

OptiPrep™ Mini-Review MV07

Comparison of gradients prepared from OptiPrep™ with those prepared from CsCl, sucrose or glycerol for the purification and analysis of viruses and viral vectors

- ◆ **OptiPrep™ is the trademark of a sterile solution of 60% (w/v) iodixanol ($\rho = 1.32$ g/ml)**
- ◆ Other OptiPrep™ Mini-Reviews described in the following text and also Application Sheets that provide detailed methodologies are all available on the following website: www.axis-shield-density-gradient-media.com or on the OptiPrep Applications flash drive. Start by clicking on either the “Mini-Review” or “Methodology” tab at the top of the home page.
- ◆ Mini-Review presents MV01 presents a summary of methodologies for the concentration and purification of viruses and viral vectors.

1. Recombinant adeno-associated virus (rAAV)

In the first paper to be published from groups at the University of Florida (Gainesville) and the University of North Carolina (Chapel Hill) on the use of density gradients for the purification of rAAV, Zolotukhin et al [1] noted the huge saving of time when employing OptiPrep™ rather than CsCl for this purpose. Two or three rounds of CsCl density gradient centrifugation were required, each of 18 h duration; moreover, in between each round it was necessary to fractionate, dialyse and analyse the gradient to determine the banding position of the vector. As a result the final product can take up to 14 days to prepare. In contrast the single iodixanol gradient required is only 1 h in duration and because of the non-ionic nature of the molecule and its lack of toxicity, electrophoresis and infectivity measurements can be executed directly on the gradient fractions without dialysis. The combination of a single iodixanol gradient and a heparin affinity column has now become a standard procedure for the purification of rAAV vectors for gene therapy investigations. The potentially toxic nature of CsCl and poor outcome of CsCl gradients contrasting with the vastly improved yields (50-70%), very high purity (ca. 99%) and high rates of transduction achievable with rAAV purified in iodixanol gradients have been emphasized in many papers [1-13].

- ◆ The considerable benefits of iodixanol are also observed with the use of the continuous gradients developed by Hermens et al [14] working at the Netherlands Institute for Brain Research in Amsterdam.
- ◆ For the purification rAAV2/6 optimal transduction was obtained with virus sequentially purified in discontinuous and continuous iodixanol gradients [15].

Moreover other problems associated with the use of multiple CsCl gradients have largely or completely been resolved by the use of the single iodixanol gradient + heparin chromatography method and other protocols using iodixanol gradients:

- a. The inability of several rounds of CsCl gradient centrifugation to remove contaminating adenovirus, adenovirus factors and proteins [16,17].
- b. Trace amounts of CsCl lead to inflammatory responses in recipient animals [10] and because of the vastly improved transduction rate per particle with iodixanol- purified rAAV [18] it is much more suited to therapeutic applications [19].
- c. Resolution of rAAV from host cellular contaminants [20,21] and contaminating proteins that lead to altered tropism profiles [22].
- d. The unsuitability of CsCl gradients for scale-up preparations [23].
- e. Use of rAAV vectors for Parkinson's disease studies identified not only the inconvenience of multiple rounds of CsCl gradients and their toxicity, but also their inability to resolve assembled virions from empty particles [24] – iodixanol overcame all these problems.
- f. Aggregation of particles observed with CsCl is avoided by the use of iodixanol gradients [25].
- g. A novel method for the purification of rAAV utilizing biotinylation requires iodixanol gradients to remove biotinylated cellular proteins [26].
- h. In studies of gene transfer to rat brain neurons CsCl-purified rAAV8 showed an unexpected astroglial transduction, while iodixanol-purified vector showed the expected transduction pattern. This was attributed to the high levels of cellular protein contamination observed with CsCl [27].

- i. In rat islet transduction studies it was necessary to increase the dose of rAAV vectors purified in CsCl compared to iodixanol because of the much-reduced infectivity in the former [28].
 - j. Several important improvements in functional parameters were observed in iodixanol gradients [29]: yields of $>1 \times 10^{14}$ genome copies per run; a capsid protein purity of $>90\%$ and separation of infectious particles from empty particles. Consequently improved transduction was obtained both in *in vivo* and *in vitro*. The methodology was found to be effective with most serotypes.
 - k. More recently Buclez et al [30] stressed the importance of simplifying the whole concentration and purification procedure by recommending the use of tangential flow filtration, followed by a modified iodixanol gradient. The authors observed that this is sufficient to purify several litres of crude lysate in one working day and yielded rAAV of high titre and good purity.
- ◆ For methodological details see OptiPrep™ Application Sheet V14. For a complete rAAV bibliography see Mini-Review MV02.

2. Adenoviral vectors

The discontinuous gradient used for rAAV vectors (see Section 1) was later adapted to adenoviral vectors by Manninen et al [31] although the method was first described in detail by Peng et al [32]. More recently a self-generate iodixanol gradient was described by Dormond and Kamen [33,34]. Compared to two rounds of CsCl gradients the combination of a single iodixanol gradient + size-exclusion chromatography improved yields fourfold [32]. This method was found to be particularly beneficial for vectors with arginine-glycine-aspartate-modified fibre proteins, which aggregated during the second round of CsCl gradients [32]. The separation of helper virus from helper-dependent adenovirus in CsCl gradients was ineffective, while two rounds of a self-generated iodixanol gradient gave efficient separation of contaminating components whose density was only marginally lower than that of the adenovirus [33]; the first round produced a 100% yield and the helper virus was reduced six-fold. Purification of capsid-modified vectors was faster on iodixanol than CsCl gradients; moreover it was unnecessary to remove the iodixanol prior to use [35].

- ◆ For methodological details see OptiPrep™ Application Sheet V07

3. Norwalk virus

Norwalk virus-like particles are unstable in both CsCl and sucrose gradients; this instability is not encountered in iodixanol gradients [36].

- ◆ For methodological details see OptiPrep™ Application Sheet V18

4. Papilloma viral vectors

CsCl gradients lead to a loss of 99% of the virus titre of these vectors, while infectivity is retained in iodixanol gradients; the authors also commented on the need to use much lower concentrations of iodixanol compared to those of CsCl because of the much lower density of the particles in iodixanol [37]. It was noted that DNA-containing capsids have a lower density than empty capsids in iodixanol, while the reverse is true for CsCl [38]. This is probably related to the much lower density of DNA in low osmolality iodinated density gradient media such as Nycodenz® and iodixanol (1.10-1.17 g/ml) compared to approx 1.7 g/ml in CsCl. The number of moles of water associated with each mole of nucleotide in DNA isolated from Nycodenz® gradients is 64; from CsCl gradients the figure is 6 [39]. Iodixanol gradients were chosen in the analysis of the formation of infectious particles because of the higher resolving power of such gradients [38].

- ◆ For methodological details see OptiPrep™ Application Sheet V10. For a complete papillomavirus bibliography see Mini-Review MV05

5. Hepatitis C virus

In vivo host viruses form complexes with plasma lipoproteins; iodixanol is superior to both sucrose and NaBr gradients in the preservation of these complexes [40].

- ◆ For methodological details see OptiPrep™ Application Sheet V19 and V20, which describe the analysis of hepatitis C virus from liver and cultured cells and the companion OptiPrep™ Application Sheets V21 and V22 which describes the isolation of viruses from the *Flaviviridae* family from cultured cells. For a complete bibliography see Mini-Review MV06

6. Retroviruses

Enveloped retroviruses used to be routinely prepared in sucrose gradients; a major problem associated with the high viscosity of these gradients was that hydrodynamic shearing at the surface of the virus caused the loss of glycoproteins that are important in the binding of the virus to host cells. Low viscosity, isoosmotic iodixanol gradients overcome this problem entirely [41-43]. Møller-Larsen and Christensen [41] also commented on the ease of handling, shorter centrifugation times and reproducibility of self-generated gradients that iodixanol offers.

- ◆ There are several OptiPrep™ Application Sheets that provide methodological details for the isolation and analysis of retroviruses:
- ◆ Human immunodeficiency virus – 1 (HIV-1) and lentivirus vectors – V34
- ◆ Moloney murine leukemia virus – V33
- ◆ Human T-cell lymphotropic virus (HTLV-1) and human endogenous retrovirus (HERV-H) –V31
- ◆ Foamy viruses (*Spumaviridae* genus) – V35
- ◆ Mason-Pfizer Monkey virus – V30
- ◆ Rous sarcoma virus – V29
- ◆ For a complete bibliography of retroviruses see Mini-Review MV04

7. Merkel cell polyoma virus

The high osmolality (low water activity) of CsCl gradients causes severe dehydration of polyoma virus and leads to an artificially high density [44]. Fully encapsidated virus has a density of 1.24 g/ml in iodixanol gradients, against 1.34 g/ml in CsCl gradients. See Application Sheet V11

- ◆ The high viscosity and osmolality of CsCl gradients is considered a big disadvantage for the purification of all viral vectors [45].

8. References

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