Count 100μl 10-26-1

meas. vol = 91 ml (est. actual vol = 92.5)

100μl → 49,845
10 min 40,782 } 4331.4 cpm

$$\frac{(4331.4\ \text{cpm})}{0.1\ \text{ml}} \times 91\ \text{ml} = 3.94 \times 10^6\ \text{cpm} \longrightarrow 1.19 \times 10^7\ \text{dpm}$$

(est cpm = 7.07×10^6)

⇒ recovery over few days in MeOH/H_2O

= 55%

so the insoluble material must have contained ~45% of adduct!

YIELD
~~Recovery of Adduct~~ "counts" from DNA:

From p. 43 — there were $1.169^{\times 10^7}$ cpm in total sample -

I estimated that there will be 7.07×10^6 cpm

$$\therefore 10^2 \frac{7.07 \times 10^6}{1.169 \times 10^7} = 42.36\%\ \text{Recovery}$$

the rest of the counts may be in the insoluble material or in the peak which eluted before the adduct on preparative chromatography.

10-25-76

Tabulation of Adduct Isolations

Sample	Volume	cpm total	µg adduct
10-19-9, 10	25.5 ml	2.88×10^5	101.1
✓ 10-21-2	20.0 ml	3.20×10^5	112.7
✓ 10-21-6	18.0 ml	1.47×10^6	514.3
✓ 10-22-1	19.0 ml	2.05×10^6	719.6
✓ 10-25-1	19.5 ml	1.47×10^6	515.4
✓ 10-25-2	16.0 ml	$1.7[illegible]6 \times 10^6$	616.7
	118 ml	7.358×10^6 cpm	2579.8 µg = 2.58 mg adduct.

→ due to [illegible] insol material, it is expected that this conc will change over time

3.96 µg/ml (same as a std)

$\frac{1.47 \times 10^6}{514.3} = 2858 \frac{cpm}{\mu g}$

✓ present in 10-26-1

10-26-76 Further Purification of Adduct.

Purpose: I will take the fractions checked (✓) above and conc. them down to remove most of the MeOH. Then, if all goes well, put the concentrate through the prep. column.

Procedure:

SAMPLE 10-26-1

- Pool the checked fracts. in 100 ml grad. cyl. volume = 91 ml (out of expected 92.5 ml)
- OBSERVATION: There is a fair amount of crystalline material floating around.
- transfer into 100 ml pear-shaped flask in (2) batches

10-26-2 = Clear SN of 10-26-1, just before separating phases

100 µl : 11538 c/4 min → 2885 cpm

$\left(\frac{2885 \text{ cpm}}{0.1 \text{ ml}}\right)(20.4 \text{ ml}) = 5.88 \times 10^5$ cpm total -

but, there was 3.94×10^6 cpm when this sample was at the 9.1 ml level.

so, % through conc =

$$\frac{5.88 \times 10^5}{3.94 \times 10^6} 10^2 = 14.9\%!$$

also, this represents a recovery of 8.3% from the column effluent!

$$10^2 \frac{5.88 \times 10^5}{7.07 \times 10^6} = 8.3\%$$

-26-76 - evaporate until the rate slowed (Rot. evap. 40°C)
- Obs - more insol. material may have formed - it is yellow grey.
  est. quant at 2-10 mg
- cool on ice. - more ppt seemed to come out
- spin pellet down on clinical centrifuge
- remove SN - meas. vol = 19.8 ml (originally was 20.4 ml)

  count 100 µl → this phase had 14.9% of the adduct soluble at the 92 ml level and only 8.3% of the adduct originally present in these supernatants.

- Separate phases

soluble material 10-26-2

insoluble material 10-26-3

(*)(*) - the adduct probably is in the precipitate. It consists of white crystals that are needle-like in shape.