described involving the binding of fluorescein isothiocyanate (FITC) to polyvinylpyrrolidone (PVP). This complex has been used as a binder during fluidised bed granulation. The resulting granules have been observed under light excitement and the distribution of PVP in the granules assessed.

The labelling of proteins with FITC[1] involves an electrophilic attack by the FITC upon the ε-amino group of lysine[2]. Since the nitrogen atom of the pyrrolidone rings of PVP is weakly basic, the FITC will form a link with this heterocyclic nitrogen atom and thus be bound, possibly as a complex, to the PVP. This was confirmed by the distinct separation of the PVP-FITC complex from the free FITC by Sephadex Chromatography.

METHOD

To bind the FITC to PVP, the method of Rinderknecht[1] was adapted. 5 mg of FITC was dissolved in 100 ml of 0.05 M sodium carbonate/bicarbonate buffer (pH 8.5) and shaken for 15 minutes at room temperature with 100 ml of 10% $^{w}/_{v}$ aqueous PVP solution.

The bound FITC was separated from the free FITC on a Sephadex G-75 column (26 mm internal diameter, 600 mm high) previously equilibrated with 0.02 M sodium phosphate buffer (pH 6.5). Twenty millilitres of the PVP/FITC solution were fed into the top of the column and developed with the sodium phosphate buffer at a flow rate of 0.35 ml/min. The eluted PVP was positioned by testing successive 8 ml samples by the B.P.C. identification method. PVP and the PVP-FITC complex were recovered in the region of the void volume (samples 9-18) whilst uncombined FITC was recovered in the region of the total fluid volume of the column (samples 39-50). The PVP concentration in each aliquot was estimated by refractive index measurements, which showed that 85% of the added PVP was in samples 9-18.

APPLICATION

A sufficient amount of PVP-FITC complex was added to unlabelled PVP to produce a 5% $^{w}/_{v}$ solution (total PVP) of suitable fluorescence. 200 ml of this solution was then used to granulate powder mixes in a fluidised bed under conditions described by Aulton and Banks[3]. Some of the granules so produced were then

sprinkled sparcely on a glass slide and observed by an incident light fluorescence technique using a Vickers fluorescence microscope (M41 Photoplan). High pressure mercury vapour light is passed through a 475 nm suppression filter and a FITC exciter filter (the peak absorption of FITC conjugates is at 495 nm), then via a FITC dichroic reflector and a fluorite objective to the sample. The emitted fluorescence passes back through the objective and the dichroic, then via a 515 nm barrier filter to the eye piece (peak emission for FITC conjugates is 520 nm). A camera system enabled permanent records to be made.

A further technique was developed for observing binder distribution across a section of an individual granule. Granules were sprinkled into molten paraffin wax and, after solidification, the wax was cut into 20 μm thick sections with a microtome. Such sections clearly showed the distribution of PVP within granules.

REFERENCES

1. H. Rinderknecht, Nature, 193, 167 (1962).
2. R.C. Nairn (Ed.), "Fluorescent Protein Tracing", E. & S. Livingstone, London, 1969.
3. M.E. Aulton and M. Banks, J.Pharm.Pharmac., 29, Suppl., P59 (1977).